RESEARCH ARTICLE

Interactions between Oxidative Stress, Lipid Profile and Antioxidants in Breast Cancer: A Case Control Study

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Abstract

Oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer. The objective of this study was to determine changes in the levels of malondialdehyde (MDA), nitric oxide (NO), total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, total antioxidant capacity (TAC), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) activities in serum samples of breast cancer patients (n=30) and healthy subjects (n=100). MDA and NO levels were found to be increased in breast cancer patients compared to the healthy subject group ($p<0.05$). Total cholesterol and triglycerides were elevated; and HDL-cholesterol level was found to be decreased in the cancer patients as compared to the healthy subjects ($p<0.05$). Compared to the healthy group, both serum TAC levels ($p<0.001$) and activity of SOD and GSH-Px ($p=0.05$) were found to be decreased in the breast cancer patients as compared to the healthy controls. Considering the data presented in this study, we suggest that free radicals induce lipid eroxidation and peroxidation of unsaturated fatty acid with decreased activity of enzymatic antioxidants in breast cancer.

Keywords: Breast cancer - oxidative stress - lipid peroxidation - lipid profile - antioxidants

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Introduction

Breast carcinoma is one of the most common neoplasms in women and is a leading cause of cancer related deaths worldwide. The etiology of breast cancer is multifactorial. Significant breast cancer risk factors include age, early age at menarche, late age of menopause, late age at first pregnancy, obesity, oral contraception, HRT (hormone replacement therapy), diet, family history, lactation and prior history of benign breast disease (Kamal et al., 2012).

Reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, and hydroxyl radicals are capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. These free radicals can evoke extensive tissue damage, reacting with macromolecules, such as membrane lipids, proteins, and nucleic acids (Emerit et al., 2001). Previously, enhanced lipid peroxidation and impairment in antioxidant defence mechanisms have been demonstrated in patients with lung and breast cancers (Kumaraguruparan et al., 2002; Aymelek et al., 2006)

Nitric oxide (NO), which is synthesized by nitric oxide syntheses, is a poly functional signalling molecule controlling processes of vasodilatation, platelet aggregation, immunocytotoxicity, and carcinogenesis. Reactive nitrogen species have been proposed to contribute to multistage carcinogenesis via DNA or tissue damage, mutations and chromosomal aberrations in inflamed tissues (Aymelek et al., 2006).

Breast cancer is considered as disease of opulence which usually funnels to high fat consumption and obesity. Weight gain and obesity causing breast cancer remains unsolved mystery (Padmanabh et al., 2011). Total cholesterol, Triglycerides, LDL-cholesterol, and HDL-C levels are correlated with development of breast cancer in women to explore their possible role prevention of breast cancer.

The levels of free radical molecules are controlled by various cellular defence mechanisms, consisting of enzymatic (glutathione peroxidase, superoxide dismutase) and non-enzymatic components (vitamin E, vitamin C, glutathione) (Senem et al., 2011).

The present study aimed to determine changes in the levels of malondialdehyde (MDA), Nitric oxide (NO), total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, total antioxidant capacity (TAC), activity of both glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in serum of breast cancer patients (n=30) and healthy subjects (n=100).

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Materials and Methods

Patients

Hundred (100) healthy female as control and thirty (30) female newly diagnosed breast cancer patients were involved in this study. The healthy subjects were aged between 56±2.4 years; and breast cancer cases were aged between 58±8.4. Patients with breast cancer were classified using the TNM system. There were 14, 8 and 8 patients classified as stages II, III and IV, respectively. All patients were studied prior to treatment. None of the patients were using oral contraceptives, hormones, or vitamins, and all were non-smokers.

Blood sample

Blood samples from both breast cancer patients and healthy subjects were collected after an overnight fast (12-24 h) into tubes. The samples were centrifuged at 3000x g for 15 min, serum was removed with pipette, and stored at -80^{0}C for the following assays.

Methods

The assays for measurement of malondialdehyde (MDA), nitric oxide (NO), total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were performed as per the manual provided in the Cayman Chemical Company, India.

Serum total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) were measured Enzymatically on the Mispa Nano autoanalyzer using a commercial kit (Agappe, Switzerland).

Statistical analysis

The data for biochemical analysis were expressed as mean±SE. Mann Whitney U-test was used to determine the significance of biochemical parameters among the patient and healthy groups. P value of <0.05 was considered as significant. Data were analyzed using the statistical package program SPSS 10.0.

Results

A total of hundred (100) healthy controls and thirty (30) women were included in the study with histologically proven cases of breast cancer. In our study, 64% patients were rural and 36% were urban. Mean age of controls were 56±2.4 years; and cases were 58±8.4 years respectively ranging from 47 years to 65 years. The age distribution was almost equal among cases and controls (Table 1).

Table 1. General Characteristic of Healthy Controls and Breast Cancer Patients (Cases)

General Characters	Healthy Control	Breast Cancer
Total No. of Subjects	100	30
Age	56±2.4	58±8.4
Age at Menarche	14±2	14±2
Premenopausal Status	25	10
Postmenopausal Status	75	20

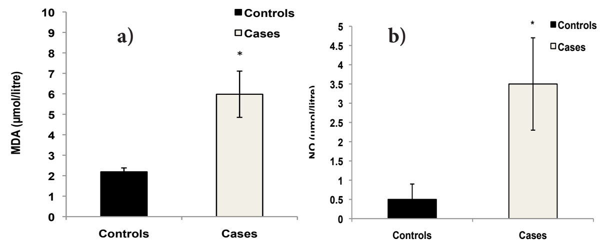

Figure 1. Extent of a) Lipid Peroxidation (MDA) and b) Level of Nitric Oxide (NO) Respectively in Serum of Cases and Controls. The bar diagram represents level of MDA and NO in controls and cases as mean±SE of three individual experiments done in triplicates

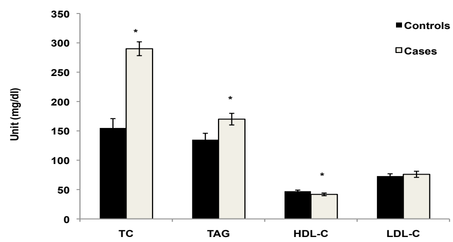

Figure 2. Status of Lipid Profile in Serum of Cases and Controls. The bar diagram represents status of lipid profile in controls and cases as mean±SE of three individual experiments done in triplicates

Majority of patients (14/30, 46.6%) presented in stage II of breast cancer and 26.6% in both stage III and stage IV; and none were in stage I indicating that even rural patients presented in advanced stages.

The level of oxidative stress was determined by measuring the extent of lipid peroxidation (MDA) and concentration of nitric oxide (NO) in cases and controls. Our study showed decreased level of malondialdehyde (MDA) (marker for lipid peroxidation) and NO (nitric oxide) in cases as compared to control. All data were statistically significant (p<0.05). Figure 1 and 2 shows the extent of lipid peroxidation (MDA) and level of NO respectively in serum of cases and control.

Total cholesterol, tryglycerides, LDL-C levels, and

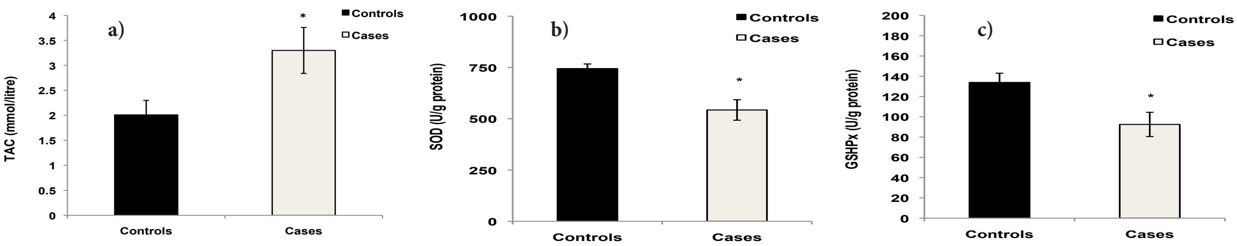

Figure 3. a) Total Antioxidant Capacity, b) Activity of SOD, and c) Activity of GSH-Px Respectively in Cases and Controls. The bar diagram represents activity of SOD and GSH-Px in controls and cases as mean±SE of three individual experiments done in triplicates

HDL-cholesterol, an important component of metabolic syndrome, were compared among cases and controls. Both total cholesterol and triglyceride levels were found to be increased in cases. Total cholesterol level among controls and cases was 155±15.9 and 290±11.8; and triglycerides level was 135±10.9 and 170±9.9. Even though HDL-C among controls and cases (47.2±1.9 and 41.83±2.3 respectively) was in normal range, serum HDL-C levels of cases were significantly lower than controls. The above data were statistically significant ($p<0.05$). However, LDL-cholesterol among controls and cases (72±3.9 and 76±5.2) was not found to be altered statistically significant ($p>0.05$). Our data thus suggest that breast cancer had comparatively low total cholesterol, tryglycerides, and HDL-C levels (Figure 3).

The total antioxidant capacity (TAC) level, activity of both SOD and GSH-Px were measured in serum of cases and was found to be decreased in cases as compared. The above data were statistically significant ($p<0.05$). Figure 3a-3c shows total antioxidant capacity, activity of SOD, and activity of GSH-Px respectively in cases and controls.

Discussion

The role of free radicals, oxidative stress, and lipid peroxidation in carcinogenesis and their contribution to the initiation and progression of the process are well documented (Himmetoglu et al., 2009). In recent years, using MDA as a marker of oxidative stress, there has been a growing interest in studying the role played by lipid peroxidation in cancer progression. MDA is low-molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids. Increased plasma MDA levels have been reported in breast cancer (Kumaraguruparan et al., 2002; Aymelek et al., 2006). Our results showed increase in MDA level in breast cancer as compared to controls thus agreeing with the previous studies, and thus suggesting increased lipid peroxidation in breast cancer patients.

Presentation of nitric oxide in human serum is a well-known phenomenon that points to a crucial role of nitric oxide in physiological and pathological processes. It exhibits a dual role, with regard to the complex mechanism of tumor invasion and metastasis. It could either mediate tumorocidal activity or promote tumor growth (Aymelek et al., 2006). Its presence has been assessed in various human malignant tumors (Thomson et al., 1995; Jannson et al., 1998). Some workers have reported a higher NO syntheses activity in tumors (Thomson et al., 1995), while some have reported a lower activity (Jannson et al., 1998). Our results support the general observation that breast malignancies are associated with an increased level of nitric oxide. In this study, we demonstrated that serum levels of nitric oxide are significantly increased in breast cancer as compared to healthy subject. Increased NO in serum of breast carcinoma may be in response of inflammation.

Breast cancer in women has been associated with changes in serum lipid and lipoprotein levels. Previous study reported that prevalence of decreased serum HDL-cholesterol levels with higher cholesterol and triglyceride levels is increasing in parallel with increasing breast cancer incidence worldwide (Anne-Sofie et al., 2004; Capasso et al., 2011). In our study, when compared with breast cancer patients, total serum cholesterol and triglyceride levels were increased in cases; whereas HDL-cholesterol, though being in the normal range, was found to be decreased in cases as compared to controls. However, LDL-C level did not differ between the two groups. Considering the data presented in this study, we suggest that free radicals induce peroxidation of unsaturated fatty acid in patients with breast cancer and that obesity might be associated with development of breast cancer.

Free radical generation is controlled by a large number of antioxidant systems that act as protection against free radicals. The disturbance of the pro-oxidant/antioxidant balance, resulting from increased free radical production, antioxidant enzyme inactivation, or excessive antioxidant consumption, is a causative factor in oxidative damage. Therefore, measurement of the total antioxidant capacity in biological samples along with activities of various antioxidant enzymes (SOD and G-Px) has been developed. The level of TAC and activity of both SOD and GSH-Px decreased in breast cancer patient as compared to the healthy control. Our report was in accordance with the previous studies (Liu et al., 2003; Badid et al., 2010). Decrease in antioxidants may be due to enhanced accumulations of free radicals.

Considering the data presented in this study, we suggest that free radicals induce lipid peroxidation and peroxidation of unsaturated fatty acid with decreased activity of enzymatic antioxidants in breast cancer; and NO may be increased in response to inflammation. However, studies with more patients and parameters related to oxidative stress, lipid profile, and antioxidants status are required, to explore the association among them, in relation to breast cancer patients and healthy controls.

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