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CLINICAL EVALUATION OF OXIDATIVE STRESS IN WOMEN WITH BREAST CANCER

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Abstract

Breast cancers are potentially life-threatening malignancies in women. Development of cancer produces oxidative stress, which increases with disease progression. Hence, studies on antioxidants may be the most promising area of research for this clinical menace. We analysed serum Uric acid (UA) and Bilirubin (BR) in women with breast cancer. The changes in the levels of serum uric acid and bilirubin are measured in breast cancer patients to assess the oxidative stress. A significant increase in the levels of uric acid and an insignificant increase in the levels of bilirubin was observed in all the three categories of breast cancer patients compared to normal individuals. The results suggested that high ROS production supports the oxidative stress in breast cancer. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy.

Keywords: Free radicals, ROS, Uric acid, Breast cancer

Introduction

Breast cancer is one of the most common cancers in women of the developed and developing countries. Experimental investigations as well as clinical and epidemiological studies implicate the involvement of oxygen derived radicals such as singlet oxygen ($^1O_2$), superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) in the etiology of cancer (Oberley and Oberley, 1989, Fisher *et al*., 1983). Free radicals are formed in both physiological and pathological conditions in mammalian tissues. In healthy conditions at cellular level, a subtle balance exists between the free radical generation and the antioxidant defense. Reactive Oxygen species (ROS) are essential for multiple normal physiological processes like cell differentiation (Abe *et al*., 2000), apoptosis (Ghosh, 1998), cell immunity (Golub *et al*., 1985) and cellular defense against microorganisms (Lajarin *et al*., 1999) at low concentrations. Excess generation of these oxygen free radicals and oxidants generate a phenomenon called oxidative stress which cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis and carcinogenesis. There is accumulating evidence from animal and human systems implicating a role of oxidative stress and lipid peroxidation in the development of breast cancer (Mianying Wang *et al*., 1996). Several studies reported that malondialdehyde; the end product of lipid peroxidation can cause cross-linking in lipids, proteins and nucleic acids (Freeman BA 1982). It is also evident that overproduction of ROS/RNS (Halliwell, 1989, Kang, 2002) plays an important role in the promotion and progression of human cancers, including breast cancer (Aghvami *et al*., 2006, Ray *et al*., 2000, Huang *et al*., 1996, Yeh, 2005). The human body is equipped with certain enzymatic and non-enzymatic antioxidant systems (Faruk Tas *et al*., 2005, Portakal *et al*., 2000). Antioxidants are known to dispose, scavenge, and suppress the formation of free radicals or oppose their action and increase with the severity of the disease (Singh *et al*., 2003, Galleotti *et al*., 1991).

The precise mechanism of the oxidative stress being induced in breast cancer is still not exactly understood and documented. Hence, in the present study an attempt has been made to determine the alteration in oxidant-antioxidant status in breast cancer patients, by estimating the antioxidants like Uric Acid and Bilirubin.

Materials and Methods

This study was conducted at Mahatma Gandhi Cancer and Research Institute and in the Department of Biochemistry, Andhra University, Visakhapatnam. 116 clinically and histopathologically proven breast cancer patients were chosen for the present study. Due permission was obtained from the management of the Mahatma Gandhi Cancer and Research Institute before the commencement of the work. The written consent of the patients was also taken. An equal number of age matched healthy subjects were considered as normal/control. The complete clinical and personal history of the patients was recorded. The subjects were ranging from 30-69 years of age. Patients suffering from diseases of any origin other than breast cancer were excluded from the study.

The study subjects are segregated into four groups so that the age group between 30-39 is referred to as group-1, followed by group-2 from40-49 years of age, then group-3 from 50-59 years and finally group-4

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from the age of 60 to 69 years. The study was carried out in three different categories of breast cancer patients in different clinical conditions.

Category I: The selected study parameters like uric acid and bilirubin levels were analyzed in untreated breast cancer patients undergoing for treatment for the first time.

Each study group (age) was classified into four stages i.e., stage-I, II, III and IV according to Manchester's classification based on a clinical evaluation of the patients case study.

Category II: The study parameters were also monitored continuously in breast cancer patients for more than 2 years time at intervals of 3, 6, 12, 18 and 24 months, irrespective of type of clinical treatment / age/ stage of breast cancer.

Category III: The study parameters were also investigated irrespective of the patient's age and stage of the cancer in 10 patients each, who were undergoing different types of clinical treatments like chemotherapy, surgical removal of breast tissue and radiation.

Blood was collected by venous arm puncture in patients and controls. The blood collected was centrifuged to separate the serum, with which the clinical investigations for the levels of selected antioxidants were carried out. Uric acid was determined by the end point assay described by Trivedi *et al*. (1978) and bilirubin by the method of diazotized sulfanilic acid by Wins ten 1969 respectively. All the chemicals used were of analytical grade.

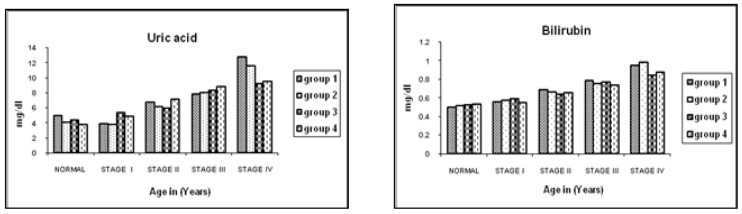
Statistical analysis between the normal individuals and patients was performed by the student *t*-test. The data expressed as Mean ± SD and $p<0.05$ were considered as significant.

Results and Discussion

The Mean values of Uric acid and Bilirubin for category I patients are indicated in the fig. 1, the mean and independent values for the category II patients are indicated in table 1 and the mean values for category III patients are shown in fig. 2 respectively.

In the present study, the association of serum uric acid levels to oxidative stress in breast cancer patients is prospectively investigated. Our results strongly reflect against the proposed antioxidant properties and protective effect of serum UA on breast cancer. The serum UA levels in the category I patients of all age groups with respect to the stage has been observed to be prominent, which suggests a positive association between serum uric acid and oxidative stress. Thus UA contributes to increased life span in humans by providing protection against oxidative stress provoked ageing and cancer. A significant rise of serum uric acid levels were observed in the stages III and IV of all age groups as shown in fig 1. This shows the protective role of serum uric acid in the ageing women with breast cancer. In category II patients the serum UA levels are insignificant compared to the untreated breast cancer patients, which may be due to high oxidative stress which may perhaps lead to the generation of a large number of free radicals was diminished by uric acid as presented in the table 1. Serum UA levels in the category III subjects was observed to be slightly higher compared to the control (fig 2).

Fig 1: Levels of various serum antioxidants in untreated breast cancer patients undergoing treatment for the first time



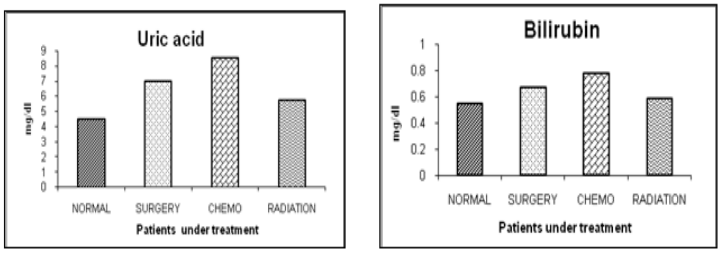
UA-Uric acid, BR-Bilirubin
Group 1: 30-39, Group-2: 40-49, Group- 3: 50-59, Group 4: 60-69
All the values are expressed as Mean.* $p<0.05$ compared to controls

Table 1: Serum antioxidant levels in continuously monitored Breast cancer patients compared to normal

Name of the Antioxidant	Normal	3 Months	6Months	12Months	18Months	24Months
UA	4.0	5.4	6.7	8.7	11.3	13.48
BR	0.53	0.63	0.7	0.76	0.8	0.84

UA-Uric acid, BR-Bilirubin
†All the values are expressed as Mean of 6 subjects

Fig 2: Serum antioxidant levels in Category III breast cancer patients under different clinical treatments like surgery, chemotherapy and radiation compared to normal



‡All the values are expressed as Mean of 10 subjects

Based on the potent antioxidant properties of bilirubin (Jaime Kapitulnik, 2004) and the proposed role of oxidative stress in carcinogenesis have been examined for an association between serum bilirubin levels and stages of breast cancer. In the present study in category I the rate of BR increase was insignificant in patients compared to the control, (fig. 1) suggesting that bilirubin is consumed to cope with oxidative stress (Sedlak Thomas, 2004) and our results also correlate with the reports of Stephen *et al.* (2004) which states that individuals with a prior history of malignancy have a lower mean bilirubin levels than those without cancer supports the hypothesis that bilirubin suppresses carcinogenesis. Serum BR levels in category II patients was observed to be almost in the normal range (table 1) and showed a very low rate of increment which might be due to oxidative stress. In category III patients slightly increased levels were observed when compared to the control (fig. 2).

From the above observations of our study, it is detected that there is an increase in the serum UA levels which show a higher free radical production and insignificant increase in the BR levels are seen. The increased levels of antioxidants like uric acid may be a compensatory regulation in response to this increased oxidative stress. Therefore, exogenous administration of antioxidants may be helpful in the management of breast cancer. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage.

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