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Alterations in Serum SOD and CAT Levels in Patients 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 superoxide dismutase (SOD), catalase (CAT) in women with breast cancer. The changes in the levels of serum superoxide dismutase and catalase are measured in breast cancer patients to assess the oxidative stress. A significant increase in the level of superoxide dismutase and a lower catalase activity 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.

Key words: Free radicals, ROS, SOD, CAT, 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 LW, Oberley TD 1986, Fisher SM *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 JI *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 B, 1989, Kang, 2002) plays an important role in the promotion and progression of human cancers, including breast cancer (Aghvami T *et al.*, 2006, Ray G *et al.*, 2000, Huang YL *et al.*, 1996, Yeh, 2005). The human body is equipped with certain enzymatic and non enzymatic antioxidant systems (Faruk Tas *et al.*, 2005, *Portakal O 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 R *et al.*, 2003, Galleotti T *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 superoxide dismutase (SOD) and catalase (CAT).

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 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 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 superoxide dismutase and catalase levels were analyzed in untreated breast cancer patients undergoing 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 at time 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 are 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. SOD activity was determined by the method of Beauchamp and Fedovich, 1976 based on the enzyme inhibition required for inhibiting the reduction of NBT by 50%. Catalase activity was measured by the titrimetric method described by Radhakrishnan and Sarma, 1963. Protein was estimated by the method of Lowry et al. (1951). 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 $p<0.05$ was considered as significant.

Results and Discussion

The Mean values of SOD and CAT for category I patients are indicated in the fig 1, the mean and independent values for the category II patients are indicated in tables 1 and the mean values for category III patients are shown in fig 2 respectively.

In the present study, we assessed the levels of serum SOD and CAT in patients with breast cancer. In category I patients a significant increase in the serum SOD levels were observed compared to the control. A steady increase in the activity of SOD was observed in the stage-I to stage-IV as reported in the fig 1. The rise in serum SOD activity in the group 2 & 3 of stages III and IV correlates with the progression of malignancy and the release of serum SOD from rapidly multiplying tumor cells may be an effort to protect themselves from the oxidative damage (Seth RK, *et al*, 2003). Superoxide anions are highly reactive and accumulation of these has also been reported in tumor cells (Devi GS *et al*, 2000). The over expression or the high levels of the SOD might be an adaptive response and it results in increased dismutation of the superoxide

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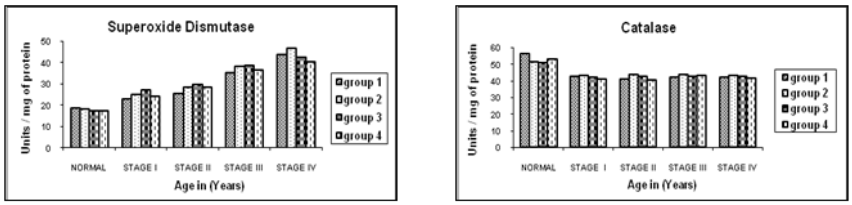
anions to hydrogen peroxide. The SOD activity was observed to have proportionally increased up till group-II with the age and decline in the later age groups. Saxena *et al*, (2006) reported that SOD activity was significantly low in the elderly people as compared to younger people which directs towards reduction in its protective and superoxide radical scavenging action in elderly people. The diminished activity of SOD could be explained on the basis of its progressive enzyme inactivation by resultant product of dismutation reaction i.e., H_2O_2 or due to increase in the glycosylation of SOD with aging. In category II patients the serum SOD activity was observed to be consistent with the above observations (table 1). These results show a correlation between redox status and tumour progression suggesting that up regulation of antioxidants enables tumour cells to counter oxidative stress as reported earlier (Kumaraguruparan R *et.,al*, 2002). Serum SOD levels in category III patients was observed to be higher compared to the normal (fig 2). A similar observation was reported by Carmia Borek (2004), who showed that oxidative stress increases with disease progression and the levels of antioxidants are further decreased in response to the treatment.

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



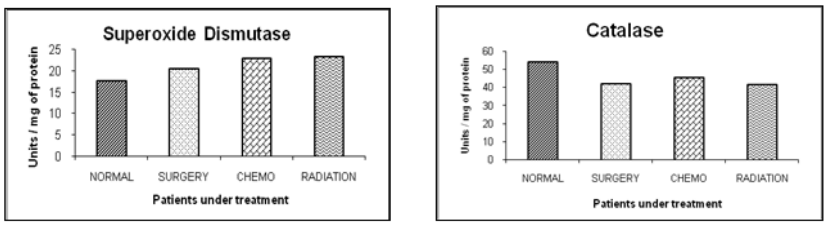
Group 1: 30-39, Group-2: 40-49, Group- 3: 50-59, Group 4: 60-69.
All the values are expressed as Mean.* $p<0.05$ compared to controls

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
SOD	17.55	23.25	26.95	33.8	41.13	43.8
CAT	54.2	47.8	47.7	46.8	46.03	45.15

SOD- Superoxide dismutase, CAT-Catalase
†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

CAT activity has been observed to be relatively insignificant in category I patients as the disease progression compared to the control. Similar studies were also reported by Kumarguruparan *et al., (*2002). However, the catalase activity in stages I, II and III were found to be reasonably low. (fig 1). The decreased activity of CAT may be a compensatory regulation in response to increased oxidative stress. It has also been reported that the low levels of CAT are due to the inactivation of the enzyme by the superoxide anions (Keno Y, 1975). Recently Tas *et al*, 2005 reported a decreased CAT activity in all stages of breast cancer and showed that lipid peroxidation in breast cancer tissue was enhanced compared to non-malignant tissue. Further they reported that higher oxygen free radical production and decreased catalase activity supporting the oxidative stress hypothesis in breast carcinogenesis. CAT activity in category II and category III patients was observed to be lower than the normal healthy women. (table 1 and fig 2). Our results are also similar to the findings of Punnonen *et al*, 1994 and these low levels might be due to treatment by anticancer drugs which reduces antioxidants and induces oxidative stress which increases with disease progression (Carmia Borek, 2004).

From the above observations of our study, it is detected that there is an increase in the serum SOD activity which show a higher free radical production and a decrease in the CAT levels are seen. The decreased levels of CAT results in accumulation of large amounts of hydrogen peroxide resulting in higher production of $OH^{\bullet}$ radicals resulting in oxidative stress in breast cancer. The increased activities of antioxidants enzymes 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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