

Blood Superoxide Dismutase and Catalase Activities in Women Affected with Breast Cancer

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(Received 30 Nov 2004; revised 9 Aug 2005; accepted revised 9 Aug 2005)

Abstract

Experimental and epidemiological evidences implicate the involvement of oxygen derived radicals in the pathogenesis of cancer development. Oxygen derived radicals are able to cause damage to membranes, mitochondria and macromolecules including proteins, lipids and DNA. Accumulation of DNA damages has been suggested to contribute to carcinogenesis. It would, therefore, be advantageous to pinpoint the effects of oxygen derived radicals in cancer development. We investigated superoxide dismutase (SOD) and Catalase (CAT) activities in the whole blood of 50 breast cancer (BC) patients and 50 healthy and age matched women. The rate of SOD and CAT activities in BC patients was significantly lower ($P < 0.001$) than controls. No effect of stage on SOD and CAT activities was observed. The results of our study have shown a higher reactive oxygen species (ROS) production and decreased SOD and CAT activities, which support the oxidative stress hypothesis in carcinogenesis. The relative lower SOD and CAT activities may not be adequate to detoxify high levels of H_2O_2 into H_2O leading to the formation of the most dangerous $^{\bullet}OH$ radical. Therefore, administration of antioxidants may be helpful in the management of BC patients. However, elaborate clinical studies are required to evaluate the role of such antioxidant enzymes (AOE) in BC management.

Keywords: Breast cancer, Catalase, Superoxide dismutase, ROS, Iran

Introduction

Breast cancer is one of the most common cancers in women of the developed and developing countries (1). Breast cancer incidence rates are increased among women of all races combined and white women since the early 1980s (2). Experimental investigation as well as clinical and epidemiological findings has provided evidence supporting the role of reactive oxygen metabolites (ROMs) such as singlet oxygen (O_2^0), super oxide anions ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) in the etiology of cancer (3). Under normal circumstances, major sources of ROMs in cell are electron leakages from electron transport chains in mitochondria and endoplasmic reticulum (4).

In the Middle East, breast cancer was the most common malignancy among women (5). In Iran, breast cancer ranks the first among cancer of women comprising 21.4 percent of all malignancy in females (6). In Tehran, Capital of Iran, breast cancer is again the most common cancer among women (25.5 percent of total) (6). In Iran, breast cancer affects women at least one decade younger than their counterparts in developed countries (7). The exact cause of breast cancer is not completely known but presently it represents a complex interplay of genetic and environmental factors (8, 9). In inflammation and other pathological conditions, stimulated polymorphonuclear leukocytes and monocytes produce a large amount of H_2O_2 in

respiratory burst pattern. Substantial amount of H_2O_2 is also produced by human tumor cells (4). The ROMs have a wide range of cellular and molecular effects resulting in mutagenicity, cytotoxicity and changes in gene expression (10). Experimental evidence reveals that ROMs are involved in initiation and promotion of carcinogenesis, where inactivation or loss of certain tumor suppressor genes is occurred (10). G-C base pairs in CpG dinucleotide sequences as a common site for point mutations in the p53 tumor suppressor gene linked to breast and other site specific cancers (11). Cellular genes are usually converted in to oncogenes, particularly ras family oncogenes in codons 12 and 13 (12). These G-C sites have been demonstrated as the main targets of oxidative damage (13), thereby resulting in the formation of 8-oxo- G, which is a mediator of mutagenesis (14). H_2O_2 is known to cause DNA breaks in intact cells and purified DNA (15). The DNA damage caused by ROMs has been demonstrated in the form of base damage (15), single - strand and double- strand breaks (16), cross-linking between DNA, chromosomal aberrations, and sister chromatid exchanges (17). Certain aldehyde such as malondialdehyde (MDA), the end product of lipid peroxidation (LPO), arising from the free radical degradation of polyunsaturated fatty acids, and cause cross-linking in lipids, proteins and nucleic acids (18). Human body is equipped with various antioxidants viz. superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) which can counteract the deleterious action of ROMs and protect from cellular and molecular damage (19). The present study was conducted to evaluate the possible role of $O_2^{\cdot-}$, H_2O_2 , and the primary antioxidant enzymes in the pathogenesis of breast cancer.

Materials and Methods

We assayed SOD and CAT activities in whole blood from 50 untreated breast cancer patients attending surgical units of Imam Khomeini

Hospital in Tehran, Iran, and 50 healthy women. Consent was taken from patients and involved doctors before blood collection. The patients aged between 28 and 69 yr (mean 44.5, $SD \pm 10.54$ yr). They were categorized as premenopausal, postmenopausal, stage I, stage II, stage III. Blood samples from 50 females, aged between 28 and 69 yr (mean 46.7, $SD \pm 9.5$) without breast disease or any other malignancy were considered as controls.

Blood lysates preparation We collected 5 ml of peripheral blood in EDTA tubes, then we removed 1 ml aliquots to determine the hemoglobin (Hb) concentration with a standard kit involving the cyanmethemoglobin method (drabkin's method).

The remaining blood was washed three times with 9 g/l NaCl solution. Then lysed with cold dionized water. Cell membranes were removed by centrifugation at $4000 \times g$ for 5 min at $4^\circ C$ and the supernates were used for determining AOE activity. The Hb concentration was also determined in the hemolysates, which were then stored in $-70^\circ C$ until enzyme assays. AOE activity of blood samples was measured within 1 week of sampling.

Enzymatic determination of CAT (Ec 1. 11. 1. 6) CAT activity was determined according to Hygo Aebi (19). Activity of CAT was determined by following the decomposition of H_2O_2 in phosphate buffer pH 7.2 spectrophotometrically at 230 nm.

One unit of CAT is defined as the amount of enzyme which liberates half the peroxide oxygen from a H_2O_2 solution in 100s at $25^\circ C$. Enzyme activity was expressed as units per mg of Hb (U/mg Hb).

Enzymatic determination of SOD (Ec 1. 15. 1. 1) SOD activity was assayed by kit RAN-SOD (cat.NO.SD 125). The role of SOD is to accelerate the dismutation of the toxic superoxide radical ($O_2^{\cdot-}$), to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthinoxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl) -3-(4-nitrophenol) -5-phenyltetrazolium

chloride (I. N. T.) to form a red formazan dye. The SOD activity is then measured by the degree of inhibition of this reaction.

One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the condition of the assay. Enzyme activity was expressed as SOD units/gHb DATA analyses.

Descriptive statistics were applied for calculating the distribution of various characteristics. An unpaired students *t*-test and one way Anova were applied to determine the significance of various biochemical changes between different groups of breast cancer patients and their respective controls.

Results

SOD and CAT activities significantly decreased in BC patients. Fig.1 shows that catalase activity was significantly ($P<0.001$) lower in BC patients (85 ± 5.8 KU/g Hb) than in controls ($134 + 4.3$ KU/g Hb) (Table 1).

We observed also that SOD activity was significantly ($P<0.001$) lower in BC patients (1132.5 ± 236 U/g Hb) than in controls (1412 ± 218 U/g Hb).

The activities of SOD and CAT in BC patients were estimated in relation to different clinical stages (Table 2). Out of the total BC patients ($n= 50$), 10 (20%) were stage I, 27 (54%) were stage II and 13 (26%) were stage III. No significant difference was observed among the clinical stages for CAT. SOD activity decreased among BC patients respective of clinical stage but this decrease was not significant.

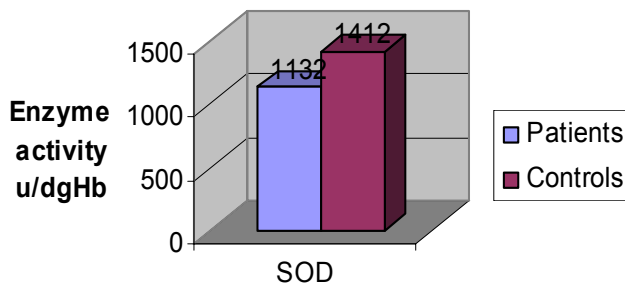


Fig. 1: Comparison of SOD activity of breast cancer patients and controls

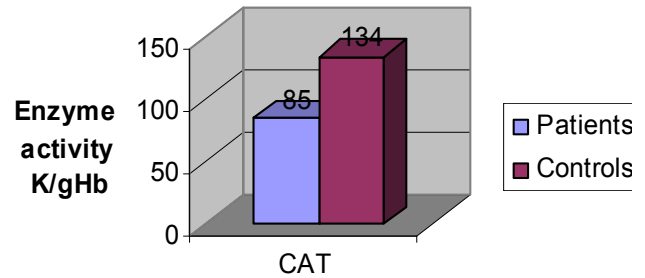


Fig. 2: Comparison of catalase activity of breast cancer patients and controls

Table 1: CAT and SOD activities in patients with breast cancer and controls

Enzyme	Controls n=50 (mean \pm SD)	Subjects n=50 Mean \pm SD	P. value
CAT (KU/g Hb)	134 ± 4.3	85 ± 5.8	$P<0.001$
SOD (U/g Hg)	1412 ± 218	1132.5 ± 236	$P<0.001$

Table 2: CAT and SOD status in different stages of breast cancer patients

Total patients n=50	Stage 1 n=10 Mean \pm SD	Stage 2 n=27 Mean \pm SD	Stage 3 n=13 Mean \pm SD
CAT (KU/g Hb)	66 ± 2.8	113 ± 7.4	86 ± 3.2
SOD (U/g Hb)	1222 ± 276	1152 ± 250	1021 ± 117

Discussion

The development of cancer in individual is the result of multiple genetic and epigenetic steps. Carcinogens and tumor promoters can act by a variety of molecular mechanisms, and repeated exposure to numerous agents is frequently necessary for the formation of cancer.

A single cell can develop from an otherwise normal tissue into a malignancy that can eventually destroy the organism. Active oxygen species and other free radical have long been known to be mutagenic. Free radical production is ubiquitous in all respiring organisms, and is enhanced in many disease states by carcinogen exposure, and under conditions of stress.

Oxygen free radicals, which are generated through several enzymatic and non enzymatic biological reactions in aerobic organisms, attack a wide variety of macromolecules such as lipid, protein, carbohydrate and DNA. Fenn and his colleagues (20) hypothesized that mutagenicity of oxygen led to chromosomal damage resulting from an increased free radical production. Several reports have demonstrated increased production of ROMs in various pathophysiological conditions (21).

In the present study the status of the antioxidants superoxide dismutase and catalase were estimated in BC patients and controls. We observed that SOD and CAT activity were decreased in BC patients showing that production of free radicals was higher. The decreased activities of SOD and CAT have also been reported in other malignancies (22, 23). The increase in erythrocyte lipid peroxidation in BC patients (24) correlates with the decline in SOD and catalase activity. SOD and CAT are considered primary antioxidant enzymes, since they are involved in direct elimination of ROMs SOD, GPX. They also can act as anti-carcinogens, and inhibitors at initiation and promotion/ transformation stage in carcinogenesis. Mutation caused by potassium super oxide in mammalian cells can be blocked by SOD (25). CAT was found to be important in the inactivation of many environmental mutagens. Plasmid DNA strand scission caused by xanthine/ xanthine oxidase was prevented by SOD and CAT enzymes. CAT also prevented chromosomal aberration caused by hypoxanthine/xanthine oxidase in Chinese hamster cells. It also prevented the onset of spontaneous neoplastic transformation in mouse fibroblast and epidermal keratinocytes (25). Our results show that BC patients have lower levels of SOD and CAT than healthy women. These findings are controversial with results that did not detect differences in SOD or CAT activities in subjects. However, these controversial findings may be partially due to differences in the enzymatic assays used and/or sample size. Same size may

be a critical factor in determining the statistical significance of the differences observed.

Recently (26) Tas et al. showed that lipid peroxidation in BC tissue was enhanced compared to nonmalignant tissues. They showed higher oxygen free radical production and decreased CAT activity supporting the oxidative stress hypothesis in breast carcinogenesis. CAT activity in our study showed a significant decrease in all the stages of BC patients.

A correlation between tissue redox status and tumor progression suggesting that up regulation of antioxidants enables tumor cells to counter oxidative stress was observed (27).

Administration of antioxidant enzymes particularly CAT may be helpful in the management of breast cancer. However further clinical studies are required before a definitive conclusion to be drawn. As discussed above, altered antioxidant enzymes have been found in many tumors. One critical question is whether this abnormality is one of the causes of cancer or is just one of the consequences of carcinogenesis, this question remains to be answered by additional research.

Acknowledgements

This work was supported by Vice-chancellor for Research, TUMS, Iran. The authors are grateful to the labs of Imam Khomeini Hospital, for providing us with blood samples.

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