Free Radical Research, Volume 38 Number 1 (January 2004), pp. 81–85

Taylor & Francis
healthsciences

Antioxidant Enzymes and Lipid Peroxidation in Different Stages of Breast Cancer

SHRUTI S. KHANZODE[a,*], M.G. MUDDESHWAR[b], SUCHET D. KHANZODE[c] and GANESH N. DAKHALE[c]

[a]*Biochemist in Medicine, Government Medical College, Nagpur, India;* [b]*Department of Biochemistry, Government Medical College, Nagpur, India;* [c]*Department of Pharmacology, Government Medical College, Nagpur, India*

Accepted by Professor F. J. Kelly

(Received 1 August 2003; in revised form 2 October 2003)

Oxidative stress resulting from an imbalance between pro-oxidants and anti-oxidants seems to play an important role in human breast carcinogenesis. There are conflicting reports regarding the tissue levels of malondialdehyde (MDA), ascorbic acid and superoxide dismutase (SOD) in breast cancer patients whereas few blood values have been reported. The present study was carried out to observe the changes in serum MDA, serum SOD and plasma ascorbic acid with the stage-wise progression of the disease. Serum MDA and serum SOD levels were found to be increased gradually from Stage I to Stage IV as compared to control group ($p < 0.001$). The maximum rise was in Stage IV patients. In contrast, mean plasma ascorbic acid levels were low in all stages compared to control group ($p < 0.001$). The decrease was more pronounced in Stage III and Stage IV. The study would be of immense help for establishing blood based biochemical marker in breast cancer patients.

Keywords: Superoxide dismutase; Ascorbic acid; Malondialdehyde; Breast cancer

INTRODUCTION

Formation of reactive oxygen species is a normal consequence of variety of essential biochemical reactions and an adequate range of anti-oxidative defences within and outside the cell has also been considered very important to offer protection against oxidative damages.[1] Therefore, balance between free radical activity and anti-oxidant defence system becomes an important requirement to prevent the damage to the cellular membrane.[2] Breast cancer is one of the most common cancers in females and is exceeded in incidence by only carcinoma of the cervix.[3] Although the incidence of breast cancer is increasing, knowledge about it's early detection, progression and spread is inadequate. Despite the recent advances in surgery, chemotherapy and radiotherapy the disease continues to be the second largest killer in females. The basic difficulty in treatment is it's late detection and rapid spread to other organs. The present clinical approaches for cancer diagnosis and management are useful only in advanced stages of disease. In view of this problem, the availability of suitable blood-based biochemical markers would be of immense help.

Previous investigators in this area have identified disturbances in particularly tissue levels of MDA, SOD results however remain inconclusive and contradictory.[4,5] Besides, tissue studies require clinical expertise and advanced laboratory setup, which is lacking in developing countries like India. In addition, blood MDA, SOD and ascorbic acid data are scant and their association with the stage wise progression of breast cancer remains unaddressed.

We undertook the present study with the purpose of describing the changes, if any, in serum levels of MDA, which represents membrane changes, serum levels of SOD which is an endogenous antioxidant enzyme and plasma level of ascorbic acid, an antioxidant vitamin and their correlation with the stage wise progression of breast cancer.

*Corresponding author. 46, S.E. Rly Colony (1), Venkatesh Apartment, Pratap Nagar, Nagpur-440 022, Maharashtra, India. Tel.: +91-0712-2228118. Fax: +91-0712-2241119/2241669. E-mail: suchetkhanzode@rediffmail.com; gndakhle@rediffmail.com

ISSN 1071-5762 print/ISSN 1029-2470 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/01411590310001637066

MATERIALS AND METHODS

Patients

This case control study was undertaken at Govt. Medical College, Nagpur, India for evaluation of oxidant–antioxidant status was comprised of 176 female subjects. Among them, 96 were newly diagnosed breast cancer patients of different clinical stages who had not received any chemotherapy or radiotherapy treatment before diagnosis. In addition, eighty age and sex matched normal healthy controls were recruited for comparison. Of the 96 untreated breast cancer patients, 20 were of stage-I, 24 were of stage-II, 32 were of stage-III and 20 patients were of stage-IV. The assessment of staging was based on clinical and histopathological evaluation. Patients with other malignancies and diseases, which affect oxidant and antioxidant status, were excluded from the study. Lipid profiles of the patients were known. We included only those patients with normal lipid profiles [Patient group: trigly cerides (1.12–2.83 μmol/l) and total cholesterol (2.30–4.32 μmol/l); Control group: triglycerides (1.03–2.64 μmol/l) and total cholesterol (2.56–4.18 μmol/l)]. We excluded patients with diabetes and hypertension.

Measurements

Five millilitre of fasting blood was taken for the routine investigation as well as estimation of MDA and SOD. Serum MDA estimation[6] was carried out using clear serum incubated in trichloroacetic acid and thiobarbituric acid and the mixture was heated in boiling water bath for 30 min. The resulting chromogen was extracted with *n*-butanol, the absorbance of which was measured at 530 nm. MDA was expressed as umol per litre of serum. Serum SOD was measured by modified method of Nischal.[7] Absorbance was recorded at 420 nm in a spectrophotometer. One unit of SOD was described as the amount of enzyme required to cause 50% inhibition of pyrogallol autooxidation per 3 ml assay mixture.

Plasma ascorbic acid was estimated by a single step colorimetric method[8] using modified acid phosphotungstate reagent. Fresh plasma was slowly mixed with the modified acid phosphotungstate and incubated at room temperature for 30 min and then centrifuged. Supernatant was used to measure absorbance at 700 nm. Standards of pure ascorbic acid obtained from Sigma Company, USA in the range of 0.10 to 0.90 mg% (5.67–51.13 μmol/l) were prepared in freshly prepared 0.5% oxalic acid solution. For every set, standard and blanks were run throughout the procedure. For all the investigations chemicals used were of analytical reagent grade. The values of serum SOD, MDA and plasma ascorbic acid of patients suffering from breast cancer were compared with those of healthy control subjects.

Protocol of the trial was submitted to institutional ethical committee. Trial was started after the approval from institutional ethical committee. After obtaining informed consent, patients were recruited in the trial.

Statistics

Results were expressed as mean ± SD. Statistical analysis of data was performed by using a one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test. The p value less than 0.05 were adjudged statistically significant. Statistical calculations were done by using Graph Pad Prism, Version 3.02. Relationship between variables was ascertained by Pearson's correlation coefficient.

RESULTS

To test whether breast cancer is associated with increased oxidative stress and lipid peroxidation, we compared values of serum MDA of breast cancer patients in different clinical stages with normal healthy controls. In all 96-breast cancer patients, the serum concentration of malondialdehyde was significantly increased as compared to normal controls ($p < 0.001$) (Table I). The concentration of serum MDA increased significantly with clinical progression of the disease from stage-I to stage-IV. The maximum rise being observed in stage-IV.

To determine whether levels of serum SOD and plasma ascorbic acid is affected with increase of staging in breast cancer patients we compared them with healthy controls. Significant change was revealed in mean serum SOD levels of breast cancer patients compared to normal subjects ($p < 0.001$) (Table II).

On the contrary mean plasma ascorbic acid level was found significantly lowered in breast cancer

TABLE I Serum MDA in control and different stages of breast carcinoma

Group	Number of patients	Mean ± SD (μmol/l)	Range
Normal control	80	1.74 ± 0.64	0.82–2.46
Stage I	20	6.93 ± 1.83*	1.36–8.65
Stage II	24	6.47 ± 2.06*	2.59–8.32
Stage III	32	6.55 ± 2.17*	2.12–9.00
Stage IV	20	7.29 ± 2.01*	3.85–10.76
One-way ANOVA		F=112.62 $p < 0.0001$	

Values are expressed as mean ± SD, degree of freedom (4,171). *$p < 0.001$ as compared to control group.

TABLE II Serum SOD in control and different stages of breast carcinoma

Group	Number of patients	Mean ± SD (μ/ml)	Range
Normal control	80	2.00 ± 0.87	1.06–4.00
Stage I	20	9.00 ± 1.02*,†	4–11
Stage II	24	10.00 ± 1.66*,#	6–14
Stage III	32	11.03 ± 2.59*,##	4–13
Stage IV	20	14.45 ± 1.70*	7–19
One-way ANOVA		F=411.9 $p < 0.0001$	

Values are expressed as mean ± SD, degree of freedom (4,171), *$p < 0.001$ as compared to control group, †, #$p < 0.001$ as compared to stage IV, ##$p < 0.01$ as compared to stage IV.

patients of all stages as compared to the control group ($p < 0.001$). The decrease was maximum in stage-IV of breast cancer patients (Table III).

Significant and positive correlation was found between serum MDA and serum SOD levels ($r = 0.27$, $p < 0.01$). Also, significant and negative correlation existed between serum SOD and plasma ascorbic acid levels ($r = -0.23$, $p < 0.05$), serum MDA and plasma ascorbic acid levels ($r = -0.26$, $p < 0.01$).

DISCUSSION

To the best of our knowledge this is the first reported study of status of serum MDA, serum SOD and plasma ascorbic acid levels with stepwise clinical progression of breast cancer with a large sample size. Breast cancer is a complex disorder resulting from oxidative stress as a result of imbalance between pro-oxidants and antioxidants. Damage to DNA, proteins, cell membrane and mitochondria play an important role in breast carcinogenesis. Although the incidence of breast cancer is on increase; knowledge about its early detection, progression and spread remains inadequate. No specific biochemical marker has been identified yet. In addition information regarding the biochemical alterations in tissue, serum and blood, particularly of antioxidant status, and its correlation with the clinical staging of disease is lacking.

TABLE III Plasma ascorbic acid in control and different stages of breast carcinoma

Group	Number of patients	Mean ± SD (μmol/l)	Range
Normal control	80	37.50 ± 2.27	30.68–44.32
Stage I	20	31.25 ± 2.84*,†	18.75–36.93
Stage II	24	28.98 ± 9.65*,#	21.60–38.07
Stage III	32	27.27 ± 4.54*	15.99–32.95
Stage IV	20	23.30 ± 13.06*	18.19–34.09
One-way ANOVA		$F = 31.67$ $p < 0.0001$	

Values are expressed as mean ± SD, degree of freedom (4,171), *$p < 0.001$ as compared to control group, †$p < 0.01$ as compared to stage IV, #$p < 0.05$ as compared to stage IV.

In the present study, serum malondialdehyde levels in the breast cancer patients were significantly higher than those of control, moreover they increased with clinical staging of the disease. This finding is in accordance with previous work that reported increased plasma MDA concentration in breast cancer patients.[9] Oxygen radical production, which increased with clinical progression of disease, involved increased lipid peroxidation as a result of which there is cellular membrane degeneration and DNA damage. Extent of lipid peroxidation is determined by estimation of malondialdehyde, a compound known to produce protein cross-linking through schiff's base formation. Malondialdehyde also forms schiff's base with DNA and damages DNA.

Increased oxidative stress plays an important role in initiation, promotion and metastasis in breast cancer.[10] Although the exact cause of breast cancer is not known; most of these cancers are hormones sensitive. Estrogen and estrogen metabolites play an important role in breast carcinoma. Effectiveness of antiestrogen therapy, the positive association of breast cancer incidence with reproductive events and the dramatic effect of oophorectomy on lowering the incidences of breast cancer suggest a close relation between estrogen and breast cancer.[11] High levels of plasma and urinary estrogens are observed in human breast cancer patients.[12] Breast being a fatty organ, role of unsaturated fatty acid in breast carcinogenesis cannot also be ruled out. Both estrogen and polyunsaturated fatty acid generate reactive oxygen species. Estrogen and its metabolites like estrone 16, –OH estrone and estradiol, increase the risk of breast cancer by generating superoxide radicals via redox cycling pathway to produce increased uncontrolled proliferation of mammary cells and DNA damage.[13] Increased formation of 8-OH-guanosine, which is mutagenic and carcinogenic, has been reported in such patients.[14]

Another important finding of this study was significant increase in serum superoxide dismutase levels in all stages of breast cancer patients as compared to the control group the maximum being at stage-IV. As a result of increased oxidative stress coupled with membrane damage due to lipid peroxidation, superoxide dismutase, which is a scavenger of oxygen radicals, might have increased in serum as a compensatory mechanism.[14] Superoxide dismutase enhances the cytotoxic ability of macrophages to scavenge superoxide radicals in these patients.[15] Antioxidant defence against free radical generation is through SOD and ascorbic acid. The ascorbic acid synthesizing capacity in man

is negligible and man totally depends on the dietary source for ascorbic acid.[16] The SOD is mainly intracellular and a very little amount is present extracellularly,[15] whereas vitamin C is present both extracellularly and intracellularly, and thus present at the site of production and also at the site of action of ROS in adequate amount. It scavenges oxyradicals intracellularly as well as extracellularly. As a chemical scavenger, it prevents oxy radical induced cellular damage. It has been observed that ascorbic acid deficiency results in accumulation of lipid peroxides, which is a resultant product of lipid peroxidation.[17]

Ascorbic acid plays an important role in synthesis of connective tissue protein such as collagen, and deficiency of it therefore affects the integrity of intracellular matrix and has a permissive effect on tumour growth. Deficiency hinders tumour encapsulation.[18] In the present study, plasma ascorbic acid levels were significantly lower in patients than that in control subjects. Levels decreased with progression of disease and were therefore highest in stage I and lowest in stage IV. Our results confirmed findings of previous study which reported maximum decrease in plasma vitamin C levels in stage IV breast cancer patients.[19]

Decrease in plasma ascorbic acid and increase in serum MDA levels indicate close correlation between plasma ascorbic acid and lipid peroxidation. Although the exact protective role of ascorbic acid or vitamin C in breast cancer is not known, several epidemiological studies have reported protective effect of fruits and vegetables, the rich source of vitamin C, in breast cancer patients.[20] The action of vitamin C may be related to its function as an antioxidant, action on immune system and collagen proliferation to control invasive potential of neoplasm.[21] Howe *et al.*[22] have found consistent decrease in risk of breast cancer with vitamin C intake. The vitamin C supplementation in these patients, however, yielded inconsistent result.[23] There is sparse literature available on association of high-risk breast cancer patients with biological markers of oxidant and antioxidant status. Although we have tried to establish a continuous link between normal, carcinoma *in situ* and cancer breast patients, but failed to reach to a concrete conclusion due to nonavailability of patients of carcinoma *in situ* because of lack of awareness in local population and late detection of breast cancer. There are reports, which suggest increased urinary MDA and 5-hydroxymethyl-2′-deoxyuridine levels in carcinoma *in situ* patients.[24,25]

Our study can be useful to establish blood based biochemical index for diagnosing and monitoring the course of breast cancer. As serum represents metabolic activity in both neoplastic and normal cells, it may be more reliable than the tissue studies. Evaluation of such markers as serum MDA, serum SOD and plasma ascorbic acid would certainly be useful and supportive for investigations in pre-cancerous and cancerous condition in addition to conventional methods of diagnosis of breast cancer. In future, more information on these biochemical changes would be valuable for developing new and innovative therapeutic strategies for prevention of breast cancer.

References

[1] Sun, Y. (1990) "Free radicals, antioxidant enzymes and carcinogenesis", *Free Radic. Biol. Med.* **8**, 583–599.
[2] Yeolekar, M.E. and Nargund, M.P. (1994) "Free radicals in human disease and the role of antioxidants", *Indian Pract.* **27**, 377–390.
[3] Robbins, S., Marcia, A. and Vinay, K. (1990) "Clinical aspects of Neoplasia", *Basic Patholog*, 3rd Ed. (Saunders WB Co., Japan), pp 564–595.
[4] Huang, Y.L., Sheu, J.Y. and Lin, T.H. (1991) "Association between oxidative stress and changes of stress elements in patients with breast cancer", *Biochem. Int.* **10**, 185–190.
[5] Punnonen, K., Ahotupa, M., Asaishi, K., Hyoty, M., Kudo, R. and Punnonen, R. (1994) "Antioxidant enzyme activities and oxidative stress in human breast cancer", *J. Cancer Res. Clin. Oncol.* **120**, 374–377.
[6] Satoh, K. (1978) "Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method", *Clin. Chim. Acta* **90**, 37–43.
[7] Nischal, H.K., Sharma, M.P., Goyal, R.K. and Kanshik, G.G. (1998) "Serum superoxide dismutase levels in diabetes mellitus with or without microangiopathic complications", *J. Assoc. Physicians India* **46**, 853–855.
[8] Kyaw, A. (1978) "A simple colorimetric method for ascorbic acid determination in blood plasma", *Clin. Chim. Acta* **86**, 153–157.
[9] Gonenc, A., Ozkan, Y., Torum, M. and Simsek, B. (2001) "Plasma malondialdehyde (MDA) levels in breast and lung cancer patients", *J. Clin. Pharmacol. Ther.* **26**, 141–144.
[10] Djuric, Z., Carleen, K.E. and Domenico, A.L. (1993) "Toxicity, single strand breaks and 5-hydroxymethyl-2′-deoxyuridine formation in human breast epithelial cells treated with hydrogen peroxide", *Free Radic. Biol. Med.* **14**, 541–547.
[11] Goldin, B.R. and Sherwood, L. (1998) "Effect of diet on the plasma levels, metabolism and excretion of estrogens", *Am. J. Clin. Nutr.* **48**, 787–790.
[12] Morreal, C.E., Dao, T.L., Nemoto, T. and Lonegan, P.A. (1979) "Urinary excretion of estrone, estradiol and estriol in postmenopausal women with primary breast cancer", *J. Natl Cancer Inst.* **63**, 1171–1174.
[13] Yager, J.D. (1996) "Molecular mechanism of estrogen carcinogenesis", *Annu. Rev. Pharmacol. Toxicol.* **36**, 203–232.
[14] Konat, G.W. and Wiggins, R.C. (1985) "Effect of reactive oxygen species on myelin membrane proteins", *J. Neurochem.* **45**, 113–1118.
[15] Umeki, S., Sumi, M., Nikki, Y. and Soejima, R. (1987) "Concentrations of superoxide dismutase and superoxide anion in blood of patients with respiratory infections and compromised immune systems", *Clin. Chem.* **33**, 2230–2233.
[16] Chaterjee, I.B. and Nandi, A. (1991) "Ascorbic acid: a scavenger of oxyradicals", *Indian J. Biochem. Biophys.* **28**, 233–236.
[17] Kucuk, O., Churley, M. and Goodman, M.T. (1994) "Increased plasma level of cholestrol-5b,6b epoxide in endometrial cancer patients", *Cancer Epidemiol. Biomark. Prev.* **3**, 571–574.

[18] Steinmetz, K.A. and Potter, J.D. (1996) "Vegetables, fruits and cancer prevention: A review", *J. Am. Diet. Assoc.* **96**, 1027–1039.
[19] Ray, G. and Husain, S.A. (2001) "Role of lipids, lipoproteins and vitamins in women with breast cancer", *Clin. Biochem.* **34**, 71–76.
[20] Freudenheim, J.O., Marshall, J.R. and Vena, J.E. (1996) "Premenopausal breast cancer risk and intake of vegetables, fruits and related nutrients", *J. Natl Cancer Inst.* **88**, 340–347.
[21] Cameron, E., Pauling, L. and Lebowitz, B. (1979) "Vitamin C and cancer", *Cancer Res.* **39**, 663–666.
[22] Howe, G.R., Hirohata, T., Hislop, T.G., Iscovich, M.J., Yuan, J.M. and Katsouyanni, K. (1990) "Dietary factors and risk of breast cancer: combined analysis of 12 case control studies", *J. Natl Cancer Inst.* **82**, 561–569.
[23] Hennekens, C.H., Buring, J.E. and Petro, R. (1994) "Antioxidant vitamins-benefits not yet proved", *N. Engl. J. Med.* **330**, 1080–1081.
[24] Boyd, N.F. and McGuire, V. (1991) "The possible role of lipid peroxidation in breast cancer risk", *Free Radic. Biol. Med.* **10**, 85–190.
[25] Djuric, Z., Heilbrun, L.K., Lababidi, S., Berzinkas, E., Simon, M.S. and Kosir, M.A. (2001) "Levels of 5-hydroxymethyl-2′-deoxyuridine in DNA from blood of women scheduled for breast biopsy", *Cancer Epidemiol. Biomark. Prev.* **10**, 147–149.