Antioxidants and Lipid Peroxidation Status In Women with Breast Cancer

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ABSTRACT

Introduction: Breast cancer is one of the most common malignancy in women and is the leading cause of cancer related death worldwide. Reactive oxygen species (ROS) play an important role in the development of tumours. Several mechanisms leading to oxidative stress have been proposed in cancer patients. In this study we aimed to measure and compare the levels of zinc, superoxide dismutase, catalase, vitamin C, vitamin E, and malondialdehyde in breast cancer patients and age matched healthy controls. **Methods**: In this comparative cross sectional study, 30 confirmed breast cancer cases and 30 age and sex matched controls were enrolled after taking their consent. Blood sample was collected in EDTA vial, and the parameters of oxidative stress were evaluated by spectrophotometric and atomic absorption spectrophotometric methods. Results: Zinc and MDA level were significantly higher ($p<0.001$) in cases (106.4 µg/dl and 7.12 nmol/ml) compared to controls (89.8µg/dl and 3.99nmol/ml). In contrast, there were lower catalase, vitamin C and E levels in cases (28.7±14.56, 0.92±0.35 and 0.68±0.13 mg/dl) compared to controls (37.1±14.7,1.62±0.59 and 1.02±0.22 mg/dl) and were statistically significant ($p<0.05$ and $p<0.001$). Whereas, superoxide dismutase was lower in cases compared to controls, but was not statistically significant. **Conclusion**: Zinc was found to be higher in our study as it is a pivotal element in all rapidly growing tissues. Enhanced lipid peroxidation observed in the circulation of breast cancer patients in our study can be attributed to over production of ROS and deficiency of antioxidant defences. Oxidative stress in cancer cells may trigger excessive consumption of antioxidants as a compensatory mechanism which lead to its lower concentration.

KEYWORDS: Breast cancer, zinc, superoxide dismutase, catalase, malondialdehyde

INTRODUCTION

The aerobic metabolism, occurring in the cell leads to generation of small amount of reactive oxygen species (ROS) in organisms.[1] ROS acts like a double edge sword. Small amount of ROS are indispensable for proper biochemical processes, however excessive production or inefficient removal leads to oxidative stress that may can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis, and carcinogenesis.[2] Body has develop an efficient method to tackle with these free radicals by various cellular defence mechanisms, consisting of enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and non-enzymatic (vit. E, vit.C, glutathione, carotenoids and flavonoids) components.[3]

Breast cancer is one of the most common cancer in women and is the leading cause of cancer related death worldwide.[4] The exact aetiology of breast cancer is still unknown, but numerous studies have shown the role of ROS in the pathogenesis of cancer.[5-10] Oxidative damage results from the interaction of ROS with cellular macromolecules such as protein, lipid and DNA.[11, 12]

ROS reacts with polyunsaturated fatty acids to induce the release of toxic and reactive aldehyde metabolites such as malondialdehyde (MDA), one of the end products of lipid peroxidation. MDA may be involved in tumor promotion by reacting with various cellular processes.[13] Superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) that catalyze the detoxification of superoxide anion (O_2) and hydrogen peroxide (H_2O_2), respectively, protect the cell against ROS-induced damage.[14] Zinc is an important element for so many enzymes in the body, also it acts as direct

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scavenger of free radicals and protects from oxidative damage. But in case of imbalance between oxidant and antioxidant, the defence mechanism loses the battle. So many enzymes are rendered function less and can leads to the sequestration of microelements in the blood.

The present study was therefore designed to examine the lipid peroxidation status by estimating MDA and the antioxidant status through SOD, catalase, vitamin C and vitamin E and Zinc level in women with breast cancer.

MATERIALS AND METHODS

Thirty newly diagnosed breast cancer patients, from B.P. Koirala Institute of Health Sciences, Dharan, Nepal, who had neither undergone any previous treatment for their tumours nor were using hormones, oral contraceptives etc were recruited in the study. All were non-smokers and none of them had concomitant diseases such as diabetes mellitus, liver disease, or rheumatoid arthritis. This comparative cross sectional study was carried out from July 2010 to August 2011. Mean age of the patients was 48.1±9.35 yrs. There were 30 female healthy controls and their mean age was 45.2±10.9 yrs. Informed and written consent was obtained from all participants. Of the 30 patients, 29 were of infiltrative ductal carcinoma and 1 infiltrative lobular carcinoma.

Blood samples were collected with EDTA as anticoagulant (1/10, v/v) before any treatment modality such as chemotherapy and surgery was instituted to the patients. Then, erythrocytes were washed with 0.9% NaCl solution three times, and washed erythrocytes were haemolysed by the addition of Tris-HCl buffer, pH 7.2, 0.015M. Haemoglobin (Hb) concentrations of the samples were measured by cyanmethaemoglobin method.[15] The haemolysate and plasma of the subjects were kept at -20ºC till biochemical analysis.

Lipid peroxidation status was determined by method of Yagi.[16] The pink colour produced by the reaction of thiobarbituric acid with malondialdehyde, was estimated at 532 nm. Assay of SOD was based on the 50% inhibition of the formation ofnicotinamide adenine dinucleotide (NADH)-phenazine methosul fate-nitroblue tetrazolium formazan at 520 nm.[17] Catalase was estimated by dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate and read spectrophotometrically at 570 nm.[18] Determination of ascorbic acid depends on the reduction of ferric ion to ferrous ion by ascorbic acid as red-orange, α, α'-dipyridal complex.[19] Vitamin E determination is based on Emmerie Engel procedure.[20] Zinc was estimated by Flame Atomic Absorption Spectrophotometry.[21]

Statistical analyses were performed using the statistical package for social sciences version 11.5 (SPSS Inc. USA). Data were expressed as mean ± SD and frequency. Student t-test and Mann-Whitney U test was performed to compare means of the biochemical parameters between cases and controls. Probability of significance was set at 5% level of significance.

RESULTS

The descriptive characteristics of cases and control is presented in Table I. Table II shows the lipid peroxidation status and zinc level in breast cancer patients and healthy controls. Both lipid peroxidation by measuring MDA and zinc level were higher in patients compared to control ($p<0.001$ and <0.05, respectively). The different antioxidant status in the study subjects is presented in Table III. There is slight decrease in SOD activity in patients but statistically insignificant, whereas catalase activity is decreased significantly in patients compared to control ($p<0.05$) as depicted in Table III. The level of vitamin E and C were significantly lower in patients when compared to control ($p<0.001$).

Table I. Descriptive characteristics of cases and controls.

Characteristics	Cases (N=30)	Controls (N=30)	P
Age (Year)	48.1±9.35	45.2±10.9	
BMI (Kg/m2)	24.3±2.70*	26.7±3.37	
Age of Menarche (Year)	12-15	12-14	
Married			
Yes (n)	26	29	
No (n)	4	1	
Dietary habit			
Vegetarian	2	9	
Non vegetarian	28	21	
Pregnancy history			
Yes (n)	24	27	
No (n)	6	3	
Menopausal status			
Premenopausal (n)	15	13	
Postmenopausal (n)	15	17	
Cancer site			
Left (n)	12	NA	
Right (n)	18	NA	
Clinical status			
Infiltrative ductal carcinoma (n)	29	NA	
Infiltrative lobular carcinoma (n)	1	NA	

*p <0.05 compare to control. NA= Not Applicable. BMI=Basal Metabolic Index

Table II. Lipid peroxidation status and zinc level in case and controls.

Parameters	Breast cancer patients	Controls	p
MDA (nmol/ml)	7.12±1.95**	3.99±1.30	<0.001
Zinc (µgm/dl)	106.4±33.3*	89.8±27.9	<0.05

*p <0.05 compare to control **p<0.001 compare to control
MDA= Malondialdehyde

Table III. Antioxidant status in case and controls.

Parameters	Case	Controls	p
SOD (U/gmHb)	1004.8±74.8	1049.2±95.5	
Catalase (U/gmHb)	28.7±14.6*	37.1±14.7	<0.05
Vitamin C (mg/dl)	0.92±0.35**	1.62±0.59	<0.001
Vitamin E (mg/dl)	0.68±0.13**	1.02±0.22	<0.001

*p <0.05 compare to control **p<0.001 compare to control
SOD= Superoxide dismutase

DISCUSSION

Breast cancer is one of the most common malignancy in women, not only in the Western world, but also throughout the worldwide.[22] Several studies conducted in recent past has showed that increase oxidative stress and lipid peroxidation is implicated in its carcinogenesis.[23-26] MDA is a marker of lipid peroxidation resulting from oxidation of polyunsaturated fatty acids in membranes induced by free radicals. Studies have shown positive association between lipid peroxidation and cancer.[10, 13, 27-29] Lipid peroxidation was measured as MDA in various studies and was found to be higher in many studies.[25, 29] Similar finding was observed in our study. The exact mechanism how these lipid peroxides induces the carcinogenesis process or it is the result of cancer, is still not clear. There have been accumulating evidence that shows the reduced level of SOD and catalase in breast cancer patients.[30-33] Yuvaraj[34] showed that the various circulating enzymatic and non-enzymatic antioxidants were low in a group of women with breast cancer. The results of the above studies are in accordance with the results obtained in our study which shows a low level of antioxidant status in the women with breast cancer. But the decrease in SOD is not significant in the breast cancer patients, which contradicts the findings of the above studies. Several studies which showed a rise in the level of antioxidant enzymes7,35 suggested that the ROS generated might induce the antioxidant enzymes in order to eliminate the excessive free radicals that were produced.These findings contradicted our results.

In this study, enzymatic and non-enzymatic antioxidants, SOD, catalase, vitamin C, vitamin E and zinc were measured. The result obtained supports the fact that the oxidative stress in cancer patients exhausts the antioxidant mechanism of our body that leads to its depletion. Vitamin C, the major circulating water-soluble antioxidant which acts as a free radical scavenger, can react with a vitamin E radical to yield a vitamin C radical while regenerating vitamin E. Several studies have shown the decrease in the vitamin C and E level in breast cancer patients, which were in agreement with the finding of the present study.[9, 10, 36] Few studies showed that risks of breast cancer recurrence and disease-related mortality were reduced among women using vitamin C and vitamin E supplements for more than three years.[36]

Zinc is a pivotal element in all rapidly growing tissues because it is an important component of DNA and RNA polymerase, and play an important role by acting as a modulatory and protective action for the growth of both normal and cancer cells.[37-38] The study done by Cavallo[39] showed that the plasma zinc level was significantly higher in patients as compared to control group and this finding is in agreement with our result. The higher zinc level and its role as cancer promoter might be attributed by the capability of tumour tissues to incorporate Zn and withhold it, as few studies have pointed out. But contradictory results have also been reported in the study of trace elements in breast cancer patients.[40-42]

CONCLUSION

In conclusion, breast cancer is related to increased lipid peroxidation in plasma with concomitant decrease of body's antioxidant capacity. In overall, findings of the present study support the importance of body's antioxidant mechanism in the aetiology of breast cancer. It may be also suggested that increased oxidative stress in breast cancer patients may also be attributed to the altered trace elements level in such patients. Thus, further larger scale studies are required to elucidate the actual relation of these components with the disease.

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