〈Original Article〉

Oxidative stress/Nitrosative stress in breast cancer

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Summary Breast cancer is one of the global public health problems. It is the third most common cancer leading to the death of women worldwide. The incidence of breast cancer is rising in developing countries such as, India. The etiology of breast cancer is multifactorial and several risk factors associated with breast cancer may exert their effects via generation of an oxidative stress status. Oxidative stress is known to occur as a result of imbalance between the production of Reactive Oxygen Species (ROS) and anti-oxidant defenses. These free radicals cause damage to lipids proteins and DNA. The present study is to examine the role of oxidative stress in Breast cancer. We examined the extent of Lipid peroxidation as evidenced by the formation of Malondialdehyde (MDA), Nitric Oxide (NO) in the Breast cancer patients. The biochemical tests of blood samples of both breast cancer patients and age matched healthy controls showed with average MDA levels were 6.90±4.59 nmol/mL in breast cancer patients were significantly higher than those of controls 2.63±0.49 nmol/mL $P < 0.001$ and plasma nitrate level in patients with breast cancer 3.74±4.0 μm/mL was higher than that of normal subjects 1.51±0.51 μm/mL higher than those of controls. Hence, identifying markers for oxidative stress may help in the early diagnosis and medical management of the patients.

Key words: Breast Cancer, Oxidative stress, Malondialdehyde, Lipid peroxidation

1. Introduction

Breast cancer is the most common form of cancer worldwide. It is a potential life-threatening malignancy in women[1]. The incidence of breast cancer is rising in developing countries such as, India.

Most breast malignancies arise from epithelial elements and are categorized as carcinomas. Breast carcinomas are a diverse group of lesions that differ in microscopic appearance and biologic behavior, although these disorders are often discussed as a single disease.

The in situ carcinomas of the breast are either ductal (also known as intraductal carcinoma) or lobular. This distinction is primarily based upon the growth pattern and cytologic features of the lesions, rather than their anatomic location within the mammary ductal-lobular system.

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The major risk factors associated to breast cancer susceptibility are related to hormonal exposure, either from endogenous sources such as early age at menarche, late age at menopause, late pregnancy or nullli-parity, overweight and obesity, or exogenous sources such as the use of hormone replacement therapy[2] and past history of breast cancer and the history of a breast biopsy, family history, ethinicity [3,4], etc. Other modifiable risk factors include alcohol intake, radiation exposure, diet and current age[5].

Oxidative stress is major risk factor for cancer initiation and progression. It is an insufficient removal of the free radicals by antioxidants, radical scavengers. It is speculated that oxidative stress causes release of ROS which initiate or aggravates many diseases such as cancer, diabetes, and coronary heart disease[6].

Oxidative stress may cause DNA, protein, and/or lipid damage, leading to changes in chromosome instability, genetic mutation, and/or modulation of cell growth that may result in cancer[7]. This can cause damage to any base or sugar moiety in DNA and SBs (strand breaks). There are 2 major types of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Oxygen radicals are associated with different steps of breast carcinogenesis, either through adducts formation, structural DNA damage, interaction with oncogenes or tumor suppressor genes or immunological mechanisms[8].

The formation of DNA adducts indirectly by initiating autocatalytic lipid peroxidation, which generates a large variety of potentially genotoxic breakdown products, including alkoxyl (ROU), peroxyl radicals (ROOU), and aldehyde, such as malondialdehyde (MDA). As a result, the DNA is constantly being damaged and oxidatively modified. Any oxidative lesion that is not repaired can lead to mutations, increasing the risk of carcinogenesis[9].

Several markers of oxidative stress are currently available, such as thiobarbituric acid reactive substances (TBARS), which have been used extensively as markers of lipid peroxidation[10].

Reactive Nitrogen species like Nitric oxide (NO) is known to be critically involved in breast carcinogenesis. Oxidative-stress-NO- induced damage to DNA includes a multitude of lesions, many of which are mutagenic and have multiple roles in cancer and aging.

The present study has been undertaken to assess the oxidative & nitrosative stress by estimating the levels of Malondialdehyde & nitrate/nitrite in Breast Cancer patients.

2. Subjects and Methods

Total 100 samples of pathologically diagnosed women with breast cancer were chosen for the study. The controls were healthy individuals matched for age, gender and socioeconomic status, and were representative of the normal population with no risk factors for breast cancer or clinical symptoms of any other systemic disease.

The study protocol was approved by the Ethical Committee of the department. As per the selection criteria in each group, informed consent of the patients was obtained. Detailed information regarding their demographic status, clinical history, family history and medication was noted.

Lipid peroxidation, as evidenced by the formation of malondialdehyde (MDA), was assayed by the method described by Gavino et al. (1981)[11] An equal amount of 0.9% saline and trichloroacetic acid were added to 0.5 mL of freshly obtained plasma. It was then incubated at 37℃ for 20 minutes and centrifuged for ten minutes at 3,000 rpm. 0.25 mL of thiobarbituric acid was added to 1 mL of protein-free supernatant and incubated for 60 minutes at 95℃, and then OD value measured using the spectrophotometer.

The nitrite/nitrate concentrations present in the reaction mixture were determined using a Griess reagent (a 1:1 mixture of 1% sulfanilamide in 5% H_3PO_4 and 0.1% N-[1-napthyl] ethylene diamine) by the method of Lepoivre et al (1990)[12]. 0.5 mL of serum was precipitated with 50 μL of 70% sulfosalicylic acid, mixed well for five minutes, vortexed and then centrifuged at 3,000 rpm for 20 minutes. 200 μL of supernatant was taken, and 30 μL of 10% NaOH, 300 μL of 50 mmol/L Tris buffer and 530 μL of

Greiss reagent were added and incubated for 10 minutes in the dark. The absorbance was read against blank (double distilled H_2O) at 540 nm using the spectrophotometer. The concentration of nitrite/nitrate in serum was determined based on the standard curve generated.

Statistical analysis was performed using SAS version 9, (SAS Institute Inc, Cary, NC, USA) and the data was expressed as mean±standard deviation. The Student's t-test was performed for lipid peroxidation (MDA) and nitrite/nitrate in order to determine the difference between the controls and BC patients.

3. Results

The demographic and clinical data of patients and controls is represented in Table 1. The risk factors for BC, smoking and non vegetarians were more prevalent in patients compared to controls.

Plasma MDA levels were found to be significantly high in the patients (6.90±4.59 nmol/mL) compared to the control (2.03±0.49 nmol/mL) group at $p<0.01$ as summarized in Table 2 and Figure 1. When serum nitrite/nitrate levels were compared with patient and control groups, nitrite/nitrate was tended to be higher in the patients (5.14±0.179 μ mol/mL) than the controls (2.29±0.37 μ mol/mL) with the difference being statistically significant at $p<0.01$ as shown in Figure 2.

4. Discussion

Among women, breast cancer is the most commonly diagnosed cancer after non-melanoma skin

Table 1 The demographic and clinical data in the control and patient groups

Characteristic	BC Patients (n)	Controls (n)
No. of Subjects	100	100
Age	47.2±10.79	39.4±5.15
Diet		
Vegetarians	16	23
Non-Vegetarians	84	77
Area of Living		
women living in rural area	67	43
women living in urban area	33	57
Family History of cancer Incidence	33	NIL
Pre/Post menopausal		
women Pre menopausal	16	86
women Post menopausal	84	14
No of women undergone Chemotherapy	100	NIL

BC: Breast Cancer

Table 2 Oxidative/nitrosative stress markers in the control and patient groups

Varibales	Mean±SD	
	Controls (n=100)	BC Patients (100)
MDA (nmol/mL)	2.03±0.49	6.90±4.59*
Nitrite/Nitrate (μmol/mL)	2.29±0.37	5.14±0.179*

*All the variables are significant at $p < 0.01$.

SD : Standard Deviation; BC : Breast Cancer ; MDA : Malondialdehyde

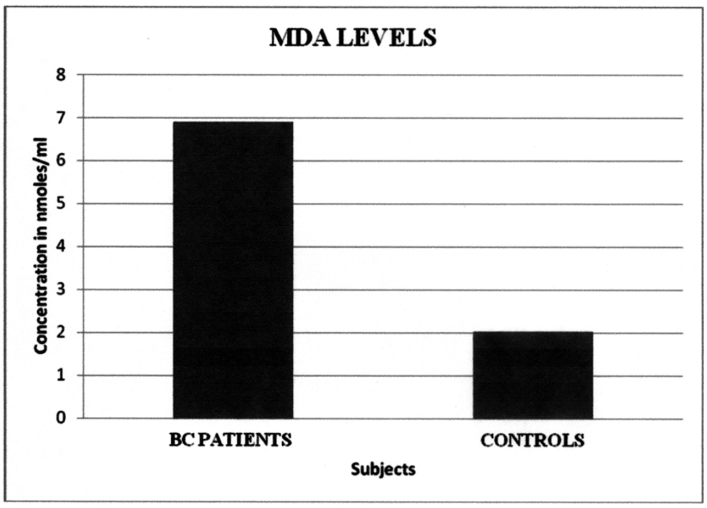

Figure 1 Plasma MDA levels in Breast Cancer patients and controls. MDA: Malondialdehyde

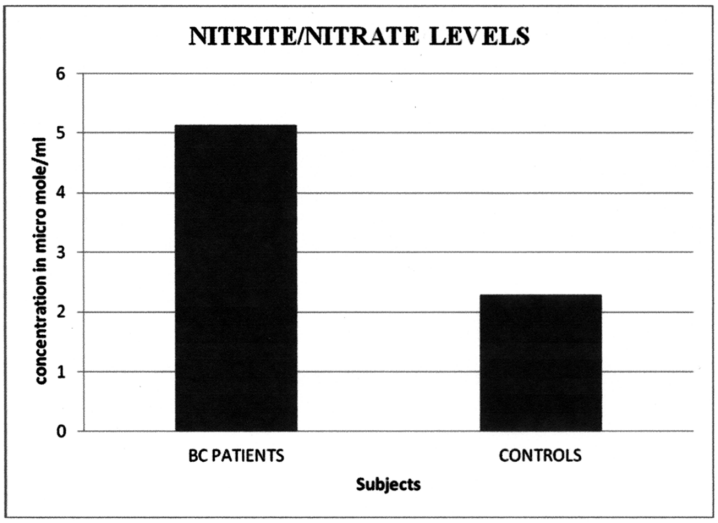

Figure 2 Plasma Nitrite/Nitrate levels in Breast Cancer patients and controls.

cancer, and it is the second leading cause of cancer deaths after lung cancer. In women with a genetic susceptibility or genetic predisposition, breast cancer, tends to occur at an earlier age than in sporadic cases. In cross-sectional studies of adult populations, 5% to 10% of women have a mother or sister with breast cancer, and about twice as many have either a first-degree relative (FDR) or a second-degree relative with breast cancer[13].

In general, breast cancer risk increases with early menarche and late menopause and is reduced by early first full-term pregnancy. Evidence suggests that reproductive history may be differentially associated with breast cancer subtype (i.e., triple-negative vs. ER-positive breast cancers)[14]. Higher levels of endogenous hormones have been hypothesized to increase breast cancer risk[15].

Previous studies show that post-menopausal

women with higher level of estrogen and testosterone have 2-3 folds risk than women with lower levels[15]. Mutations in Breast cancer susceptibility genes BRAC1 and BRCA2 account for the majority of families have been associated with more than one affected individual. Women with natural menopause at age 55 or older had twice the breast cancer risk experienced by those whose menopause occurred before age 45.

Reactive oxygen species (ROS) have been known to play an important role in the initiation and promotion of carcinogenesis and have been implicated in carcinogenesis in humans as well as animal models[16]. Previous studies have demonstrated that permanent modification of genetic material resulting from the oxidative damage leads to carcinogenesis[17].

It has been shown that ROS are associated with the different steps of carcinogenesis either through structural DNA damage or interaction with oncogenes or tumor suppressor genes or immunological mechanisms[18]. Pervious findings suggest that the depletion of estrogen in postmenopausal state could cause oxidative stress in addition to the known symptoms[19].

MDA is a physiologic ketoaldehyde produced by peroxidative decomposition of unsaturated lipids as a byproduct of arachidonate metabolism. The excess MDA produced as a result of tissue injury can combine with free amino groups of proteins (MDA reacts mainly with Lys residues by Michael addition), producing MDA-modified protein adducts. Modification of proteins by MDA could conceivably alter their biological properties. Moreover, MDA-modified proteins are immunogenic, and autoantibodies gainst MDA-modified Lys residues have been detected in the sera of rabbits and humans[20].

The increases in MDA levels were found even in early stages of cancer[21]. MDA, the end product of lipid peroxidation, owing to its high cytotoxicity has been suggested to act as a tumor promoter and a co-carcinogenic agent[22].

The elevated levels of MDA in carcinoma could be used as an important parameter in patients at risk for this disease mainly due to its dual role as a mutagen and a tumor promoter. Our study also has also documented higher levels of MDA in Breast Cancer patients compared to healthy controls.

Increased amounts of lipid peroxidation end products have been demonstrated in tumor tissue itself clearly pointing to the source of increased MDA levels in cancer patients[23]. Similarly Aghvani et al,[24] (2006) and Gonenc et al, (2001)[25] showed average MDA levels were in breast cancer patients.

Among well documented effects, free radicals like ROS and RNI are known to induce DNA damage and genomic instability favouring the acquisition of mutations that contribute to cellular transformation and cancer cells survival[17] (Jose' et al, 2011). The concept of oxidative stress originally con˚ned to free radical such as ROI-hydroxyl and superoxide radicals, hydrogen peroxide and singlet oxygen‰and now has been extended onto reactive nitrogen species (RNI) as nitric oxide (NO), peroxynitrite and, recently, S-nitrosothiols[26].

NO is a key molecule involved in many physiological functions. NO is a free radical, it is a highly reactive molecule within biological systems, reacting with other free radicals, molecular oxygen and heavy metals. It has been suggested that the biological effects of NO can be mediated by the products of different NO metabolites.

NO can cause DNA damage via the generation of peroxynitrite ($ONOO^-$) and N_2O_3. Peroxynitrite can oxidise and nitrate DNA and may potentially cause single-strand DNA breaks through attack on the sugar-phosphate backbone. Various studies have shown that NO levels can be involved in promoting or inhibiting the etiology of cancer[27].

NO may also increase metastatic ability in human cancers[28]. However, evidence is accumulating that sustained high levels of NO over extended periods of time contribute to carcinogenesis[29].

Studies by Tran et al (1988)[30] showed that plasma nitrate level in patients with breast cancer was higher than in normal subjects. Our study is also in accordance with earlier studies showing high plasma Nitrite/Nitrate levels in Breast Cancer patients compared with controls.

Increased NO-generation in a cell may select mutant p53 cells and contribute to tumour angiogenesis by up-regulating VEGF. In addition, NO may

modulate tumour DNA repair mechanisms by up-regulating p53, poly (ADP-ribose) polymerase (PARP). An understanding at the molecular level of the role of NO in cancer will have profound therapeutic implications for the diagnosis and treatment of disease[27].

ROI and RNI act as "toxic" substances that may react with proteins, carbohydrates and lipids, with consequent alteration both in the intracellular and intercellular homeostasis, leading to possible cell death and regeneration[29] (Alessandro et al, 2007).

Therefore, the current concept of "oxidative stress" should also include the pathways related to the "nitrosative stress" and, for their implication in cellular and extracellular metabolic events, to the "metabolic stress".

Therefore, the study also suggests that the increased oxidative, nitrosative stress markers may also act as prognostic predictors and potential targets for therapeutic strategies in Breast cancer.

Conflict of Interest: There is no conflict of interest
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References

1. Veni VK, Rao DB, Kumar DM, Usha B, Krishna VM, Rao TR: Clinical evaluation of oxidative stress in women with breast cancer. Rec Res Sci Tech, 3(1): 55-58, 2011.
2. Hulka BS, Moorman PG: Breast cancer: hormones and other risk factors. Maturitas, 38(1): 103-113; discussion 113-116, 2001.
3. Lushchak, VI: Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol, 101: 13-30, 2010.
4. Nelson HD, Zakher B, Cantor A, Fu R, Griffin J, O'Meara ES, Buist DS, Kerlikowske K, van Ravesteyn NT, Trentham-Dietz A, Mandelblatt JS, Miglioretti DL: Risk factors for breast cancer for women aged 40 to 49 years: a systematic review and meta-analysis. Ann Intern Med, 156(9): 635-648, 2012.
5. Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. Epidemiol Rev, 15(1): 36-47, 1993.
6. Cooke MS, Evans MD, Dizdaroglu M, Lunec J: Oxidative DNA damage: mechanisms, mutation, and disease. FASEB J, 17(10): 1195-1214, 2003.
7. Klaunig JE, Kamendulis LM, Hocevar BA: Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol, 38(1): 96-109, 2010.
8. Kang DH: Oxidative stress, DNA damage, and breast cancer. AACN Clin Issues, 13(4): 540-549, 2002.
9. Arsova-Sarafinovska Z, Eken A, Matevska N, Erdem O, Sayal A, Savaser A, Banev S, Petrovski D, Dzikova S, Georgiev V, Sikole A, Ozgök Y, Suturkova L, Dimovski AJ, Aydin A: Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem, 42(12): 1228-1235, 2009.
10. Hao Y, Montiel R, Huang Y: Endothelial nitric oxide synthase (eNOS) 894 G>T polymorphism is associated with breast cancer risk: a meta-analysis. Breast Cancer Res Treat, 124(3): 809-813, 2010.
11. Gavino VC, Miller JS, Ikharebha SO, Milo GE, Cornwell DG: Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures. J Lipid Res, 22(5): 763-769, 1981.
12. Lepoivre M, Chenais B, Yapo A, Lemaire G, Thelander L, Tenu JP: Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. J Biol Chem, 265: 14143-14149, 1990.
13. Yang Q, Khoury MJ, Rodriguez C, Calle EE, Tatham LM, Flanders WD: Family history score as a predictor of breast cancer mortality: prospective data from the Cancer Prevention Study II, United States, 1982-1991. Am J Epidemiol, 147(7): 652-659, 1998.
14. Phipps AI, Chlebowski RT, Prentice R, McTiernan A, Wactawski-Wende J, Kuller LH, Adams-Campbell LL, Lane D, Stefanick ML, Vitolins M, Kabat GC, Rohan TE, Li CI: Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. J Natl Cancer Inst, 103(6): 470-477, 2011.
15. Key T, Appleby P, Barnes I, Reeves G: Endogenous Hormones and Breast Cancer Collaborative Group: Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. Natl Cancer Inst, 94(8): 606-616, 2002.
16. Chen X, Ding YW, Yang Gy, Bondoc F, Lee MJ, Yang CS: Oxidative damage in an esophageal adenocarcinoma model with rats. Carcinogenesis, 21(2): 257-263, 2000.
17. Farias JW, Furtado FS, Guimar菊s SB, Silva Filho AR, Vasconcelos PR: Oxidative stress parameters in women with breast cancer undergoing neoadjuvant chemotherapy and treated with nutraceutical doses of oral glutamine. Acta Cir Bras, 26 Suppl 1: 82-87, 2011.

18. Battisti C, Formichi P, Tripodi SA, Vindigni C, Roviello F, Federico A: Vitamin E serum levels and gastric cancer: results from a cohort of patients in Tuscany, Italy. Cancer Lett, 151(1): 15-18, 2000.
19. Sánchez-Rodríguez MA, Zacarías-Flores M, Arronte-Rosales A, Correa-Muñoz E, Mendoza-Núñez VM: Menopause as risk factor for oxidative stress. Menopause, 19(3): 361-367 2012.
20. Stocker R, Keaney JF Jr: Role of oxidative modifications in atherosclerosis. Physiol Rev, 84(4): 1381-1478, 2004.
21. Akbulut H, Akbulut KG, Icli F, Büyücelik A: Daily variations of plasma malondialdehyde levels in patients with early breast cancer. Cancer Detect Prev, 27(2): 122-126, 2003.
22. Seven A, Civelek S, Inci E, Inci F, Korkut N, Burçak G: Evaluation of oxidative stress parameters in blood of patients with laryngeal carcinoma. Clin Biochem, 32(5): 369-373, 1999.
23. Bitla AR, Reddy EP, Sambasivaiah K, Suchitra MM, Reddy VS, Rao PVLNS: Evaluation of plasma malondialdehyde as a biomarker in patients with carcinoma of stomach. Biomed Res, 22(1): 63-68, 2011.
24. Aghvami T, Djalali M, Keshavarz A, Sadeghi MR, Zeraati H, Sadrzadeh Yeganeh H, Negahdar M: Plasma level of antioxidant vitamins and lipid peroxidation in breast cancer patients. Iranian J Publ Health, 35(1): 42-47, 2006.
25. Gönenç A, Ozkan Y, Torun M, Simsek B: Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J Clin Pharm Ther, 26(2): 141-144, 2001.
26. Kröncke KD: Nitrosative stress and transcription. Biol Chem, 384(10-11): 1365-1377, 2003.
27. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG: The role of nitric oxide in cancer. Cell Res, 12(5-6): 311-320, 2002.
28. Nakamura Y, Yasuoka H, Tsujimoto M, Yoshidome K, Nakahara M, Nakao K, Nakamura M, Kakudo K: Nitric oxide in breast cancer: induction of vascular endothelial growth factor-C and correlation with metastasis and poor prognosis. Clin Cancer Res, 12(4): 1201-1207, 2006.
29. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C: Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer, 121(11): 2381-2386, 2007.
30. Tran M, Wu Y, Chillar R, Vadgama JV, Nitric oxide levels in breast cancer patients is related with clinical pathology and advanced disease. 1988 ASCO Annual Meeting, Abstract No: 2196