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EVALUATION OF THE EXTENT OF OXIDATIVE STRESS IN BREAST CANCER PATIENTS OF INDIAN POPULATION

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ABSTRACT

Background and purpose: Oxidative stress in a cell is evident due to surplus production of oxidants because of the hysterical functioning of the system that regulates them. One such secondary product produced due to oxidative stress is malondialdehyde (MDA), a product of lipid peroxidation. To quench the effect of oxidants, antioxidant system in the cell has a significant role. The imbalance between these two creates oxidative stress.

Methods: The present study, focused on assessing the oxidative stress ratio and evaluating the levels of malondialdehyde and total antioxidant status (TAS) in breast cancer patients and healthy controls. Blood samples from breast cancer patients and age matched controls (n= 30 each. MDA and TAS estimated by pursuing Thiobarbituric Acid Reactive Substance (TBARS) Assay and Ferric Reducing Antioxidant Power (FRAP) Assay respectively.

Results: The level of MDA in patients was significantly higher (172.7 ± 81.4 nM/mL) than that of controls (77.9 ± 49.5 nM/mL) (*p=0.009*) whereas the level of TAS in the patients (2551 ± 1298µM/L) was significantly lower to that of the controls (3631 ± 1123µM/L) (*P=0.001*). In addition, MDA and TAS levels correlated with respect to chemotherapy cycles in patients. Patients undertaking the final stage of chemotherapy treatment had shown reduced oxidative stress than the patients in initial stage of chemotherapy, presenting a promising recovery pattern.

Conclusion: The oxidative stress was evident in patients but the effectiveness of chemotherapy drugs. Foods rich in antioxidants could elevate the health and morale of the patients.

KEYWORDS : Breast cancer, Cellular damage, Lipid peroxidation, Malondialdehyde, TAS, Oxidative stress.

INTRODUCTION

Breast cancer is the atypical and ungovernable growth of cells occurring in the mammary glands. The International Agency for Research on Cancer (IARC) studied the global cancer burden with a variability of 20 geographical regions estimated and reported 18.1 million cancer incidences and 9.6 million cancer deaths in 2018. Among women, breast cancer ranks the highest in both incidence (24.2%) and in mortality (15.5%) followed by cervical cancer and ovarian cancer [1]. The breast cancer stands first with high incidences in Indian women followed by cervical cancer [2]. It is estimated that women who survive upto the age of 85 years, would have 1in 9 lifetime chance of being affected by breast cancer [3]. Now that the studies reveal mutations in genes like BRCA might make the condition heritable and so the burden of inheriting the genetic mutation is soaring[4].

Oxidants are the reactive components that remove electrons or oxidize the reactants resulting in damage. Lipid peroxidation is a process in which the oxidant would rip-off electrons from lipids inside a healthy cell. This effect of the lipid peroxidation prominently occurs in the lipid bilayer of cell membrane causing potential cellular damage[5]. The common secondary product of lipid peroxidation is the formation of malondialdehyde (MDA). The problem with accumulating MDA is that it might react with nucleotides forming DNA adducts [6]. Hence, MDA is used as a noteworthy marker for oxidative damage. Antioxidants play a very significant role in protecting DNA, proteins, lipids and cellular components from the annoyance of oxidants[26 7]. This study hypothesizes that in breast cancer, the level of oxidants might level up due to nuisance in the metabolic pathways and the elevated levels of oxidants might present a huge burden on the antioxidant store of the cell resulting in oxidative stress. Oxidative stress is the measure of imbalance in the level of antioxidant and that of the oxidant in the system. Oxidative stress is an added liability to many other abnormalities in cancer patients and this problem needs effective management through chemotherapy.

The objective of this study was to estimate the amount of Malondialdehyde (MDA) in the study subjects, to evaluate the Total Antioxidant Status (TAS) in the study subjects and to calculate the oxidative stress ratio in the subjects.

MATERIALS AND METHODS

SAMPLE COLLECTION

An overall of 60 blood samples (2ml) were collected, out of which 30 samples were from breast cancer patients who were undergoing different cycles of chemotherapy and the remaining 30 were from healthy women volunteers who were free of disease condition and metabolism issues. The samples were screened and certified free of HIV and Hepatitis B. Individuals in the study were between 29 to 58 years of age and the controls were age matched. The plasma separated from peripheral blood of the subjects was stored at -20° C. Consent was obtained from the subjects and study approved by Institutional Review Board (IRB).

ESTIMATION OF LIPID PEROXIDATION

The extent of lipid peroxidation was assessed by Thiobarbituric Acid Reactive Substance Assay (TBARS) by Ohkawa *et al.,* [8] (1979). Initially, 50µl of 2% butylated hydroxyl toluene was added to the 100 µl of plasma sample, then1.5 ml of 0.67% thiobarbituric acid and 0.5 ml of 20% trichloroacetic acid was added to the mixture which is then incubated at 100°C for an hour and allowed to cool. Additionally, 2 ml of n-butanol was added to the mixture and mixed until the pink MDA adducts were dissolved into the upper organic layer. With n-butanol as blank, the absorbance of the organic layer formed was read at 532 nm (Specord 210, Analytik Jena, Germany). The concentration of MDA level was expressed in nM/ml. The standards were prepared by dissolving 4.167µl of 1, 1, 3, 3,-tetramethoxypropane with concentrations ranging from 1nM to 5nM and the standards were treated as that of the samples.

ESTIMATION OF TOTAL ANTIOXIDANT STATUS

Total antioxidant status was estimated through Ferric Reducing Antioxidant Power Assay (FRAP) by Benzie and Strain *et al.,* [9] (1999). To 30µl of plasma, 1 ml of FRAP reagent was added, (which contains acetate buffer 300mM, 2, 4, 6- tris (2-pyridyl) s-triazine (TPTZ) in 40mM HCl, and ferric chloride 20mM respectively in a 10:1:1 ratio). Standards were prepared using ascorbic acid in the range of 100 µl - 1000 µl concentration). After the reagent were added, the absorbance was taken at 0 minute initially and after 4 minutes of incubation in 37° C, the final absorbance was taken at 593 nm (Specord 210, Analytik Jena, Germany). The value of FRAP was estimated as,

$$FRAP\ value = \frac{\Delta\ sample\ (0-4\ minutes)}{\Delta\ standard\ (0-4\ minutes)} \times 2 \times 1000\ \mu M/l$$

EVALUATION OF OXIDATIVE STRESS RATIO

Oxidative stress in the study subjects were calculated by the formula put forth by Suresh et al., 2000 10.

$$Oxidative\ Stress = \frac{level\ of\ oxidant\ in\ the\ sample\ (MDA)}{level\ of\ antioxidant\ in\ the\ sample\ (TAS)}$$

STATISTICALANALYSIS

The data from the biochemical analysis of the samples were expressed as Mean ± Standard Deviation. The obtained data was tabulated and analyzed for its significance through the Graphpad Prism software using Mann-Whitney Rank Sum U-test. The data on oxidative stress was subjected to unpaired t-test. The p-value of < 0.05 was considered statistically significant.

RESULTS

In the present study, about 90% of the patients were found to have invasive ductal carcinoma, which is the most common type of breast carcinoma that occurs in the milk producing ducts of the breast and 10% of the patients had non-invasive ductal carcinoma, which is localized. Considering the location of tumor, 46.6% patients had tumor on their right side of the breast, 43.4% patients had it on left proximity and 10 % had the tumor on both right and left sides of the breast. The patients had to undergo chemotherapy in an interval of 21 days. During this study, around 10% of patients underwent I cycle of chemotherapy, 10 % underwent II cycle, around 13.4% of patients underwent cycle III, 16.6% were in cycle IV, 16.6% were in cycle V and 33.4% were in cycle VI of chemotherapy.

LIPID PEROXIDATION IN THE STUDY SUBJECTS

The levels of MDA in controls were 77.9 ± 49.5 nM/ml (Mean ± SD) and that of patients was 172.7± 81.4 nM/ml. The level of MDA among patients was significantly higher than that of controls (P<0.0001) (Table 1). The MDA levels were considered for evaluation in patients who have undergone chemotherapy cycles I-III and IV-VI. Around 33.4% of the total patients had undergone I-III cycles of chemotherapy treatment with the mean MDA level estimated to be 190.50 ± 81.62 nM/ml, whereas 66.6% patients had undergone chemotherapy cycle IV- VI with the mean level of MDA as 165.12 ± 85.11 nM/ml. The patients in their early stage of chemotherapy cycles had higher levels of MDA when compared to that of the patients with late cycles of chemotherapy. However, the difference was not statistically significant (p=0.4547) (Table 2).

TABLES

Table 1. Total MDA, TAS and OS levels in controls and breast cancer patients.

Sample	Age in years (Range)	MDA (nM/ml) (Mean ± SD)	TAS (µM/L) (Mean ± SD)	OS (Mean ± SD)
Controls (n=30)	21-56	77.9 ± 49.5	3631 ± 1123	0.02 ± 0.01
Patients (n=30)	29-58	172.7 ± 81.4*	2551 ± 1298*	0.22 ± 0.54*

* Statistically significant MDA- Malondialdehyde; TAS- Total Anti-Oxidant Status; OS- Oxidative Stress

Table 2. Total MDA, TAS and OS levels in the breast cancer patients according to the chemotherapy cycle.

Chemotherapy cycle	Number of patients	MDA (nM/ml) (Mean ± SD)	TAS (µM/L) (Mean ± SD)	Oxidative stress (Mean ± SD)
I – III	10	190.50 ± 81.62	2256 ± 1530	0.25 ± 0.44
IV – VI	20	165.12 ± 85.11	2864 ± 1131	0.21 ± 0.59

MDA- Malondialdehyde; TAS- Total Anti-Oxidant Status; OS- Oxidative Stress

TOTALANTIOXIDANT STATUS OF THE STUDY SUBJECTS

The mean level of TAS estimated for controls was 3631 ± 1123µM/L and that of the patients was 2551 ± 1298µM/L. The levels of TAS in controls were significantly higher when compared to that of patients (*p= 0.0011*) (Table 1). The TAS of patients who had undergone chemotherapy cycles from I to III and from IV to VI were grouped for comparison. The mean TAS level in patients in initial chemotherapy cycles was estimated to be 2256 ± 1530 µM/L and that of TAS level in patients at later chemotherapy cycles was found to be 2864 ± 1131 µM/L. Although, TAS level was higher in the latter group, there was no significant difference between the TAS levels of patients at initial chemotherapy stages and later chemotherapy cycles (*p=0.2245*) (Table 2).

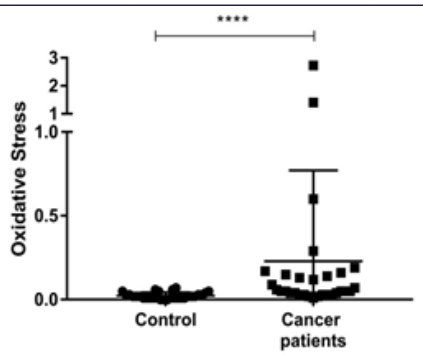

Figure 1: Scatter plot indicating a significant increase in oxidative stress (p= <0.0001) in cancer patients due to reduced antioxidant levels.

EVALUATION OF OXIDATIVE STRESS RATIO

The oxidative stress ratio in the controls was 0.02 ± 0.01 whereas in patients it was amounted to be 0.22 ± 0.54, which was shown to be significantly elevated in patients than that of the control and was statistically significant (*p<0.0001*) (Table 1). Oxidative stress ratio calculated for the patients in the initial chemotherapy cycles was 0.25 ± 0.44 and that of patients in later chemotherapy cycles was 0.21 ± 0.59. The oxidative stress ratio in late chemo cycle patients was lower when compared to the oxidative stress in patients with early chemo cycle, though this data was not statistically significant (p=*0.6404*) (Table 2).

DISCUSSION

The lipid peroxidation could typically be a subtle indicator of prominent oxidative cellular damage in any disease condition because, higher the damage, faster will be the progression of the disease. The inevitable secondary products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [6]. MDA can be highly mutagenic when there is an abrupt boost in their production as well as when there is not a satisfactory quantity of antioxidants to quench them [5]. MDA is more chemically stable and easily membrane permeable. Biosynthesis of thromboxane leads to formation of MDA and other secondary products through an enzymatic process. Once MDA is enzymatically produced, it reacts with cellular proteins and DNA to form adducts and will gain the ability to cause cellular devastation. MDA adducts crosslinking with protein or DNA will alter the biochemical property of a cell and accumulate on aging and supplementing in chronic diseases [11]. Thus, when production of MDA was elevated in a cell, it signifies the homeostasis of the cellular mechanism has been disrupted and a disease condition has been induced. As the condition progresses, the level of free radicals would keep elevating unless it was controlled with sufficient treatment [22]. Thus, the customary levels of antioxidants cannot aid in trapping free radicals when there was an evident boost [23]. This corresponds to the present study where the amount of the MDA upsurges in the patients than the controls group and articulating to this fact, the amount of TAS was higher in controls than in patients group, which explains that when the antioxidants are not sufficient then the free radicals causes inevitable damages [12]. Other studies also suggest almost two folds increase in the amount of MDA in breast cancer patients [1321]. Oxidative stress corresponds to the same factors, that when the antioxidants and the oxidants are in equilibrium, oxidative stress is low and cellular damage is less prominent. But, when this equilibrium was disrupted, an imbalance in one of them occurs and oxidative stress along with cellular impairment would be evident. Studies confirm that there would be an increase in certain enzymatic antioxidants during cancer ailment, such as Catalase, Glutathione peroxidase (GPx) and SuperOxide Dismutase (SOD) for restoration of the damage as an adaptive response [15,27,28].

Coherent to a study by Ray *et al.,* [14] *(*2000), this study also shows that the patients in the later stages of the breast cancer have reduced MDA when compared to the patients in the early stages of the ailment and vice versa for the total antioxidant level [24]. The effect of drugs taken during chemotherapy such as, tamoxifen and cisplatin could aid as a potential lipid peroxide suppressor [15.] Tamoxifen aids in increasing the enzymatic and non-enzymatic antioxidants along with suppression of lipid peroxides in a span of three to six months [1626]. Certain antioxidant rich diets that were suggested to the patients as a supplement by the medical experts were to improve the efficiency of chemotherapy.

Many citrus fruits were said to be abundant in antioxidants which could help in eliminating the further damage [17]. There would be many efficient antioxidant derived pathways that could be existent to inhibit lipid peroxides [28]. The most notable ones were the GPx or glutathione peroxidase derived enzymes that could render the hyperoxides insignificant [18 27]. The drug paclitaxel is known to significantly lower the level of lipid peroxides[19]. Even natural compound like curcumin is prevalently known to reduce lipid peroxidation and in elevating antioxidant levels[20]. Foods rich in flavonoids, carotene, tocopherols, and ascorbates are said to be rich in antioxidants and while taken along with proper chemotherapy regime, it restores the antioxidant store in all the cells, thus minimizing the effect of oxidative stress [25]. Nuts, fruits, dry fruits and spinach are highly loaded with the goodness of antioxidants and it is highly advisable to include all these foods in correct proportion. Corresponding to the reduction in MDA levels in later stages of chemotherapy, the oxidative stress was also reduced when compared to that of the earlier patients in the initial cycles of chemotherapy. Hence, it was evident that reduction in oxidative stress in the final stages of chemotherapy was a good indication of an adaptive response.

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