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ORIGINAL

The role of multi-layer spiral CT based perfusion imaging in lung cancer radiotherapy assessment in athletic patients

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ABSTRACT

Background and purpose: Breast cancer is one of the chief causes for escalating mortality among female athletes. 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, an 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 were estimated by pursuing Thiobarbituric Acid Reactive Substance (TBARS) Assay and Ferric Reducing Antioxidant Power (FRAP) Assay respectively.
Results: The level of MDA in athletic 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 athletic 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. Athletic Patients undertaking the final stage of chemotherapy treatment had shown reduced oxidative stress than the athletic patients in initial stage of chemotherapy, presenting a promising recovery pattern.
Conclusion: The oxidative stress was evident in athletic patients but the effectiveness of chemotherapy drugs. Foods rich in antioxidants could elevate the health and morale of the athletic patients.

KEY WORDS: 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, which also happens to acquire changes in the normal physiological process in the cells. 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 female athletes, breast cancer ranks the highest in both incidence (24.2%) and in mortality (15.5%) followed by cervical cancer and ovarian cancer (Bray et al., 2018). The breast cancer stands first with high incidences in Chinese female athletes followed by cervical cancer (Dhillon et al., 2018). It is estimated that female athletes who survive up to the age of 85 years, would have 1in 9 lifetime chance of being affected by breast cancer(Singletary, 2003). 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(Vaidyanathan et al., 2009).

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, especially polyunsaturated fatty acids and creates various break down products like aldehydes and ketones. This effect of the lipid peroxidation prominently occurs in the lipid bilayer of cell membrane causing potential cellular damage. In disease condition, the overproduction of oxidants leads to formation of secondary products(Esterbauer, Eckl, & Ortner, 1990). 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(Repetto, Semprine, & Boveris, 2012). 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. Furthermore, variation in the gene expression due to the presence of mutant allele may further

decrease the activity of antioxidant enzymes which may increase the susceptibility to oxidative stress in cancer athletic patients. Antioxidants could be obtained through diet, and a few are produced by the body itself(Rahal et al., 2014). 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 athletic 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 female athletic patients who were undergoing different cycles of chemotherapy and the remaining 30 were from healthy female athletes 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, Ohishi, & Yagi). 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 & Strain, 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(Manjari, Suresh, Devi, & Das, 2001).

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

STATISTICAL ANALYSIS

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 athletic 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 Athletic 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% Athletic patients had it on left proximity and 10 % had the tumor on both right and left sides of the breast. The Athletic 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 Athletic 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 level of MDA was evaluated in controls and Athletic patients to examine

the extent of cellular damage due to lipid peroxidation. The levels of MDA in controls were 77.9 ± 49.5 nM/ml (Mean ± SD) and that of Athletic patients was 172.7± 81.4 nM/ml. The level of MDA among Athletic patients was significantly higher than that of controls (P<0.0001) (Table 1).

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

In addition, the cumulative data, on the MDA levels were considered for evaluation in athletic patients who have undergone chemotherapy cycles I-III and IV-VI. Around 33.4% of the total athletic 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% athletic patients had undergone chemotherapy cycle IV- VI with the mean level of MDA as 165.12 ± 85.11 nM/ml. The athletic 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).

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

Chemotherapy cycle	Number of Athletic 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

TOTAL ANTIOXIDANT STATUS OF THE STUDY SUBJECTS

Total Antioxidant status was estimated to obtain insights on the protective role of antioxidant levels. The mean level of TAS estimated for controls was 3631 ± 1123µM/L and that of the athletic patients was 2551 ± 1298µM/L. The levels of TAS in controls were significantly higher when compared to that of athletic patients (*p= 0.0011*) (Table 1).

In addition to the cumulative data, the TAS of athletic patients who had undergone chemotherapy cycles from I to III and from IV to VI were grouped for comparison. The mean TAS level in athletic patients in initial chemotherapy cycles was estimated to be 2256 ± 1530 µM/L and that of TAS level in athletic 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 athletic patients at initial chemotherapy stages and later chemotherapy cycles (*p=0.2245*) (Table 2).

EVALUATION OF OXIDATIVE STRESS RATIO

Oxidative stress ratio indicates the extent of damage due to excessive levels of oxidants to that of the depleted level of antioxidants. The oxidative stress ratio in the controls was 0.02 ± 0.01 whereas in athletic patients it was amounted to be 0.22 ± 0.54, which was shown to be significantly elevated in athletic patients than that of the control and was statistically significant (*p<0.0001*) (Table 1).

Oxidative stress ratio calculated for the athletic 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 athletic patients was lower when compared to the oxidative stress in athletic 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). 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(Zarkovic, Cipak, Jaganjac, Borovic, & Zarkovic, 2013). 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(Erten Şener,

Gönenç, Akıncı, & Torun, 2007). Thus, the customary levels of antioxidants cannot aid in trapping free radicals when there was an evident boost (Gönenç, Özkan, Torun, & Şimşek, 2001). This corresponds to the present study where the amount of the MDA upsurges in the athletic patients than the controls group and articulating to this fact, the amount of TAS was higher in controls than in athletic patients group, which explains that when the antioxidants are not sufficient then the free radicals causes inevitable damages (Ray et al., 2000). Other studies also suggest almost two folds increase in the amount of MDA in breast cancer female athletic patients(Tas et al., 2005). 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.

Coherent to a study by Ray *et al., (*2000), this study also shows that the athletic patients in the later stages of the breast cancer have reduced MDA when compared to the athletic patients in the early stages of the ailment and vice versa for the total antioxidant level. The effect of drugs taken during chemotherapy such as, tamoxifen and cisplatin could aid as a potential lipid peroxide suppressor. Tamoxifen aids in increasing the enzymatic and non-enzymatic antioxidants along with suppression of lipid peroxides in a span of three to six months. Certain antioxidant rich diets that were suggested to the athletic 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. There would be many efficient antioxidant derived pathways that could be existent to inhibit lipid peroxides. The most notable ones were the GPx or glutathione peroxidase derived enzymes that could render the hyperoxides insignificant. The drug paclitaxel is known to significantly lower the level of lipid peroxides. Even natural compound like curcumin is prevalently known to reduce lipid peroxidation and in elevating antioxidant levels. 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. 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 athletic 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.

CONCLUSION

The oxidative stress is at its peak in an ailment like breast cancer and it seems to attain a reduction after certain courses of chemotherapy that includes drugs like tamoxifen, cisplatin and paclitaxel and antioxidant rich diet. The oxidative stress is just one of the distresses among many anomalous changes in the body of athletic patients and could be managed by chemotherapy drugs in a required dose at specific intervals of time. Also suggesting a suitable antioxidant rich diet plan would improve the quality of life and the morale in athletic patients.

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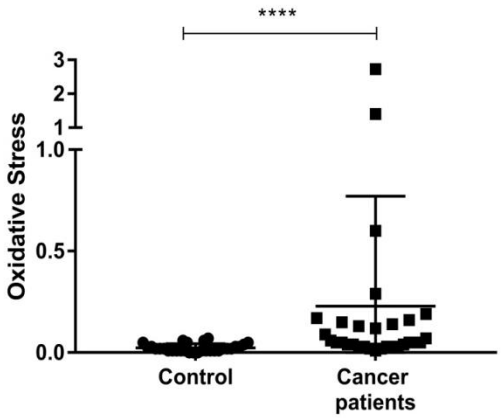

Figure 1: Scatter plot indicating a significant increase in oxidative stress (p= <0.0001) in cancer patients due to reduced antioxidant levels.