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Effect of oxidative stress markers in breast carcinoma patients: A comparative cross sectional study in tertiary care hospital, Madya Pradesh, India

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Abstract---Background: Curiosity in view of the literature regarding breast cancer especially in our country, there has been little study on breast cancer individuals during and progression of the cancer on oxidative stress markers. Aim: To determine the effect of MDA, TAC, SOD, Catalase, reduced glutathione in breast cancer subjects and to compare them with the apparently healthy controls. Methods: Lately diagnosed female subjects with breast cancer in the age group of 40-60 years were included in the study. Apparently healthy controls were selected from the group of people who were attending for annual health check-up and found to be healthy. Results: On comparison between the two groups, the present study observed significant differences in the values of MDA, TAC, and reduced glutathione. Interestingly, we observed higher levels of MDA in the subjects suffering with breast carcinoma, whereas lower levels in the apparently healthy controls incorporated in the study. Comparing between the groups of LDL and HDL level, the present study observed

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significant difference. Conclusion: The present study concludes that alterations in the study parameters in breast cancer group are due to dys-balance oxidants production and lower expression of antioxidants.

Keywords---oxidative stress markers, MDA, TAC, glutathione, breast carcinoma.

Introduction

Numerous free radicals are concerned to trigger an amount of oncogenic signaling molecules that cause injury to deoxyribonucleic acid (DNA) and tumor suppressor genes, or encourage activation, conversion, and expression of proto-oncogenes. Reduced level of combating molecules and increases oxidative stress molecules are associated with the initiation and development of numerous types of cancer. Breast cancer is a disease in which cells in the breast grow out of control. There are different kinds of breast cancer. Breast cancer is the most common cancer in women worldwide, with nearly 1.7 million new cases diagnosed in the year 2012, representing about 25 percent of all cancers in women[1]. Incidence rates vary widely across the world, from 27 per 100,000 in Middle Africa and Eastern Asia to 92 per 100,000 in Northern America[2].

Oxidative stress exists when there is dys-balance between the oxidants including reactive oxygen species (ROS), Reactive Nitrogen Species (RNS) their combat forces such as anti-oxidants. These anti-oxidants are enzymatic including Superoxide Dismutase (SOD), Catalase, and glutathione peroxidase, cellular molecules like reduced glutathione, uric acid, albumin, vitamin C etc. When more oxidants are produced constantly than anti-oxidants, it leads to damage to bio-molecules such as carbohydrates, proteins, DNA, and proteins. Such damage to eventually leads to the production adducts, glycation, and if DNA damage then leads to the configuration of new proteins that generates abnormal molecules. Lipid peroxidation causes the production of Malondialdehyde (MDA), a lipid by-product due to the attack of ROS on lipid membranes.

MDA is a by-product which is formed non-enzymatically, via schiff base, with free amine groups present on protein and DNA on reaction with glucose. Piling of MDA in individuals is correlated with many disease states including liver injury, human immunodeficiency virus, cancer, and diabetes mellitus. MDA formation is stimulated in the plasma of various carcinomas and obese patients[3,4,5,6,7,8,9,10,11] . Similar increase in animal models was seen upon induction with carcinogens into the tissues of the mice[6,8]. In a case controlled study on breast cancer, the authors have observed increased amount of MDA than the healthy counter-parts[9]. Anti-oxidant enzymes are vital that converts superoxide to hydrogen peroxide, a less reactive ROS. These enzymes are necessary molecules to maintain the reduced state inside the cell. Alteration of the intra-cellular state may lead to the conversion of proto-oncogenes to oncogenes. In a study on breast cancer[4], observed lower levels of anti-oxidants in the subjects who are not performing regular exercise than the subjects of breast cancers who are performing regular exercises.

Cholesterol is an essential ingredient in the membranes and has many vital functions including protecting the cell from stress, acts like insulator, precursors for the steroid hormones in the body etc. Cholesterol is transported from the liver to the extra-hepatic tissues via LDL molecules for its usage. After utilization, the left over cholesterol is taken back to the liver through reverse cholesterol transport for its fate. The cholesterol is now excreted through the liver in the form of bile. Recent reports[12,13] have suggested a devastating role of LDL in breast cancer, distressing cell proliferation and migration, thereby encouraging disease progression. Lately, some reports have shown that improper clearance of cholesterol form tissues is the initial stage for the development of cancer[12,13,14,15] .On the contrary, there are some reports that have shown non-involvement of LDL in the breast cancer progression.

Materials and Methods

This study titled "Effect of Oxidative Stress Markers in Breast Carcinoma Patients: A Comparative Cross Sectional Study in Tertiary Care Hospital, Madya Pradesh, India" was carried out during the period of from January 2019 to January 2022.The study was conducted on 100 subjects admitted during the above period in research centre of Index Medical College and Hospital, Indore, Madya Pradesh, India affiliated to Malwanchal University with an aim to evaluate the effect of oxidative stress markers in breast carcinoma patients

Inclusion Criteria

Lately diagnosed female subjects with breast cancer in the age group of 40-60 years attending to research Centre in Index Medical College and Hospital, Indore, Madya Pradesh, India affiliated to Malwanchal University were included in the study. These breast cancer subjects were chosen irrespective of type and stage of the pathology. Apparently healthy controls were selected from the group of people who were attending for annual health check-up and found to be healthy. These control subjects were eventually selected only when they cleared and notified by the attending physician of them to include in the control group. The age matched control subjects are selected from apparently healthy women attending for health check up in the research Centre in Index Medical College and Hospital.

Exclusion Criteria

The breast cancer group subjects or apparently healthy control group subjects suffering from co-morbid conditions which affect serum levels of oxidative stress markers and other malignancies, and those undergoing treatment for breast cancer were excluded from the study. Serum was separated and tests were performed according to standard procedure for each marker on the same day.

Ethics

This study was approved by the Institutional Ethics Committee of Index Medical College and Hospital, Indore, Madya Pradesh, India affiliated to Malwanchal University. An informed written consent was taken from all the patients involved in the study after explaining regarding the study.

Study Procedure

Fasting venous blood (5ml) were drawn into the plane vials, after informed written consent from all the study group subjects. Serum was separated by centrifuging the blood at 3000 rpm for 20 minutes and stored in aliquots at -20° C until assayed. Serum was processed to assess the levels of oxidative stress markers.

Statistical analysis

SPSS statistical software was used to perform statistical analysis. Unpaired 't' test was performed to compare the means of variables between two groups. Percentages were also calculated. Chi-Square test was used to check the relative risk & Odds ratio. P <0.05 was considered significant.

Sampling population

Sample size is calculated[16] on the basis of incidence of breast cancer individuals using the formula :
Where σ_1 = 2 units, σ_2 = 5 units, the SD of number of breast cancer individuals
d = mean(σ_1, σ_2) the minimum mean difference consider to be clinically significant
Type I error α = 5% corresponding to 95% confidence level
Type II error β = 20% for detecting results with 80% power of study
So the required sample size n = 50

Results

In the table 1, we have shown the values of MDA, TAC, SOD, Catalase and reduced glutathione in both the groups of the present study. On comparison between the two groups, the present study did not observe significant differences in the values of SOD and catalase.

Table 1
Showing the values of MDA, TAC, SOD, Catalase, & reduced glutathione in both the groups

S.No	Parameter	Breast carcinoma Group (n=50)	P-value	Controls Group (n=50)
1	Malondialdehyde (µmol/L)	9.2 ± 1.4	<0.001	5.2 ± 0.32
2	Total Antioxidant capacity (µmol/dL)	2.8 ± 0.2	<0.05	4.6 ± 0.8
3	Superoxide Dismutase (U/mL)	5.2 ± 0.4	>0.05	6.2 ± 0.9
4	Catalase (U/mL)	2.1 ± 0.2	>0.05	2.9 ± 1.2
5	Reduced Glutathione (µmol/L)	3.8 ± 0.2	<0.001	7.4 ± 1.1

In the figure 1, we have shown the bar diagram of MDA, TAC, SOD, Catalase and reduced glutathione values in both the groups of the present study. On comparison between the two groups, the present study observed significant differences in the values of MDA, TAC, and reduced glutathione. Interestingly, we observed higher levels of MDA in the subjects suffering with breast carcinoma, whereas lower levels in the apparently healthy controls incorporated in the study.

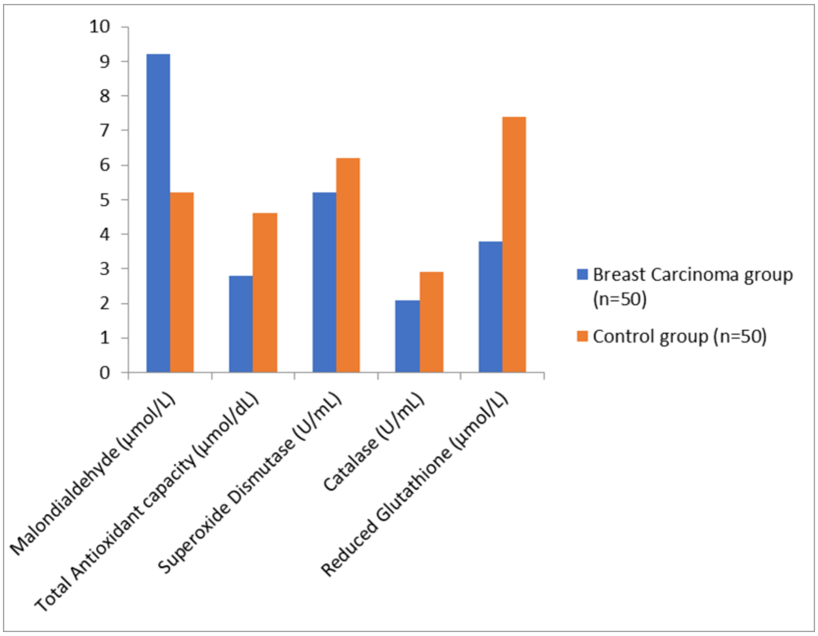

Figure 1. Bar diagram depicting the MDA, TAC, SOD, Catalase and reduced glutathione values in both groups

Age of the present study subjects of both groups have been shown in figure 2 & figure 3. No significant difference was observed in age distribution in breast cancer group and control subjects as well.

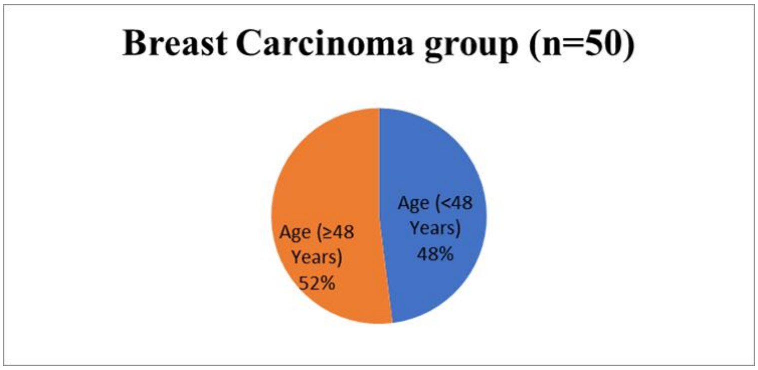

Figure 2. Pie chart depicting the age distribution of subjects of Breast cancer group

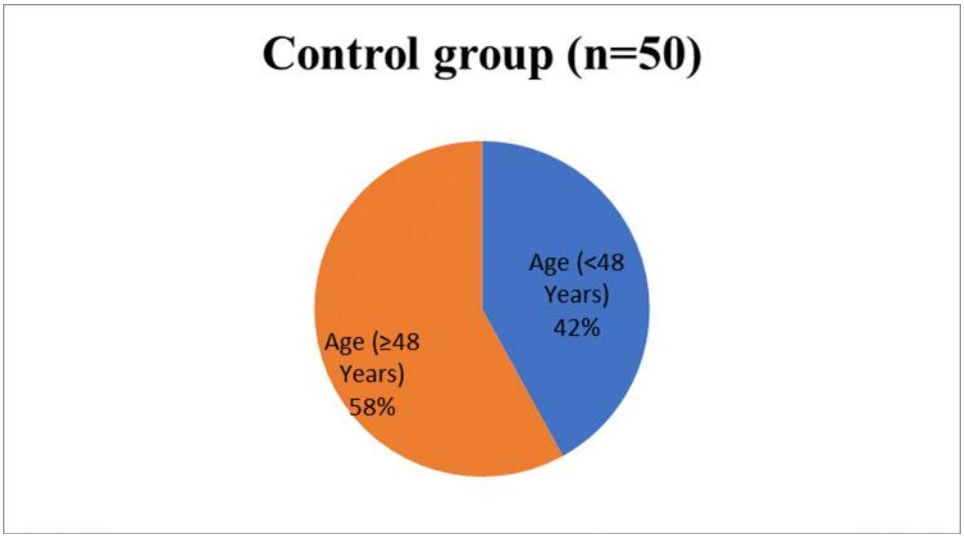

Figure 3. Pie chart depicting the age distribution of subjects of control group

A chi-square test of independence showed that there was no significant association between age and breast cancer, X^2 (1, N = 50) = 0.7, p = .41. Similarly, the presence of breast cancer did not differ by the SOD levels of breast carcinoma patients, X^2 (1, N = 50) = 0.1, p = .12. On the contrary, there is a significant relationship between the age and SOD variables in apparently healthy controls. The levels of SOD are more likely to be associated with healthy nature of the control subjects X^2 (1, N = 50) = 10.7, p < .01. Figure 4 show the relationship of parameters in the present study group subjects. Pertaining to breast cancer group subjects, a positive correlation (y=0.029x+2.687 & R^2=0.026) between Age (x axis) with SOD value (y axis) was established as evident from the graph shown in the figure .

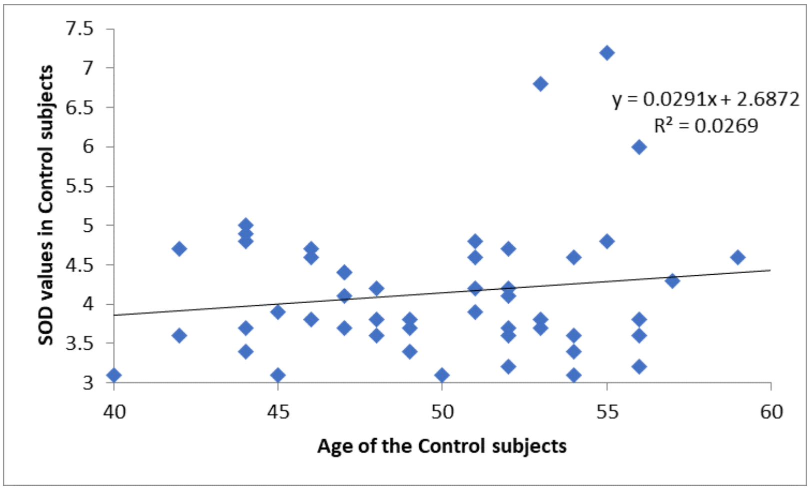

Figure 4. Scatter diagram showing relationship between Age & SOD value in control subjects group

In the figure 5, we have shown the serum values of HDL and LDL in both the groups of the present study. Serum HDL level was found to be higher in case of apparently healthy controls than breast cancer subjects. Whereas, LDL was lower in case of apparently healthy controls than breast cancer subjects. Comparing between the groups of HDL level, the present study observed significant difference. Similarly, the LDL level was also found to be significantly differed when compared between the two groups.

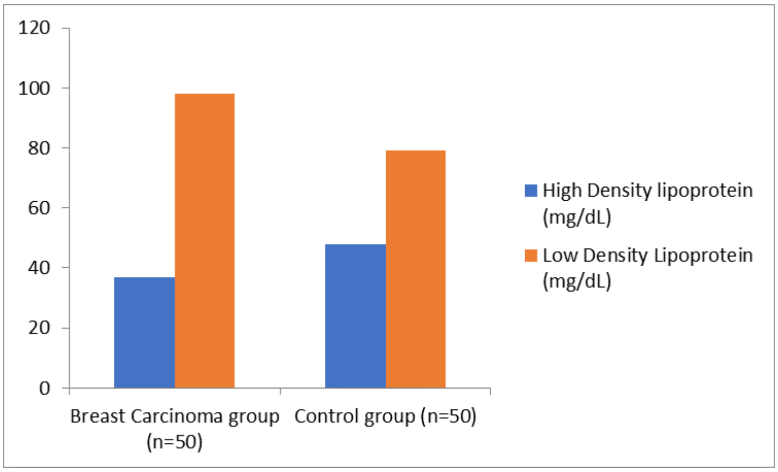

Figure 5. Bar diagram depicting the HDL and LDL values in both groups

Discussion

In studies, it was reported that breast cancer can affect anyone irrespective of age, but it is most likely to appear between 41 to 60 years of age[17,18,19]. The reports also reported that breast cancer disease affects the female gender at the higher rate[17,18,19]. Piling of MDA in individuals is correlated with many disease states including liver injury, human immunodeficiency virus, cancer, and diabetes mellitus. MDA formation is stimulated in the plasma of various carcinomas and obese patients[3,4,5,6,7,8,9,10,11]. In a case controlled study on breast cancer, the authors have observed increased amount of MDA than the healthy counter-parts[9]. In randomized-doble blind study the authors observed increase in the concentration of MDA (TAC) in 40 subjects who are affected with breast carcinoma. In another study on breast cancer[4] observed increased MDA in the subjects who are not performing regular exercise than the subjects of breast cancers who are performing regular exercises. In correlation with the cited literature above, the present study authors[20] also observed increased levels of MDA in comparison with the apparently healthy subjects. We infer the increase of MDA to the increase in LDL levels in breast cancer subjects as there is decrease in the removal of accumulated lipids in the tissues.

Glycation can take place with any bio-molecule including protein, lipids, nucleic acids, and carbohydrates. To prevent this glycation, glutathione is the major intracellular anti-oxidant and anti-oxidant enzymes like SOD, catalase and glutathione peroxidase that acts like policemen. In addition, some studies quotes that glycation ligands in a close linkup with reduced glutathione may prove to be effective therapeutic markers of severity of breast cancer and for angiogenesis of tumor[21,22,23,24] .Some studies in their report demonstrated that the impact of membrane lipid peroxidation is directly proportional to the lipid by-products and adducts (MDA) concentrations in vitro and also is highly prognostic for long term cancer related complications. The present study observed significant change in the values of TAC when compared between breast cancer subjects and apparently healthy control subjects. Similarly, the present study authors also observed significant change in the values of reduced glutathione in breast cancer subjects than apparently healthy subjects. Across the globe similar results to us have been reported. In randomized-double blind[20] study the authors observed increase in the concentration of TAC in 40 subjects who are affected with breast carcinoma. In bench-based experimental studies also observed reduced glutathione levels when compared to healthy counter-parts[6,8].

Breast cancer is influenced by cholesterol content in the membrane as the cell membrane has many signaling molecules embed into and to it[25]. Cholesterol is transported from the liver to the extra-hepatic tissues via LDL molecules for its usage. After utilization, the left-over cholesterol is taken back to the liver through reverse cholesterol transport for its fate. The cholesterol is now excreted through the liver in the form of bile. Recent reports[12,13] have suggested a devastating role of LDL in breast cancer, distressing cell proliferation and migration, thereby encouraging disease progression. Lately, some reports have shown that improper clearance of cholesterol form tissues is the initial stage for the development of cancer[14,12,13,15]. This improper clearance of cholesterol by HDL enhances the production of hormones that are responsible for the progression of breast cancer[25]. On the contrary, there are some reports that have shown non-involvement of LDL in the breast cancer progression. A study on breast cancer subjects has shown increased cholesterol levels when compared to control[26]. Another study reported an increase in the total lipids of breast cancer serum[27]. In another study[28] it has been shown that dyslipidemia existed in breast cancer subjects but these altered levels were significantly prominent in HDL levels when compared to control. The present study observed significant difference in the LDL and HDL concentrations when compared between the breast cancer subjects and also apparently healthy controls.

SOD is the vital anti-oxidant enzyme that converts superoxide to hydrogen peroxide, a less reactive ROS. This enzyme is one of the necessary molecules to maintain the reduced state inside the cell. Alteration of the intra-cellular state may lead to the conversion of proto-oncogenes to oncogenes. In a study on breast cancer[4] observed lower levels of SOD in the subjects who are not performing regular exercise than the subjects of breast cancers who are performing regular exercises. A study[7] on Iraqi breast cancer women observed lower levels of SOD activity in comparison to healthy controls. In another study[10] the authors observed lower levels of SOD and Catalase as well in breast cancer subjects. In opposition with the cited literature above, the present study authors did not observe significant differences in the levels of SOD and Catalase when compared between breast cancers and apparently healthy control subjects. This might be due to over-expression of SOD and Catalase to exert a protective effect on the cancer due to lack of antioxidants in response to increased lipid peroxidation.

Conclusion

The present study concludes that alterations in the study parameters in breast cancer group are due to dys-balance oxidants production and lower expression of antioxidants. This dys-balance is due to low production of SOD, Catalase, and reduced glutathione in breast cancer subjects than healthy controls. The present study also concludes that there is association of age with SOD in healthy controls. The alterations in the cholesterol and HDL in breast cancer subjects due to deficient scavenging action of HDL. Finally, a large multicentric study which should include all ethnic population is required to validate the findings as the present study parameters are inexpensive and efficacious to control the financial costs associated with breast cancer as an add-on investigative procedure to the existing investigative procedures to prevent the initiation and progression of the disease.

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