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Original Article

Chemotherapy on hematological and biochemical parameters in breast cancer patients

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Abstract

Background: Drugs used in chemotherapy specifically target and kill the cancer cells during the breast cancer treatment. However, the majority of anti-cancer therapies are non-specific, which will harm the innate cells. Our research work assessed the impact of chemotherapy with adriamycin/cytoxan (AC) on the influence of antioxidant enzymes and hematopathological profiles in the diagnosis and prognosis of breast cancer treated with chemotherapy.

Methods: 40 breast cancer patients treated with AC chemotherapy (Adriamycin 60 mg/m2, Cytoxan 600 mg/m2) between July 2020 and March 2021 are part of this prospective study. The first sample was taken prior to chemotherapy, the second after the intervention's three cycles, and the third after the intervention's last cycle. Spectrophotometric technique was used to evaluate the amounts of antioxidant enzymes in serum samples. Patients' demographic variables, clinical features, biochemical andhematogical parameters data were noted. The data was compared before and after treatment using the Paired-t test.

Results: 55% of the patients were detected with carcinoma on left breast and majority was in Grade 3 clinical stage 37.5%. Most of the patients express estrogen and progesterone receptors 72.5%. Our findings demonstrated that a significant decrease in the mean values of antioxidant enzymes MDA, NO, TAS, CAT, GPx, GR, SOD and GST along with hematological parameters after three cycles of AC treatment in breast carcinoma individuals. The p-value is < 0.05.

Conclusion: Our research demonstrates that the body's oxidant/antioxidant system, particularly reduction levels and antioxidant enzyme activity, is drastically altered by AC chemotherapy in breast carcinoma individuals.

Keywords: Breast carcinoma, Antioxidants, AC chemotherapy, Hematological parameters.

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Breast cancer is the overgrowth of breast tissue and because of an interaction of an external environmental element with a genetically vulnerable host (1, 2). In addition to being the second largest cause of death worldwide, breast cancer is the most frequent cancer in women (3). There are many predisposing variables in particular lifestyles that have led to an increase in breast cancer incidence in the Indian population (4). Approximately 60% of breast cancer fatalities and 50% of new cases currently are recorded in developing nations. Many genes, including HER2/neu, p53, BRCA1 and BRCA2, have been associated with the development of breast carcinoma (5-7). There is evidence that suggests that oxidative stress is a contributing factor to the development of the disease (8). When there is a significant reduction or absence of antioxidant defence, oxidative stress occurs (9, 10).

Moreover, it occurs when the antioxidant defence mechanism is overpowered by an excessive synthesis of reactive oxygen species (ROS). Free radicals are chemical entities with an unpaired electron that are extremely reactive. They are either products of metabolism or produced during phagocytosis in the extra-nuclear compartment by the mitochondrial respiratory chain and the mixed function oxidase system. Due to oxidative stress, free radicals and ROS are responsible for the damageof breast cells and these factors can cause lipids, cell membranes, proteins, and genetic content to deform severely, acting as a carcinogen (11). A combination of the anticancer chemotherapy medicines doxorubicin and cyclophosphamide is used to treat the majority of individuals with breast cancer (12). Because of their toxicity, these anti-cancer drugs lower antioxidant levels by accelerating the peroxidation of membrane phospholipids' unsaturated fatty acids (12-13). Numerous cancer therapies, including radiotherapy and specific chemotherapy drugs, destroy tumor cells by activating oxidative stress pathways and producing ROS. (14, 15). Antioxidants in the body can decrease via chemotherapy treatments. The antioxidant status can, however, be enhanced by some medication combinations (16). Antioxidants are substances that counteract free radicals in vivo or in vitro to stop oxidative damage. During chemotherapy, a number of anti-cancer medications stimulate the body's enzymatic and non-enzymatic antioxidants, which modify the biological activity of the cell (17). Chemotherapy's impact on the catalase (CAT) and superoxide dismutase (SOD) enzymes in breast carcinoma patients was studied by Bindary et al. They found that chemotherapy lowers the levels of the CAT and SOD enzymes (18). The effects of breast cancer on the concentrations of various antioxidant-active enzymes and oxidative stress variables were investigated. In addition to lower levels of glutathione (GSH) and catalase, they found that breast cancer patients had higher NO and lipid peroxidation. Free radical production led to cellular destruction, which is what happened in this situation. Antioxidants, which are used to quench free radicals, are present in lower levels in breast cancer patients (19, 20). The current study compared the levels of antioxidant enzymes and hematological indices between the patients with breast cancer and healthy individuals at various treatment intervals.

Methods

Study design and study period: 40 breast cancer patients between the ages of 31 and 74 participated in the present cross-sectional study. The American Joint Committee on Cancer staging system was used to confirm the cancer disease stage. The Omega Cancer Hospital and GayatriVidyaParishad Institute of Health Care and Medical Technology in Visakhapatnam served as the referral source for all of the patients. Between July 2021 and March 2022, the study was carried out. Every participant gave written, informed consent, and the study was approved by the Institutional Ethics Committee.

Exclusion criteria : Participants who used contraceptive pills, smoked, drank coffee or alcohol, used vitamins or other antioxidant supplements, or had any genetic abnormalities or other malignant disorders were excluded from the study.

Inclusion criteria: The study comprised 40 patients with histopathologically confirmed breast cancer and belongs to curative group. Adriamycin 60 mg/m2 and cytoxan 600 mg/m2 were given to the patients as part of the AC protocol. These patients also received PEG and GCSF drugs as these drugs preserves and increases WBC counts and they become neutrophils.

Methods: An International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEC) questionnaire (21) was completed by all study participants. The questionnaire inquired about standard demographic information (age and gender), medical conditions (x-ray exposure, vaccinations, and medications), lifestyle (smoking (22), alcohol and coffee consumption, diet, etc.), and employment (including the number of hours worked per day and protective measures taken). Based on the data collected for demographic variables, medical conditions and life style choices, we selected the study population. All 40 breast cancer patients had information on their clinical characteristics, breast cancer stages, grade, tumor origin, surgery type, progesterone, estrogen, and HER2 receptor status recorded from their hospital medical records. The study included a test group of 40 patients who had been diagnosed with breast cancer and a control group of 40 persons who were healthy age-matched females. Blood samples were collected from each patient before undergoing any type of treatment and after receiving three cycles of AC Chemotherapy. The 1st sampling was done four weeks following surgery, prior to the start of chemotherapy n=40 (C0), and 2nd sampling was done after three courses of chemotherapy (usually, after 9 weeks from first chemotherapy (C2) and similarly followed by third sampling (C4). Peripheral blood samples were sent to the biochemical lab and preserved at 50^{o}C for biochemical analysis following serum separation. Malondialdehyde (MDA), nitric oxide (NO), antioxidant enzyme activities

(total antioxidant status, catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase, and glutathione transferase), and hematological indices like white blood cell count, platelet count, hemoglobin level, neutrophil level, and lymphocyte level were all measured in the serum samples. The WHO standard range and the results of the CBC SYSMEX XK -21N hematology analyzer were used to pinpoint the specific hematologic abnormalities. Using the Griess method, nitric oxide (NO) was measured, and the estimate of thiobarbituric acid reactive substances (TBARS) method was used to evaluate lipid peroxide (malondialdehyde) (23). The Aebi method, was used to detect the catalase activity (24) and ferric reducing antioxidant power (FRAP) assay, was used to evaluate the overall antioxidant state (25, 26) with spectrophotometric analysis. As per protocol, the serum SOD, GR, GPx, and GST activity in the specimens (U/mL) was measured using a spectrophotometric assay (27, 28). Following the injection of the prepared standards and samples into the device, the concentration of the samples was determined in parts per billion (ppb) using the standard curve.

Statistical analysis: The data was examined using social sciences (SPSS) 16.0 Version. A descriptive analysis was used to assess the sociodemographic characteristics of the research participants. The paired-t test was used to compare the mean±SD of antioxidant enzymes, hematological indices, and oxidative biomarkers. A $p < 0.05$, statistical value was considered significant.

Results

The demographic data and clinical features of breast carcinoma patients were depicted in Figure 1 and Figure 2. Our demographic data and clinical features of breast carcinoma patients were depicted in figures 1 and 2. Our study included 40 breast cancer patients and 40 healthy controls. The average age of the study participants ranged from 30 to 75. In 55% of the breast cancer cases, there was a familial history. Most of the breast cancer patients 65% were in premenopausal stage. There were 55% of controls and 60% of married cases in terms of marital status. 25% of the controls and patients were finished secondary school and 22.5% of cases were not able to read and write (figure 1). Clinical variables such as cancer site, clinical stage, and HER-2/neu, ER (oestrogen receptor), and PR (progesterone receptor) status were determined by reviewing medical records (figure 2). All patients were staged clinically according to the AJCC 8th edition TNM classification into stages 1, 2 and 3 in the current study (figure 2). 55% of the patients were detected with carcinoma on left breast and majority was in Grade 3 clinical stage 37.5%. Most of the patients express estrogen and progesterone receptors 72.5%. Invasive ductal carcinoma was present in 66.3% of the 40 individuals, aged in between 30-75. Currently, table 1 displayed the comparisons of various biochemical characteristics between healthy individuals and those with breast cancer. The findings demonstrated a highly significant decrease in the mean values of MDA, NO, TAS, CAT, GPx, GR, SOD, and GST over the course of several subsequent cycles of therapy ($p < 0.05$). Table 2 compares the hematological parameters of breast cancer patients and healthy individuals during different anticancer therapy cycles. The mean values of hemoglobin, platelet count, neutrophil count, and lymphocyte count all significantly decreased over the course of the various treatment cycles ($p<0.05$), but the mean value of TWBC count varied insignificantly over the course of the anticancer treatment cycles ($p>0.05$). The tumor cells exhibited homogeneous intense membrane HER2/neu and IHC Ki 67 positivity, as well as strong nuclear estrogen receptor (ER) and strong nuclear progesterone receptor (PR) in figure 3. Images of breast cancer were displayed in figure 4.

Table 1. Comparison of biochemical variables between control and breast cancer patients

Biochemical Variables		Mean	SEM	SD	P-value
Malondialdehyde (MDA)	Control	82.93	5.94	18.77	0.001
	1st cycle	62.15	7.05	22.29	
	2nd cycle	57.04	5.86	18.52	
	3rd cycle	42.80	5.09	16.08	
Nitric Oxide	Control	20.91	1.31	4.13	0.01
	st cycle	11.94	2.23	7.06	
	2nd cycle	9.84	2.14	6.96	
	3rd cycle	6.12	0.63	1.98	

Biochemical Variables		Mean	SEM	SD	P-value
Total antioxidant status level	Control	865.00	19.82	62.67	0.03
	1st cycle	691.48	27.86	88.09	
	2nd cycle	536.16	19.25	60.86	
	3rd cycle	525.24	27.61	87.32	
Superoxide dismutase	Control	37.75	2.03	6.42	0.001
	1st cycle	25.21	2.34	7.41	
	2nd cycle	19.98	1.33	4.19	
	3rd cycle	16.79	2.66	8.40	
Catalase	Control	565.98	21.24	67.18	0.00001
	1st cycle	417.72	62.73	198.37	
	2nd cycle	397.54	46.93	148.41	
	3rd cycle	268.44	15.99	50.56	
Glutathione Peroxidase (GPx)	Control	94.26	7.92	25.05	0.01
	1st cycle	66.57	9.23	29.18	
	2nd cycle	67.16	7.52	23.79	
	3rd cycle	52.37	6.63	20.95	
Glutathione Reductase	Control	5.23	0.20	0.62	0.001
	1st cycle	8.11	0.23	0.71	
	2nd cycle	7.39	0.39	1.23	
	3rd cycle	6.22	0.28	0.87	
Glutathione transferase (GST)	Control	299.11	31.46	99.48	0.001
	1st cycle	537.36	36.65	115.89	
	2nd cycle	494.91	40.48	128.02	
	3rd cycle	388.37	23.38	73.9	

Table 2. Comparison of hematological variables between control and breast cancer patients

Hematological Variables		Mean	SEM	SD	P-value
Hemoglobin	Control	12.1375	1.12105	0.17725	0.01
	1st cycle	11.3800	1.05956	0.16753	
	2nd cycle	10.3175	1.31459	0.20785	
	3rd cycle	8.4550	0.90948	0.14380	
Platelet count	Control	3.5100	0.38551	0.06095	0.01
	1st cycle	3.4725	0.44949	0.07107	
	2nd cycle	2.5800	0.64498	0.10198	
	3rd cycle	2.4675	0.62199	0.09834	
Total white blood count	Control	10.0375	0.61590	0.09738	0.06
	1st cycle	10.9800	0.85521	0.13522	
	2nd cycle	10.0750	0.76519	0.12099	
	3rd cycle	9.3125	0.84039	0.13288	

Hematological Variables		Mean	SEM	SD	P-value
Neutrophils	Control	56.1250	9.29623	1.46986	0.01
	1st cycle	59.6750	7.51200	1.18775	
	2nd cycle	55.7250	8.44587	1.33541	
	3rd cycle	51.6750	9.32707	1.47474	
Lymphocytes	Control	33.8750	7.73665	1.22327	0.02
	1st cycle	34.8750	7.71009	1.21907	
	2nd cycle	28.6000	7.30578	1.15514	
	3rd cycle	27.8500	6.48292	1.02504	

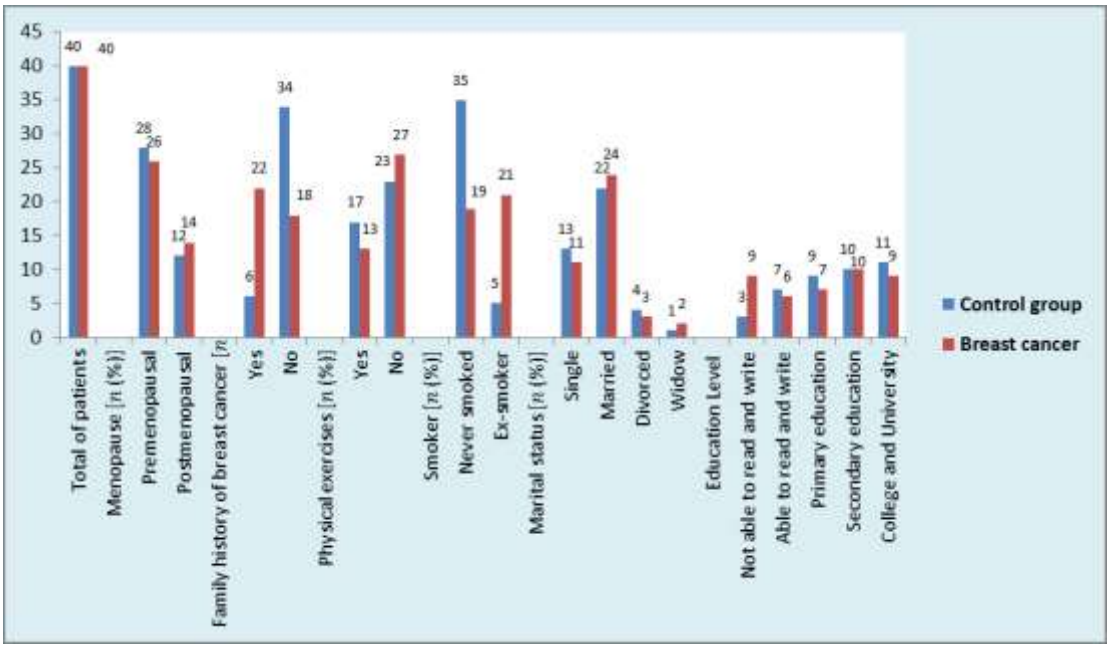

Figure 1. Demographic variables of the study

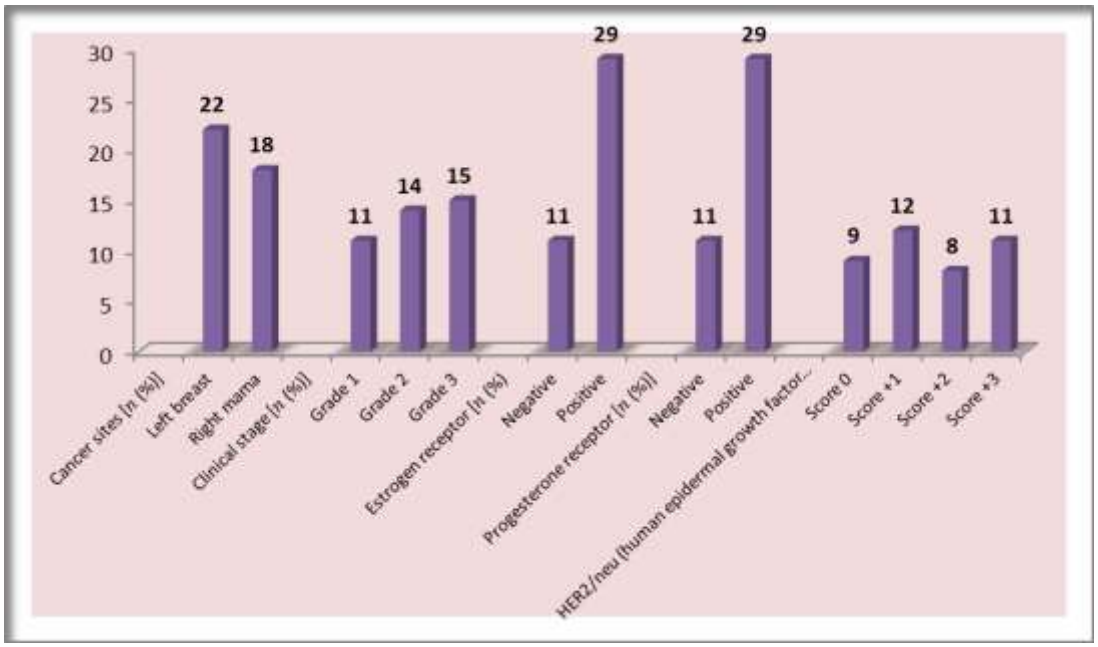

Figure 2. Clinical features of the study participants

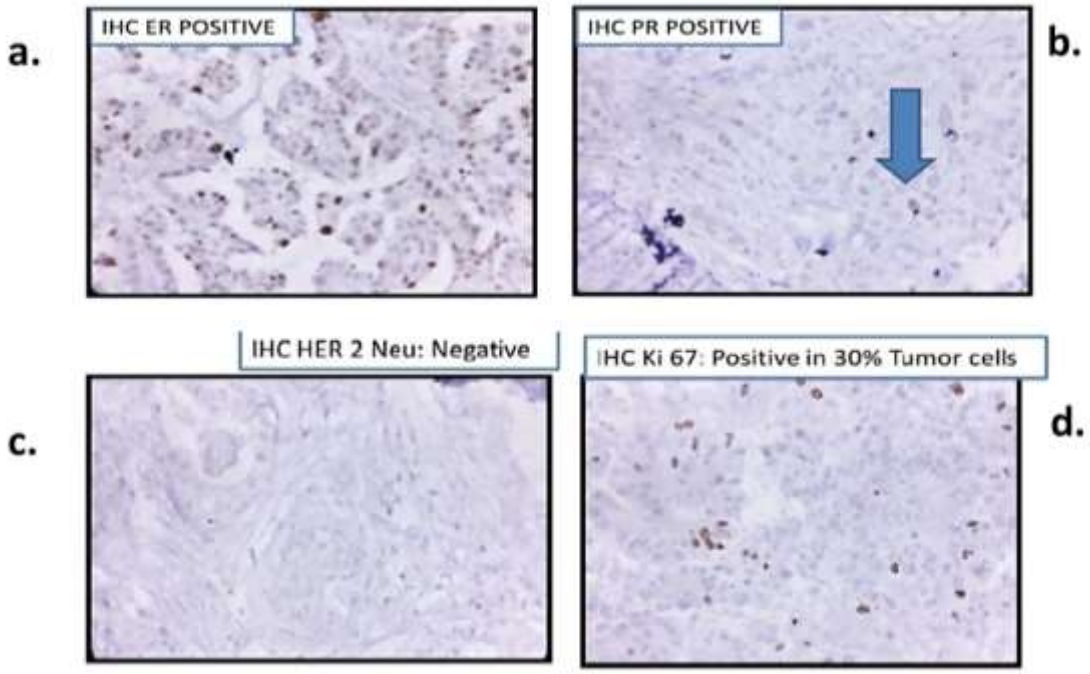

Figure 3. a-d) Showed strong nuclear estrogen receptor (ER), strong nuclear progesterone receptor (PR) and uniform intense membrane HER2/neu and IHC Ki 67 immunoreactivity in tumor cells

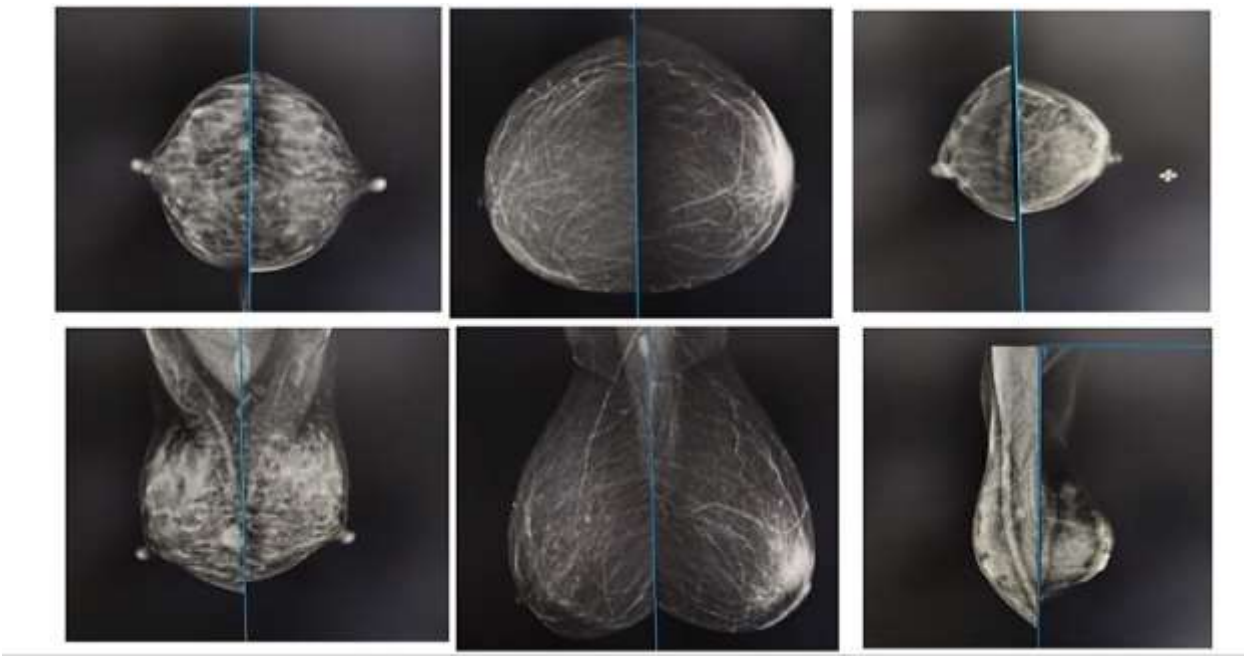

Figure 4. A) Hypoechoic lesion extending from 7'0 – 8'0 clock in right breast 3cm present malignant lesion BRADS-6. B) Well defined oval hypo echoic lesion from 8'0 clock -9'0 clock position BRADS-5. Enlarge left axillary lymph nodes with thickened cortex likely metastatic BRADS-4. Figure 4c: Lobulated hyper dense lesion with multiple clusters of tiny calcific foci in left retroaerolar region. Malignant lesion BRADS-6.

Discussion

Thousands of people are diagnosed with cancer each year, and chemotherapy is still the preferred anti-cancer treatment (27). Medication used in chemotherapy can alter body tissues and metabolic processes in a number of ways, which can raise oxidative stress and lower antioxidant capacity. The most potent anti-neoplastic therapy medications currently available are cyclophosphamide, Adriamycin, and (CA), which are administered to millions of women worldwide as an adjuvant or palliative treatment

for breast cancer. Nowadays neoadjuvant therapy was introduced for the treatment of high-risk breast carcinoma patients where three drug regimens were followed for the treatment. For example, gemcitabine is added to accelerated paclitaxel with epirubicin or cyclophosphamide was used (28). Our results showed that following three sessions of AC treatment, the mean levels of MDA, NO, TAS, CAT, GPx, GR, SOD, and GST in breast cancer patients decreased significantly. It appears that the vulnerability of tumors to oxidative stress during anticancer pharmaceutical treatment is dependent on their antioxidant level (29-33). Similar to this, our research validates these findings regarding the negative effects of chemotherapy on hematological and biochemical markers. According to the study, which was conducted during the cycles, hemoglobin increased during cycles and decreased after therapy. Following treatment, there was a decrease in several antioxidant enzymes in the white blood cells (WBC), neutrophils, platelets, and lymphocytes. It has been proposed that carcinogenesis is significantly influenced by oxidative stress, which is created by either increased free radical formation or a decreased antioxidant level in the target cells and tissues. ROS involved in both the development and spread of cancer (34, 35). Nearly all malignancies have been found to contain elevated levels of ROS, which support a number of features of tumor growth and development. Increased ROS levels can start DNA damage, which could eventually cause carcinogenesis (36). Our research suggested that individuals with breast cancer who received AC chemotherapy experienced higher levels of oxidative stress. We demonstrated that a lipid peroxidation measure called malondialdehyde significantly increases in more advanced stages of breast cancer (37). One research study claims that the concurrent use of antioxidants and chemotherapy factors may reduce the effectiveness of the chemotherapy or promote the development of enzymes that can reduce the toxicity of cytotoxic factors (28). Millions of women with breast cancer worldwide receive prescriptions for cyclophosphamide and Adriamycin (AC), the most potent anti-neoplastic therapy medications now available, as adjuvant or palliative treatments for breast cancer. The concentrations of many antioxidant-active enzymes, as well as oxidative stress markers such lipid peroxidation and NO. The current investigation found that the chemotherapy (AC regimen) had a detrimental effect on hematological and biochemical indicators, causing liver dysfunction, neutropenia, thrombocytopenia, and anemia (38). The highly reactive, short-lived free radical nitric oxide has the ability to either trigger or prevent apoptosis. In the current study, nitric oxide levels were shown to be significantly greater in postoperative stage III and stage IV breast cancer patients compared to healthy controls, both before to and throughout chemotherapy. Numerous researchers have discovered increased nitric oxide levels in serum from breast cancer patients when their disease was still operable (39, 40). The activities of antioxidant enzymes like GPx and GR significantly decreased in the study by Aturkeren et al. on 30 patients utilizing adjuvant anthracycline-based chemotherapy before, during, and after treatment (41). After the AC treatment, GPx and GR significantly decreased in our study. Our analysis was conducted following the third chemotherapy cycle, and we also looked at the levels of the hematological profiles.CAT and GR levels were shown to have significantly decreased in Ragab et al.'s study from 2014 (42). In our study, patients with breast cancer were treated, and catalase activity was considerably reduced after therapy compared to before. When compared to control groups, the antioxidant levels of breast cancer patients receiving chemotherapy are significantly lower (43). Another investigation demonstrated that chemotherapy dramatically reduced r-CAT activity and increased oxygen free radical generation (44). It was found that after receiving chemotherapy, human polymorphonuclear leukocytes, whether stimulated or unstimulated, produced higher levels of hydrogen peroxide and superoxide anion. As shown by the thiobarbituric acid assay, this was accompanied by a rise in the production of lipid peroxidation products (45-46). Instead of only affecting cancer cells, the majority of chemotherapy medications also affect normal cells. Alopecia, fatigue, a generalized rash, diarrhea, and dizziness are just a few of the unpleasant side effects it causes in practically all bodily tissues.

Our findings showed that three sessions of AC chemotherapy altered the body's oxidant/antioxidant system and dramatically reduced antioxidant capacity. After chemotherapy is finished, it is suggested that the body's antioxidant system be strengthened with antioxidant supplements and dietary natural antioxidant substances. The difficulties brought on by high oxidative stress in other tissues and organs appear to be avoidable in this way. For patients with breast cancer, monitoring serum oxidative stress indicators may be useful in assessing the effects of treatment. Our findings showed that AC treatment increased malondialdehyde, a measure of lipid peroxidation, and reduced the overall antioxidant status of breast cancer patients. Increased lipid peroxidation and oxidative stress are the main causes of breast cancer spread. At all projected risk levels, our findings largely confirm the significance of endogenous antioxidants in the development of breast cancer.

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Authors' contribution: Tejaswi Pullakanam: conception and design, data collection, analysis and interpretation of results and manuscript preparation. Murugan Mannangatti: guiding the whole research work and approved the final version of the manuscript. Alamuri Ramesh reviewed the results. B. Pradeep Kumar: manuscript preparation. Ramakrishna Nekkala: manuscript preparation. Payala Vijayalakshmi: data analysis.

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