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Research Article

Evaluation of Oxidative Stress and Clinical Parameters among Female Breast Cancer Patients of Sargodha, Pakistan

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Abstract

Background: Breast cancer is the most prevalent malignancy and is one leading cause of death among women all over the globe. This study was conducted to assess the clinical parameters including different biochemical and hematological parameters along with oxidative stress among the female breast cancer patients of Sargodha, Pakistan.

Methods: Total 55 females were recruited for this study through informed consent. Among them 40 was female breast cancer patients from DHQ hospital Sargodha and 15 were control subjects. Blood was taken and estimation of oxidative stress index along with hematological and biochemical parameters were done in subjects under study.

Results: Decreased activity of antioxidant enzymes (Catalase, Super oxide dismutase and Glutathione peroxidase) was observed among the females suffering from breast cancer. While MDA, a product of lipid peroxidation was found significantly higher ($p<0.05$) in cancerous group (2.96 µmol/L ± 2.27 µmol/L). Decreased total antioxidant capacity and increased per-oxidant concentration was also found in cancerous patients. Among hematological parameters significant difference was observed in the levels of MCH, MCHC, Lymphocytes number, and percentage of lymphocytes, MPV, PDW and MXD.

Conclusion: It is obvious from the study that oxidative stress and alterations in hematological indices are associated with breast cancer. Oxidative injury might be involved in the disease progression.

Keywords: Breast cancer; Antioxidants; Catalase; SOD; Glutathione

Introduction

Breast cancer is the most commonly diagnosed malignancy that leads to death in females across the world and the highest cause of deaths in female with almost 2.30 million cases being diagnosed in 2020 [1]. Breast cancer being undiagnosed and untreated in developing economies is a major cause of deaths among females [2]. The main cause high death rate is scarcity of awareness and late prognosis of disease [3]. Incidence of breast cancer is rising in most areas of the world, but there are enormous variations between developed and developing states [4]. Currently, more than half cases of breast cancer patients are being reported in the developing countries [5]. In most of the developing countries the prevalence of breast cancer is less than 40 out of 100,000 females [6]. In many low- and middle-income countries, the rate of breast cancer is increasing gradually due to variations in some factors like age at menarche, age at menopause, number of children, age at pregnancy and sedentary lifestyle [7]. Breast carcinoma is the maximum prevailed cancer among 140/184 countries and it is the reason of 15% deaths among female in the whole world [8]. Globally 5, 21900 deaths have been reported due to breast cancer [9]. It is anticipated that cases of breast cancer will reach up to 22 million in next two decades [10].

The prevalence rate of breast cancer is maximum in Europe, North America and Oceania while it is lowest in Africa [11]. In European Union, deaths due to breast cancer increased by 21.3% from 2005 to 2015 [12]. In Eastern Africa, the prevalence of breast carcinoma is 19.3 out of 100,000 females and 89.7 out of 100,000 females in Western Europe.

Breast carcinoma is the major type of cancer after lung cancer responsible for female mortality among Asian countries like Russia, China and India [13]. Asian females have lower prevalence rate of breast cancers as compared to Western white females [14]. The frequency of breast cancer among women in East and Southeast Asia has risen sharply in recent 40 years [15]. The incidence rate of breast cancer is highest among females of 50 years in Hong Kong, Japan, Taiwan and Korea while it is highest among females of 70 years in the United States [16].

Pakistan is also facing the awful rise in the occurrence of breast cancer due to late-stage clinical presentation being a common aspect. However, in Pakistan there is rare nationwide breast cancer incidence, death, or risk factor information existing, yet it has been stated as the common malignancy, reporting of 34.6% of women cancers. The rate of incidence of breast cancer in Pakistan is 2.5 times more than Iran [17]. Age Standardized Incidence Rate (ASIR) of breast cancer among the women in Pakistan is 61.9 per 100,000 which are

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the highest ASIR in Asian people after Jews [18]. Pakistani females have greater (50/100,000) incidence rate as compare to the females in India (19/100,000). More than half of the breast cancer patients have advanced stages of cancer (stages III and IV). Regular medical breast examination and mammography of females according to the globally acknowledged instructions can cause down-staging of breast cancer of asymptomatic females. In the Pakistani framework, educating the females about the risk factors of breast cancer can establish first step to early prognosis of breast cancer, so that females would be able to evaluate their risk and take appropriate actions [19].

Early diagnosis of breast cancer and its cure can lead to the recovery of patients and high survival rates. Regardless of progressive screening and detection methods, this disease often remains unnoticed until it has got an advanced stage. Pakistani females exhibit less knowledge regarding the risk factors and signs associated with breast cancer [20]. The females have less awareness about screening tests and examination related to breast cancer [21]. Awareness regarding risk factors associated with breast cancer depends upon academic and professional status of women [22]. Presently a small fraction of females has better perception about Breast self-examination. Majority of females have positive attitude for breast self-examination, but they experienced BSE once-a-month [23]. Low-income women performed BSE lower than high income women, per month. Females with poor awareness about risk factors associated to breast cancer have low BSE performance than women with good knowledge. Women with cancer relatives have low BSE performance as compared to women without cancer relatives. Close relative with breast cancer, income, knowledge about risk factors and marital status affect the occurrence of BSE performance. Perception regarding risk factors in public health campaigns can encourage BSE performance [24]. Urban women have encouraging attitudes about breast carcinoma diagnosis performance than rural women [25]. Women require more knowledge regarding breast carcinoma. Women need more access to breast cancer examinations, mammography and occurrence of breast cancer [26]. Community awareness programs are needed to prevent an increasing problem of breast cancer among females [27]. In Sargodha risk of breast cancer in females is growing day by day but no such research has been conducted in this area. The aim of this research was to assess the knowledge about breast cancer.

Change in hematological parameters during cancer can be used to predict severity disease and death risk among breast cancer patients. Poor hematological parameters result in poor outcomes of breast cancers [28]. Breast cancer patients have high blood leukocyte count as compared to the healthy females [29]. Values of platelet count, red blood cell distribution width, number of neutrophils/lymphocytes ratio and Platelet count/Lymphocytes ratio (PLR) become also greater among breast cancer female's patients than healthy females [12]. Erythrocyte Sedimentation Rate also prolonged among breast cancer patients than to the healthy persons [30]. The value of PCV becomes low in breast cancer patients while it is high among healthy females. The mean of MCV, MCHC, and MCH also become less among breast cancer patients while it is high among healthy females [28].

Differences in biochemical parameters can also be used to predict the severity of disease. Level of urea, uric acid and bilirubin become greater among breast cancer female's patients than healthy females while the level of creatinine, Serum Glutamic Pyruvic Transaminase (SGPT) and glucose level remains normal [31]. Higher metabolism of breast cancer cells is usually related with a rise in ROS. Antioxidants are responsible for cellular mechanism of defense which results in increase in reactive oxygen species. The ROS have ability to stop tumorigenesis and can raise life span. Apart from their defensive role as protective mediators against breast cancer growth, there is sign that antioxidant reduce toxicities in breast tumor. Some specific antioxidant enzymes play defensive roles in breast cancer are Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPXs) and Peroxiredoxins (PRXs) [32].

Oxidative stress occurs in breast cancer patients if there is no balance between generation of ROS and their removal by antioxidant defense system. Normal cellular metabolic process produces ROS which play a vital role by triggering the signaling path in results of changings in intracellular and extracellular environments [33]. Stress is increased due to high amount of ROS and low number of antioxidants [34,35].

ROS is mainly produced by mitochondria. Lesser amount of ROS is also produced by Peroxisomes, inflammatory cells, cytochrome P450 system inflammatory cells [36,37]. UV, xenobiotics, anti-estrogen tamoxifen and X-ray radiations can also produce ROS [38,39]. 3×1022 free radicals are produced in a normal individual per hour [40]. Oxidative variations are due to the constant generation of ROS with the help of mitochondria. A great diversity of redox sensors is existed in the cells to compensate the ROS generation by triggering the antioxidant defense system. High amount of ROS or inadequate antioxidants produced oxidative stress [41,42]. Pathogenesis of any type of cancer is due to the contribution of superoxide anion (O_20-), hydroxyl radical (OH-) and hydrogen peroxide (H_2O_2) [43]. Catalase carries out the reduction of H_2O_2, but this enzyme is suppressed by the superoxide anion (O_20-) [44]. H_2O_2 are the reason of cellular damages as it can cross the cellular membranes [33]. Catalase and glutathione peroxidase breakdown the H_2O_2 into molecular oxygen and water. OH, produce lethal damages i.e., DNA damage in mammals [45]. There is positive relation of H_2O_2 and O_20- with breast malignancy [46]. Cancer is originated by constant oxidative stress which is involved in suppression of genes that inhibit tumors [39].

Damage caused by ROS help as an indication for the triggering of disease development by beginning the inflammatory process. Metastasis progression and neoplastic progression is due to the oxidative stress [47]. For increasing the survival rate cancer cells must support metabolic changes. Warburg Effect [48] the change in metabolism of cancers. This effect proposes that cancer cells favor the process of glycolysis rather than oxidative phosphorylation to achieve the energy necessities [49]. Due to mutations in mitochondrial DNA and oncogenic indications, tumor cells express dysfunction of mitochondria and are more reliant on glycolytic pathway [50]. Reverse Warburg Effect has been detected among breast cancer patients [51]. It shows compartmentalization of glycolytic metabolism and oxidative metabolism in mitochondria which recommended elevation in breast cancer [52]. Owed to malfunctioning of oxidative phosphorylation, reactive oxygen species lead to tumor stimulation, promotion and growth [53].

Reactive species produce many chemical changes that provoke DNA damages through the processes of methylation and oxidation [41]. Poly-unsaturated fatty acids are primarily targeted by ROS which cause the lipid peroxidation [54,55]. Antioxidants defense system is the more effective method of defending the body from the deadly effect of ROS [45]. Defense mechanisms of cell comprises of non-enzymatic antioxidants (Vitamin A, C, E and glutathione)

and enzymatic one (Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase and Peroxiredoxins) [45]. Oxidative stress is estimated by products of the reaction bio-molecules with ROS [56].

Some antioxidant enzymes (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPXs) and Peroxiredoxins (PRXs)) can suppress the rise in oxidative stress. Substantial rise in the level of SOD, CAT, GPx, GSH and GST in samples from breast cancer patients are present [57]. SOD carries out the conversion of the superoxide anion into H_2O_2 which breakdown into water and oxygen with the help of catalase (Figure 1). H_2O_2 can also carry out the oxidation of 4 Glutathione (GSH) into Glutathione Disulfide (GSSH). Reduced thioredoxin (Trx red) is changed into the oxidized thioredoxin (Trx ox) by using H_2O_2 and reaction rate is increased by glutathione peroxidase and peroxidases for thioredoxin turnover (PRX). Reduced glutathione is stored by glutathione reductase. Glutathione and thioredoxin both are used to reduce the oxidized proteins [58].

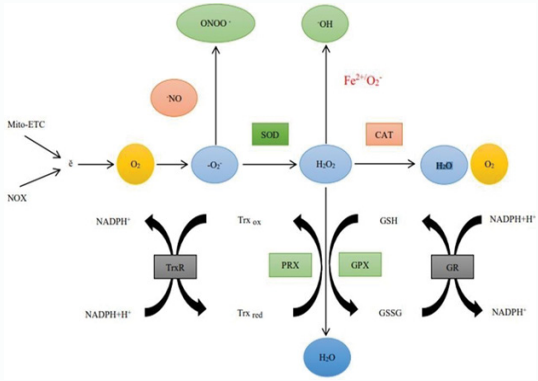

Figure 1: Generation and Elimination of ROS [58].

Superoxide Dismutase (SOD) is responsible for breakdown of superoxide anions into hydrogen peroxide. Excessive accumulation of reactive species can cause deadly damage and producing the reduction in activity of SOD [39]. Catalase is a homo-tetrameric anti-oxidant enzyme responsible for defending the cells from ROS induced effects by decaying the hydrogen peroxide (H_2O_2) [59]. It changes the hydrogen peroxide into oxygen and water [60]. Cancer expansion and metastasis is extremely related with reduced activity of catalase [61]. Catalase and SOD act as the anti-toxins, anti-carcinogens and suppressors for the development and expansion of carcinomas [62].

MDA is produced by the action of ROS on poly un-saturated fatty acids [63]. Elevated MDA levels have been determined in breast cancer patients [64]. Some biomarkers are used to estimate the oxidative stress like Melanodialdehyde, 8-F2-isoprostanes and 4-hydroxynonenal [65,66]. Glutathione peroxidases anti-oxidants are responsible to convert the H_2O_2 and increase the oxidation of GSH while H_2O_2 is at the same time convert into molecular oxygen and water [45].

Materials and Methods

Sampling

The study was carried out to assess the clinical parameters and oxidative stress among breast cancer patients. Samples were collected from the District Headquarter hospital, Sargodha. All the subjects in control and experimental group were women. Forty five breast cancer patients and 25 healthy individuals were agreed to participate but 40 diseased subjects and 15 healthy were recruited for research that fall under inclusion criteria. All study subjects were given a questionnaire and had face-to-face interviews, particularly to have demographic data regarding their age, residential status and questions relating to different medical issues (vaccinations, exposure to X-rays, medication, etc.) along with lifestyle (exercise and diet preferences.), history of different chronic diseases, cancer history and occupation After assessing the clinical reports for mammography and histopathology features such as diagnosis, cancer stage, tumor grade, lymph node association and metastasis, 10 patients were eliminated from experimental group and 5 from control group.

Blood samples were collected by venous arm puncture from control and study group with the help of sterile injections. Total 6 ml of blood from each patient was drawn in EDTA tubes which are further used for Complete Blood Count (CBC) test and experimental analysis for oxidative stress. Samples were immediately placed in the ice boxes where ice bags were placed to maintain the temperature at 4°C. After collection of samples, blood CBC tests have been performed through automated hematology analyzer. For plasma extraction, Samples were centrifuged for 1 minutes at 4100 rpm in Humax-4K centrifuge at DHQ laboratory. Micropipette was used to separate the plasma present on the upper layer and then placed into labeled Eppendorf. Plasma samples then immediately stored at -20°C in ultra-freezer. UV-Visible spectrophotometer was used for investigating the levels and measuring the absorbance of antioxidants in plasma (Figure 2).

Figure 2: Sampling site as DHQ Sargodha (Source-Google maps).

Exclusion criteria

Subjects with any genetic disease history, smokers and alcohol consumers were excluded from the present study. The individuals aged less than 33 years and more than 65 years were also excluded from this study to minimize the effect of age on the results.

Oxidative stress index and anti-oxidant assays

Total Antioxidant Capacity (TAC) and Total Peroxides (TP) are collectively called Oxidative Stress Index (OSI). Frap assay was used to determine TAC. FRAP assay principle is that Ferric Tripyridyl Triazine (Fe III TPTZ) gets reduced to Ferrous Tripyridyl Triazine and gives intense blue color on low PH. FOX_2 method was used to measure (TP) of samples. The method is based on oxidation reaction; ferrous ions oxidize to ferric ion because of peroxides present in sample and for ferric xylenol complex and give orange color.

Oxidative stress index is calculated as follow: OSI=[Total Peroxides (TP) × 100]/Total Antioxidant Capacity (TAC).

Catalase assay principle is based on H_2O_2 decomposition rate which was measured through spectrophotometer at wavelength of 240 nm. Superoxide Dismutase (SOD) assay principle is based on inhibition of Nitro Blue Tetrazolium (NBT) caused by SOD. Lipid Peroxidation assay principle is that when lipid peroxidation product MDA is heated with Thiobarbituric Acid (TBA) their reaction produces orange to pink colored product and absorbance of this supernatant is recorded at 535 nm. Glutathione Peroxidase assay principle is based on oxidizing ability of glutathione peroxidase to use NADPA during conversion of lipid peroxidase and H_2O_2 wavelength of 340 nm.

Statistical analysis

The 25th version of Statistical Package for Social Sciences (SPSS) has been used for analysis of data. Independent sample t-test was applied to evaluate the significance of clinical and oxidative stress parameters.

Results

Hematological parameters

Different hematological parameters were assessed among control and cancer patients by using independent sample t test. The mean levels of white blood cells (103/µl) in cancerous group and non-cancerous group were recorded as 7.83 ± 3.77 and 7.97 ± 2.17. The mean values observed for red blood cells (106/µl) in cancerous group and non-cancerous group were 4.37 ± 1.14 and 4.15 ± 0.76 respectively. Higher number of red blood cells was recorded higher in cancerous group. Level of hemoglobin (g/dl) in cancerous group and non-cancerous group was measured as 10.46 ± 1.98 and 11.20 ± 2.05 respectively. The mean values for Hematocrit % in cancerous group and non-cancerous group was 33.46 ± 6.16 and 34.74 ± 6.02 respectively. Mean level noted for MCH (pg) in cancerous group and non-cancerous group was recorded as 24.24 ± 2.63 and 26.25 ± 1.80. Level of MCHC (g/dl) in cancerous group, non-cancerous group was observed as 30.96 ± 1.54, 33.28 ± 1.44. Mean values for MCV (fl) in cancerous group, non-cancerous group were 78.17 ± 8.23, 81.41 ± 5.34. Level of Lymphocytes in cancerous group and non-cancerous group was measured as 1.75 ± 0.92 and 2.52 ± 0.29. The lymphocytes were recorded higher in non-cancerous group. Mean values found for Lymphocytes % in cancerous group and non-cancerous group were 21.34 ± 12.98 and 31.92 ± 6.85 respectively. Platelets count (103/µl) in cancerous group and non-cancerous group overall was recorded as 262.07 ± 100.79 and 314.20. Platelets count was found higher in non- cancerous group. The mean value for PLCR % in cancerous group, non-cancerous group and overall were 33.25 ± 16.47 and 32.5 ± 9.94. Mean level of MPV (fl) in cancerous group was 8.90 ± 1.74 and non-cancerous group was 10.55 ± 1.36. Mean level for RDW (%) in cancerous group and non-cancerous group were observed as 18.95 ± 8.87 and 16.45 ± 3.04. The mean values for Neutrophils in cancerous group, non-cancerous group were 5.80 ± 3.18 and 4.71 ± 2.13 respectively. Percentage of neutrophils was found higher in non-cancerous group. The mean values for Neutrophils % in cancerous group, non-cancerous group were 67.21 ± 3.51 and 62.69 ± 7.43 respectively. Mean level observed for MXD# in cancerous group and non-cancerous group was 1.40 ± 0.87 and 0.51 ± 0.32 respectively. Percentage of MXD was found higher in non-cancerous group. Mean values noted for MXD % in cancerous group and non-cancerous group were 0.20 ± 6.09 and 6.34 ± 2.12 Statistically significant difference ($p<0.05$) was found in the level of MCH, MCHC, Lymphocytes numbers, percentage of lymphocytes, MPV, PDW, MXD# while no significant difference ($p>0.05$) was observed in the level of WBC, RBC, hemoglobin, hematocrit, MCV, platelets, PLCR%, RDW, percentage of neutrophils, neutrophils#, MXD% among cancerous and non-cancerous females (Table 1).

Oxidative Stress index and antioxidant analysis

Oxidative stress was calculated by estimating the level of antioxidants. Level of catalase in cancerous group and non-cancerous group was recorded as 65.80 ± 28.69 and 126.80 ± 13.50 respectively. Catalase level was higher in noncancerous group as compared to cancerous group which means a substantial decrease in activity of catalase was found in breast carcinoma. Mean SOD level was measured in U/L. The mean values for SOD in cancerous group and non-cancerous group were 35.07 ± 22.22, 128.60 ± 14.02 respectively. SOD level was higher in noncancerous group as compared to cancerous group. GPx level was noted in (U/L). Level of GPx in cancerous group, non-cancerous group and overall was recorded as 73.99 ± 55.67 and 332.66 ± 75.49 respectively. GPx level was higher in noncancerous group as compared to cancerous group. Values of MDA were measured in µmol/L. Mean values for MDA are higher in cancerous group and non- cancerous group were 2.96 ± 2.27 and 0.77 ± 0.28, respectively. Cancerous group showed elevation in MDA level compared to the non-cancerous group. The mean values for TAC in cancerous group and non-cancerous group were 40.64 ± 33.33 and 116.6 ± 46.85. TAC level was higher in noncancerous group as compared to cancerous group. Level of TPC in cancerous group, non-cancerous group measured as 0.48 ± 0.40 and 0.17 ± 0.18 respectively. Oxidative stress index was calculated by given formula. OSI was higher in cancerous females than non-cancerous females. OSI level in cancerous group and non-cancerous group was recorded as 1.95 ± 1.87 and 0.40 ± 0.83 respectively. Statistically significant differences ($p<0.05$) were found in level of catalase, SOD, MDA, GPx, TAC, OSI and TPC between cancerous and non-cancerous group (Table 2).

Biochemical parameters

Level of glucose (mg/dl) in cancerous group and non-cancerous group were recorded as 158.26 ± 104.50 and 106.46 ± 26.12 respectively. Glucose level was higher in cancerous group as compared to non-cancerous group. The mean values for bilirubin (mg/dl) in cancerous group, non- cancerous group was measured as 0.85 ± 0.28 and 0.78 ± 0.18, respectively. Bilirubin level was higher in cancerous group as compared to non-cancerous group. Mean values for urea (mg/dl) in cancerous group and non-cancerous group were 30.39 ± 7.75 and 28.93 ± 5.29 respectively. Urea level was higher in cancerous group as compared to non-cancerous group. The mean values for creatinine (mg/dl) in cancerous group and non-cancerous group were as 0.81 ± 0.24 and 0.76 ± 0.14 respectively. Creatinine level was higher in cancerous group as compared to non-cancerous group ($t=0.852$). Mean level of Alkaline phosphatase (u/l) in cancerous group and non-cancerous group measured as 185.27 ± 23.43 and 179.73 ± 23.98 respectively. Alkaline phosphatase level was higher in cancerous group as compared to non-cancerous group. Level of SGPT (u/l) in cancerous group, non-cancerous group was observed as 28.92 ± 4.25, 42.40 ± 14.78 respectively. Statistically no significant differences ($P>0.05$) were found in biochemical parameters except SGPT was higher ($P<0.05$) in non-cancerous group (Table 3).

Discussion

Hematological profile of breast cancer patients in our studies have shown non-significant difference in WBC, RBC, hemoglobin, hematocrit, MCH, Lymphocytes, MCV, platelets, PLCR%, RDW,

Table 1: Statistical Analysis of Hematological Parameters (Independent sample t-test).

Hematological Parameters	Cancerous	Non-Cancerous	p-value
White blood cells (10^3/µl) (Mean ± SD)	7.83 ± 3.77	7.97 ± 2.17	0.895
Red blood cells (10^6/µl) (Mean ± SD)	4.37 ± 1.14	4.15 ± 0.76	0.495
Hemoglobin (g/dl) (Mean ± SD)	10.46 ± 1.98	11.20 ± 2.05	0.228
Hematocrit % (Mean ± SD)	33.46 ± 6.16	34.74 ± 6.02	0.492
MCH (pg) (Mean ± SD)	24.24 ± 2.63	26.25 ± 1.80	0.009
MCHC (g/dl) (Mean ± SD)	30.96 ± 1.54	33.28 ± 1.44	0
MCV (fl) (Mean ± SD)	78.17 ± 8.23	81.41 ± 5.34	0.163
Lymphocytes # (Mean ± SD)	1.75 ± 0.92	2.52 ± 0.29	0.008
Lymphocytes % (Mean ± SD)	21.34 ± 12.98	31.92 ± 6.85	0.004
Platelets (10^3/µl) (Mean ± SD)	262.07 ± 100.79	314.20 ± 87.09	0.083
PLCR % (Mean ± SD)	33.25 ± 16.47	32.5 ± 9.94	0.874
MPV (fl) (Mean ± SD)	8.90 ± 1.74	10.55 ± 1.36	0.002
PDW (fl) (Mean ± SD)	12.25 ± 3.86	14.66 ± 3.08	0.035
RDW % (Mean ± SD)	18.95 ± 8.87	16.45 ± 3.04	0.294
Neutrophils # (Mean ± SD)	5.80 ± 3.18	4.71 ± 2.13	0.229
Neutrophils % (Mean ± SD)	67.21 ± 3.51	62.69 ± 7.46	0.225
MXD # (Mean ± SD)	1.40 ± 0.87	0.51 ± 0.32	0
MXD % (Mean ± SD)	9.20 ± 6.09	6.34 ± 2.12	0.082

Table 2: Statistical Analysis of Oxidative stress Parameters (Independent sample t-test).

	Cancerous Group	Non-Cancerous	P value
Catalase (Mean ± SD) (U/ml)	65.80 ± 28.69	126.80 ± 13.50	0
SOD (Mean ± SD) (U/L)	35.07 ± 22.22	128.60 ± 14.02	0
FOX (µmol/L) (Mean ± SD)	0.48 ± 0.40	0.17 ± 0.18	0.01
GPx (Mean ± SD) (U/L)	73.99 ± 55.67	332.66 ± 75.49	0
FRAP (Mean ± SD) (µmol/L)	40.64 ± 33.33	116.6 ± 46.85	0
MDA (Mean ± SD) (µmol/L)	2.96 ± 2.27	0.77 ± 0.28	0.001
OSI (Mean ± SD)	1.95 ± 1.87	0.40 ± 0.83	0.003

Table 3: Statistical Analysis of Biochemical Parameters (Independent sample t-test).

	Cancerous Group	Non-Cancerous	t-value	p-value
Glucose (mg/dl) (Mean ± SD)	158.26 ± 104.50	106.46 ± 26.12	1.887	0.065
Bilirubin (mg/dl) (Mean ± SD)	8575 ± .28001	0.78 ± 0.18	0.993	0.325
Urea (mg/dl) (Mean ± SD)	30.3950 ± 7.75093	28.93 ± 5.29	0.672	0.505
Creatinine (mg/dl) (Mean ± SD)	0.81 ± 0.24	0.76 ± 0.14	0.852	0.398
Alkaline phosphatse (u/l) (Mean ± SD)	185.27 ± 23.43	179.73 ± 23.98	0.776	0.441
SGPT (u/l) (Mean ± SD)	28.92 ± 4.25	42.40 ± 14.78	-5.28	0

PDW, percentage of neutrophils, Neutrophils number and MXD% while significant decrease in the levels of MCHC, percentage of lymphocytes, MPV, MXD. Shrivastava et al. [67], have also examined the decrease in the levels of percentage of lymphocytes, hemoglobin, WBC and RBC.

It has been certified experimentally that ROS cause deterioration in cells by damaging the membrane, mitochondria, lipids and DNA which is accountable for the onset of inflammatory response and growth of tumor cells [68]. Proliferation potencies of breast cancer cells are highly associated with ROS activities [69]. Reactive oxygen intermediates give benefit by acting as intracellular messengers and by giving protection against microorganisms but excessive amount of reactive species cause damage to the cells [70]. Prevalence rate is higher among the Pakistani women due to the oxidative stress conditions and is listed at the top among the Asian countries [64].

Conferring to our results catalase and SOD levels were decreased in breast cancer patients significantly. Level of catalase in cancerous group was much lower as compared to in control group. Levels of SOD were significantly lower in cancerous patients in comparison with the control group. Sahu et al. [63] have also examined the similar results and described the levels of catalase much lower in cancerous group as compared to control group. Decrease in the level of SOD and increased levels of MDA were also reported in their studies. MDA analysis in our results has confirmed that stage of cancer has positive relation with the level of MDA. Level of MDA in cancerous patients was higher in cancerous. Seraj et al. [71], evaluated the same results. He examined that MDA level was considerably higher in case group as compared to the control group under study.

It has been observed that levels of glutathione peroxidase also decreased as the level was in cancerous group as compare to in control group. Investigations of Yeh et al. [72], have also reported the increased levels of lipid peroxidation products and superoxide radicals while Rajneesh et al. [57], evaluated the decreased level of glutathione in breast cancer and lipid peroxidation products level in human plasma. These findings have supported the hypothesis that breast carcinogenesis is due to the involvement of oxidative stress.

The overall decrease in Total Antioxidant Capacity (TAC) as Zowczak-Drabarczyk et al. [73], have reported the similar results and have demonstrated the decrease in plasma TAC in breast carcinoma. It has been proved from experimental analysis of their research that level of TAC decreased because of higher consumption of antioxidants. Amin [22] have also reported the decrease in total antioxidant capacity and increased MDA level in breast cancer patients. They have evaluated the decrease in status of TAC from 1.48 ± 0.04 µmol/L in control to 0.99 ± 0.02 in case group.

Differences in biochemical parameters among breast cancer female patients were also studied. In the present study, level of glucose (mg/dl) (158.26 ± 104.50 and 106.46 ± 26.12), bilirubin (mg/dl) (0.85 ± 0.28 and 0.78 ± .18), creatinine (mg/dl) (0.81 ± 0.24 and 0.76 ± 0.14), urea (mg/dl) (30.39 ± 7.75 and 28.93 ± 5.29), Alkaline phosphate (u/l) (185.27 ± 23.43 and 179.73 ± 23.98) was higher in cancerous group as compared to non-cancerous group. The mean values for SGPT (u/l) (28.92 ± 4.25 and 42.40 ± 14.78) was higher in non-cancerous group than cancerous group. Nandhini et al. [31] found that the mean values of urea (32.58 ± 19.7 mg/dl), uric acid

(27.9 ± 10.24 U/L) and bilirubin (0.30 ± 1.3 mg/dl) were found more than normal in cancerous patients while the level of creatinine (1.05 ± 0.59 mg/dl), Serum Glutamic Pyruvic Transaminase (SGPT) (111 ± 2 4.04 U/L) and glucose level (110 ± 25 mg/dl) was testified to be normal. Similar results were found by Pushpa et al. [74] that the level of glucose, urea, creatinine, bilirubin and alkaline phosphatases was more in cancerous patients as compared to non-cancerous patients.

It is obvious from above discussion that breast cancer is endemic to Pakistan and has high prevalence among females. It is associated with remarkable hematological changes and oxidative stress. Lacks of basic knowledge of breast cancer are increasing its burden.

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