Decreased catalase activity and glutathione concentration levels in women patients with breast cancer

Ruqayah Ali Salman[1, 2]; Genan Adnan Abdullatef AlBairuty[3;] Omar F. Abdul-Rasheed[4]

[1] University of Baghdad, College of Education for Pure Science-Ibn AL Haitham, /Iraq
[2]Al-Furat Al-Awsat Technical University/Iraq
[3]Department of Microbiology, College of Science, Al-Karkh University of Science/Iraq
[4]Departement of chemistry and Biochemistry, College of Medicine, Al-Nahrain University/Iraq

*Corresponding author:
Ruqayah Ali Salman (rokayaalibio@gmail.com)

Abstract:
Breast cancer is caused by malignant cells in the breast tissue and is still one of the main medical concerns, especially for women, with socio-economic significance. Oxidative stress plays an important role in the development, growth, and invasion of breast cancer. Glutathione and Catalase deficiency contributes to oxidative stress, which plays a key role in aging and pathogenesis of many diseases, one of which is cancer. The aim of the study is to determine CAT activity and GSH concentration as an antioxidant in women having breast cancer and compared it with healthy control women. A serum of (40) breast cancer patients has been taken to estimate the levels of CAT and GSH. All studied patient's samples were female with a mean age (47.88±10.92) years old. The result of the study revealed that serum CAT activity in breast cancer patients was 4.17×10-4 nmol/L (range 0-20.83×10-4) which was far much lower than that of the control (median 12.5×10-4, range 0-125.63×10-4 nmol/L). Whereas, GSH concentration in patients was 6350 mmol/L (range 850-16450 mmol/L) which appeared significantly lower than the control groups (7950 mmol/L; range 7350- 15150 mmol/L).

Keywords: Breast cancer Catalase, Glutathione, Reactive Oxygen Species (ROS)

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Introduction

Breast cancer (BC) has become a significant risk to the health of women in Iraq, where it is the leading cause of death from heart disease among women, with a cancer-related death rate of 23% [1, 2]. Coverage of differences in the incidence of breast cancer in different populations in different areas of the Asian continent may be due to multiple factors, including geographical variability, racial/ethnic background, genetic variation, lifestyle, environmental factors, the prevalence of known risk factors, and the use of screening mammography, diagnostic stage of disease, and appropriateness [3, 4]. Reactive oxygen species (ROS), such as superoxide anions and lipid peroxidation induced by hydrogen peroxide, could play a major role in the spread and invasion of malignant transformations and tumor cells [5].

Oxidative stress happens when an imbalance exists between the reaction ability of ROS and antioxidants that promote the development of a disease such as breast cancer [6, 7]. Oxidative stress can be a barrier to the growth of tumors because of its ability to cause deleterious changes in cancer cells [8]. It is also antioxidants classify into two systems: enzymatic and non-enzymatic. The enzymatic system includes enzymes formed by the organism itself as catalase (CAT) superoxide dismutase (SOD). The SOD enzyme functions as a buffer against superoxide, while the catalase enzyme acts against H2O2 [9]. Superoxide dismutase, catalase, and peroxidase of glutathione are top of the list [10].

Catalase is among the most active antioxidant defense enzymes known to cooperate highly with SOD and other hydrogen peroxide producers. Catalase (EC1.11.1.6; hydrogen peroxide: oxid-reductase hydrogen peroxide; CAT) is an iron porphyrin enzyme that catalyzes the breakdown of H_2O_2 into water

and oxygen molecules. CAT is used together with glutathione peroxidase and superoxide dismutase as an effective ROS scavenger to protect cell damage [11]. CAT is a common antioxidant enzyme found in nearly every living tissue that uses oxygen. The enzyme utilizes either iron or manganese as a cofactor and catalyzes hydrogen peroxide (H_2O_2) a breakdown or reduction to water and molecular oxygen, thereby completing the SOD-imitated detoxification process [11]. Many research studies found that CAT enzymes function as anti-carcinogens, antitoxins, and inhibitors in carcinogenesis initiation, promotion, and transformation [12, 13].

The main protective roles of glutathione (GSH) against oxidative stress are: (i) Act as a cofactor of several detoxifying enzymes against oxidative stress; (ii) Participates in amino acid transport through the plasma membrane; (iii) Scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase; (iv) Is able to regenerate the most important antioxidants back to their active forms (Figure 1)[14, 15].Data supporting this indicated that elevated GSH promotes metastasis in both melanoma and liver cancer [16]. Such data illustrate the dual role of ROS and GSH in the initiation of cancer and progression [17].GSH chemical structure determines its possible roles, and its wide distribution across all living organisms represents its significant biological role [18].

The maintenance of the intracellular redox balance and the basic protein thatstatus [19] is another important GSH feature. In addition, GSH is correlated with growth in liver cancer and metastatic melanoma cells [20, 21] and a strong association between GSH rates associated with cell proliferation and metastatic activity has also been shown [20]. In the presence of nitrosative and oxidative stress, a high percentage of tumor cells with high GSH content were able to survive, thus representing the main task force in the metastatic invasion [22]. Therefore, it is possible that maintaining high levels of GSH intracellular could be critical to metastatic cell extravascular development. Cancer cell lines with low GSH have been shown to be much more sensitive to the effect of irradiation than the control cells [18].The present study aimed to investigate changes in the levels of catalase (CAT) activity and glutathione (GSH) concentration in serum of breast cancer patients and to compare with healthy women as a control.

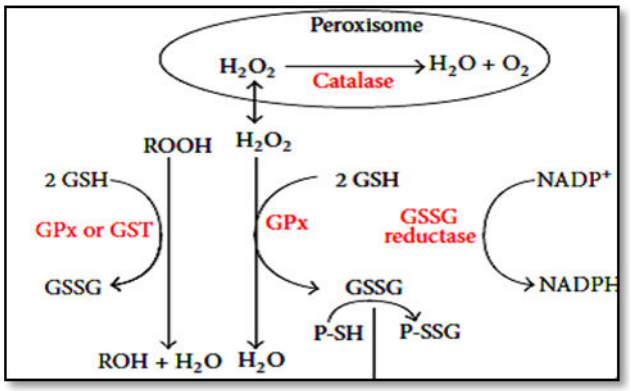

Figure 1: Antioxidant function of GSH.The hydrogen peroxide, produced during the aerobic metabolism, can be metabolized in the cytosol by GSHperoxidase (GPx) and catalase in peroxisomes. In order to prevent oxidative damage, the GSSG is reduced to GSHby GSSG reductase at the expense ofNADPH, forming a redox cycle [23]. Organic peroxides can be reduced both by GPx and GSH-transferase (GST).

Material and Methods

The study population comprised breast cancer patients who were consecutively recruited from the Oncology Teaching Hospital in the Medical City Teaching Hospital in Baghdad and National Cancer Center between Januarys to June 2019. The breast cancer group included patients whose mean age was 47.88±10.92 years, while such a mean age in healthy women's group was 46.2±9.94 years. The study was approved by the Ethical Committee of the institute. Informed consent was taken from patients before drawing blood. Blood samples (about 5 ml) were collected from each healthy women and cancer patient in a plain tube. The tube was centrifuged at 3000 rpm for 10 minutes. Serum was collected carefully and used for biochemical analysis (catalase activity, CAT and glutathione concentration level, GSH). The CAT activity was estimated by the method of assay Kit (Colorimetric) (ab83464). It was

measured in serum samples, the catalase present in the sample reacts with hydrogen peroxide to produce water and oxygen. The unconverted H2O2 reacts. One-unit catalase activity equals the amount of catalase that will decompose µmole of H2O2 per minute at pH 4.5 at 25℃. GSH was measured calorimetrically using DTNB, i.e. 5, 5-dithiobis (2-nitrobenzoic acid) in aqueous solution at pH 7.8. The reaction between GSH and DTNB was analyzed at 420 nm wavelength [24].

Data Analysis

The statistical analysis was performed using SPSS program version 24. Results were expressed as mean ± SD. Less than 0.05 is considered a significant level. The normality of distribution has been checked by Shapiro-Wilk and Kolmogorov-Smirnov tests. The normality test revealed the non-normal distribution of either catalase activity of GSH concentration. Mann Whitney U test was used to compare the median of these markers between patients and controls.

Results

Demographic Characteristics of the Study Population

The mean age of the BC patients was 47.88±10.92 years compared to 46.2±9.94 years for controls with no significant difference. Likewise, there was no significant difference between the patients and control groups with respect to menarche (13.03±1.07 years and 12.8±1.16 years respectively). However, BC patients had significantly higher body mass index BMI than controls (27.88±4.74 kg/m2 versus 25.89±3.94 kg/m2). Postmenopausal women were more frequent among BC (35%) than healthy controls (22.5%) without a significant difference as shown in table 1.

Table 1: Demographic characteristics of the study population

Variables	Breastcancer patients (n=40)	Controls (n=40)	p- value
Age, years(mean±SD)	47.88±10.92	46.2±9.94	0.475
BMI, kg/m^2(mean±SD)	27.88±4.74	25.89±3.94	0.044
Menopausal status **Premenopausal** **Postmenopausal**	26 (65%) 14(35%)	31(77.5%) 9(22.5%)	0.217
Menarche(mean±SD)	13.03±1.07	12.8±1.16	0.371
History of BC **No** **Yes**	25(62.5%) 15(37.5%)	……	……
Estrogen receptor (ER) **Positive** **Negative**	24(60%) 16(40%)	……	……
Progesterone receptor (PR) **Positive** **Negative**	21(52.5%) 19(47.5%)	……	……
Her2 neu **Positive** **Negative**	20(50%) 20(50%)	……	……

BMI: body mass index, SD: standard deviation, Her2 neu:human epidermal growth factor receptor 2

Family history for BC was reported in 37.5% of the patients. Regarding steroid receptors, 60% of patients were positive for ER, while 52.5% of them were positive for PR. Finally, 52.5% of the patients were positive for Her2 (Table 1).

Catalase Activity

Median catalase activity in patients was 4.17×10^{-4}nmol/L (range 0-20.83×10^{-4}) which was far much lower than that of the control group (median 12.5×10^{-4}, range 0-125.63×10^{-4}nmol/L) with a highly significant difference (Figure 2).

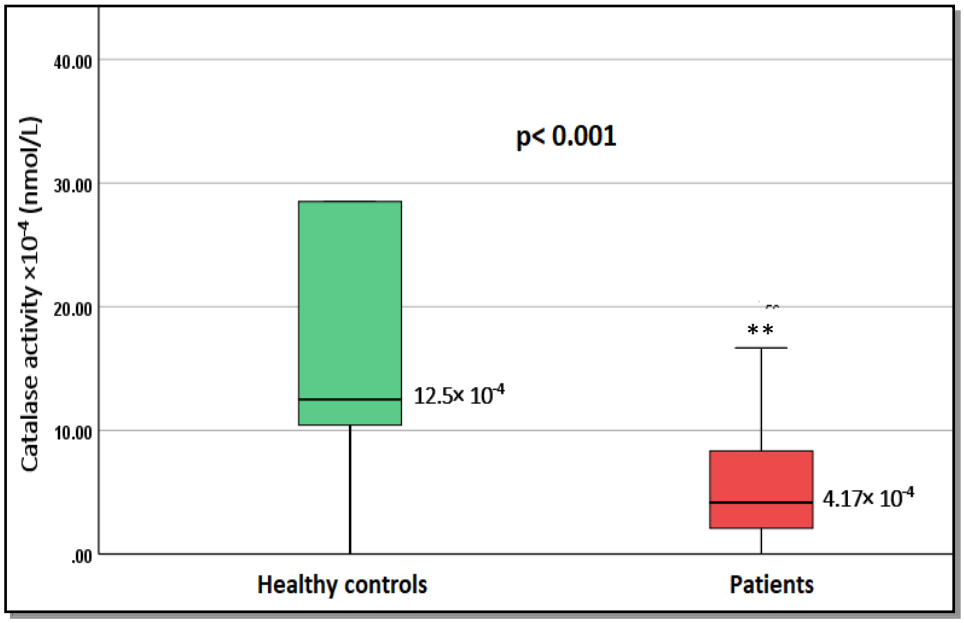

Figure 2: Median catalase activity in patients and healthy controls. ** means significant difference at p<0.001

Serum Level of Glutathione

Median glutathione concentration in patients and healthy controls was 6350 mmol/L (range 850-16450 mmol/L) and 7950 mmol/L (range 7350- 15150 mmol/L) respectively. Mann Whitney U test revealed a highly significant difference between the two groups (Figure 3).

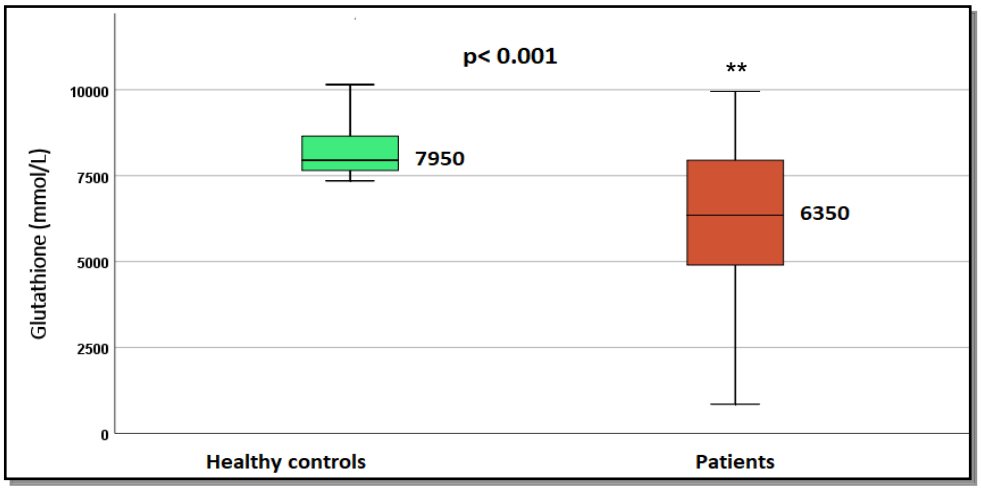

Figure 3: Median glutathione concentration in patients and control group.** means significant difference at p<0.001

Diagnostic Value of Different Biomarkers

The receiver operating characteristic (ROC) curve was used to estimate the diagnostic value of each biomarker in the context of discrimination between BC patients and healthy controls. For catalase activity, the area under the curve (AUC) was 0.831, 95%CI=0.741-0.921, p<0.001. The sensitivity and specificity of the test at the cut-off value of catalase activity = 7.29×10-4 nmol/L was 80% and 70% respectively (Figure4).

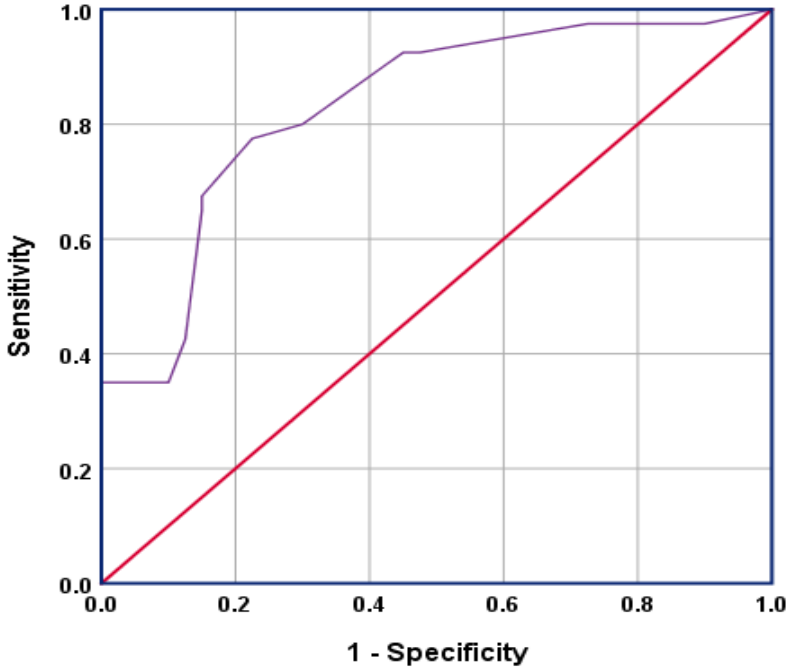

Figure 4: Receiver operating characteristic curve for catalase activity in the context of discrimination between BC and healthy controls

The value of AUC in ROC of glutathione was 0.762, 95%CI=0.654-0.870, p< 0.001. The sensitivity and specificity of the test at the cut off value of catalase activity= 7600 mmol/L were 82.5% and 62.5% respectively (Figure 5).

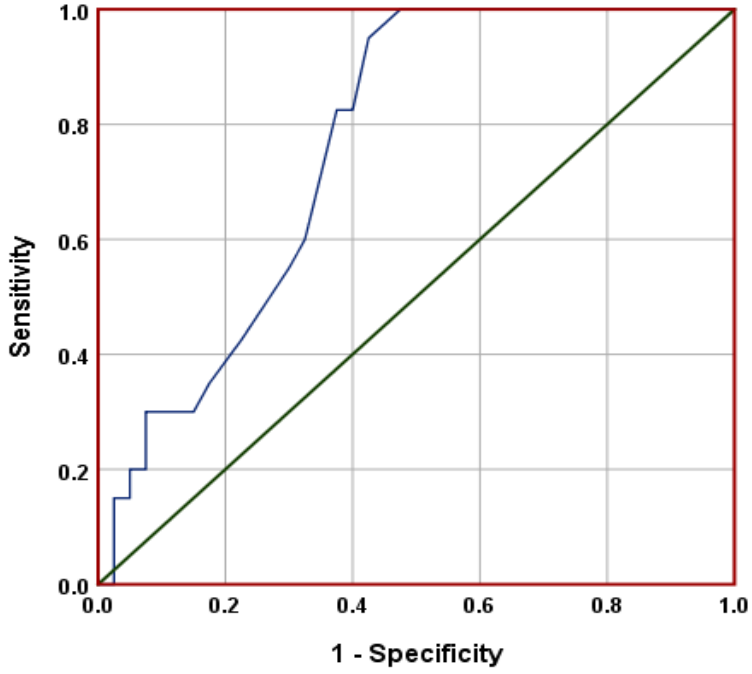

Figure 5: Receiver operating characteristic curve for glutathione in the context of discrimination between BC and healthy controls.

Discussion

Relative to a healthy person, this analysis shows a large BMI for breast cancer patients. Increased body mass index (BMI) is an adult malignancy risk factor [25]. Excess body weight was associated with an increased risk of postmenopausal breast cancer, and increasing evidence also indicates that obesity is associated with poor prognosis in women diagnosed with early-stage breast cancer [26, 27, 28]. There is no clear link between menopausal status and the production of catalase and glutathione.

It is believed that anticipated mechanisms for increased oxidative stress in breast cancer will trigger genetic changes in antioxidant enzymes, estrogen therapy, and reactive oxygen generation and damaged antioxidant systems [29]. Our results showed that in patients with breast cancer there was a significant decrease in CAT activities compared to the controls. A significant increase in GSH and CAT activity was observed in patients with postoperative or/and postoperative chemotherapy, which could be due to free radical scavenging activity [30].

This research is in accordance with whom Sahu, A. et. al (2013) [31], showing reduced CAT activity in breast cancer serum patients relative to healthy controls, indicating substantial decreases in serum CAT activity. GSH in the serum blood of patients had shown a decrease in patients with breast cancer compared to control according to the U-test of two sample means. And the mean of GSH in the serum blood of controls $p<0.001$, the difference may be due to the continuous consumption of pH in the GSH pool contained in the serum blood of those patients with cancer in order to compete with the oxidation stress in the tumor cell. [32]. Previous findings show that GSH inhibition could be the first step towards apoptosis induced by selective nanoradiation[33].

On the other hand, high levels of GSH improve antioxidant capacity and resistance to oxidative stress, and this is observed in many types of cancer[34]. Our approach, combined with the delivery of ROS-generating endogenous radiation, to exploit the specific GSH levels in normal cells and cancer cells [35].Many studies using primary cancer tissue, however, have shown increased levels of ROS-scavenging enzymes and antioxidant compounds [36]. This increase can result from an adaptive response to the intrinsic ROS stress.

It has been shown to be toxic to tumor cells to increase ROS or to decrease free radical scavengers such as GSH. [37] It has been demonstrated that combining GSH depletion with 1, 3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) chemotherapy with superoxide dismutase (SOD) gene therapy could be highly successful in breast cancer treatment.Certain factors may play a major role in the process of GSH therapy and when using GSH modulation medications, consideration should be given. A group of enzymes called Glutathione-S-transferases (GSTs) is an important factor. In many tumor cell types, high levels of GST have been shown to reduce the effectiveness of chemotherapy [38].

On the other side, the cells are more vulnerable to ROS attacks when intracellular GSH levels are low (using certain drugs such as BSO). Increased ROS may activate different oncogenic intracellular pathways or mutate a pathway of the tumor suppressor gene that activates a process of tumorigenesis[39]. Since the rise in ROS in cancer cells may be part of cancer initiation and development, this intrinsic oxidative stress is often seen as an adverse event. Moreover, excessive levels of ROS stress also can be toxic to cancer cells and cells are probably to be more vulnerable to further damage from exogenous drug-induced ROS and make them more responsive to cancer-producing ROS therapies. Changing levels of ROS by modulation of GSH is, therefore, a way of selectively killing cancer cells without causing significant toxicity to normal cells [40, 41].

In conclusion, Breast cancer is associated with a reduction in the ability to defend antioxidants. Overall, our data support the significance of endogenous antioxidant in breast cancer etiology across all expected risk levels. In order to demonstrate our current results, prospective studies should be carried out in a larger population. It is concluded that the biochemical changes in catalase and GSH levels can be viewed as biomarkers for the early detection of recurrent disease as well as tracking the patients ' efficient therapeutic follow-up. These are the strongest biomarkers of breast cancer diagnosis, prognosis, and treatment.

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