

**How to Cite:**

Abbas, M. H., & Alsamarai, A. T. S. (2022). Evaluation of serum fibronectin, glutathione and malondialdehyde in breast cancer women. *International Journal of Health Sciences*, 6(S3), 6393–6403. <https://doi.org/10.53730/ijhs.v6nS3.7415>

## Evaluation of serum fibronectin, glutathione and malondialdehyde in breast cancer women

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**Abstract**--Cancer is a term used to describe a group of disorders in which the body's cells alter and grow out of control. Most cancer cells eventually join together to create a mass known as a tumor, and are named according to the name of the part of the body from which the tumor originates. The Cancer is one of the diseases that have the ability to grow in unlimited fission of the body's cells and the ability of these cells to attack and destroy nearby tissues or move through the blood to distant tissues and this is called a malignant tumor, and there is a second type characterized by specific growth and its inability to attack and move called "Benign tumor", which can develop into a malignant tumor. The goal of this research was to find out the levels of fibronectin and glutathione (GSH) as antioxidants and malaondialdehyde (MDA) as oxidative stress indicator in a group of patients of breast cancer and to compare the results with healthy one. The results showed a significant increase ( $P \leq 0.05$ ) in the level of fibronectin in breast cancer women ( $3918.237 \pm 982.667 \text{ ng/ml}$ ) compared to Control ( $1170.357 \pm 304.787 \text{ ng/ml}$ ) as well as a high level of fibronectin in patients with breast cancer before radiotherapy as compared to other groups with breast cancer. The results also showed a significant increase ( $P \leq 0.05$ ) in the level of malondialdehyde in patients with breast cancer ( $1.380 \pm 0.714 \mu \text{ mol/L}$ ) compared with the control group ( $0.720 \pm 0.089 \mu \text{ mol/L}$ ). While level of glutathione showed a significant decrease ( $P \leq 0.05$ ) in breast cancer patients ( $4.841 \pm 1.472 \mu \text{ mol/L}$ ) compared to the healthy group ( $8.970 \pm 2.936 \mu \text{ mol/L}$ ).

**Keywords**--breast cancer, oxidative stress, fibronectin, antioxidants, malondialdehyde, glutathione.

## Introduction

Breast cancer disease is one of the malignant tumor kinds. that occur in the breast tissue and appears in the tubes that carry milk to the nipple (1), it occurs in women and men, but the incidence of women is very large compared to men, as every 200 cases of women correspond to one case of men (2) . One of the most prevalent malignant tumors is breast cancer and one of the main causes after lung cancer that leads to death (3), and the most common type of breast cancer is adenocarcinoma and is usually detected either during the examination before symptoms appear or after the woman notices the presence of the tumor (4). The incidence of breast cancer in Western societies is greater than the rate of infection in Eastern societies, because women in Western societies use hormones as well as contraceptive methods in addition to the different nature of life (5). The incidence of cancerous diseases increases every year due to the presence of uncontrolled canned food and environmental pollution, and the lack of culture among some, which may contribute to the high number of cancer and the important role of preparing for the genetic factor in the family (6). Breast cancer begins when breast cells grow abnormally, as lumps of tissue form as a result of rapid cell division and multiplication, and these lumps are called tumors, and these tumors may be cancerous and are called malignant tumors, or these tumors may be non-cancerous and are called tumors Benign, malignant tumors multiply and destroy healthy tissues in the body and also it is possible for some tumor cells to separate and spread to other areas of the body and this spread is called metastasis (7).

Fibronectin is a glycoprotein with a high molecular weight of about 270 KD(8,9), and it contains in its structure more than 5% of carbohydrates bound to a protein receptor called integrin it also interacts with extracellular components like fibrin., collagen, and heparin (10). Fibronectin usually plays a vital role in the body, as it helps in the formation of supporters that support the cells of the connective tissues in the body, which facilitates tissue repair after injury, but after a heart attack, the reaction of fibronectin is very strong, so it polymerizes and helps in the production of a lot of intercellular substance macrophages; It also causes an overproduction of clumped and ineffective cardiomyofibroblasts that are harmful to the heart (11). A recent study discovered a link between fibronectin and vitamin D2 in breast cancer patients. (12). In the field of oxidation and reduction science, oxidative stress is a term used to describe the state of being overexposed to oxygen, medicine has witnessed a tremendous development in the past thirty years, and it is a global concept defined as an imbalance between antioxidants and free radicals so that radicals increase above their normal level and this arises through increased production of reactive oxygen species – ROS. Or because of the deficient of defensive antioxidants, as this deficiency develops when eating low amounts of antioxidants such as vitamin E- "tocopherol", and vitamin C "ascorbic acid", or a decrease in the rate of synthesis of antioxidant enzymes such as super oxide dismutase or glutathione peroxidase-GPX, and a deficiency may occur in antioxidants via its extensive use (13). Additionally, oxidative stress arises in biological systems when the creation and elimination of free radicals are out of equilibrium. Therefore, the production of free radicals in large quantities, which is responsible for the events of many biochemical changes in cells, and there are also many factors that lead to the occurrence of oxidative stress, the most

important of which are shocks, heat, ionic radiation, toxins and violent exercise (14,15).

## **Materials and Methods**

### **Sample collection**

Samples were taken from 50 women who had been diagnosed with breast cancer, and (40) women without cancer and were considered as a control group ,5 ml of blood was withdrawn and placed in plastic test tubes and left for 10 minutes and then placed in a centrifuge with a rotation force of 3000/rpm for the purpose of obtaining the serum.

### **Determination of Serum Fibronectin**

The level of serum fibronectin was determined by using an enzyme linked immunosorbent assay (ELISA) (16).produced by ELISAGenieCompany, which take the number HUDL01098

### **Determination of serum MDA level**

#### **The principle of the method**

The method of reaction of the modified thiobarbituric acid (TBA) used by Guidet&Shah(17) was used to measure the level of MDAwhich is one of the main lipid peroxidation products. The approach relies on the interaction of lipid peroxides, particularly MDA, with thiobarbitic acid. This reaction occurs in an acidic media and produces a colorful product with a 532 nm absorbance.

### **Determination of Serum (GSH) level**

#### **The principle of the method**

Serum glutathione level was determined using the modified Elllman reagent method (18).

### **Statistical analysis**

The results of the current study data were statistically analyzed using the statistical program (SPSS) version (23) through the mean and standard deviation test and using the T test to determine the difference between the two groups at the level of probability ( $P \leq 0.05$ ) (19).

## **Results and Discussion**

Table (1) shows that the mean of serum fibronectin  $\pm$  standard deviation in patients with breast cancer was (3918.237  $\pm$  982.667 ng/ml), and compared to the control group, it was (1170.357  $\pm$  304.787 ng/ml).

Table 1  
Mean  $\pm$  standard deviation of the level of fibronectin in the two group's patients and control

Groups	Sample	Mean $\pm$ SD ng\ml	P- value
Patients	50	3918.237 $\pm$ 982.667	P $\leq$ 0.05
Control	40	1170.357 $\pm$ 304.787	

The results showed a significant increase (P $\leq$  0.05) in the level of fibronectin in the serum of patients with breast cancer (3918.237 $\pm$ 982.667 ng\ml) compared with control group (1170.357 $\pm$ 304.787ng\ml). The results agreed with what was indicated by Libring. et al (20), Zhuoran Gong et al (21) and AparnaShinde (22), which showed higher levels of fibronectin in women with breast cancer compared to healthy control Where fibronectin usually plays essential role in the body, it helps in the formation supporters that support the connective tissue cells in the body, this facilitates tissue repair after injury, but in cancer, the reaction of fibronectin is very strong, so it polymerizes and helps produce a lot of intercellular connective material. It also causes an overproduction of the ineffective fibroblasts found in breast cancer (11).

Fibronectin is a protein that helps vertebrate creatures operate normally. (23). It is involved in "cell adhesion, growth, migration, and differentiation. Soluble cellular fibronectin is assembled to form extracellular matrix, the insoluble network that separates and supports the organs and tissues of an organism. Fibronectin plays an essential role in the wound healing process" (24) Plasma fibronectin is deposited at the site of the injury, together with fibrin, to create a blood clot that prevents bleeding and saves the underlying tissue. As the wounded tissue heals, fibroblasts and macrophages begin to remodel the area, breaking down the proteins that constituted the momentary blood clot and replacing them with a matrix that is comparable to that of the surrounding normal tissue. The fibroblasts secrete the proteolytic enzyme (Protease), including matrix metallolytic enzymes that digest plasma fibronectin, The fibroblasts then produce cellular fibronectin, which is assembled into an insoluble matrix by the fibroblasts. Protease fragmentation and lysis of fibronectin causes wound constriction and its important and critical step in wound healing (25). Other studies have also proven that plasma fibronectin levels increase after trauma caused by vascular tissue damage, after inflammation, and in many diseases such as some other cancers types , atherosclerosis, ischemic heart disease and stroke (26).

Table (2) shows the subdivision of breast cancer group  
G1, which included (15) newly diagnosed breast cancer.  
G2, which included (35) did not receive radiotherapy.  
G3, which included (15) did not receive radiotherapy.  
G4, which included (11) relieved 2-4 chemotherapy doses.  
G5, which included (24) relieved 5-8 chemotherapy doses.  
G6, which included (27) urban.  
G7, which included (23) rural

Table 2  
Mean  $\pm$  standard deviation of fibronectin in subgroups

GROUP	NOMBER	Mean $\pm$ SD ng\ml
Control	40	1170.357 $\pm$ 304.787d
G <sub>1</sub>	15	3509.344 $\pm$ 974.674bc
G <sub>2</sub>	35	4014.011 $\pm$ 1903.708bc
G <sub>3</sub>	15	4929.221 $\pm$ 1092.207a
G <sub>4</sub>	11	3131.507 $\pm$ 485.307c
G <sub>5</sub>	24	3770.180 $\pm$ 796.712bc
G <sub>6</sub>	27	3994.270 $\pm$ 980.599bc
G <sub>7</sub>	23	3828.981 $\pm$ 999.451bc

\*Similar letters mean there are no significant differences.

\*Different letters mean there are significant differences.

Results showed that a significant increase in patients with breast cancer without exposure to any radiation doses compared to other groups with breast cancer, although it remained high compared to the control group. Chemotherapy drugs probably contributed to increased survival rates among breast cancer patients. Despite these achievements, cancer cells continue to be resistant to treatments and relapse is a typical occurrence. While a lot of factors lead to chemotherapeutic resistance, fibronectin has gotten a lot of attention. The efficacy of tamoxifen, an estrogen receptor antagonist, was utilized to investigate how the microenvironment restricts the sensitivity of cancer cells to tamoxifen in a study by Pontiggia et al. (27). Fibronectin plays a major role in the processes associated with tumor development (28). Chemotherapy is the standard treatment in clinics for breast cancer, while chemotherapy agents are underdiagnosed in the long term (29). As a first-choice treatment for cancers, chemotherapy patients generally have lower delivery efficiencies to the tumor site with significant variance between different patients (30,31). It is believed that the reason for the decrease in fibronectin in other groups that were treated with chemical or radiological doses is due to the effect of radiation treatment on the action of fibronectin that occurs in breast cancer that increases fibronectin level. Table (3) shows that the level of malondialdehyde  $\pm$  standard deviation in patients with breast cancer was (1.380  $\pm$  0.714) mg / dL compared to the control group, it was (0.720  $\pm$  0.089) mg / dL.

Table 3  
Mean  $\pm$  standard deviation of the level of malondialdehyde in the two groups of the study

Groups	Sample	Mean $\pm$ SD $\mu$ mol/L	P- value
Patients	50	1.380 $\pm$ 0.714	P $\leq$ 0.05
Control	40	0.720 $\pm$ 0.089	

The results showed a significant increase ( $P \leq 0.05$ ) in the level of malondialdehyde in patients with breast cancer ( $1.380 \pm 0.714 \mu\text{mol/L}$ ) compared to the control group ( $0.720 \pm 0.089 \mu\text{mol/L}$ ). The results of the current study agree with the findings of Lilo et al (32). and Khalaf et al. (33) Nsaif, et al (34), who showed in their study an elevated level of MDA in the serum of patients with breast cancer compared with the control group. MDA is one of the types of oxidative stress results that is a major driver of "breast cancer initiation" and development and is often associated with a high cancer risk, as the level of MDA is elevated in patients with breast cancer (35,36)

Table 4  
Mean  $\pm$  standard deviation of malondialdehyde in subgroups

GROUP	NOMBER	Mean $\pm$ SD $\mu\text{mol/L}$
Control	40	$0.720 \pm 0.089e$
G <sub>1</sub>	15	$0.983 \pm 0.197de$
G <sub>2</sub>	35	$1.668 \pm 1.063bc$
G <sub>3</sub>	15	$1.234 \pm 0.436cde$
G <sub>4</sub>	11	$2.944 \pm 0.617a$
G <sub>5</sub>	24	$1.179 \pm 0.275cde$
G <sub>6</sub>	27	$1.498 \pm 0.902bcd$
G <sub>7</sub>	23	$1.243 \pm 0.370cde$

The results showed a significant increase ( $P \leq 0.05$ ) in the level of MDA in the serum of breast cancer patients (G<sub>4</sub>) ( $2.944 \pm 0.617 \mu\text{mol/L}$ ) compared with the control group ( $0.720 \pm 0.089 \mu\text{mol/L}$ ) and the other groups, where the results of the study corresponding with Gupta et al. (37), who showed in their study a higher level of MDA in women with breast cancer after chemotherapy compared to the control group. The action of free radicals on unsaturated lipids in red blood cells causes the membranes of red blood cells to decompose. As stated by Motoyoshi et al.(38), they observed a significant increase in MDA in the serum of patients with breast and cervical cancer compared with healthy controls. They attributed the reason for the increase in lipid peroxidation, This could be due to an increase in the production of reactive oxygen species; or the inhibition of the antioxidant defense mechanism in metabolically active tissues. Table (5) shows that the mean of glutathione  $\pm$  standard deviation of patients with breast cancer which was ( $4.841 \pm 1.472 \mu\text{mol/L}$ ) compared to the control group, which it was ( $8.970 \pm 2.936 \mu\text{mol/L}$ ).

Table 5  
Mean  $\pm$  standard deviation of the level of glutathione in the two groups of the study

Groups	Sample	Mean $\pm$ SD $\mu\text{mol/L}$	P- value
Patients	50	4.841 $\pm$ 1.472	P $\leq$ 0.05
Control	40	8.970 $\pm$ 2.936	

The results showed a significant decrease (P $\leq$  0.05) in the level of glutathione in patients with breast cancerwomen(4.841 $\pm$ 1.472  $\mu\text{mol/L}$ ) compared with the control group(8.970  $\pm$  2.936  $\mu\text{mol/L}$ ). The results of the current study agreed with the findings of Enrico et al. (39), Chih-Ching et al. (40) and Luke et al. (41), as glutathione is one of the reducing agents to contain a thiol group, which has the ability to donate a hydrogen atom (42). It is also considered an antioxidant, as it prevents and delays cell damage and detoxifies chemicals within the liver(43). It is also an essential component of the defense system in plants and humans exposed to various environmental stresses. The protective mechanism includes sequestration of minerals and scavenging of reactive oxygen species by glutathione in plants facing environmental stresses (44). Due to its effective participation in the prevention of oxidation, its level will decrease as a result of its consumption in cases of oxidative stress, as well as it will decrease in the animal's plasma and tissues with age.

GSH has a main role in aging and is required for carcinogen detoxification, therefore alterations in this pathway can have a significant impact on cell survival. Furthermore, cancer cells with high GSH levels are able to protect these cells in cancers of the "bone marrow, breast, colon, larynx, and lung" by conferring resistance to several chemotherapeutic drugs (45,46). In addition, glutathione plays important roles in antioxidants (including: "gene expression, DNA and protein synthesis, cell proliferation and signal transduction, cytokine production and the immune response") (47).

Table 6  
Mean  $\pm$  standard deviation of glutathione in subgroups

GROUP	NOMBER	Mean $\pm$ SD $\mu\text{mol/L}$
Control	40	8.970 $\pm$ 2.936a
G <sub>1</sub>	15	4.128 $\pm$ 0.949b
G <sub>2</sub>	35	5.332 $\pm$ 3.009b
G <sub>3</sub>	15	5.151 $\pm$ 1.321b
G <sub>4</sub>	11	4.042 $\pm$ 1.003b
G <sub>5</sub>	24	5.317 $\pm$ 1.588b
G <sub>6</sub>	27	4.576 $\pm$ 1.365b
G <sub>7</sub>	23	5.152 $\pm$ 1.560b

There were no significant differences in glutathione level among breast cancer subgroups but it was decreased in patients with breast cancer in all groups under study compared with the control group. Due to the fact that glutathione (GSH) and oxidized glutathione (GSSG) are the major insulators of intracellular antioxidants against oxidative stress, To maintain intracellular glutathione and redox balance, glutathione synthesis, transport, usage, and metabolism are all closely regulated. (48). As for cancer cells, they need a greater level of reactive oxygen species than normal cells to promote metabolism and hyper-proliferation (49, 50).

Moreover Greater amounts of ROS can be mitigated by increasing the antioxidant defense system's activity, which responds to the higher oxidation state. The GSH system is a major cellular antioxidant system that cooperatively maintains and synergizes redox balance (51). Elevated GSH level has also been observed in various human tumor cells and is an important factor in cancer pathogenesis and resistance to anticancer therapy (52). Conversely, depletion of GSH increases the susceptibility of cancer cells to various forms of apoptosis and sensitivity to chemotherapies (53).

### **Conclusion**

- Fibrinectin levels in the blood serum of women with breast cancer are significantly higher than in the control group.
- Significant increase in the level of oxidative stress index Malone Dialdehyde (MDA) in the blood serum of women with breast cancer compared with the control group.
- A significant decrease in the level of the antioxidant glutathione (GSH) in the blood serum of women with breast cancer compared with the control group.

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