Assessment of the Efficiency of Specialization Therapies of Breast Cancer by Evaluation of Oxidative Stress

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Abstract

Background: Breast cancer is one of the leading causes of cancer related death, when it is the second most common cancer in women after l*ung, when it constitutes 23% of all cancer cases in women, moreover it presents the first in global mortality (18.6%) of cancer According to the latest statistics, breast cancer ranks the first number (2,088,849 new cases) of recorded cases worldwide, annually. Breast cancer is a significant and common disease that has a negative effect on women health, and deaths, 626,679 cases). When an imbalance between free radical production and detoxification occurs, ROS production may overwhelm antioxidant defenses, leading to the generation of a noxious condition called Oxidative Stress and overall to the impairment of the cellular functions. Subjects: Seventy four females were included in the current study, they were classified into three groups depending on their health and the type of tumor suffered by patients. The first included 25 females with malignant breast tumors, the second group included 24 women who had benign breast tumors, and the last group included 25 women who appeared to be healthy. Results: All of NO and MDA showed significant statistical significant increase (p=0.000), in contrast to SOD which illustrated significant decrease (p=0.000) in patients with breast cancer when compared with benign tumor patients as well as healthy controls group. The current study recorded an increase in the level of MDA and SOD concentrations in all samples diagnosed as breast cancer patients, which make the sensitivity of this parameters up to 100%, while the sensitivity percentage of NO reached to 96% . When the levels of studied parameters were experiential during the chemotherapy stage, a gradual decrease of the NO was observed to be proportional to the number of doses received by the patients, from other side; all of MDA and SOD showed significant increase agreed with progression of chemotherapy stages.*

Key words: **breast cancer, chemotherapy, oxidative stress, tumor marker, NO, SOD, MDA.**

Introduction

Cellular energy is mainly produced *via* oxidative phosphorylation taking place within mitochondria, which are crucial organelles for numerous cellular processes, such as energy metabolism, calcium homeostasis, lipid biosynthesis, and apoptosis [1]. More than 90% of the body's oxygen is consumed by the electron transport chain in mitochondria, and about 1–5% of it is released as superoxide ($O2^{\bullet-}$) and hydrogen peroxide (H_2O_2) [2]. Free radicals are molecules contain unpaired electrons, usually in outer orbitals, and have important functions in normal cellular physiology, including oxidative phosphorylation and cellular signaling. About 1-2% of the oxygen

consumed by mitochondria normally results in the formation of **Reactive Oxygen Species (ROS)** which are fundamental in the maintenance of cellular homeostasis. In physiological conditions, low to moderate concentrations of ROS are involved in processes such as immune response, inflammation, synaptic plasticity, learning, and memory [3]; but any mitochondrial dysfunction results in an increase in ROS. In addition, exogenous oxidants and acute body stress, such as sepsis, tissue hypoxia, and ischemia-reperfusion, enhance the additional formation of free radicals [4]. ROS may also be generated from estrogen metabolism through catechol estrogen redox cycling. This chain reaction is propagated by the formation of lipid hydroperoxides, lack of histone protection, low level of DNA repair, and usually involves lipid bilayers. Mitochondrial DNA, intracellular membranes, proteins, and others nucleic acids are particularly vulnerable to oxidative damage and these complex biochemical events contribute to the development of multiorgan failure [1,3]. The human body is equipped with a two systems of antioxidant, **Enzymatic** which block the initiation of free radical chain reactions andincluded:superoxide dismutases (SODs), catalase (CAT) and the glutathione peroxidase (GSHPx), and **Non-enzymatic**; that serve to counterbalance the effect of oxidants. When an imbalance between free radical production and detoxification occurs, ROS production may overwhelm antioxidant defenses, leading to the generation of a noxious condition called **Oxidative Stress** and overall to the impairment of the cellular functions [2,4,5]. This phenomenon is observed in many non-cancerous pathological cases [6,7] as well as cancers [8,9]. During carcinogenesis, ROS cause DNA damageresulting in gene mutation and abnormal cell proliferation, oncogenesis,and induce apoptosis. Under the pressure of cytotoxicity, cancer cells develop antioxidant systems [6].

NO (NO) a free radical is formed from L-arginine by converting it to L-citrulline via at least two physiological pathways: NO synthase (NOS) dependent and NOS independent enzymes. The last product (L-citrulline) has been indicatedto be a secondary NO donor in the NOS-dependent pathway, since itcan be converted to L-arginine. Moreover, nitrate and nitrite are the main substrates to produce NO via the NOS-independent pathway; these anions can be reduced *in vivo* to NO and other bioactive nitrogen oxides. Other molecules, such as the dietary supplement glycin propionyl-L-carnitine (GPLC), have also beensuggested to increase levels of NO, although the physiological mechanismsremain to be elucidated [9-15].The family of NO synthases (NOS) comprise inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS (nNOS) [16]. NO is as apleiotropic, signaling molecule that regulates many functions, such as vasodilatation, blood pressure, neurotransmission, macrophage-mediated immunity, mitochondrial respiration, platelet function, and oxidation-sensitive mechanisms. NO may act as an autocrine or paracrine messenger, and its production and degradation are cell type dependent [16-18]. NO having both cytoprotective as well as tumor promoting agent. The reaction product of NO with superoxide generates potent oxidizing agent "peroxynitrite" which is the main mediator of tissue andcellular injury. Peroxynitrite is reactive towards manybiomolecules which includes amino acids, nucleic acidbases and metal containing compounds. In general, NO metabolitesmay play a key role in mediating many of the genotoxic/carcinogenic effects as DNA damage, protein and lipid modification [9,17,19,20]. The basic reactions of NO can bedivided as direct effect of the radical where it alone plays arole in either damaging or protecting the cell milieu and anindirect effect in which the byproducts of NO formed by convergence of two independent radical generatingpathways play the role in biological reactions whichmainly involve oxidative and nitrosative stress. NO is also capable of directly interacting with mitochondriathrough inhibition of respiration or by permeabilitytransition. Excessive production of NO can be studied byinhibiting the synthetic pathway of NO using bothselective or specific NO synthase inhibitor or nonselective NO synthase inhibitor with respect to isoforms of NO [9, 19].

Arachidonic acid is the primary source of free-radical generation through a series of synthesis and conversion via cyclooxygenases reactions of a number of intermediates whose final product is Malondialdehyde (MDA) [22]. MDA is a colorless organic compound with the formula $CH_2(CHO)_2$, with 72 °C as melting point, 72.063 g/mole molar mass and density of 0.991 g/mL. It is a

highly reactive compound that occurs as the enol [23]. The reactivity of MDA molecule is mainly based on its electrophilicity making it strongly reactive toward nucleophiles, such as basic amino acid residues. More than, the reactivity of MDA is not only based on its aldehydic nature but is also influenced by its 1,3-dialdehydic structure making it possible to form mesomerically stabilized Schiff bases [22]. Particularly, MDA's high reactivity and capability of forming adducts with multiple biological molecules such as proteins and DNA has attracted major attention over the last decades, when MDA became widely used as a biomarker for assessing oxidative stress in biomedical fields. Biomonitoring of MDA has been used in both *in-vivo* and *in-vitro* studies as a key biomarker for various disease patterns including hypertension, diabetes, atherosclerosis, heart failure and cancer[24-28]. Higher MDA levels were observed in various cancerous and noncancerous illness [24,29-34]; as well as non-illness cases [24,35-37]. This finding suggest the validity of the MDA assay as a reliable tool in finding out the oxidative stress in different status [22].

Superoxide Dismutases (**SODs, E.C.1.15.1.1**) are metalloproteins, whichfirstly discovered by McCord and Fridovich[1].They are subdivided into four different categories, as they contain different metals [38]. SODs are important antioxidant enzymes when they are formed group of the main enzymes in the first line of defense against formation of reactive oxygen species and their derivatives, as well as, they are eliminated superoxide radicals from cell environment. They are catalyzed superoxide anion dismutation on hydrogen peroxide and oxygen,and then removal of superoxide free radicals[39]. SODs are present throughout all orders of life and that the expression of the Mn-SOD isoform is essential for the survival of aerobic higher eukaryote organisms demonstrate the importance of $O_2^{\bullet-}$ detoxification. $O_2^{\bullet-}$ readily inactivates iron–sulfur-containing proteins like aconitase *via* the disruption of its [4Fe–4S] cluster, which results in the release of free iron. Additionally, $O_2^{\bullet-}$enzymatically dismutase to yield H_2O_2 (kobs $\sim 10^9$ $M^{-1}\cdot s^{-1}$ at pH 7.4), which can either oxidize biomolecules, be a substrate of different enzymes (peroxiredoxins, glutathione and heme-peroxidases, and myeloperoxidase), or act as a signaling molecule [39,40]. SODs may be only damaged by some xenobiotics, e.g., azide, cyanides, chloric acid or diethyl-dithio-carbamate and hydrogen peroxide [1].

Materials and Methods

- **Subjects**

Seventy four females were included to participate in the current study. The participator women were classified into three groups depending on their health and the type of tumor suffered by patients. The first included 25 females between the ages of 25 and 73 (47.520±12.613) who were diagnosed as patients with malignant breast tumors. The second group included 24 women who had benign breast tumors between the ages of 12 and 70 years (27.170±16.090), and the last group included 25 women who appeared to be healthy, aged between 25 and 68 years (47.730±11.885).

Thirteen of the malignant tumor patients had a tumor location in the right breast and 11 of them had left breast tumor location while the tumor was in both breasts in one of cases only. The stages of the patients were divided between the first and the third, where they were divided as follows: 3 females were in the first stage of cancer, 14 of them were in the stage Π, finally the remained cases of them were in the stage ΙΙΙ. All women with cancer who participated in the study, except one patient, were mothers of a number of children, and the number of births ranged between 1-11times. The study required exclusion of all breast cancer patients who had suffered from renal and cardiovascular diseases, diabetes or hypertension from participating in the current study. Moreover; the study excluded smoker women with breast cancer, as well as, those whose cancer symptoms coincided with taking oral or intravenous contraceptives or who took oral contraceptives for 3 consecutive years before the onset of symptoms. Additionally; the study excluded cancer patients who underwent surgery within 5 years of onset of symptoms. The study included women who took contraceptives during their lifetime but all these women had stopped taking contraceptives at least 3 years before the onset of the symptoms of cancer. The study included

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All patients with benign breast tumors were married and had more than a healthy birth (2-7 children), in addition to that, they were non-smokers as well, all of whom did not take contraceptives during the onset of the tumor. Ten of the women with benign breast tumors, the right breast was the location of the tumor and the remaining left breast was the site of the tumor. All women with diabetes, cardiovascular disease or kidney disease are excluded from the current study in the group of women with benign breast tumors. Finally, some patients in this group underwent Cesarean delivery only as a surgical intervention prior to injury.

Selection of healthy females as a control group based on several criteria included: normal menstrual cycle for at least 6 consecutive months "for healthy pre-menopausal women ". They might at approximate age range with the patients group, with similar food style, without major medical or surgical illness in the previous 5 years, no hospital admissions, no current medication, and a subjective perception of good health as determined by health questionnaire.

- Colorimetric indirect method by detecting of nitrate or nitrite was applied to determine NO concentration in the serum samples of breast tumors patients and healthy individuals groups.
- Thiobarbituric Acid Reactive Substances (TBARS) assay was applied to determine MDA concentration in sera samples of malignant and benign breast tumors as well as control groups.
- Competitive – ELISA technique was applied to estimate SOD concentration in serum samples of patients and healthy groups.

Results and Discussion

- **Assessment of NO Concentration in the Serum Samples of the Study Groups**

Pre-treatment, levels of NO concentration were evaluated in the sera samples of the current studyparticipants. The statistical analysis using ANOVA test showed a respectable significant increase (**p=0.000**) of NO levels in patients with breast cancer when compared with benign tumor patients as well as healthy controls group, on the other side; no significant variations were shown when benign breast tumors and controls groups compared together; as recorded in **Table 1**

Table 1: Levels of NO (Mean ± S.D.) Concentration (µmol/L) in Sera of Tumoral Patients and Controls Subjects

Study Groups (n)	NO Concentration (µmol/L) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	59.877 ± 6.499	45.395 - 75.555 30.160	0.000 MT *vs* BT
Benign Tumors 24	24.358 ±9.719	10.793 - 37.619 26.826	0.000 MT *vs* C
Controls 25	25.331 ± 11.730	10.793 - 53.565 42.772	0.772 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

NO scored a significant statistical ability to distinguish between women with malignant breast tumors and healthy women, with a sensitivity percentage of 96%, when 24 of the 25 cases registered in the current study showed levels exceeding the upper limit of the same parameter in the

control group, while 18 of 24 (75%) women with benign breast tumors recorded levels of NO within the normal range of this standard in the control group.

According to previous studies [9,17,19], NO having both cytoprotective as well as tumor promoting mediator. NO is reactive towards many biomolecules which includes amino acids, nucleic acid bases and metal containing compounds. In general, NO metabolites may play a key role in mediating many of the genotoxic/carcinogenic effects as DNA damage, protein and lipid modification.The current study is based on the hypothesis of high levels of NO production as a result of the high activity of enzymes that regulate synthesis of this compound. Previous studies have shown activity in the gene expression of enzymatic forms that induce NO synthesis in the different tissues.

On the other hand, studies have shown that all three forms can be involved in promoting or inhibiting the occurrence of cancer. In addition, previous studies indicate NO synthesis activity in tumor cells of different histopathological origins. The degree of tumor, prevalence rate and expression of the important signal components associated with cancer growth such as estrogen receptors were associated. The significant increase in NO levels in the samples of breast cancer to increase the efficiency of enzymes organized for its synthesis, which increases the level of production concurrently with the occurrence of cellular change processes during the stage of carcinogenicity, which corresponds to the level of production with the severity of the tumor and the extent of the progress of infection and the spread of the disease. Results of the present study were agreed with previous studies that referred to increase of NO levels in the malignant diseases [41,42,43,44].

When the levels of NO were experiential during the chemotherapy stage, a gradual decrease in the levels of this parameter was observed to be proportional to the number of doses received by the patients with a slight abnormality recorded in only three of the patients with a higher NO level in their serum after receiving the first dose of chemotherapy followed by a decrease in those values with progress in the treatment stages (**Figure 1**).

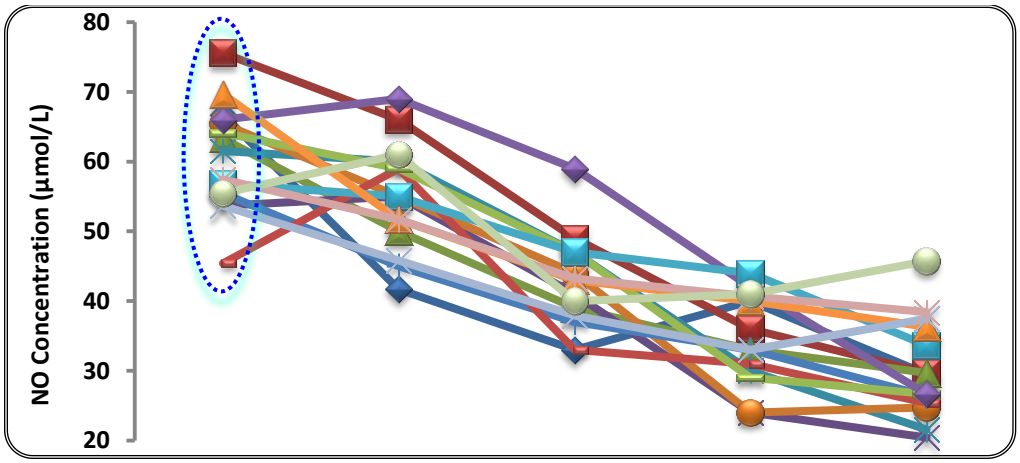

Figure 1: Follow-up of NO Levels during Consecutive Chemotherapy Stages

After the fourth dose of chemotherapy, a slight (statistically insignificant) increase was observed at NO level in only **two** of the older patients. From the operative observation it was found that women in the advanced malignancy stage (**III**) are showed more decreasing in the levels of NO after receiving the first dose of chemotherapy followed by gradual declines relatively close.

The gradual decrease in NO levels with the progression of the chemotherapy period may indicate to the body's response for treatment, moreover,it may be refer to the decrease in the number of cancer cells, thus causing in the deficiency of the production of induce enzymes for the NO formation. Based on the results obtained, NO can be used as an efficient tool to monitor

patients' response to treatment during successive chemotherapy phases. To compare the levels of NO in breast cancer as tool of cure after receiving a planned dose of follow-up in the present study with levels in the sera of the group of women with benign breast tumors as well as with the control group. Although the levels of this parameter were significantly reduced in the cancer patient group (the levels of the NO after chemotherapy were approximately half that of the same patients at the time of diagnosis), but the levels remained higher than those in the group of patients with benign tumors and in the control group; where the study showed a significant increase in NO levels in the group of cancer patients compared to the other two groups, as shown in the **Table 2**.

Table 2: Concentration (Mean ± S.D.)of NO (µmol/L) in Sera of Malignant Tumor Patients After Treatment, Benign Breast Tumors and Controls Subjects

Study Groups (n)	NO Concentration (µmol/L) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	31.877 ± 5.970	20.428- 45.714 25.286	0.027 MT *vs* BT
Benign Tumors 24	24.358 ±9.719	10.793 - 37.619 26.826	0.016 MT *vs* C
Controls 25	25.331 ± 11.730	10.793 - 53.565 42.772	0.772 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

The levels of NO in the samples of patients with breast cancer may be higher than that recorded in the group of women with benign breast tumors and healthy women, although cancer patients received at least four regular doses of chemotherapy, such as: **(1)** The survival of a number of cancer cells in the bloodstream or different tissues that are attributed to this supernatural production of NO . **(2)** The abnormal level of this compound is due to cellular damage caused by exposure to chemotherapy, as it has negative effects on healthy cells, leading to an increase in oxidative stress products, including NO. **Figure 2** shows great reduce in levels of NO in all patients in the malignant breast tumor group (100% of cases) after receiving the last planned dose of chemotherapy compared to their levels in the same samples at diagnosis. The statistical analysis indicates that there is a significant difference (**p<0.001**) between levels of this parameter before and after receiving chemotherapy, which contributes to the proposal of this criterion as a significant function to infer the patient's response to the treatment provided.

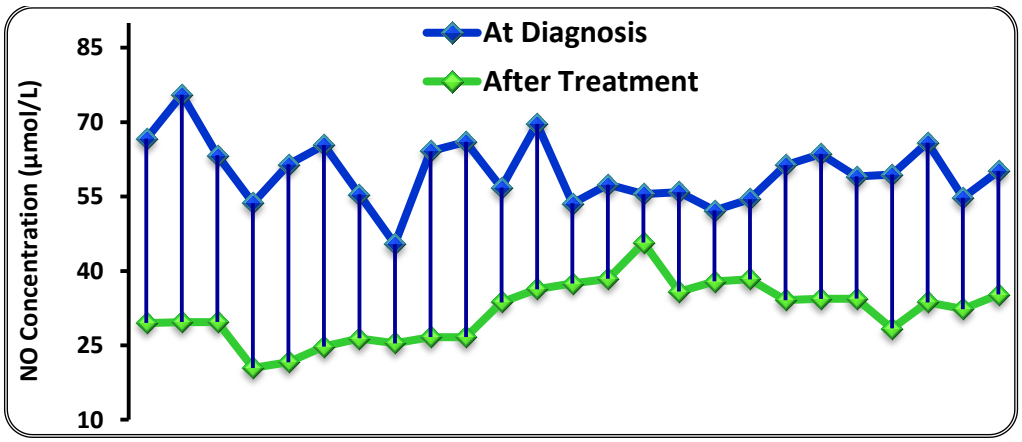

Figure 2: Comparison Levels of NO in The Sera Samples of Cancerous Patients Before and After Treatment with Chemotherapy (Radiotherapy)

The outresults of the present work illustrated that the largest differences between levels of NO before and after chemotherapy were in the first stage of breast cancer patients.Moreover; when the patients at the same stage were compared, it was found that women show same response to chemotherapy in one stage of the breast cancer, in addition; age does not make significantly affect in the NO levels.

In the current study, previous findings showed NO as a very good diagnostic and follow up tool for breast cancer, but not as indicator to the complete recovery. On the other hand, it is possible that NO cannot be used to indicate complete recovery of breast cancer patients because the follow-up period in the current study was relatively short, while, in general, most women with breast cancer need between 3 - 8 doses of chemotherapy and sometimes a number need doses of radiation therapy. Moreover; women who are in premenopausal age, in most these cases, breast cancer patients need supportive hormonal therapy. So, NO may be more effective in distinguishing between those who have reached full recovery from the others, if the present was more extensive.

- **Assessment of MDA Concentration in the Serum Samples of the study Groups**

Table 3 shows a significant increase (**p=0.000**) of serum MDA levels in malignant tumors group when compared with those of benign tumors group, and healthy individuals groups; respectively. With the same manor, highly significant variations (**p=0.000**) were recorded when the ladies in the benign breast tumors group compared to those in the control group.

The current study shows that the levels of MDA in all breast cancer patients are higher than the average of this parameter in the control group, which makes the sensitivity % of MDA in the distinction between breast cancer patients and healthy ones up to 100%. While the present study recorded a significant rise in the levels of Malondialdehydethan the average of this parameter in the control group in 19 out of 24 patients with benign breast tumors, indicating that this criterion lacks the specialized distinction between the benign and malignant tumor, on the other hand; that can enhance that the elevation of MDA were synchronous with any excitation caused by abnormal cell turnover.

Table 3: Levels of MDA (Mean ± S.D.) Concentration (nmol/mL) in Sera of Tumoral Patients and Controls Subjects

Study Groups (n)	MDA Concentration (nmol/mL) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	30.137 ± 2.485	25.507 - 38.312 12.805	0.000 MT *vs* BT
Benign Tumors 24	21.477±5.374	12.312 - 29.682 17.370	0.000 MT *vs* C
Controls 25	12.784±1.874	9.412 – 15.898 6.486	0.000 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

The arise in the level of MDA in the samples of patients with breast cancerin comparison to those of benign breast tumors and healthy controls could be attributed to the abnormal activity of

aerobic metabolism as well as surviving pathways in the malignant cells, leads to overproduction of ROS molecules, MDA especially. ROS overload leads to imbalance in the oxidants-antioxidants system, that promote alteration redox-homoeostasis within cell occurring. The rise in the level of MDA in the neoplasm may be due to reduction in the synthesis of endogenous antioxidant systemcomponents, thatwill cause inefficiency of cellular and extracellular antioxidant defense system. Several studies, which focused on evaluating the levels of MDA at patients with cancer tumors[24,30,45-47]recorded results similar to those recorded in the current work.

In order to verify the changes of MDA concentration with the advancing of chemotherapy treatment, estimation of this parameter was carried out after each dose of chemotherapy. **Figure 4** shows a relative but statistically insignificant increase inMalondialdehydelevels after receiving the latest chemotherapy doses planned within the current study design. Results of the current work showed a decrease in levels of Malondialdehydeafter receiving the first dose of chemotherapy in the group of women with breast cancer, ***exceptthree cases***, which were elderly women in the advanced stage (stage III) of cancer compared to the rest of the patients of this group.

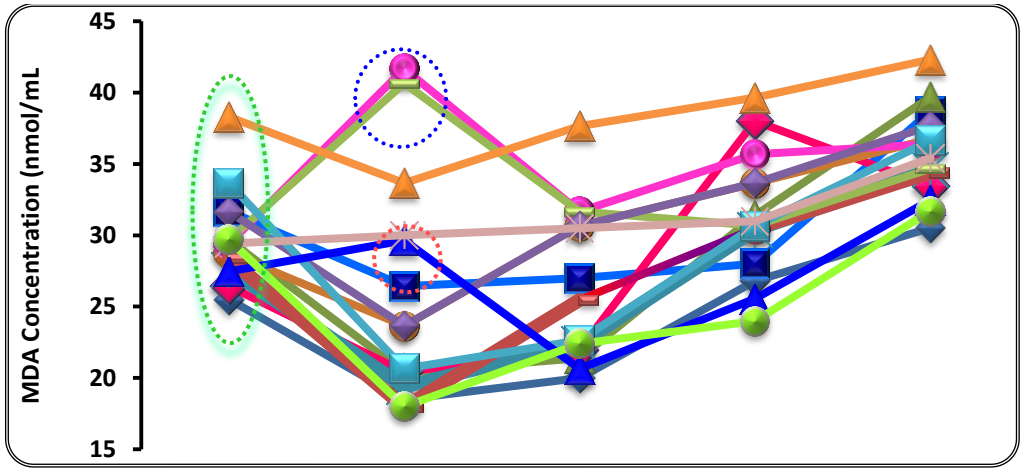

Figure 4: Follow-up of MDA Levels during Consecutive Chemotherapy Stages

MDA levels were gradually raised after receiving the second and third doses of chemotherapy until reached to the highest level at the last dose received.

After completion the number of specific chemotherapy doses in the design of the present work, levels of MDA in the group of ladies with breast cancer were compared with their analog in the group of benign breast tumors group and healthy women in the control group, full results were summarized in the **Table 4**.

Table 4: Concentration (Mean ± S.D.) of MDA (nmol/mL) in Sera of Malignant Tumor Patients After Treatment, Benign Breast Tumors and Controls Subjects

Study Groups (n)	MDA Concentration (nmol/mL) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	34.977± 2.712	30.507- 42.312 11.805	0.000 MT *vs* BT
Benign Tumors 24	21.477±5.374	12.312 - 29.682 17.370	0.000 MT *vs* C
Controls	12.784±1.874	9.412 – 15.898	0.000

25		6.486	BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

Analysis of data showed significant elevation (**p=0.000**) in the levels of MDA in the cancerous patients group when compared to both of benign tumors group and healthy individuals group, same results were observed when benign breast tumor and control groups comparison together, as shown in **Table 4**. This result can be attributed to the side effects of chemotherapy therapy, which is a toxic compound for both malignant and natural cells, resulting in an oxidative stress condition that causes excessive free-radical production and high levels of MDA as a final result of the condition.

Student's t-test was applied to compare the results of serum MDA levels in the malignant tumor group at diagnosis and post-treatment with chemotherapy. Non-significant (**p>0.05**) elevations in sera samples of post-treated group comparison to those in the pre-treated group were noted in **Figure 5**.

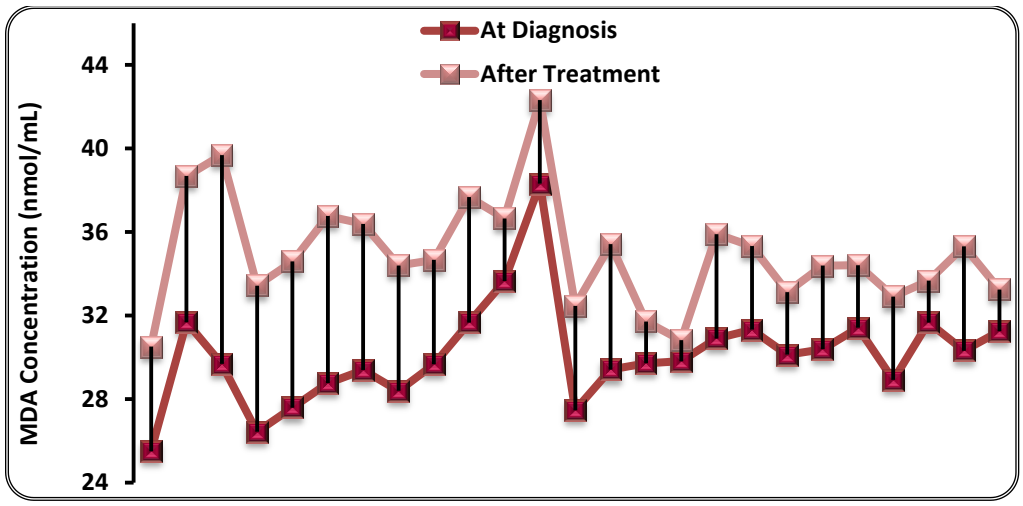

Figure 5: Comparison Levels of MDA in the Sera Samples of Cancerous Patients Before and After Treatment with Chemotherapy (Radiotherapy)

Depending on the results recorded in the current work, Malondialdehydecan be used as a tool to distinguish between patients with breast tumors (regardless of the fact that the tumor is malignant or benign) than healthy women only, while not a tool to diagnose the type of tumor. This criterion is influenced by the age factor, which is consistent with the age of both patients and healthy people. In addition to the progress of the current work results, the levels of this parameter rise simultaneously with the progress of the stage of cancer at the same age of the participants in the study.

The present results were agreed with finding of Ali and his team[48,49] when they studied effect of chemotherapy and radiotherapy treatment in the groups of patients with breast cancer, when they observed an elevation in the MDA levels after treatment regardless the stage of tumor. Same observations were recorded in the studies of nor [50]and Humam[30].

On the other side, present study was disagreed with the study of Dariusz that carried out on the small cell lung cancer [51].

- **Assessment of SOD Concentration in The Serum Samples of the Study Groups**

Results of the present study showed significant statistical differences (**p=0.000**) between women in the group of breast cancer patients and women in the group of benign breast tumors on the one hand, and between cancer patients and healthy women on the other, as illustrated in **Table 5**.

Table 5: Levels of SOD (Mean ± S.D.) Concentration (pg/mL) in Sera of Tumoral Patients and Controls Subjects

Study Groups (n)	SOD Concentration (pg/mL) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	2207.122 ± 322.425	1873.845 - 3002.565 1128.720	0.000 MT *vs* BT
Benign Tumors 24	332.711 ± 73.749	192.502 - 457.090 264.588	0.000 MT *vs* C
Controls 25	401.606 ± 107.070	223.145 - 738.802 515.657	0.368 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

Comparison failed to find respectable significant variances in the levels of SOD between women with benign breast tumors andhealthy individuals in the control group (data shown in **Table 5)**. The current study recorded an increase in the level of SOD concentrations in all samples diagnosed as breast cancer patients, which makes **the sensitivity** of this parameter up to **100%**. In contrast, the study demonstrated that the levels of SOD concentration in the samples of benign breast tumors were all within the limits recorded for this criterion in the control group, this finding reinforces the hypothesis that SOD cannot be used to differentiate between those injured with benign tumors of the healthy ones, actually this observation make **the specificity of SOD** reach to **100%**.

SOD is one of the most important organs of the endogenous antioxidant system, which is composedthe first line of resistance against the cellular over-oxidationproducts, so the high production of this enzyme is a natural response to the state of oxidative stress caused by the carcinogenesis. The transformation of the natural cellular form to the cancerous cell by peroxidation iscontributed in several nuclei changes, *i.e.*, DNA methylations, histone deacetylation, chronic estrogen stimulation, single nucleotide polymorphisms (SNPs), loss of heterozygosity (LOH), and microRNA-21 (miR-21). Total nuclei alterations can be stimulate abnormal production of this enzyme, which in turn acts to guide a number of cellular changes associated with the growth and development of cancer like oxidative tumor microenvironment, tumor growth, metastasis, and recurrence[52]. The present outcomes agreed with previous studies that focused on the evaluation of SOD in the cancer of beast [5],[53], ovary and cervical [52,54], brain [55], pancreas [56], and gastric [6].

Overall, the study showed a gradual increase in the level of SOD during the stages of receiving chemotherapy, especially after the first two doses and the results of this parameter has fluctuated after the third dose, although it did not fall below the levels in the same patients before the use of chemotherapy. As well-known in the **Figure 6** after the last dose of treatment, the levels of SOD concentration were higher than the levels of diagnosis at the same sample, ***except for one case*** which was the youngest patients (25 years old) who was in the first stage of malignancy.

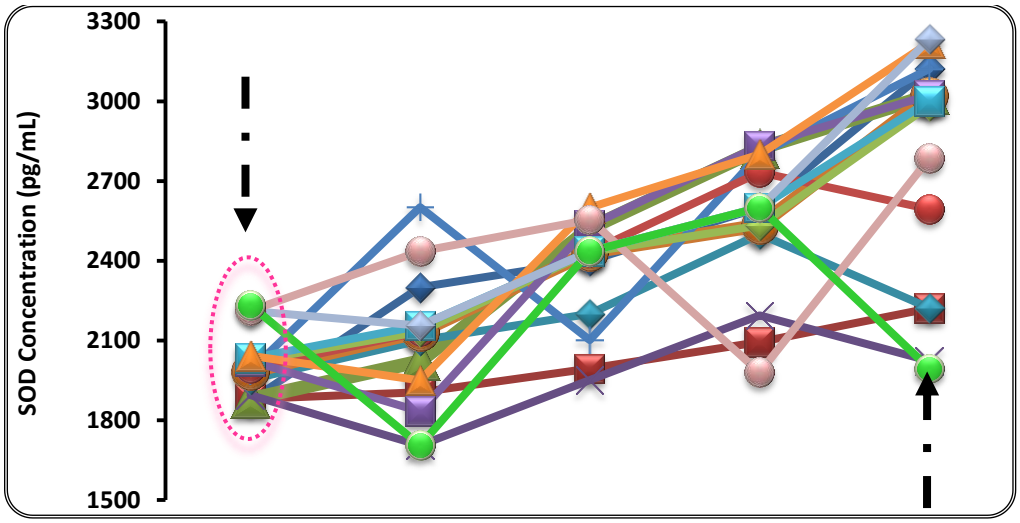

Figure 3.6: Follow-up of SOD Levels During Consecutive Chemotherapy Stages

Based on the fact that the levels of SOD were not consistent during successive chemotherapy stages, this parameter could not be used as a tool for tracking patients' response to chemotherapy. After the period of chemotherapy planned in the current study and for the assessment of SOD levels in the group of breast cancer patients after treatment, the study groups were compared using ANOVA test.

It is possible to explain the significant increase in levels of the production of the SOD enzyme after the completion of the fourth dose of chemotherapy as a reaction to the accumulation of toxicity of chemotherapy, especially after the experience of relative stability in the levels of this enzyme, especially after receiving the two intermediate doses, which followed a significant rise in levels after the last dose. The statistical analysis showed significant differences in levels of SOD when comparing the group of women with breast cancer after receiving chemotherapy and the group of patients with benign breast tumors; and same resultwas recorded when chemotherapy treatments group were compared with women in the control group, as illustrated in **Table 6**.

Table 6: Concentration (Mean ± S.D.) of SOD (pg/mL) in Sera of Malignant Tumor Patients After Treatment, Benign Breast Tumors and Controls Subjects

Study Groups (n)	SOD Concentration (pg/mL) Mean ± S.D.	Min.-Max. Range	p-value
Malignant Tumors 25	2665.394 ± 438.913	1991.188- 3234.271 1243.083	0.000 MT *vs* BT
Benign Tumors 24	332.711 ± 73.749	192.502 - 457.090 264.588	0.000 MT *vs* C
Controls 25	401.606 ± 107.070	223.145 - 738.802 515.657	0.368 BT *vs* C

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

The results of this study were similar to the results of the Jine study and his team [6] who indicated elevated levels of this enzyme after receiving doses of chemotherapy in the group of patients with stomach cancers. While the results were contrasted to the results of Kaya team which illustrated significant raise in the free radical levels and significantly decreased in the levels of

SOD in the serum of patients with Hodgkin's lymphoma after treatment with ABVD chemotherapy [57].

Using independent student t-test for comparison the results of SOD in the diagnosis stage (**2207.122 pg/mL**)with its counterparts in the post-receiving stage of the fourth dose of chemotherapy (**2665.394 pg/mL**), the statistical treatment showed no significant differences (**p=0.071**) between two stages. **Figure 7** illustrates that number of samples showed an increase in the SOD values after completion of treatment, this finding was recorded in the cases of patients in the first stage of cancer, while the cases which showed deficiency in the levels of SOD after receiving chemotherapy comprising to the diagnosis stage, they were elderly women with advanced stage **(III).**

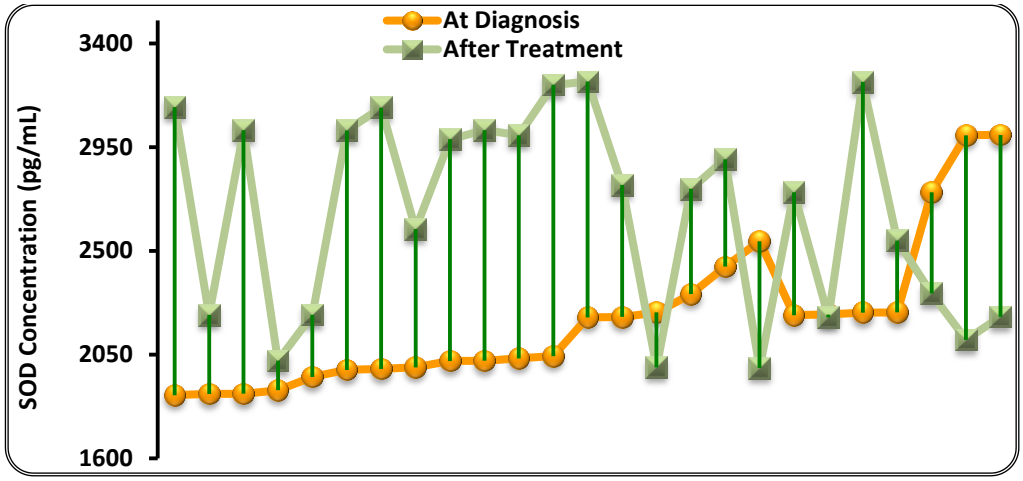

Figure 7: Comparison Levels of SOD in the Sera Samples of Cancerous Patients Before and After Treatment with Chemotherapy (Radiotherapy)

Figure 7 shows an increase in the levels of SOD enzyme in 80% of the samples that received chemotherapy (20 out of 25 cases) indicating that this parameter can be used as a statistically significant tool for evaluating the efficacy of chemotherapy in treating breast cancer patients.

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