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EFFECTS OF BREAST CANCER AND COMBINATION OF CHEMOTHERAPY ON OXIDATIVE STRESS IN BASRA, IRAQ

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ABSTRACT : Recent studies are interested in the effects of breast cancer and chemotherapy combination on blood biomarker. The objective of our study is to determine the effects oxidative stress status represented by measurement of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and malondialdehyde (MDA) in sera of women with breast cancer before and after 2 and 4 cycles of receiving chemotherapy composed of anthracycline, cyclophosphamide and docetaxel (ACD) and comparing them with healthy females. A cohort study used 25 women that diagnosed with breast cancer after surgery and they underwent chemotherapy (ACD), with an age range of (20- 65) years and (25) women with the same age range as a healthy control group. Serum samples of patients were obtained before receiving chemotherapy, after 2 cycles and after 4 cycles of treatment with (ACD). The results showed that the activities of SOD and (GR) and the concentration of MDA were significantly decreased; whereas the activity of (CAT) was unchanged, in serum of women with breast cancer untreated with the chemotherapy (ACD) comparing to the control group. Treating with (ACD) chemotherapy led to opposite the above results. Treating with ACD chemotherapy combination led to overcome the oxidative stress status of the women with breast cancer.

Key words : Breast cancer, antioxidant enzymes, anthracycline, cyclophosphamide and docetaxel.

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INTRODUCTION

Breast carcinoma is the major type of cancers in women and also the main cause of death worldwide (Siegel *et al*, 2012). The variable risk factors of breast cancer include alcohol intake, age, diet, smoking, family history and lactation (Torre *et al*, 2015).

Many studies proposed that oxidative stress is involved in etiology of breast cancer. The Oxidative stress was defined as an imbalance between production of reactive oxygen species (ROS) and antioxidants leading to probable cellular damage. Conversely, oxidative stress can produce a significant ROS which affects the defense system of antioxidant or decrease defense of antioxidant (Sosa *et al*, 2013). A variety of mechanisms of cellular defense be made up of enzymatic components for instance catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and non-enzymatic components for instance vitamin C, vitamin E and glutathione, organize the standard oxidant/antioxidant imbalance of the human body (Matés *et al*, 2000). The too much formed of ROS could modify and damage of many intracellular components include nucleic acids, lipids and proteins (Sosa *et al*, 2013; Al-Jassani *et al*, 2019).

Most types of chemotherapeutic agents are not specific to kill neoplastic cells, and may be have an effect on the normal cells of human body (S´anchez-Su´arez *et al*, 2008). They give adverse wide range of reactions in practically most of tissues of the human body for instance alopecia, bone marrow suppression, generalized rash, fatigue, dizziness and diarrhea (Walko *et al*, 2014).

Cyclophosphamide, is one of the mainly used as anticancer compounds, is having two functional alkylating main member of the family of nitrogen mustard that contain different kinds of DNA damage, for example DNA adducts, a chromosomal aberrations and mutation of genes (S´anchez-Su´arez *et al*, 2008). Cyclophosphamide makes combination with doxorubicin, an agent of anthracycline to be used, and they capable of

intercalating into DNA (Marsh *et al*, 2009). The (AC) mechanism is to make cytotoxicity to cells, contain intracellular creation of free radicals, subsequent inhibition of DNA topoisomerase II, and DNA intercalation (Marsh *et al*, 2009). Free radicals can stimulate damage of oxidative DNA, causative to mutagenesis, which is necessary to the method of tumor start proliferation (Freeman *et al*, 2012). Damage of DNA associated with a diversity of disorders in the human body including neurodegenerative diseases and neoplasm (Rodriguez-Rocha *et al*, 2011). The level of the damage caused by ROS can increase or decrease by the enzymatic antioxidants (SOD, CAT and GR) or nonenzymatic antioxidant (vitamins A, C and E, selenium and reduced glutathione (GSH)) (Glorieux *et al*, 2011).

SOD is one of the major important of enzymatic antioxidants. It can catalase the switch conversion of superoxide to hydrogen peroxide (H_2O_2) and molecular O_2 (Carocci *et al*, 2018).

The major important mechanism of the activity of (GR) was derivative as of oxidation of NADPH. The unit of GR activity is the amount for the enzyme that catalyzes change of 1 μmol of substrate per one minute (Elena *et al*, 2010).

Malondialdehyde is a reactive organic species occurs naturally and has the formula of $CH_2(CHO)_2$, produced by the lipid metabolism in the body (Arshad *et al*, 2014). MDA has a potential toxicity and can stimulate the immune and inflammatory response if it is found in an elevated levels (Evans *et al*, 2014). It is the most important aldehyde that arises from the peroxidation of polyunsaturated fatty acids (PUFA) such as linolenic and arachidonic (Shakir *et al*, 2016). MDA can cause protein damage by its reactions with amino groups of the protein amino acids leads to alteration of protein structure. Lipid peroxidation participates in neurodegenerative disorders and can introduce mutagenic lesions (Kota *et al*, 2014). Malondialdehyde measurement is an indicator of lipid peroxidation, which used as a biomarker for oxidative stress (Jarc *et al*, 2018).

Aim of the study

The study aimed to determine the effects of chemotherapy combination consist of Anthracycline, Cyclophosphamide and Docetaxel, on the oxidative stress status in women with breast cancer.

MATERIALS AND METHODS

Area of study

A cohort study made on 50 women, with an age range of (20- 65), consists of (25) women with breast cancer, they were diagnosed and classified generally by the physicians and followed up after 2 cycles and 4 cycles of treatment with ACD combination of chemotherapy and (25) healthy women as a control group.

Samples collection

A 5 ml of venous blood samples from each of the patients and control groups were withdrawn using one-use syringes and needles under aseptic technique, transferred into gel tubes and centrifuged at 3500 rpm for 4 minutes. The collected sera were frozen at -20^0 C until they used for the determination of each of (SOD), (CAT), (GR) and (MDA).

Method

Serum SOD, CAT and GR activities and MDA concentration were determined by using Elabscience, USA kits by Enzyme-Linked Immunosorbent Assay (ELISA).

The statistical analysis

The data obtained from the study was analyzed using SPSS (Statistical Package for Social Sciences) version(25). Ordinal and nominal data were presented as frequencies. Results obtained were expressed as (mean ± standard deviation) and *P*-values less than 0.05 were regarded as statistically significant. A NOVA was used to compare more than two means, followed by post hoc.

RESULTS AND DISCUSSION

Oxidative stress status in breast cancer

The oxidative stress characteristics as listed in Table 1 showed that the serum activities of SOD and GR as well as the MDA concentration were significantly decreased, while CAT activity was unaffected in women with breast cancer untreated with ACD chemotherapy as compared with the control group ($P<0.05$).

The results of this study in agreement with many previous studies by Prabasheela *et al* (2011), Kamal *et al* (2012), Mostafa *et al* (2017) and Pinar Atukeren *et al* (2010).

Oxidative stress has been shown to play an important role in breast cancer pathogenesis and is associated with cancer complications. Enzymatic systems (SOD, GR and CAT) function directly or successively to remove of ROS, thereby terminating the ROS activities (Kamal *et al*, 2012). The deficiency of this enzymatic antioxidants may be resulted from the uncontrolled ROS production which often leads to damage of the cellular macromolecules (DNA, proteins and lipids) (Alacacioglu *et al*, 2013).

Several studies showed that oxidative stress

Table 1 : Oxidative stress status in patients untreated and healthy control groups.

Variable	Healthy control group Mean ±SD	Untreated women with breast cancer Mean ±SD	p. Value
Serum SOD ng/mL	41.40±3.52	34.08±3.74	0.0001
Serum Catalase ng/mL	7.90 ±0.96	7.90 ±1.35	1.000
Serum GR pg/mL	418.20±9.05	410.27±9.70	0.009
Serum MDA ng/mL	123.20±2.99	117.02±.96	0.0001

Table 2 : Effects of ACD chemotherapy treatment on oxidative stress parameters.

Variable	Untreated women with breast cancer Mean ±SD	After C2 of ACD treatment	p. Value
Serum SOD ng/mL	34.08 ±3.74	41.86 ±1.60	0.0001
Serum Catalase ng/mL	7.91 ±1.30	5.00 ±0.20	0.0001
Serum GR pg/mL	410.20 ±5.90	424.90 ±9.80	0.0001
Serum MDA ng/mL	117.06 ±0.95	145.03 ±7.70	0.0001

Table 3 : Effects of ACD chemotherapy treatment on oxidative stress parameters.

Variable	After C2 of ACD treatment	After C4 of ACD treatment	P.Value
Serum SOD ng/mL	41.86 ±1.60	47.30 ±1.70	0.0001
Serum Catalase ng/mL	5.00 ±0.20	3.10 ±0.50	0.0001
Serum GR pg/mL	424.90 ±9.80	437.54 ±10.90	0.0001
Serum MDA ng/mL	145.03 ±7.70	188.41 ±6.60	0.0001

increased in**;** cerebrovascular accident (Al-khalifa *et al*, 2018) and rheumatoid arthritis (Mahdi *et al*, 2019).

Breast cancer can start in different sites of the breast, for instance lobules and ducts. Ductal carcinoma in situ (DCIS) is the majority familiar (Rakha *et al*, 2010). Some studies have reported that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are but in the etiology and series in different cancers (Vallejo *et al*, 2013).

These reactive species (ROS) have been related to the maturity and tumorigenesis by activating varied kinds of damage of DNA, leading to the emergence of mutations and aberration of chromosomal to the inflammatory reaction and leading to make intense injuries and disorganization for tissues (Gupta *et al*, 2012).

The major site to generate reactive oxygen species (ROS) is the mitochondria, also are may be the main intracellular target to oxidative damage. "Agents of anticancer can enhanced generation lipid peroxidation, and once the mitochondrial membrane barrier function is lost, several factors contribute to cell death. furthermore, Mitochondrial permibilization was facilitated by lipid peroxidation and antioxidant enzyme inhibited it" (Imad *et al*, 2013).

Effect of ACD chemotherapy treatment on oxidative stress

The effects of ACD chemotherapy on oxidative stress characteristics are listed in Tables 2 and 3.

The activities of serum SOD and GR and the concentration of MDA as in Tables 2, 3 were significantly elevated in women with breast cancer treated with a ACD combination after cycle 2 and cycle 4 of treatment, whereas CAT activity was significantly decreased as compared with the group of women before treatment ($p<0.05$). The results of this study are in agreement with many previous studies (Kamal *et al*, 2012; Mostafa *et al*, 2017; Antonio *et al*, 2015).

This study showed an increase in serum MDA concentration after cycle 2 and cycle 4 of ACD treatment, which agreed with the results of Mostafa *et al* (2017). Other previous studies showed a decrease in serum MDA concentration (Suhail *et al*, 2012). Similarly, the activity SOD was increased after cycles 2 and 4 of ACD treatment. This result agreed with the results of Suhail *et al* (2012). On the other hand, Gupta *et al* (2012) showed a decrease in SOD activity in serum.

Increasing in GR activity that was reported in a study that express elevated in lipid peroxidation in women with breast cancer in addition to elevated in antioxidant enzymes. Signifying that there might be upregulation for antioxidant enzymes induced by ROS (Deng *et al*, 2007). Other study by Pinar Atukeren *et al* (2010) suggested that decreased in the activity of GR and catalse.

Our study showed also decrease in serum CAT activity after cycle 2 and cycle 4 of ACD treatment. ACD for the breast cancer gave a significant decreased of

serum CAT activity. Heart has low antioxidant activity, given that it low catalase level, and many researcher have made known that anthracycline selectively down regulate GR that may be cardiomyocytes are showing high levels of hydrogen peroxide. Besides, cardiomyocytes are loaded in mitochondria, that signify the mass of cardiomyocyte up to 50% that serve as together target and source of ROS. Also, the significant role has been certified to exogenous (NADH) dehydrogenase. Finally, elevated NO and decreased CAT and GSH that stimulate lipid peroxidation level (Deng *et al*, 2007).

Some chemotherapies are thought to work in part by increasing the oxidative stress on cancer cells. Oxidative stress, however, is not always detrimental. Selective oxidative stress sometimes is desirable and can be utilized therapeutically also.

Severe oxidative stress leads to apoptosis. Anthracyclines have been described to be able to induce significant oxidative and nitrosative stress to different kinds of cells (Alacacioglu *et al*, 2013).

The data exposed that oxidative stress and antioxidant improvement may make certain breast cancer series, definitely mediated with SOD, GR and CAT activity as well as serum MDA level.

CONCLUSION

Treating with ACD chemotherapy lead to change in the antioxidant status of the women with the breast cancer during treatment duration.

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