DISCUSSION

Breast cancer is by far the most common cancer among women of both developed and developing countries accounting for 22.9% of all female cancers. It is also the leading cause of cancer death in females accounting for 13.7% of their cancer related mortality. The favorable incidence to mortality ratio (3:1) can be attributed to the more favorable survival in developed regions (3.7:1) than the less developed regions (2.7:1) [166]. In Egypt, breast cancer is estimated to be the most common cancer among females accounting for 37.7% of their total with 12,621 new cases in 2008. It is also the leading cause of cancer related mortality accounting for 29.1% of their total with 6546 deaths. The incidence to mortality ratio is poor (1.9:1) [166]. These estimates are confirmed in many regional Egyptian cancer registries [167,168] as well as in hospital-based frequencies [169]. The etiology of breast cancer is multifactorial and several risk factors associated with breast cancer may exert their effects via generation of an oxidative stress status [4].

Oxidative stress is caused by an unfavorable balance between reactive oxygen species (ROS) and antioxidant defenses. ROS are generated during normal cellular metabolism, as a result of the influence of various environmental factors, as well as during pathological processes [170] .Oxidative stress is responsible for DNA, lipid and protein damage and it plays an important role in the development and progression of many human diseases, including breast cancer[171]. Furthermore, there is a great interest in the measurement of oxidative stress biomarkers in breast cancer patients. Several markers of oxidative stress are currently available, such as lipid hydroperoxides and thiobarbituric acid reactive substances, which have been used extensively as markers of lipid peroxidation, as well as protein carbonyl, that is the most frequently used biomarker of protein oxidation in epidemiological and clinical studies [172-175].

Radiotherapy and certain chemotherapy agents act through various oxidative stress mechanisms to produce free radicals that damage tumor cells [176] . Oxidative stress during cancer therapy also harms healthy tissue. Antioxidant supplements may help in protecting normal cells from oxidative damage and reduce the short- and long term harmful effects of cancer treatment [176-180]. Several antioxidants such as vitamin A and its precursor (beta-carotene, lycopene, lutein, etc), vitamins E, C, and selenium, have drawn a lot of attention to the scientists and the public alike [177,178] . They have been shown in experimental studies to neutralize or trap reactive oxygen species (also known as free radicals), thereby preventing cellular damage caused by the reaction of these species with proteins and nucleic acids [179-184].

On the other hand, concern has been raised that antioxidant supplements may also protect tumor cells during radiotherapy and chemotherapy, thereby compromising treatment efficacy [176, 180-185]. This has resulted in controversy over guidelines for the use of vitamin supplements during cancer treatment [176, 178,186-188]. While many investigators and clinicians recommend that vitamin supplements, in particular antioxidants in high doses, should not be used by patients during cancer treatment [176, 186-188], others have supplemented with vitamins during chemotherapy [179,189-192].

Accordingly the present study is undertaking to explore whether vitamin A and E supplementation during 5-fluorouracil, adriyamicin, and cyclophosphamide (FAC) therapy will have its impact on chemotherapy-induced oxidative stress.

This study is carried out, after the approval of Ethics Committee – Medical Research Institute, on 45 breast cancer patients who were divided into two groups; group (I) (20 patient) which was subjected only to chemotherapy and group (II)(25 patient) which was subjected to chemotherapy plus supplementation with vitamin A and E. Markers of oxidative stress including serum MDA, β-carbonyl protein (β -CP) and total antioxidant capacity (TAOC) are measured in patients of those two groups before and after chemotherapy with or without vitamins supplement.

In group (I), vitamins supplemented –ve(20 breast cancer pateint), the results showed that chemotherapy with FAC resulted in a significant elevation in MDA and β-CP accompanied by a significant decrease in TAOC. Anthracyclines rank among the most effective anticancer drugs ever developed [193]. Most patients with breast cancer are treated with a combination of the anticancer chemotherapy drugs of 5-fluorouracil, Adriyamicin, and cyclophosphamide (FAC). These antineoplastic agents cause a reduction in antioxidant levels because their toxicity increases the peroxidation of the unsaturated fatty acids of membrane phospholipids [194] .These chemotherapeutic drugs are hydrophilic and cannot penetrate the inner membrane of cells where they would be reduced by NADH located on the inner membrane surface [195,196] . Chemotherapeutic drugs, particularly doxorubicin used in FAC treatment are able to enter the outer mitochondrial membrane and enter the cytosol. Intramolecular rearrangements result in the formation of a lipophilic deoxyaglycone that can penetrate the inner membrane of the mitochondria. In mitochondria, doxorubicin competes with coenzyme Q10 as an electron acceptor and diverts electrons to molecular oxygen resulting in the formation of superoxide radicals [196]. Doxorubicin intercalates DNA coils and interferes with normal cellular metabolism through a diverse set of biochemical mechanisms that may explain its toxicity. It causes an increase in the peroxidation of unsaturated fatty acids of membrane phospholipids which lead to a decrease in the level of antioxidants and generate a high level of oxidative stress. In addition, doxorubicin is able to divert electrons from the mitochondrial electron transport system in addition to generating ROS at cellular sites [195,196] .

Recently, it has been reported that the levels of catalase and glutathione (GSH) were significantly reduced in breast carcinoma and FAC treated breast cancer patients. The lipid peroxidation and nitric oxide (NO) levels were significantly enhanced in both untreated and FAC treated breast cancer patients. The creatin kinase (CK) and lactic dehydrogenase (LDH) were significantly enhanced in the FAC group. Therefore, it was concluded that oxidative stress is implicated in breast carcinoma and chemotherapy aggravates this oxidative stress which causes damage to many cellular targets and has the main side effect of cardiotoxicity [197]. Alshabanah and his associates [198], reported a decrease in the gene expression levels of glutathione peroxidase (GSHPx), catalase, glutathione reductase (GR), and glutathione transferase (GST) in liver tissue with the cumulative dose of doxorubicin with a decrease in their activity in the serum. Thus, doxorubicin not only increased free radical formation but also decreased its ability to detoxify the ROS. The formation of superoxide radicals together with NO might form peroxynitrite induced by doxorubicin which causes tissue damage leading to an increase in the levels of thiobarbituric acid substance i.e., increased lipid peroxidation.

Moreover, antineoplastic agents induce oxidative stress leading to protein and DNA damage. It has been observed that after chemotherapy, a marked raise in DNA damage and protein carbonyl levels. Therefore, it has been suggested that chemotherapy induces a certain level of systemic oxidative stress, which is maintained along successive clinical interventions and could influence the clinical outcome of the patients [178]. This may explain, in part, the observed reduction in the total antioxidants capacity level in breast cancer patients who received chemotherapy alone without antioxidant vitamins supplementation.

On the other hand, several studies have pointed out to the increased levels of carbonyl protein as results of chemotherapy. Protein carbonyl content (PCC) is widely used as a measure of total protein oxidation in cells. Carbonyl groups formed by ROS on amino acid side chains are chemically stable moieties making them useful in detecting oxidative damage [199]. Carbonyl modifications are not repaired and the extent of carbonylation depends upon factors that influence oxidant status. Thus, the plasma protein carbonyl biomarker captures the net of pro-oxidant exposures and antioxidant status. The most commonly oxidized amino acids are Arg, Lys, Pro, Thr, and the plasma protein, fibrinogen, is highly susceptible to free radical attack [١٠٤,١٩٩]. It should be noted that the present study revealed appositive correlation between the mean concentration level of MDA and β-CP, meanwhile the mean level of TAOC was correlated negatively with the mean concentration level of both MDA and β-CP. Intracellular oxidized proteins are rapidly degraded by the 20S proteosome [٢٠٠]. However, it is unclear whether extracellular oxidized proteins are actively degraded or simply eliminated by normal turnover. Plasma protein carbonyl levels have been shown to increase with fat overload and exercise, and decrease with weight loss, and vitamin treatment [٢٠١–٢٠٤].

In a study based on doxorubicin chemotherapy as a clinical model for oxidative results a progressive elevation in protein carbonyl content was observed. The study indicated that (1) plasma MDA is not a sensitive biomarker in humans; (2) protein carbonyl content potentially may be used, if antioxidant reserves are taken into account; (3) antioxidant reserves play an important role in the reaction to oxidative stress [٢٠٥]. Other cytotoxic agent, e.g., Topoteca, exerts its cytotoxic effect by inhibiting topoisomerase I and causes double-strand DNA breaks which inhibit DNA function and ultimately lead to cell death. It has been reported that Topoteca increases oxidative stress in breast cancer cell line (MCF-7). This was evident by increased lipid peroxidation and protein oxidation (carbonyl content), and decreased GSH and sulfhydryl levels in MCF-7 [٢٠٦].Thus, the results of the present study are in accordance with some previous studies and confirm the observation that breast cancer patients are subjected to oxidative stress not only due to the disease but also as result of FAC chemotherapy.

Oxidative stress is manifested by elevation in free radicals production and decreased antioxidant capacity which is evident by increased levels of MDA and β-CP accompanied by a significant decrease in total antioxidant capacity. This may have its impact on the breast cancer patients render them subjected to different side effects including chronic fatigue which is the most common side effects[٢٠٧-210]. Chronic fatigue caused by cancer therapy can reduce therapeutic efficacy [210-211]. These all could lead us to the important question which is "would antioxidant vitamins such as A and E could attenuate the chemotherapy-induced oxidative stress?"

The answer of the previous question may be obtained from the results of group II(25 breast cancer pateint). In this group, patients are supplemented with vitamin A and E during FAC chemotherapy. It should be noted that before chemotherapy, the levels of the studied biomarkers of oxidative stress; MAD, β-CP and total antioxidant capacity were not significantly different from that in group I. After chemotherapy, a significant reduction in the mean concentration levels of MDA and β-CP accompanied by a significant elevation in the mean total antioxidant capacity level. Moreover, in group II after chemotherapy and vitamin supplementation, the mean concentration levels of MDA and β-CP are significantly reduced when compared to those in group I after chemotherapy.

The observed reduction in free radical production; manifested by the decrease in the concentration levels of MDA and β-CP, and the interesting increase in total antioxidant capacity level could be attributed to the antioxidant properties of both vitamins A and E. Britton in (1995) [212]defined that for carotenoids to be an effective antioxidant, it would have to remove the free radicals from the system either by reacting with them to yield harmless products or by disrupting free radical chain reactions. The electron rich conjugated double bond structure is primarily responsible for the excellent ability of β - carotene to physically quench singlet oxygen without degradation and for the chemical reactivity of β-carotene with free radicals and for its instability toward oxidation [212,213]. The maximum protection to quench singlet oxygen is given by those carotenoids having nine or more double bonds [214]. Handelman, in (1996) [215] suggested that the following structural properties could contribute to antioxidant functions of carotenoids:

- ►► A multiplicity of closely spaced energy levels between the excited state and ground state of the carotenoids, such that the carotenoid can dissipate excited state energy via small collisional exchanges with the solvent.
- ►► Minimal tendency for the excited state carotenoid to sensitize other molecules.
- ►► Resonance states in the excited state carotenoid allowing delocalization and stabilization of the excited state.
- ►► Multiple potential sites on the carotenoid for attack by active oxygen.

On the other hand, vitamin E (VE) is one of the most important lipid-soluble primary defense antioxidants. Its molecule can be divided into two parts, a hydroxyl-bearing aromatic system (one phenolic and one heterocyclic ring, called the chroman head) that is responsible for its antioxidant properties, and either a saturated (tocopherols) or polyunsaturated (tocotrienols) hydrocarbon tail for the orientation of VE in the lipid membrane [216]. In its function as a chain-breaking antioxidant, VE rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a VE radical [216,217]. The VE radical can be reduced to VE by vitamin C or reduced glutathione or alternatively it is further oxidized to VE quinone [218].

The pervious question can be put in other words "whether antioxidant therapy will increase the quality of life of breast cancer patients through protection of normal tissues and possibly slow disease progression by lowering oxidative levels or interfere with the eventual clinical outcome of their disease". It could be suggested that the patient outcomes

may be improved by antioxidants through improving the therapeutic index of coadministered chemotherapy drugs, i.e., increasing a patient's ability to tolerate full doses of antineoplastics with uninterrupted treatment schedules. The toxic side effects of chemotherapy, as chronic fatigue, often lead to dose reductions, interruptions and delays in chemotherapy treatment, and incomplete courses of treatment. A reduction in these side effects, via supplementation with antioxidant vitamins A and E, might result in an improved quality of life for the patient, and possibly better survival rates.