INTRODUCTION

Epidemiology

Breast cancer is the most common cancer in women in developed western countries[1], and is becoming ever more significant in many developing countries [2]. Although incidence rates are increasing, mortality rates are stable, representing an improved survival rate. This improvement can be attributed to effective means of early detection, mainly mammography, as well as to significant improvement in treatment options [2].

Breast cancer, the most common cancer among women worldwide, accounts for the highest morbidity and mortality [3]. Annually, around 1 million new patients are diagnosed with breast cancer and 400,000 women die from the disease. The etiology of breast cancer is multifactor and several risk factors associated with breast cancer may exert their effects via generation of an oxidative stress status [4].

In Egypt, breast cancer is the most common cancer among women, representing 18.9% of total cancer cases (35.1% in women and 2.2% in men) among the Egypt National Cancer Institute (NCI) series of 10,556 patients during the year 2001 [5] ,with an age-adjusted rate of 49.6 per 100,000 population. However, this represents hospital-based data of referral tertiary centers and does not represent all breast cancer cases in Egypt [5].

In Alexandria, Egypt, the total number of cancer cases is 5010, 4662, 5064 in 2008, 2007 and 2006, respectively. The statistics of most common cancers in 2008 according to Cancer Registry Department in Medical Research Institute were as follow: 1136 from breast, 456 liver, 403 lungs, and 221 bladders [6].

Risk factors

Breast cancer should be largely viewed as a disease predominantly influenced by risk factors related to lifestyle, as only approximately 15% of all breast cancer cases can be attributed to familial and genetic influences [7].Most known risk factors for breast cancer can be linked to hazardous effects of hormonal exposures [8], although other risk factors such as exposure to ionizing radiation are also relevant in some populations [9, 10] .

Reproduction-Related Exposures

Early age at menarche, late age at menopause [8],small number of children and null parity, late age at first birth[11],and little or no breastfeeding[12,13] have all been associated with an increased risk of developing breast cancer. Although several retrospective studies have suggested that induced abortion is related to an increased risk of this disease, this is not seen in prospective studies [14], the period of exposure to sex hormones before the first full-term pregnancy is a time when the breast tissue is especially susceptible to carcinogenesis. Long-term use of hormone replacement therapy, but apparently not long-term use of oral contraceptives, is also related to increased risk of breast cancer [15-18]. Despite the use of mega-doses of hormones in fertility treatments, there is no current evidence that these treatments are hazardous to the breast[19,20] .Of major interest are risk factors for which there is a potential to reduce risk at the population level. Use of external

hormones and breastfeeding probably are the 2 best candidates. Meta analyses have demonstrated that long-term breastfeeding can be linked with up to a 30% reduction in breast cancer risk [20].

Benign Breast Disease

History of benign breast disease is also related to increased risk of breast cancer [21]. The risk, however, is mostly restricted to women who underwent biopsies, and especially those in whom atypical hyperplasia was found in such biopsies [22].

Nutritional Factors

The role of diet and nutrition in the etiology of breast cancer has been under debate for decades .Dietary fat has been the most investigated food constituent studied in this regard. It is currently believed that a high-fat diet is not directly related to the risk of breast cancer [23,24]. Overall caloric intake and obesity in particular with certain weight-gain patterns, are related to increased breast cancer risk, with different effects between pre- and postmenopausal women [25, 26] .This is also in line with a proven role of regular physical activity in reducing breast cancer risk [27]. High fruit and vegetable consumption is related to decreased breast cancer risk in most, but not all, studies [28]. Specifically, the consumption of cruciferous vegetables has been shown in vitro and in vivo to be related to such protection [29]. Of all food items studied, regular alcohol consumption, even at moderate levels, has consistently been found to be related to a mild increase in breast cancer risk in women [30, 31].

Smoking and radiation

Active and passive smoking have been shown to be related to breast cancer risk [31,32]. Ionizing radiation has been shown to increase the risk of breast cancer in studies following cohorts exposed to the A-bomb as well as in studies of women exposed to medical radiation [9, 10].

Numerous studies have failed to show that environmental hazards, such as exposure to specific pollutants, are substantially related to breast cancer risk. Some studies have suggested that exposure to polychlorinated biphenyls (known as PCBs) and organ chlorines carries increased risk of breast cancer [33-35].

High Breast Density

High breast density, as reflected on mammography films, has been shown to be one of the most significant markers of breast cancer risk [36]. Dense breast tissue probably reflects high hormonal exposure and is typical of young women, women using hormone replacement therapy, and those who are breast cancer A gene (BRCA) gene carriers.

Genetic Factors

An established proportion of all breast cancer cases is caused by mutations in specific genes, mainly the BRCA genes. This proportion differs between different ethnic groups, and is especially high among Jewish women of Ashkenazi and Iraqi origin [37-41]. In the latter group, up to 10% of all newly diagnosed breast cancers are due to mutations in

these genes. In addition to BRCA gene mutations, other genes such as Adenine thymine (AT) and Protein 53 or tumor protein 53 (p53) are also involved in the development of breast cancer [7]. A variety of single nucleotide polymorphisms in genes encoding phase I and phase II enzymes, as well as other enzymes involved in the hormonal metabolism, are thought to interact with hormonal, nutritional, and radiological exposures to increase the risk of breast cancer [7].

Age

The risk of developing breast cancer increases with age. The disease is uncommon in women younger than 40 years of age; only about 0.8% of breast cancers occur in women <30 years old and approximately 6.5% develop in women between 30 and 40 years old[42] .

Gender

Breast cancer is relatively uncommon in men; the female-to-male ratio is approximately 100:1. It accounts for < 1% of all cancer cases in men, and an estimated 460 men will die of the disease in 2005. The incidence of breast cancer in men has remained relatively stable over the past decades, except in Africa, where for unclear reasons, the incidence is rising. BRCA mutations are associated with an increased risk for breast cancer in men. The most common presentation in men is asymmetric gynecomastia, often related to a single effect of drug therapy (such as from digoxin) or liver failure. All palpable masses in men should be carefully examined. Based upon the findings on physical examination, mammography and breast ultrasonography should be considered. Fine-needle aspiration (FNA) or core biopsy can be used to distinguish between gynecomastia and breast cancer. Core biopsy may be performed if the FNA is non diagnostic [42].

Race

Caucasian women have a higher overall rate of breast cancer than African or American women; however, this difference is not apparent until age 50 and is marked only after menopause. The incidence of breast cancer in American Asian and Hispanic women is approximately half that in American Caucasian women. Breast cancer risk is extremely low in Native-American women [42].

Geography

There is at least a fivefold variation in the incidence of breast cancer among different countries, although this difference appears to be narrowing. The incidence of breast cancer is significantly lower in Japan, Thailand, Nigeria, and India than in Denmark, the Netherlands, New Zealand, Switzerland, the United Kingdom, and the United States. It has been suggested that these trends in breast cancer incidence may be related, in some way, to dietary influences, particularly dietary fat consumption [42].

Socioeconomic status

The incidence of breast cancer is higher in women of higher socioeconomic background. This relationship is most likely related to lifestyle differences, such as age at first birth [42].

Disease site

The left breast is involved slightly more frequently than the right, and the most common locations of the disease are the upper outer quadrant and retroareolar region. The risk of contralateral breast cancer in women with *BRCA* mutations is approximately 40% at 10 years after the initial diagnosis of breast cancer. This risk is higher in *BRCA1* than *BRCA2* mutation carriers and those first diagnosed at age < 50 [(42)].

Survival

Survival rates for patients with nonmetastatic breast cancer have improved. These improvements may be secondary to advances in adjuvant chemotherapy and radiation therapy. The contribution of screening mammography to breast cancer-specific survival is variable, favoring a reduction in breast cancer mortality of up to 25% in some series [(42)].

Menstrual and reproductive factors

Early onset of menarche (< 12 years old) has been associated with a modest increase in breast cancer risk (two fold or less). Women who undergo menopause before age 30 have a twofold reduction in breast cancer risk when compared with women who undergo menopause after age 55. A first full-term pregnancy before age 30 appears to have a protective effect against breast cancer, whereas a late first full-term pregnancy or null parity may be associated with a higher risk. There is also a suggestion that lactation protects against breast cancer development [(43)].

Radiation exposure

An increased rate of breast cancer has been observed in survivors of the atomic bomb explosions in Japan, with a peak latency period of 15-20 years.

It has been noted that patients with Hodgkin's lymphoma who are treated with mantle irradiation, particularly women who are younger than age 20 at the time of radiation therapy, have an increased incidence of breast cancer [(43)].

Alcohol consumption

Moderate alcohol intake (two or more drinks per day) appears to modestly increase breast cancer risk [(43)].

Obesity

Alterations in endogenous estrogen levels secondary to obesity may enhance breast cancer risk. Obesity appears to be a factor primarily in postmenopausal women [(44)].

Lactation

Although it has been suggested that lactation may protect against breast cancer, it is unclear whether lactation reduces breast cancer risk. studies failed to demonstrate any

breast cancer risk reduction in women who breast-fed and showed no dose-response effect in women who breast-fed for longer periods [44].

Alterations in diet

A reduced incidence of breast cancer has been observed in countries where the diet is typically low in fat. However, no reduction in breast cancer risk has been observed in the United States when women followed low-fat diets [44].

Epstein –Bar virus (EBV)) infection

Previous studies indicated that EBV may play a role in the development and behavior alteration of some aggressive BC. These studies the presence of EBV genome in considerable subset of invasive in Egyptian women .In light of new approaches in treating EBV associated malignancies ,this studies give hop that substantial presence of invasive BC could be treated with antiviral agents or with immunotherapy [45].

Pathology of breast cancer

Many BCs arise from a sequence that begins with an increase in the number of breast (hyperplasia) to the emergence of atypical breast cells (atypical hyperplasia) followed by carcinoma in situ (noninvasive cancer) and finally, invasive cancer. Not all BCs necessarily follow this progressive pattern; however, the speed of progression for those that do is highly variable. It also appears that some cancers may never progress beyond in situ disease [46].

A- Noninvasive:

1- **Ductal carcinoma in situ (DCIS)** is the most common type of noninvasive BC, accounting for about 15% of all new BC cases in the U.S. It refers to an uncontrolled growth of cells that are confined to the breast duct. Women with DCIS are also at higher risk of developing cancer in the opposite breast. With early detection and treatment, the five-year survival rate for DCIS is nearly 100%, providing that the cancer has not spread past the milk ducts.

2- **Lobular carcinoma in situ (LCIS)** is characterized by abnormal changes in the cells that line the milk-producing lobules, or lobes, of the breast. LCIS is much less common and carries slightly less risk of invasive cancer than DCIS.

B-Invasive:

1- **Invasive ductal carcinoma (IDC)** is the most common type of BC. About 80% of invasive BCs are classified as invasive ductal carcinoma. Tumors can cause skin and nipple retraction.

2- **Invasive lobular carcinoma (ILC)** begins in the milk-producing lobules where it extends into the adipose tissue of the breast. It is relatively uncommon, comprising about 10% of invasive BCs.

3- **Tubular carcinoma** is a highly differentiated invasive carcinoma whose cells are regular and arranged in well-defined tubules. Its incidence ranges from about 8% - 27%. Pure tubular carcinoma has limited metastatic potential and better than average prognosis.

4- **Medullary carcinoma** is a relatively uncommon type of invasive carcinoma, accounting for less than 5% - 7% of all invasive BCs. Histologically, the tumor is characterized by larger than average cancer cells, and with immune system cells present on the edges of the tumor.

5- **Mucinous carcinoma (colloid carcinoma)** is an invasive form of BC characterized by large amounts of extracellular mucin production. Less than 5% of invasive BCs show a mucinous component. It usually occurs in postmenopausal women.

6- **Metaplastic carcinoma** is uncommon, representing less than 5% of all BCs. Lesions contain several different types of cells that are not typically seen in other forms of BC.

7- **Invasive cribriform carcinoma** is a well-differentiated cancer comprised of small and uniform cells. It shares some features with tubular carcinoma and is also associated with better than average prognosis. It represents 5% - 6% of invasive BCs.

8- **Invasive papillary carcinoma** is very rare, comprising less than 1% - 2% of invasive BCs and predominantly in postmenopausal women.

9- **Invasive micropapillary carcinoma** is poorly recognized variant of BC, usually presenting as a firm, immobile mass. It is uncommon, with an incidence of less than 3%.

c- Other Types:

1- **Inflammatory BC** is a form of locally advanced BC associated with a rapid onset of clinical features including breast inflammation, warmth, thickening or dimpling and a palpable ridge at the margin of induration. Often mistaken as an infection, symptoms result from the blocking of lymphatic vessels near the surface of the skin by cancer cells. Inflammatory BC is relatively rare, representing about 1% - 5% of all BCs in the U.S.

2- **Paget's disease** of the nipple begins in the milk ducts as either an in situ or invasive cancer; prognosis is excellent when associated with carcinoma in situ. Early stage symptoms include erythema and mild scaling of the nipple skin. Symptoms of more advanced disease may include nipple tingling, itching, increased sensitivity, burning, pain. It accounts for approximately 1% of all BCs.

3- **Phylloides tumors (phyllodes)** can be either benign or malignant. Malignant tumors are very rare. Phylloides tumors are biphasic and composed of benign epithelial elements and cellular connective tissue stroma[47,48].

Staging

The stage of a BC describes its size and the extent to which it has spread. The staging system ranges from Stage 0 to Stage IV.

The seriousness of invasive BC is strongly influenced by the stage of the disease the extent or spread of the cancer when it is first diagnosed. There are two main staging systems for cancer. The American Joint Committee on Cancer's classification of tumors uses information on tumor size (T), lymph node involvement (N), and the presence or absence of distant metastases (M), and is commonly used in clinical settings. Once the T,

N, and M are determined, a stage of I, II, III, or IV is assigned, with stage I being an early stage and stage IV being the most advanced. A simpler system used for staging cancers is known as the SEER summary stage system and is used more commonly in reporting to cancer registries and for public health research and planning. According to this system:

- Local-stage tumors are cancers confined to the breast.
- Regional-stage tumors have spread to surrounding tissue or nearby lymph nodes.
- Distant-stage cancers have metastasized (spread) to distant organs [48].

Treatment:

I- Surgery

In theory, cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. The goal of the surgery can be either the removal of only the tumor, or the entire organ. Examples of surgical procedures for cancer include mastectomy for BC.

1. Partial Mastectomy (Lumpectomy).
2. Total or Simple Mastectomy.
3. Modified Radical Mastectomy.
4. Radical Mastectomy [48-50].

II- Systemic therapy

Systemic therapy includes chemotherapy, hormonal and targeted therapy. Systemic treatment given to patients before surgery is called neoadjuvant therapy. Neoadjuvant therapy has been found to be as effective as therapy given after surgery in terms of survival, disease progression, and distant recurrence [51].

1- Chemotherapy:

Chemotherapy is any treatment involving the use of drugs to kill cancer cells. Because chemotherapy affects healthy cells as well as cancerous ones, side effects are common as leading to hair loss, nausea, vomiting and fatigue in addition to premature menopause and infertility. Another recently described side effect is "chemobrain, memory and concentration problems that happen to some people during and after chemotherapy.

There are many classes of antineoplastics drugs:[48]

Alkylating agents.

a. Antimetabolites.
b. Anti-tumor antibiotics.
c. Mitotic inhibitors.
d. Topoisomerase inhibitors.

a- Alkylating agents:

Alkylating agents directly damage DNA to prevent the cancer cell from reproducing. These agents are not phase-specific and used to treat many different cancers, including cancers of the lung, breast, and ovary.

There are many different alkylating agents including:

- Nitrogen mustards: such as mechlorethamine (nitrogen mustard), chlorambucil, cyclophosphamide (Cytoxan), ifosfamide, and melphalan
- Nitrosoureas: which include streptozocin, carmustine (BCNU) and lomustine
- Alkyl sulfonates: busulfan
- Triazines: dacarbazine (DTIC), and temozolomide (Temodar)
- ethylenimines: thiotepa and altretamine (hexamethylmelamine)

b- Antimetabolites:

Antimetabolites are a class of drugs that interfere with DNA and RNA growth by substituting for the normal building blocks of RNA and DNA. These agents damage cells during the S phase. They are commonly used to treat leukemias, tumors of the breast, ovary, and the intestinal tract, as well as other cancers. Examples of antimetabolites include 5-fluorouracil (5-FU), capecitabine (Xeloda), 6-Mercaptopurine (6-MP), Methotrexate, Gemcitabine (Gemzar), cytarabine (Ara-C), fludarabine, and pemetrexed (Alimta).The pyrimidine analogue 5-fluorouracil (FUra), is a drug with multiple mechanisms of cytotoxicity. These include: inhibition of DNA synthesis as a result of thymidylate synthase inhibition by FdUMP (I), increased DNA strand breaks as the result of FdUTP incorporation into DNA, and alterations in the structure and/or function of RNA as a result of FUTP incorporation into RNA. General inhibition of pre-mRNA processing, with a subsequent decrease in the level of mRNA within the cell, could contribute to FUra's RNA-mediated cytotoxicity by altering the level of proteins required for growth and viability. Also 5-FU affects other cellular responses such as cell cycle regulation and induction of apoptosis [52,53].

Methotrexate (amethopterin) competitively and irreversibly inhibits dihydrofolate reductase (DHFR) causing deficiency of folic acid which is needed for the de novo synthesis of the nucleoside thymidine and purine base. Therefore, it inhibits the synthesis of DNA, RNA and proteins. Moreover, a new mechanism of methotrexate is evaluated whereas it inhibits endothelial cells (ECs) proliferation in vitro and blocks ECs growth factor-induced neovascularization[54] .

Gemcitabine is a nucleoside analog in which the hydrogen atoms on the 2' carbons of deoxycytidine are replaced by fluorine atoms. The triphosphate analogue of gemcitabine replaces one of the building blocks of cytidine, during DNA replication resulting in apoptosis and arrests tumor growth. Another target of gemcitabine is the enzyme ribonucleotide reductase (RNR). The diphosphate analogue binds to RNR active site and inactivates the enzyme irreversibly. Thus, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair, and cell apoptosis is induced [55]. Gemcitabine plus paclitaxel is superior for overall survival as well as prevention of disease progression

compared with paclitaxel alone for the treatment of women with metastatic BC. The combination of gemcitabine plus doxorubicin can be safely administered every 21 days with promising response as first-line therapy for metastatic breast cancer (MBC). The response rate, time to disease progression and overall survival rates of this regimen are comparable to other standard therapies for MBC, as well as other gemcitabine including taxanes and cisplatin [56].

c- Anti-tumor antibiotics:

Mitotic inhibitors are often plant alkaloids and other compounds derived from natural products. Anthracyclines, a class of drugs derived from Streptomyces bacteria, used as components of adjuvant polychemotherapy for BC as they interfere with enzymes involved in DNA replication. Anthracyclines such as doxorubicin (Adriamycin), epirubicin, and idarubicin, can stop mitosis or inhibit enzymes from making proteins needed for cell reproduction. These drugs work during the M phase of the cell cycle, but can damage cells in all phases. They are used to treat many different types of cancer including breast, lung, myelomas, lymphomas, and leukemias. Disadvantages of these drugs are known for their potential to cause peripheral nerve damage, which can be a dose-limiting side effect [57].

d- Mitotic inhibitors:

These are often plant alkaloids and other compounds derived from natural products. They inhibit mitosis via inhibition of enzymes from making proteins needed for cell replication. They work primarily in the M phase, but can damage cells in all phases. They are used to treat many different cancers including breast cancer, lung cancer, myelomas, lymphomas, and leukemias. They often cause peripheral nerve damage [58].

Examples of mitotic inhibitors include:

The taxanes: Paclitaxel (Taxol) and Docetaxel (Taxotere)

Epothilones: Ixabepilone (Ixempra)

The vinca alkaloids: Vinblastine (Velban), Vincristine (Oncovin) and Vinorelbine (Navelbine) [59].

2-Targeted therapies:

They are more specifically than traditional chemotherapy drugs for cancer cells. Most attack cells with mutant versions of certain genes, or cells that express too many copies of a particular gene. Examples include Imatinib, Gefitinib, Erlotinib, Sunitinib and Bortezomib. The most popular of them are as follows:

- Drugs act on receptors, their ligands, enzymes, etc., mediating signal transmission to tumor cells (antibodies, small molecules, etc.);
- Drugs inhibit tumor microenvironment critical for tumor survival;
- Antibodies eliciting immune responses and/or delivering toxic substances (radioactive materials, cytostatic drugs, etc.) to tumor cells [60].

a- Immunotherapy:

b- Tyrosine kinase inhibitors:

c-Anti-angiogenic therapy:

d- Histone deacetylase inhibitors (HDI):

e- Gene therapy:

3-Hormonal therapy:

They are hormone-like drugs that alter the action or production of female or male hormones. They are used to slow the growth of breast, prostate, and endometrial (uterine) cancers, which normally grow in response to natural hormones in the body. They do not work in the same ways as standard chemotherapy drugs, but rather by preventing the cancer cell from using the hormone that it needs to grow, or by preventing the body from making the hormones. Examples include[48]:

- Anti-estrogens; fulvestrant (Faslodex), tamoxifen (Nolvadex) and toremifene (Fareston)
- Aromatase inhibitors; anastrozole (Arimidex), exemestane (Aromasin), and letrozole (Femara)
- Progestins; megestrol acetate (Megace)
- Anti-androgens; bicalutamide (Casodex), flutamide (Eulexin), and nilutamde (Nilandron)
- LHRH agonists; leuprolide (Lupron) and goserelin (Zoladex).

Hormonal therapy is often used to treat women whose cancers are sensitive to hormones estrogen ER +ve and progesterone receptor positive PR +ve cancers. The main classes of medications are used in hormone therapy are selective estrogen receptor modulators (SERMs) and Aromatase Inhibitors (AIs)[61].

a- Selective Estrogen Receptor Modulators (SERMs):

b- Aromatase Inhibitors (AIs):

III- Radiotherapy

Radiotherapy of BC patients is usually recommended after a lumpectomy and mastectomy to the region of the tumor bed in order to destroy microscopic tumors that may have escaped surgery. Radiation can reduce the risk of recurrence by 50-66% (1/2 - 2/3rds reduction of risk) when delivered in the correct dose. Radiation may be used to destroy cancer cells remaining in the breast, chest wall, or underarm area after surgery or to reduce the size of a tumor before surgery. There are two types of radiation therapy.

External radiation is the usual type of radiation for women with BC.

Internal radiation therapy (brachytherapy) uses a radioactive substance sealed in needles, seeds, wires, or catheters that are placed directly into or near the cancer[62].

Oxidative stress

Oxidative stress is defined as a disturbance in the equilibrium between free radicals (FR), reactive oxygen species (ROS), and endogenous antioxidant defense mechanisms[63], or more simply, it is a disturbance in the balance between oxidant-antioxidant states,

favoring the oxidant environment [64]. Both of the oxidant and antioxidant species are very important for normal metabolism, signal transduction and regulation of cellular functions. Therefore, each cell in the human body maintains a condition of homeostasis between the oxidant and antioxidant species [65].

Oxidative stress can result in injury to all the important cellular components like proteins, DNA and membrane lipids, which can cause cell death. Oxidative stress has been demonstrated to be involved in various physiological and pathological processes, including DNA damage, proliferation, cell adhesion, and survival. Moreover, there are several experimental and clinical data providing compelling evidence for the involvement of oxidative stress in large number of pathological states including carcinogenesis [66]. The broad definition of the ROS is oxygen-containing, reactive chemical species. Up to 1–3 % of the pulmonary intake of oxygen by humans is converted into ROS [68] .There are two ROS subgroups; free radicals such as super oxide radicals ($O^{\cdot}_{2}$) and non-radical ROS such as hydrogen peroxide (H_2O_2). Both radicals and non-radical ROS are common in the presence of an oxygen atom , which differentiates them from the reactive nitrogen specie (RNS)(Figure1)[67].

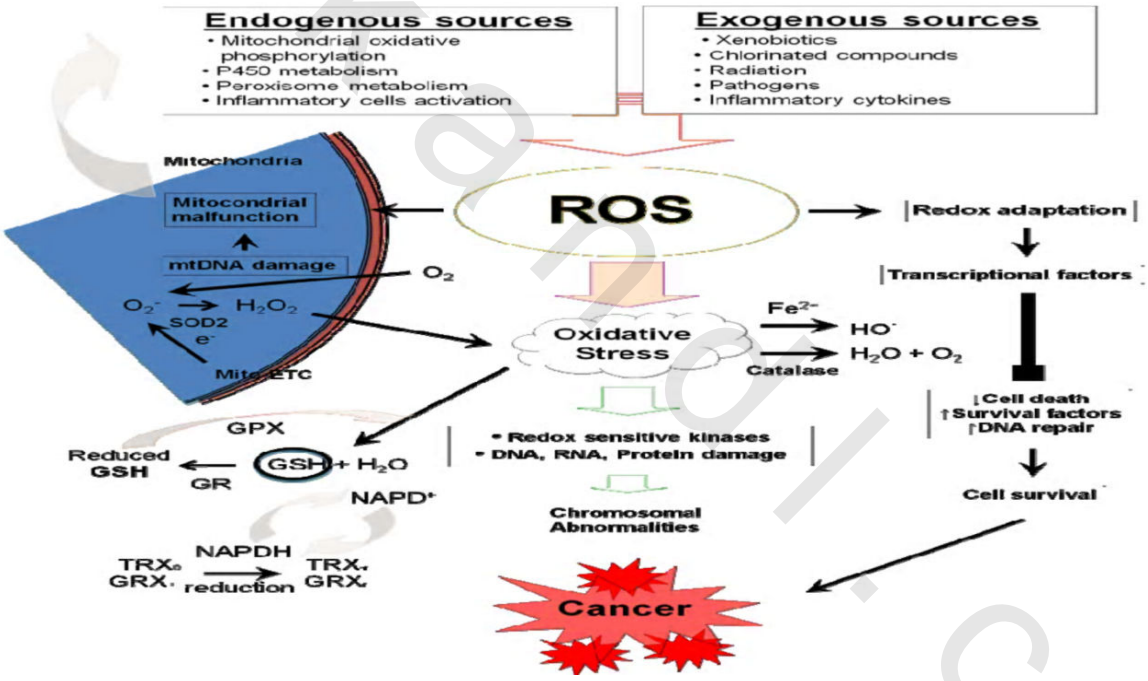


Figure (1): The signaling pathways induced by oxidative stress [67].

Sources of free radicals

Free radicals (oxidants) come from two major sources: (a) endogenous and (b) exogenous.

Endogenous free radicals

Are produced in the body by four different mechanisms. First, from the normal metabolism of oxygen-requiring nutrients, mitochondria the intracellular powerhouses which produce the universal energy molecule, adenosine triphosphate (ATP) normally

consume oxygen in this process and convert it to water. However, unwanted by-products such as the superoxide anion, hydrogen peroxide and the hydroxyl radical are inevitably produced, due to incomplete reduction of the oxygen molecule. It has been estimated that each cell produces more than 20 billion molecules of oxidants per day during normal metabolism [68].

Second, white blood cells destroy parasites, bacteria and viruses by using oxidants such as nitric oxide, superoxide and hydrogen peroxide. Consequently, chronic infections result in prolonged phagocytic activity and increased exposure of body tissues to the oxidants [68].

Third, other cellular components called peroxisomes produce contrast to the mitochondria, which oxidize fatty acids to produce ATP and water, hydrogen peroxide as a byproduct of the degradation of fatty acids and other molecules. In peroxisomes oxidize fatty acids to produce heat and hydrogen peroxide. The peroxide is then degraded by an enzymatic antioxidant called catalase. Under certain conditions [69].

Finally, an enzyme in the cells called cytochrome P450 is one of the body's primary defenses against toxic chemicals ingested with food. However, the induction of these enzymes to prevent damage by toxic foreign chemicals like drugs and pesticides also results in the production of oxidant by-products [69].

Exogenous sources of free radicals include

Air pollution, of which industrial waste and cigarette smoke are major contributors. Cigarette smoke literally bristles with oxidants. Radiation and trace metals, notably lead, mercury, iron and copper, are also major sources of free radical generation. Normal diets containing plant foods with large quantities of certain compounds such as phenols .

The combination of oxidative damage by exogenously and endogenously produced free radicals has continues consequences for body tissues. The oxidants induce alterations in the structures of tissues and in their functions, which manifest as aging and chronic degenerative diseases like arthritis, atherosclerosis, and cancer [69].

Implication of oxidative stress in breast cancer

I-Role of ROS in tumor growth:

There is strong relationship between ROS-induced BC and oxidative stress either directly during the inflammation process or as products of ROS metabolism [70-74]. The start of carcinogensis mediated by ROS following chronic inflammation may be direct oxidation, nitration, halogenation of nuclear DNA, RNA and lipids and can also increase the expression of transcription factors including c-fos and c-jun oncogenes involved in neoplastic transformation. Of the major oxidatively modified DNA base products, 8-Hydroxy-2'-deoxyguanosine, is almost 10 times more prevalent in invasive ductal breast carcinoma cells than in normal control samples from the same patient [75]. The role of ROS in BC may not be limited to early mutagenic events, carcinoma cells are frequently under persistent oxidative stress [76]. Consequently, markers of constitutive oxidative stress have been detected in samples from in vivo breast cancer [77].

In human studies, elevated levels of cholesterol peroxides, were observed in the breast fluid of BC patients [78]. Another consequence of elevation of ROS in cancer patients is induction of changes in antioxidant status via decreasing catalase levels, increased Glutathione Peroxidase (GPx), Superoxide Dismutase SOD (I) and (II) and Glucose-6-phosphate dehydrogenase activities in addition to a marked decrease in ability for MCF-7 cells metabolites peroxide inducing transformation in normal breast epithelial cells. In this aspect, in chemical carcinogen-induced mammary tumor animal models, high-fat diets are associated with increased tumor incidence, and this effect is diminished by antioxidants [79,80].

On the other hand, Epidemiological studies suggest that a diet that is rich in antioxidants may help to prevent the development of breast carcinoma. Antioxidants depletion in plasma may be due to increased scavenging of lipid peroxides by antioxidants as well as their sequestration by tumor cells as a trial to meet their demands for growing. Antioxidant vitamins have a number of biological activities such as immune stimulation, and an alteration of metabolic activations of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the ROS [81,82].

***Causes of carcinoma cell oxidative stress:**

i- Alterations to metabolic pathways in tumor cells.

ii- An inadequate tumor vascular network.

iii- Macrophage infiltration of the tumor.

iv- Therapeutic interventions.

***Consequences of carcinoma cell oxidative stress:**

i- Increased mutation rate and accelerated tumor progression.

ii- Activation of growth-promoting signalling pathways.

iii- Adaptation to oxidative stress, resulting in increased resistance to therapy.

iv- Increased blood supply to tumor cells.

v- Increased risk of metastasis[83].

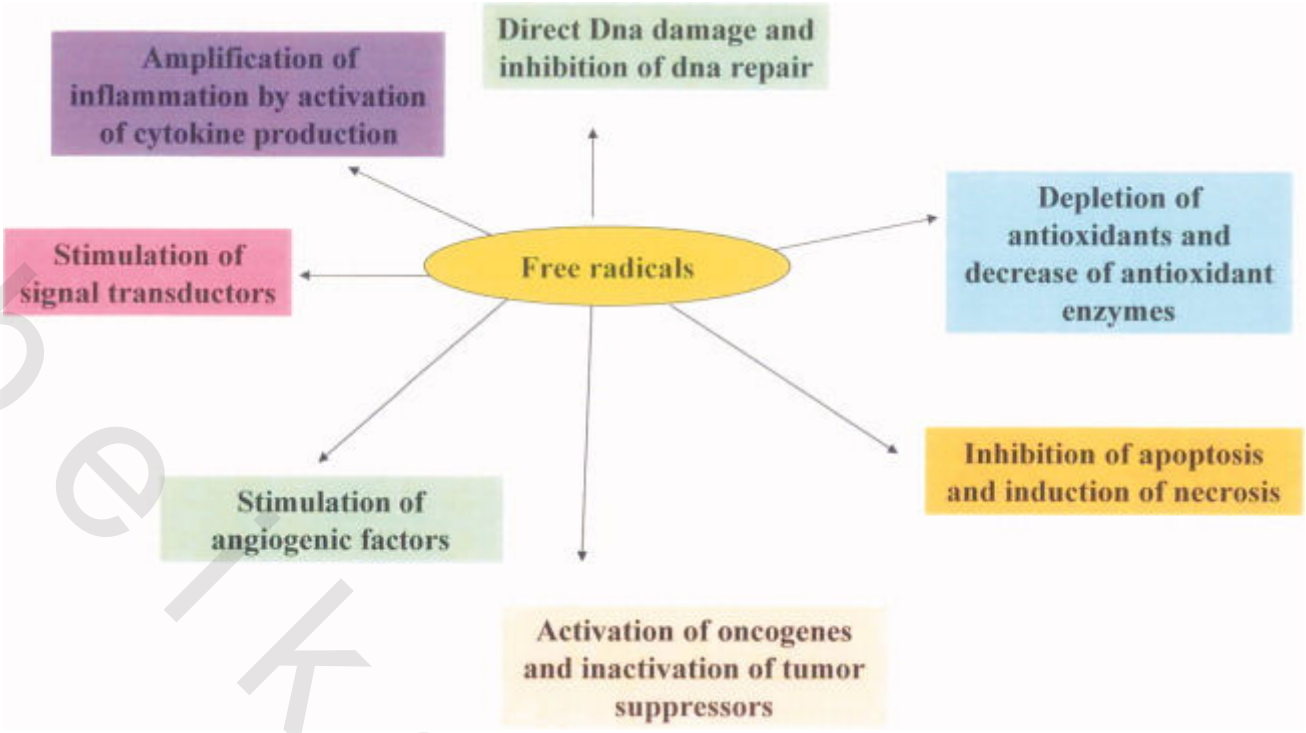
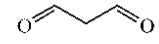
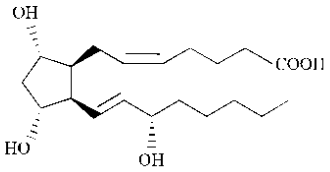
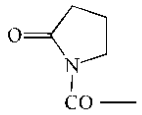
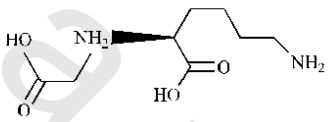
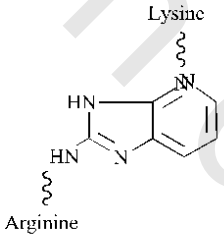
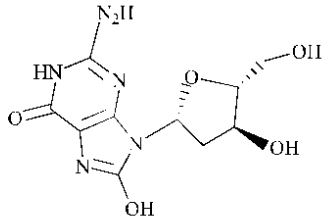


Figure (2): The role of free radical on carcinogenesis [83].

Oxidative Damage in DNA, Lipid, and Protein

It is well-documented that significant oxidative stress carries out severe damage to lipids, proteins, sugars and nucleic acid bases (Table 1), which compromises cell viability and functions [84-87].

Table (1): Validated Biomarkers of Oxidative Stress in Serum, Plasma and/or Urine[87]

Markers of Oxidative Stress		Chemical Structure	Detection Methods
Lipids	Malonaldehyde	 Malonaldehyde (1)	• TBARS spectrophotometric assay • HPLC-based TBARS assay • GC-MS
	F2-isoprostanes (8-iso-PGF2α)	 8-iso-PGF2α (2)	• Immunoassays • GC-MS, LC-MS
Proteins (carbonyl groups)	2-pyrrolidone	 2-pyrrolidone (3)	• DNPH spectrophotometric assay • One- and two-dimensional electrophoresis • MS • Immunoassays
Sugars	Carboxymethyl-lysine	 Carboxymethyl-lysine (4)	• HPLC • GC-MS • Immunoassays
	Pentosidine	 Pentosidine (5)	
DNA	8-hydroxy-2'-deoxyguanosine	 8-hydroxy-2'-deoxyguanosine (6)	• HPLC-ECD • LC-MS, GC-MS • Immunoassays

1. DNA

The formation of oxidative stress may result in damage to critical cellular macromolecules including DNA, lipids, and proteins. Oxidative DNA damage may participate in ROS-induced carcinogenesis[88]. A common form of damage is the formation of hydroxylated bases of DNA, which are considered an important event in chemical carcinogenesis [88,89]. This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Several different pathways by which oxidative DNA damage leads to mutations have been proposed, including chemical

modification of nucleotide moieties in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase-specific hot spots, conformational change in the DNA templates, and the induction of a DNA polymerase conformation that is error prone [90]. Formation of 8-hydroxy-2'- deoxyguanosine (8-OHdG) [an oxidative modification of DNA produced by hydroxylation in the C-8 position of deoxyguanosine residues by the hydroxyl radical] [91] has been used as a measurement of oxidative**.**

2. Lipid

Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides [92]. Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA). The formation of lipid damage (lipid peroxidation) may result in several possible sequelae including protein oxidation [92].These lipid radicals can diffuse through membranes,thus modifying the structure and function of the membrane and resulting in a loss of cell homeostasis. In addition, lipid peroxides may result in the interaction with cellular DNA and cause the formation of DNA-MDA adducts [93].

Lipid peroxidation and MDA

MDA is a free-radical mediated chain of reactions that, once initiated, results in an oxidative deterioration of polyunsaturated lipids. The most common targets are components of biological membranes. When propagated in biological membranes, these reactions can be initiated or enhanced by a number of toxic products, including endoperoxides and aldehydes[94].

MDA:

It is a three-carbon, low-molecular weight aldehyde that can be produced by different mechanisms (Dahle and his assistants in (1962) [95]), Postulated a mechanism of MDA formation based on the fact that only peroxides that possessed α or β unsaturations to the peroxide group could be capable of undergoing cyclization to finally form MDA.

A MDA formation route is described in Figure 3. The target of reactive species is the carbon-carbon double bond of polyunsaturated fatty acids (I). This double bond weakens the carbon-hydrogen bond, allowing easy abstraction of the hydrogen by a free radical. Then, a free radical can abstract the hydrogen atom and a lipid free radical is formed (II), which suffers oxidation generating a peroxyl radical (III). The peroxyl radical can react with other polyunsaturated fatty acids, abstracting an electron and producing a lipid hydroperoxide (IV) and another lipid free radical. This process can be propagated continually in a chain reaction [96,97]. The lipid hydroperoxide is unstable and its fragmentation yields products such as malondialdehyde (V) and 4-hydroxy-2-nonenal.

In physiological conditions, oxy-radicals are part of the normal regulatory course of the organism and the cellular redox state is closely controlled by antioxidants. When the levels of free radicals increase and both the enzymatic systems and low molecular antioxidants are not sufficient to protect the organism, these radicals attack membranes and cells.

Various reactive electrophilic compounds from lipid peroxidation, in particular MDA, have been showed to be mutagenic and gen5otoxic [98], which can lead to cancer

formation. MDA can react with nucleic acid bases at physiological pH and forms adducts with deoxyuanosine (dG), deoxyguanosine (dA) and deoxycytidine (dC) [99].

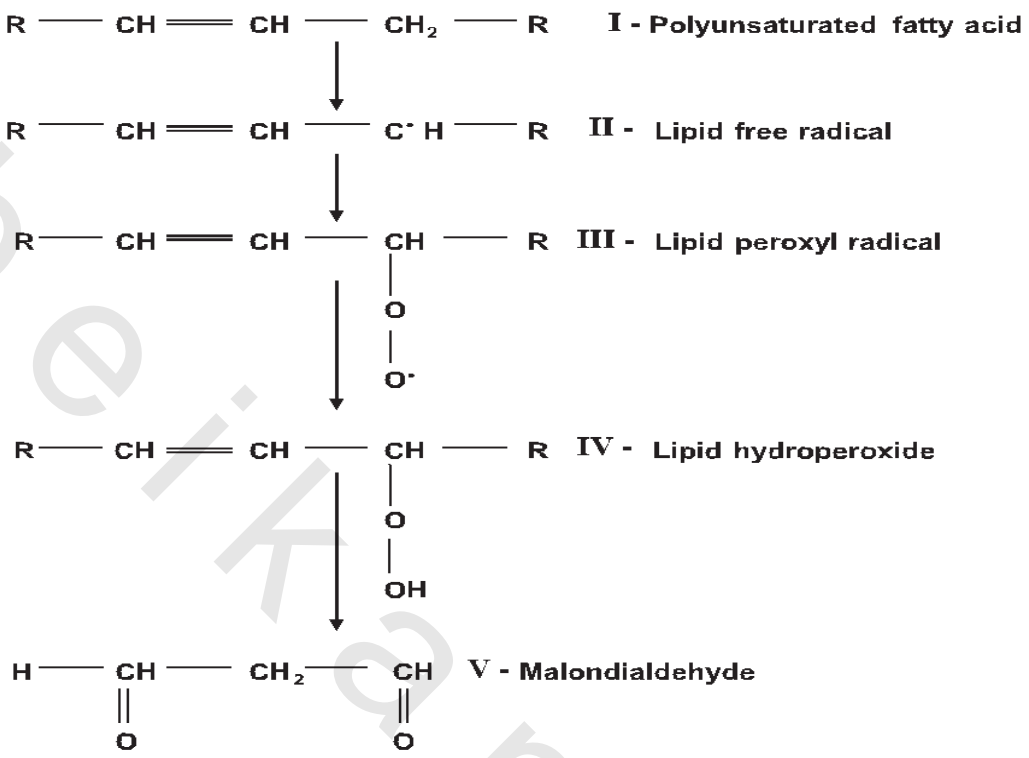


Figure (3): Schematic steps of MDA formation from polyunsaturated fatty acids[99]

3. Protein

Proteins are also easily attacked by ROS directly or indirectly through lipid peroxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modification of enzyme activity (stimulation or inhibition) [100]. In addition to enzymes, damage to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intercellular calcium and potassium that will trigger a series of changes in cells [100]. Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases alteration of protein structure may allow the target protein to be further attacked by proteinases [101]. Thus protein oxidative damage can result in the modifications in structure, enzyme activity, and signaling pathways.

Protein carbonyl biomarker

Proteins are major targets of reactive oxidants in cells and oxidized proteins that accumulate during aging, accompanied with oxidative stress and some pathological conditions [102,103]. Protein carbonyl content is actually the most commonly used marker of protein oxidation ROS leading to protein oxidation include radical species such as superoxide ($O2^{-\cdot}$), hydroxyl (OH^{-}) peroxyl ($RO2^{-\cdot}$), alkoxyl (RO^{-}), hydroperoxyl (HO_2^{-}), and non radical species such as hydrogen peroxide (H2O2), hypochlorous acid (HOCl), ozone (O3), singlet oxygen (1O2), and peroxynitrite ($ONOO^{-}$) [103,104].

Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains (especially of Pro,Arg, Lys, and Thr) when they are oxidised (Fig. 1).These moieties are chemically stable, which is useful for both their detection and storage. Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins by either the a-amidation pathway or by oxidation of glutamyl side chains, leading to formation of a peptide in which the N-terminal amino acid is blocked by an a-ketoacyl derivative [105].In addition, CO groups may be introduced into proteins by secondary reaction of the nucleophilic side chains of Cys, His, and Lys residues, with aldehydes (4-hydroxy-2-nonenal, malondialdehyde, 2-propenal [acrolein]) produced during lipid peroxidation or with reactive carbonyl derivatives (ketoamines,ketoaldehydes, deoxyosones) generated as aconsequence of the reaction of reducing sugars, or their oxidation products with lysine residues of proteins(glycation and glycoxidation reactions), with the eventual formation of the advanced glycation/lipoxidation end products (AGEs/ALEs), that is, glycoxidation products, such as carboxymethyllysine and pentosidine, and lipoxidation products, such as malondialdehyde-lysine and 4-hydroxy-nonenal-protein adduct (for exhaustive reviews showing detailed chemical structures[106-107].

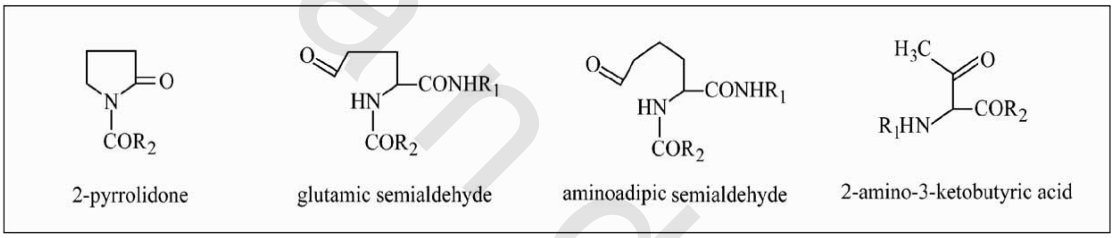


Fig. 4, The structure of carbonyl derivatives produced by direct oxidation of amino acid side chains: 2-pyrrolidone from prolyl residue, glutamic semialdehyde from arginyl and prolyl residue, a-aminoadipic semialdehyde from lysyl residue, *and 2-amino-3-ketobutyric acid from threonyl* residue.

Protein carbonyl circulates in detectable levels in plasma and is a well established marker of oxidative stress that has been applied in epidemiological and clinical studies. Increased concentrations of protein carbonyl have been associated with increased risk of cancer, including pediatric malignancies [108] , bladder cancer,[109] Hodgkin's lymphoma[113], and breast cancer [110], one study of lung cancer and protein carbonyl found no association[111]. Protein oxidation may cause structural changes and loss of function in proteins related to cancer initiation, promotion, and progression. It is known that protein oxidation can lead to cellular malfunction and/or death by oxidizing amino acid residue side chains, cleaving peptide bonds, forming covalent protein-protein cross-linked derivatives, and oxidizing the backbone of protein molecules which causes protein fragmentation [112].

Relation between oxidative stress and carcinogenesis:

Active oxygen may be involved in carcinogenesis through two possible mechanisms:

1- The induction of gene mutations that result from cell injury.

2- The effects on signal transduction and transcription factors. Which mechanism it follows depends on factors such as the type of active oxygen species involved and the intensity of stress? [113].

Cellular targets affected by oxidative stress include DNA, phospholipids, proteins, and carbohydrates on the cell membrane. Oxidized and injured DNA has the potential to induce genetic mutation. That some telomere gene (A **telomere** is a region of repetitive nucleotide sequences at each end of a chromosome genes) are highly susceptible to mutation in the presence of free radicals is now apparent and it is known that tumor suppressor genes such as p53and cell cycle-related genes may suffer DNA damage. In addition, oxidized lipids react with metals to produce active substances (e.g., epoxides and aldehydes) or synthesize malondi-aldehyde, which has the potential to induce mutation [114].

Active oxygen species act directly or indirectly via DNA damage on gene expression (DNA binding of transcription factors) and signaling at the cellular level. Some antioxidants play a role in such signal transduction. Two examples are glutathione and thioredoxin, working in the mechanisms of redox regulation [115].

Antioxidants as defense mechanism

Antioxidative networks in the biological body

The antioxidative network (Fig.5) acts as a defense mechanism against stress [115,116]. The human body has antioxidative enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH), and catalase, which scavenge free radicals. These enzymes make up a preventive type of antioxidative network.

General types of antioxidative substances are able to respond directly to and eliminate free radicals and are therefore called radical-scavenging antioxidants. These are divided into water-soluble substances, such as vitamin C, and fat-soluble substances, such as vitamins A and E as well as coenzyme 10. The water- and fat-soluble antioxidants mutually react with each other and individually form sophisticated networks that protect the body against oxidative damage.

Systems that repair and regenerate lipids, proteins, and DNAs by free radicals (repair/regeneration type of antioxidative activities) also exist. In these systems, phospholipase, protease, transferase, and DNA repair enzymes are the primary workhorses.

Antioxidative stress actions:

Antioxidative stress actions include

(1) Avoiding the sources of free radicals

(2) Increasing one's own antioxidative capacity[116].

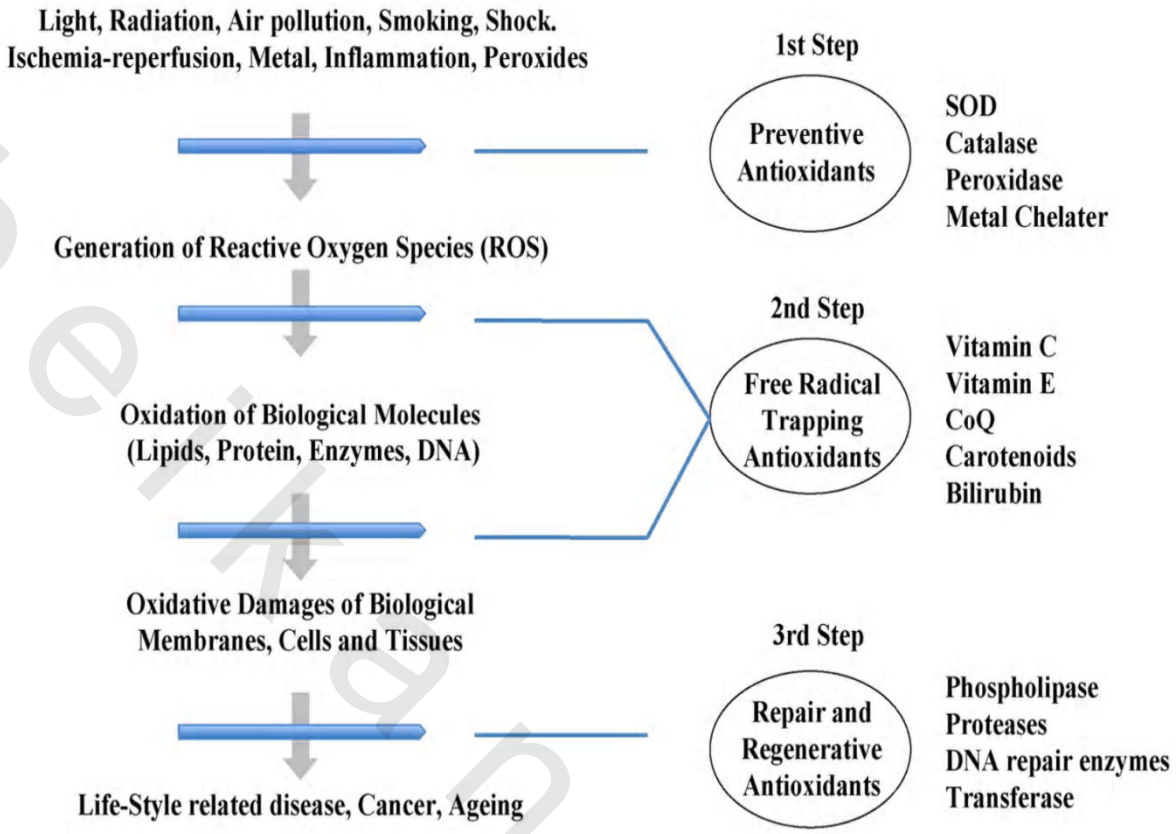


Figure (5): Antioxidative network in biological system[116]

Vitamins supplementation and breast cancer treatment

There is a considerable number of in vitro and animal data showing that vitamins and other antioxidants can protect cells against radiation and chemotherapy. It is also sometimes claimed that antioxidants are directly cytotoxic and/or actually increase the effectiveness of cytotoxic treatments. For example in vitro studies have reported that vitamin A, C, and E as well as caroteiniods, can enhance the effectiveness of chemotherapy [117].Of great interest is the potential of drug-drug or drug –antioxidant combinations to act in additive or synergistic fashion. Synergistic drug drug and drug antioxidant interactions should allow clinicians to achieve therapeutic effects comparable to that achieved with single agents , but at sustainably lower doses [117] .A huge literature is available on the modulation of apoptosis by putative cancer chemopreventive agents .either datary principles , vitamins ,or pharmacological agents [118].

Vitamin A

Vitamin A refers to a group of compounds called retinoids which play an important role in vision, bone growth, reproduction, cell division and cell differentiation [119]. Retinoids are active as retinal, retinoic acid and retinol. Retinal is actively involved in supporting vision, retinoic acid regulates cell differentiation, growth and embryonic development and retinol is responsible for transport and storage [120].

Dietary source of vitamin A

Two forms of vitamin A are found in plants and animals, provitamin A and preformed vitamin A, respectively. Vitamin A found in fruits and green leafy vegetables is called provitamin A carotenoid which is converted into retinol once ingested. The most common provitamin A carotenoids found in foods include betacarotene,alpha-carotene and beta-cryptoxanthin. Among these, beta-carotene is the most efficiently converted into retinol. Vitamin A found in foods derived from animal sources is called preformed vitamin A and is absorbed as retinol. Liver, whole milk, and select fortified foods are good sources. Interestingly, non-fat milk lacks adequate vitamin A and therefore non-fat milk is fortified with vitamin A. Breakfast cereals are also fortified with vitamin A. Preformed vitamin A is the form supplied by most supplements [121].

Metabolic aspects of beta-carotene

The enzymatic cleavage of β- carotene has been established to be a central cleavage and to produce retinal through action of the enzyme β- carotene- 15, 15'-dioxygenase in the small intestine [122-124]. β- Carotene is not efficiently metabolized to vitamin A (retinol) and it is mostly absorbed intact along with the metabolized parts. In the intestinal mucosa, retinal is reduced to vitamin A (retinol) by retinaldehyde reductase and free retinol is taken up by the enterocytes, perhaps involving both diffusion and protein-mediated facilitated transport. In cells, retinol is complexed with cellular reinol-binding protein type-II and this complex is believed to help re-esterification process of retinol by the enzyme retinol acyltransferase and free retinol is also esterified by acyl-CoA and acyltransferase and these esters are then incorporated into chylomicrons and finally secreted into the lymph 77, 78 and transported to the target tissues (Figure 6). It may be suggested that the different metabolic patterns of vitamin A and β-carotene play significant roles in cancer modulation by these compounds at variable degrees [125].

The various forms of vitamin A are solubilized into micelles in the intestinal lumen and absorbed by duodenal mucosal cells . Both retinyl esters and provitamin A carotenoids are converted to retinol, which is oxidized to retinal and then to retinoic acid . Most of the body's vitamin A is stored in the liver in the form of retinyl esters[125].

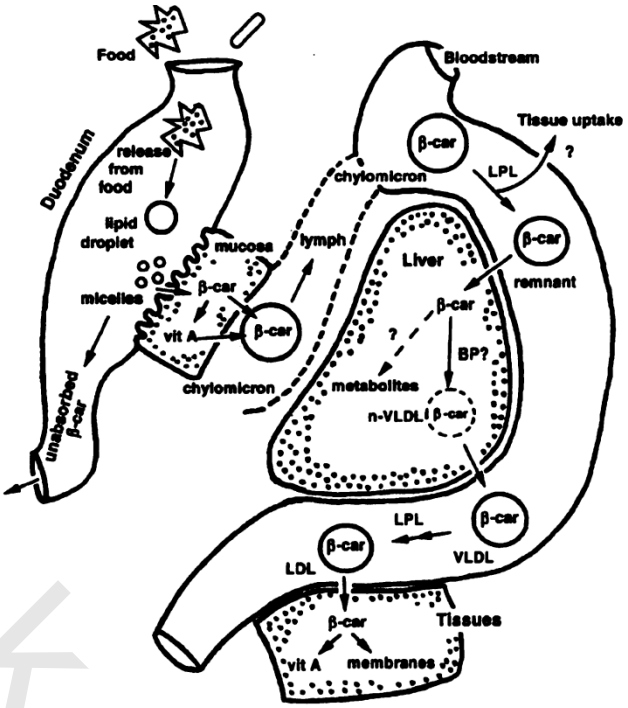


Figure (6): Pathways and processes involved in the absorption, plasma transport, and tissue uptake of carotenoids such as β-carotene. Not illustrated is the potential transfer of carotenoids from chylomicrons to high-density lipoproteins during lipolysis or the potential recycling of LDL- or VLDL-β-carotene into liver. β-car, β -carotene; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; n-VLDL, nascent VLDL; LPL, lipoprotein lipase; vit A, vitamin A (retinyl ester); BP, binding protein[125].

Vitamin A and breast cancer

Vitamin A and its derivatives have been long implicated in development and tumor suppression [126]. Retinoid have been implicated in the induction of cell death in many tumor delivered cultured cell systems through retinoid receptor dependant or retinoid receptor independent mechanism [127]. Retinoid have been shown to be important in carcinogenesis for many tissues, and alteration in vitamin A and retinoid homoeostasis are found in many tumors, including leukemia, breast, oral prostate and carcinoma of cervix [128,129].

Many longitudinal cohort studies have assessed carotenoid intake and endogenous retinol levels and the risk for developing breast cancer among postmenopausal women from multiple ethnic groups and geographic locations worldwide [120,130-134].Among postmenopausal women, select studies have concluded that intake of carotenoids and retinol levels are not associated with breast cancer risk [135] .When individual carotenoids were evaluated, however, lycopene was associated with a reduction in breast cancer risk among postmenopausal women [136].

Retinoic acid (RAs) ,the natural and synthetic derivatives of vitamin A , are able to induce the proliferation arrest ,differentiation ,and apoptosis of cancer[138] .Studies in vitro and in vivo have shown that RAs might b e effective in prevention and treatment of breast cancer [137,138] .

Vitamin E

Vitamin E is a lipid soluble group of compounds with similar biological activity to RRR- α tocopherol. This includes the stereoisomer α- tocopherol, β -tocopherol, γ -tocopherol , δ-tocopherol,along with the four tocotrienoids α , β ,γ , δ tocopherol structure shown in figure (7) [139] .

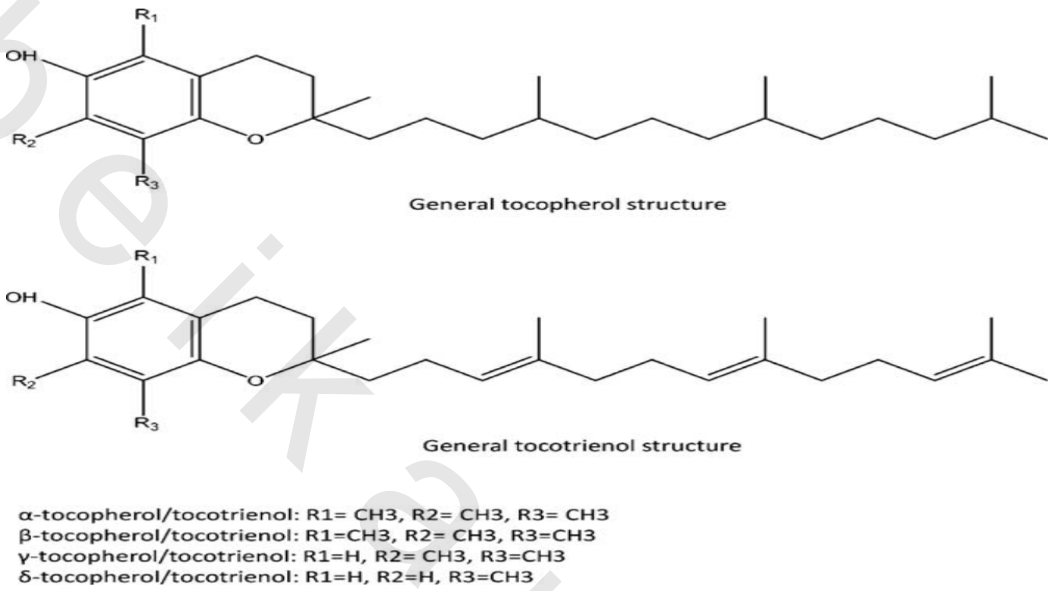


Figure (7): Generat tochopherol structure

Dietary source of vitamin E

Vitamin E is a fat-soluble vitamin that can be obtained through dietary intake of nuts, seeds, vegetable oils, green leafy vegetables and fortified cereals or as a supplement .While Vitamin E exists in several chemical forms, it is the alpha-tocopherol form that is preferentially found in human plasma and the most widely studied in clinical trials .Some of the pharmacologic properties of Vitamin E include inhibition of oxidation, inflammation and protein kinase C. Vitamin E also increases the release of prostacyclin which promotes blood vessel dilation and reduces platelet aggregation [120].

The tocopherols are exclusively synthesis in photosynthetic organism including higher Plant ; significant amount are found in all green tissues but predominantly occur in seeds. Plant derived oils represent the major sources of vitamin E in humans diet , and because these oils contain the four tocopherols in different relative amount , the overall intake of each vitamin E analogue depends in large on dietary oil performance in different countries [140].The tocopherols are the major vitamin E component of palm oil , significant amount are also found in barely , oat and rice bran[141] .

Cellular effect of vitamin E

In human, some of symptoms of vitamin E deficiency, such as increases liposuctions most likely originate from the lack of scavenging ROS in the lipid phase [142].where as other symptoms such as dying back sensory axonopathy may also be explainable by other

ways, e.g. by modulating gene expression in the brain [143-145], and by acting survival factors for specific neurological cells [146].

Vitamin E could serve a ducal function ;primary ,as an essential cofactor acting as a protein bound molecule in redox-dependant manner, and secondary ,when all the other redox active molecule are exhausted or when excess vitamin E is supplemented ,by preventing the oxidation of lipid and progression of their damaging effect ,depending on continuous recycling by L-ascorbic acid . Thus, the continuous removal of oxidized lipids by the vitamin E/vitamin C system may prevent their accumulation in the membrane and consequent rapid depletion of the essential factor ((vitamin E)) [147].

Currently, the molecular and cellular effect of vitamin E has been explained either by acting as a more antioxidant preventing damage or proteins and regulating their activity by specially scavenging ROS and NOS [148]. Or by interacting and regulating specific enzymes and transcription factors and influencing cellular structure such as membranes and lipid domains [148,149].

Metabolism of vitamin E

Vitamin E is a fat soluble and it is absorbed in the same manner as fat. Specifically unique tiny spheres with a water – loving (hydrophilic) outer layer called micelles engulf the vitamin E and help ferry it across the gut .Chylomicrons, produced by the small intestine ,carry the micelles into the lymph ,the milky fluid containing white blood cells ,proteins , and fats . In the lymph the enzymes lipoprotein lipases break down the majorty of the chylomicrons to produce chylomicrons remnant, which go into the blood. The majority of the chylomicron remnant reach the liver , which strips away the vitamin E from the remnant and puts it into the freshly produces very low density lipoprotein (VLDL) .VLDL is broken down by lipoprotein lipase to produce the low density lipoprotein (LDL , bad cholesterol). In our blood LDL is the largest carrier of vitamin VLDL E . LDL is freely exchanges vitamin E with high density lipoproteins HDL and LDL seems to deliver Vitamin E in our tissues [150].

Our blood and tissues contains much more α-tochopherol than any of the other tocopherols and tochotrinols .At the hreart of this mechanism is a special protein , called the alpha - tochopherol transfer protein .it recognize the alpha tochopherol and preferentially puts more of it in the blood [151] .

Vitamin E and cancer

Cancer chemoprevention is presently acceptable as a strategy for the reduction of cancer occurrence not only in high risk groups but also in the general public. In addition to the search for the identification of chemopreventive agents, the development of effective therapies for cancer is also under thorough investigation. Nevertheless, the search for a single compound to treat all types of cancer has been unsuccessful to date. Current chemotherapeutic treatments are usually not completely selective for carcinogenic cells with consequent cytotoxic effects on normal cells and therefore decreased quality of life for patients with cancer. Interestingly, evidence in the literature suggests that more effective treatments of cancer could be possibly achieved if chemotherapeutic drugs were used with other adjuvant agents with known antitumorigenic activities [152].

During the past decade much evidence has accumulated demonstrating the anticancer activity of specific form of vitamin E [153,154].In last few years, there has been an explosion of research activity surrounding the potential anti-cancer effect of different vitamin E vitamers. These studies suggest that not only α- tocopherol and both γ, δ tocopherol might have potent anticancer activity, possibly mediated via anti-angiogenic effect, anti-proliferate, and proapoptotic effect [155,156].These apparent anticancer effects appear to be mediated independently of antioxidant properties [157,158].

The anti-tumor activity of tocopherols may not be associated with their activity only bases on the findings, the anticancer effect can be addressed through at least four mechanisms: antiangiogenic, antiproliferation, induction of apoptosis, and improving immunological function [159].