

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE



**INVESTIGATION OF AMOUNTS OF VITAMINS, STRESS
BIOMARKERS, GLUTATHIONE AND AMINO ACID IN BLOOD
SERUM OF PATIENTS WITH BREAST CANCER**

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Master's Thesis

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THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Firat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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..... / / 20

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Investigation of Amounts of Vitamins, Stress Biomarkers, Glutathione and Amino acid in Blood serum of patients with Breast Cancer” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

24 March 2021

Awat Hamad AWLA BRAIM



PREFACE

First, my deepest gratitude to God for all his mercy and blessings, and for giving me the strength and ability to complete this search in these difficult times.

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The studies were carried out with the permission of the Ethics Committee .22/September/ 2019 ... and numbered 355284

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ABSTRACT

Investigation of Amounts of Vitamins, Stress Biomarkers, Glutathione and Amino acid in Blood serum of patients with Breast Cancer

Awat Hamad AWLA BRAIM

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences
Department of Chemistry

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Summary: Breast cancer is the most common type of cancer among women in the world, in this study, blood samples were drawn from 45 breast cancer and 32 healthy control group individuals and their serums were separated

The amounts of fat-soluble vitamins (A, E, β -carotene), water-soluble vitamins (C, B₁, B₂, B₃, B₅, B₆, B₉ and B₁₂), stress biomarkers (4-HNE, MDA), GSH, GSSG and amino acids in blood serums were determined by HPLC.

The decrease in the amount of fat and water-soluble vitamins in patients with breast cancer compared to the control group, excluding vitamins C, B₅ and B₁₂, was statistically significant ($p < 0.001$).

The amounts of lipid peroxidation products 4-HNE and MDA were higher in cancer patients compared to the control group ($p < 0.05$). Compared to the control group, there was a decrease in the amount of GSH and an increase in the amount of GSSG in cancer patients ($p < 0.01$). The GSH/GSSG ratio of breast cancer patients was found to be lower than the control group ($p < 0.01$).

Levels of valine, tryptophan, glycine, histidine, threonine, proline, cysteine and asparagine were increased in the serum of cancer patients compared to the control group, while leucine, isolatinine, phenylalanine, methionine, lysine, alanine tyrosine, glutamic acid, aspartic acid, arginine, glutamine and decrease in the amount of serine amino acids were observed ($p < 0.0001$).

As a result, it was observed that there was a decrease in the amount of water and fat-soluble vitamins and GSH in the serum of breast cancer patients, an increase in the amount of stress markers (MDA, 4-HNE) and GSSG, and changes in the amount of amino acids. From these results, it can be said that a serum-based screening test may be useful for the diagnosis of breast cancer.

Keywords: Breast Cancer, Stress Biomarkers, Glutathione, Vitamins, Amino Acids

ÖZET

Meme kanserli hastaların kan serumundaki vitaminler, stres biyomarkırları, glutatyon ve amino asit miktarlarının araştırılması

Awat Hamad AWLA BRAIM

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ÖZET: Meme kanseri, dünyadaki kadınlar arasında en yaygın kanser türü olup, bu çalışmada 45 meme kanserli ve 32 sağlıklı kontrol grubu kişilerden kan örnekleri alınarak, serumları ayrılmıştır.

Kan serumlarındaki yağda çözünen vitaminler (A, E ve β -karoten), suda çözünen vitaminler (C, B₁, B₂, B₃, B₅, B₆, B₉ ve B₁₂), stres biyomarkırları (4-HNE, MDA), GSH ve GSSG ile amino asitlerin miktarları HPLC ile tayin edilmiştir.

Meme kanserli hastaların yağda ve suda çözünen vitaminlerin miktarları C, B₅ ve B₁₂ vitaminleri hariç kontrol grubuna göre azalma istatistiksel olarak anlamlıdır ($p<0.001$),

Lipit peroksidasyon ürünlerinden 4-HNE ve MDA'nın miktarları kontrol grubuna göre kanserli hastalarda daha yüksek bulunmuştur ($p<0.05$). Kontrol grubuna kıyasla kanserli hastaların GSH miktarında azalma ve GSSG miktarlarında ise artma gözlenmiştir ($p<0.01$). Meme kanserli hastalarının GSH/GSSG oranı ise, kontrol grubuna göre düşük bulunmuştur ($p<0.01$).

Kontrol grubuna göre kanserli hastaların serumlarındaki aminoasitlerden valin, triptofan, glisin, histidin, thronin, prolin, sistein ve asparajinin miktarlarında artma gözlenir iken, leusin, izoleusin, fenilalanin, metiyonin, lizin, alanin, tirozin, glutamik asit, aspartik asit, arginin, glutamin ve serin aminoasitlerin miktarlarında ise azalma gözlenmiştir ($p<0.01$).

Sonuç olarak, meme kanserli hastaların kan serumlarındaki suda ve yağda çözünen vitaminler ile GSH miktarlarında azalma, stres markerleri (MDA, 4-HNE) ile GSSG miktarlarında ise artma olduğu, aminoasit miktarlarında da değişimlerin olduğu gözlenmiştir. Bu sonuçlardan göğüs kanseri teşhisi için serum temelli bir tarama testinin yararlı olabileceği söylenebilir.

Anahtar Kelimeler: Meme kanseri, Stres biomerkerleri, Glutatyon, Vitaminler, Amino asitler

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ABBREVIATIONS

Abbreviations

BC	:Breast Cancer
ER	:Estrogen Receptor
PR	:Progesterone Receptor
DNA	:Deoxyribonucleic Acid
MDA	:Malondialdehyde
4-HNE	:4-Hydroxynonenal
GSH	:Glutathione
GSSG	:Oxidize Glutathione Disulfide
AC	:Antioxidant Capacities
AAs	:Amino Acids
HPLC	:High Performance Liquid Chromatography
HER2	:Human Epidermal Growth Factor 2
EMR	:Eastern Mediterranean
WHO	:World Health Organization
TNM	:Tumor, Node And Metastasis
BSE	:Breast Self - Examination
CBE	:Clinical Breast Examination
MRI	:Magnetic Resonance Imaging
•NO	:Nitric Oxide
OH•	:Hydroxyl Radical
O ₂ ⁻	:Superoxide Anion
LOO•	: Lipid Peroxide radical
H ₂ O ₂	:Hydrogen Peroxide
ROS	:Reactive Oxygen Species
ROIs	:Reactive Oxygen Intermediates
H•	:Hydrogen Radical
RNS	:Reactive Nitrogen Species
SOD	:Superoxide Dismutase
PUFAs	:Polyunsaturated Fatty Acids
ONOO ⁻	:Peroxynitrite

NO ₂	:Nitrogen Dioxide
GSS	:Glutathione Synthase
NOS2	:Nitric Oxide Synthase 2
NADPH	:Nicotinamide Adenine Dinucleotide Phosphate
CAT	:Catalase
GPx	:Glutathione Peroxidase
GRx	:Glutathione Reductase
LOOH	: Lipid hydroperoxides
TEA	:Triethylamine
PITC	:Phenyl isothiocyanate



1. INTRODUCTION

Breast cancer is the most prevalent cancer among women in the world, is responsible for the highest morbidity and fatality. Every year about 1 million new breast cancer patients are diagnosed and 400,000 women die due to this disease. Breast cancer etiology is multifactorial and several breast cancer-related risk factors may exert their impact through the generation of an oxidative stress status. Pathogenesis of various chronic diseases like cancer, diabetes, and cardiovascular diseases as well as overage aging has been affected by oxidative stress [1, 2]. Oxidative stress may generally be described as an oxidant / antioxidant imbalance that may lead to damage. If the amount of reactive species is high and defeats the human body's antioxidant defense mechanisms the oxidative damage may occur to lipids, proteins, or instantly to DNA, and this damage is considered to play an important role in the advancement and progression of many human disorders such as carcinogenesis initiation including breast cancer [3, 4]. In addition, the measurement of oxidative stress biomarkers in breast cancer patients is of significant interest. There are several markers of oxidative stress, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which have been applied broadly as markers of lipid peroxidation, and protein carbonyl that is the biomarker of protein oxidation mostly used in epidemiological and clinical studies [5]. Cells are preserved from oxidative stress through antioxidant detoxification pathways that are containing non-enzymatic antioxidants like reduce glutathione (GSH) and varied antioxidant enzymes.

Glutathione has been frequently used as a biomarker of oxidative stress because GSH is a reducing agent that is widely spread in cells. Because of the nature of the characteristic measurements and interactions between the various antioxidant compounds, various methods were developed to test serum and plasma antioxidant capacities (AC) [6, 7]. These methods give an analysis of the biological associations between individual antioxidant substances and their effectiveness in preserving the host cells during oxidative stress periods [8]. In patients suffering from breast cancer, it is becoming extremely important to identify possible modifiable factors affecting oxidative stress [2].

In addition, vitamins which play an important role as coenzymes or enzymes in many important processes for the proper functioning of the body so they are nutrients necessary for human metabolism. In recent years, because of much research that studied this relation, it has become clear that vitamins are important for health and human disease [9, 10] so it is important to know the relation of vitamins with breast cancer.

Amino acids (AAs) are an essential part of most analysis of metabolomics so because of their involvement in the synthesis of proteins and metabolic regulators, can be excellent markers of disease in the body and provide an opportunity for an integrated evaluation of

several pathways and their biological effects by measure downstream output rather than upstream gene and protein products [11].

The profiling of amino acids has shown variations in amino acid levels between patients with cancer and healthy controls; although some of the results published remain to be verified. Further, a diagnostic test was recently reported by a Japanese group that compared plasma amino acid levels between lung cancer patients and healthy controls. The metabolic change from respiration to fermentation in the tumor cells will lead, among other substances, to increased demand for and consumption of amino acids. In fact, several publications have shown this [12-14].

Besides metabolite consumption by cancer, the role of amino acids in the pro-and anti-tumorigenic influence of immune cells is also important. Immune cell activation also leads to increased requirements for baseline amino acids, and immune effector cells often depend on the external supply of some amino acids. Here, a lack of amino acid will negatively affect the immune response in several ways like cell division, differentiation, migration, maturation, and production of effector functions. Because cancer cells are detected through altered metabolic pathways so the determination of low-molecular metabolites, including free amino acids, could be a reduced invasive approach related with high diagnostic potential in biological fluids [15]. Free amino acid profiles have been shown to differ depending on the type of cancer and its level. Moreover, In the case of breast cancer, the ability of amino acids as indicators of this disease has not been widely investigated so far.

The analysis of 20 amino acids, fat and water-soluble vitamins, MDA, 4-HNE as oxidative stress biomarker with glutathione is performed in this study and if differences in the profiles between breast cancer patients and controls were detected, indicates the ability to improve breast cancer screening.

1.1. Anatomy of the breast

Breasts are an important organ. The breast is the tissue overlying the chest (pectoral) muscles. Breasts will undergo several and distinct periods of growth, development, differentiation, and involution within a lifetime of women. Breast development takes place after birth, unlike most other types of tissue. The apparent purpose of breasts is to supply milk to descendants. The conical shape of the breast helps human infants nurse without suffocation. The breasts of women consist of specialized tissues producing milk (glandular tissue) and fatty tissues. The fat amount determines the breast size. The breast milk-producing part is constructed into 15 to 20 lobes. There are smaller structures within every lobe, called lobules, which produce milk [16]. The milk passes through a network of small tubes called ducts that join in larger ducts and eventually exit the skin in the nipple. That lobule consists of cavities

that store milk called the alveoli, which are covered with hundreds of milk-secreting acini. The mammary gland is stimulated to release stored milk from the alveoli through the ducts and out through the nipple by oxytocin, produced during pregnancy and childbirth and in response to infant suckling. Both ducts and lobules consist of epithelial cells, surrounded by the contractile myoepithelial cells, which contract to stimulate oxytocin. Lymph is drained by a rich supply of the vessels from the mammary tissue; those lymph vessels are linked to a network of lymph nodes around the edges of the breast. These lymph nodes have a major role to play in breast cancer spread, such as axillary lymph nodes, which are among the earliest places in which cancer spreads from the breast [17].

1.2. Cancer

Cancer is the uncontrolled growth of the body's abnormal cells. Arising from a series of molecular events that fundamentally alter the cells' normal properties. The normal control systems which prevent cell overgrowth and invasion of other tissues in cancer cells are disabled. Such altered cells divide and expand, show uncontrolled growth, invasion, and sometimes metastasis by dividing and increasing cells in the presence of signals that normally inhibit cell growth. Cancer cell disorders typically result from mutations in protein-encoding genes that control cell division. With time, more genes are mutated; that's often because the genes that produce the proteins that commonly repair DNA damage do not function normally themselves because they are mutated as well [18]. Additionally, mutations in the cell continue to increase, causing additional anomalies in that cell and the daughter cells as in **Figure 1.1**. Some of these mutated cells die, but other alterations may give a reproductive advantage to the abnormal cell that allows it to multiply much faster than normal cells, this improved growth describes most cancer cells that have obtained repressed functions in normal, healthy cells [19]. If these cells stay at their original location, they are considered benign; while they are considered malignant if they become invasive. Cancer cells may often metastasize in malignant tumors, spread from one organ or portion to another, which is not directly related to it [20].

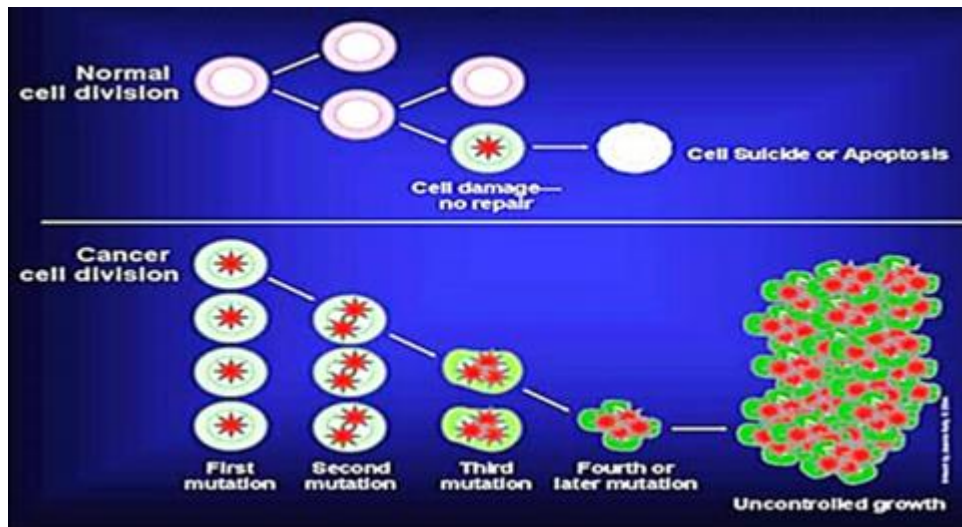


Figure 1.1. Comparison of a normal cell with malignant cells [21].

1.3. Breast cancer

Breast cancer is the second most commonly diagnosed cancer in the world after lung cancer. Breast cancer is the second leading cause of cancer death among women worldwide. About one in nine women develop the disease over their lifetime. While it is understood that breast cancer affects women, it can also be equally damaging for men. Male breast cancer constitutes 1% of all breast cancer diagnosed [22]. These cancer cells continue to develop into a tumor. Though more than 80% of the lumps in the breast are not carcinogenic, the only way to diagnose them is through a biopsy method. Breast cancer occurs in the breast tissue consisting of the milk production glands, known as lobules, and the duct attaching the lobules to the nipple. There are fatty, connective, and lymphatic tissues in most of the breast. Also, this is a very heterogeneous group of cancers caused by different cell types and each has its own clinical consequences [23]. Now, the presence of Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Receptor2 (HER2/neu) protein is being studied on all breast cancers. The immunohistochemistry usually performs ER and PR tests while FISH accesses HER2/neu. These tumor profiling proteins help predict the ultimate prognosis and can help determine the most suitable treatment for the individual. In about 70 percent of breast cancer cases, ERs are over-expressed and are referred to as "ER-positive" tumors [24]. Binding estrogen to ER induces the growth of mammary cells by increasing the division of the cells and replication of the DNA and increasing the rate of mutation. This causes cell cycle disruption, apoptosis, and processes of repairing DNA which ultimately leads to tumor formation. In addition, the metabolism of estrogen contributes to the production of genotoxic by-products which could potentially affect DNA, resulting in point mutations. Approximately 65 percent of ER-positive breast cancers are also PR-positive, and about 5 percent are ER-negative and PR-

positive. .when cells contain receptors for both hormones or receptors for one of these two hormones, then cancer is considered a positive hormonal receptor [25]. HER2/neu is a protein in breast cancer that gives higher aggressiveness. It is a member of the ErbB family of proteins, most commonly known as the receptor family of the epidermal growth factor. Around 30 percent of breast cancers have an accumulation or over-expression of the HER2/neu gene in their protein product [24].

1.3.1. Epidemiology of Breast cancer

Breast cancer is the world's most common cancer, with an estimated 1.57 million new cases in 2012 accounting for 25 percent, among women. it is also the leading cause of death among women with cancer. Although the survival of this disease was substantially improved in countries with high-resource, there was an increased risk of high deaths in the middle and low-income countries [26]. Breast Cancer is the fourth-largest cause of death in Eastern Mediterranean (EMR) countries after cardiovascular disorders, infectious diseases, and accidents. The EMR is composed of 21 member states in the Middle East, North Africa, and Central Asia according to the WHO classification. Breast cancer has become the main threat to women's health in Iraq, with a cancer-related mortality rate of 23 percent, leading to death after cardiovascular diseases of women. Breast cancer has become the highest-ranking malignancy among the general Iraqi population since 1986. The most recent list of Iraqi cancer [27]. The frequency and prevalence of breast cancer in western Turkey in 1992 were 24.4/100,000 and 0.3 percent respectively in 1992. Distribution of breast cancer occurrence in the 2000s changes due to geographic, environmental, social, cultural factors in Turkey's distinct regions. The prevalence of breast cancer in the western part of Turkey (50/100,000) is more than double in the eastern part of Turkey (20/100,000) due to " secularization life" (late menopause, early menarche, first birth > 30 years, less breastfeeding, etc.) [28].

1.3.2. Types of Breast cancer:

Breast cancer is classified into five different types

- Ductal carcinoma: occurs in the cells that line the duct of the breast, under the nipples and areola. Ductal cancer is nearly 90% of all breast cancers. If ductal carcinoma is in situ, the cancer is well-contained, not invasive, and can be successfully treated.
- Lobular carcinoma: occurs with lobes or glands that produce the milk. These lobes are located under the duct inside the breast. Around 8 % is lobular in breast cancers. If the cancer is in situ lobular carcinoma, it has not spread and is limited to the lobe.

- Invasive breast cancer: occurs when cancer cells from inside the ducts or lobules reach the breast tissue. Cancer cells can spread through the bloodstream or lymphatic system from the breast into other parts of the body.

- Inflammatory breast cancer: it takes the form of nests and sheets in place of lumps, being the least common, but severe breast cancer. It can begin in the breast's soft tissue, just below the skin or it appears in the skin.

- Nipple Areola Paget's disease: it often appears like skin rash or gritty skin.

It looks like eczema [29].

1.3.3. Staging of breast cancer

The cancer staging system explains how far cancer has anatomically spread and attempt to bring patients with similar prognosis and diagnosis into the same staging group This staging system is usually called TNM [30], a tumor, node, and metastasis that is used in breast cancer. Thus the staging of TNM takes into account the tumor size, whether the lymph nodes are compromised or if cancer transferred to other parts of the body (metastasis) [31].

- Early breast cancer-stage 0: in situ carcinoma or disease which has not defeated the membrane in the basement.

- Stage I: Small primary tumor with no involvement in lymph nodes.

Phase II: Local lymph node involvement;

- Locally advanced breast Cancer-Stage III: usually a big tumor with significant nodal activity in which node or tumor is located to the chest wall; often involves increasingly progressing inflammatory breast cancer.

- Metastatic or advanced breast cancer: Stage IV: Metastases in organs away from the tumor [32].

1.3.4. Diagnosis

Diagnosis Breast Cancer detected in the early stage is more likely to be treated effectively before it has had the chance of growing up or spreading. Breast cancers detected when they can be noticed appear to be bigger and are more likely to have spread out of the breast already. However, screening tests also detect cancer of the breast when they are small yet still limited to the breast. Some of the most important factors in predicting a woman's outlook (prognosis) with this disease are the size of breast cancer and how far it has spread. Many doctors believe that early breast cancer detection testing saves thousands of lives each year. if even more individuals and their health care providers taking advantage of these tests, many more lives will possibly be saved. Breast self-examination (BSE) is a process that allows women to monitor breast modification and to help detect cancer at an early stage following further investigations.

Screening mammography is used to diagnose patients with breast cancer [33]. When identified abnormal mass by using screening technique or palpation, three diagnostic procedures are used: clinical breast examination and loco-regional lymph nodes, the radiological analysis combined with ultrasounds, and magnetic resonance imaging (MRI) used in some cases, and clinical examination of fine-needle aspirate, often accompanied with core biopsy. All diagnostic work-up is obligatory and confirmatory biopsy is required for diagnosis [34, 35].

1.3.5. Risk factors of breast cancer

A risk factor is something that increases the chance of developing a disorder or disease. The risk of breast cancer is increased with age, although in women under 45 years the disease is quite rare. Life-time exposure to female sex hormones is the most essential risk factor as they encourage cell growth, so hormone influences are important. During the years that women reproductive, higher levels of hormones appear to increase the risk that genetically damaged cells will develop and become cancerous, particularly when they are not interrupted by hormonal changes of pregnancy. Age at first Menarche, menopause, and first birth. There is a greater risk for women who began menstrual periods early (< 12 years) or who have gone through late menopause (after 55 years). However, there is a higher risk for women who never had children or who only had them after 30 years. The increased risk also is related to hormone replacement, oral contraceptives, obesity, and height. By comparison, breastfeeding and fruit and vegetable diets are also involved in reducing the risk of breast cancer [36]. In addition, an increased risk for previous benign breast disease was related to risk factors associated with exposure to sex hormones. Intake of alcohol. The increased risk of breast cancer is associated with excessive alcohol consumption (over 1-2 drinks per day), with a possible increase in the risk of breast cancer in chemical products and other industrial products [37]. 5% exposed to radiation, especially during childhood, may be exposed to increased risk in adulthoods. Another significant risk factor is a family history of breast cancer. About 7 percent of all breast cancers were estimated to be hereditary [38], mutations in the BRCA1 or BRCA2 gene are the most commonly known high-risk genetic predisposition. The frequency of these mutations in women with breast cancer varies with geographic location; Age and family history of a patient. In young patients with breast cancer (<14). These findings indicated that the proliferation of genetically damaged cells causes breast cancer. This genetic damage is known to accumulate gradually over time in the body cells. People with mutated BRCA1 and BRCA2 genes have a head start in this process, and the possibility of a woman developing breast cancer rises by a factor of two if she has a first-degree relative with the disease. The more family cases and the younger the relatives who developed breast cancer has the higher the risk [39].

1.3.6. Prevention in breast cancer

The most likely causes of breast cancer are several risk factors and enhance them. For example, age and gender are some of these risk factors almost unavoidable. Governments have engaged in many awareness-raising programs to implement strategies of prevention to reduce and avoid breast cancer. To prevent breast cancer, weight control, physical activity, and diet are necessary [40]. New research has demonstrated that the risk of exposure to breast cancer is decreased for women who give birth and feed their baby's with their own milk. The development of breast cancer is also influenced by obesity and low physical exercise. The frequency of breast cancer may be decreased significantly in developed countries if mothers are childbirth at their right age and supplying their kids with their own milk in the early stages after childbirth [41]. More efforts are being made around the world to eliminate breast cancer, though prevention can minimize some risk, most breast cancers in low- and middle-income countries can't be eliminated by these strategies, thus early detection to improve the outcome of breast cancer and its survival stay the key of breast cancer control [42].

1.3.7. Available treatments for breast cancer

The main aim of all cancer treatments is to cure it completely through the clearing of malignant cells and leaving the remaining cellular structure as intact as possible and then prolonging life and improving patient quality of life if this isn't possible. Depending on the stage, the size, the position, whether it has been transmitted to other parts of the body, and the physical health of a patient [43]. The treatments for breast cancer may be varied. Current breast cancer treatments involve surgery, radiation therapy, chemotherapy, hormonal, and targeted therapy. Depending on the stage of the disease these therapies may be used alone or in combination. Surgery is still the main form of treatment for most patients, the tumor is localized, With the possibility of adjuvant hormonal therapy, all breast (mastectomy) or some part (lumpectomy), depending on the size, stage, and location of a tumor, is removed by surgery [44]. Axillary surgery is a preferred method for the evaluation of axillary metastases in early breast cancer, from the release through node sampling to the biopsy of a sentinel node. Neo-adjuvant treatment is used primarily for evaluating pharmacologic effectivity in people and minimized the degree of the surgery required in successful responders, originally carried out in down-stage inoperable cancers. Sometimes the surgery is performed using a combination of radiation and systemic therapy [45]. Radiotherapy is a cancer treatment that uses high-energy x-rays which are carefully monitored and managed. It is intended to destroy all the cells of cancer left behind in the breast area following the surgery in primary breast cancer. Chemotherapy involves the use of drugs to break down cancer cells. Chemotherapy plays an important part in the treatment of gynecologically malignant patients [29]. Systemic therapies

are used in the advanced state alone or combined. Systemic treatment involves chemotherapy, immune treatment, and hormone therapy. Chemotherapy is typically less therapeutic than other medical products; thus, the potential for serious adverse chemotherapy-related effects has made the selection of patients and medications relevant. Chemotherapy also causes other inconveniences. Most chemotherapy agents are harmful to healthy cells, and chemotherapy can cause serious and dangerous side effects such as nausea and vomiting, depression of the bone marrow and immunosuppression. Even with chemotherapeutic agent combinations, many tumor cells are immune or resistant to chemotherapeutic agents. Hormonal therapy is the opposite of type of female hormone sometimes used for reduction of menopause symptoms (also called hormone therapy or antiestrogen therapy). Hormonal therapy is prescribed for most women suffering from breast cancer, and is sometimes used by women who have not been diagnosed with breast cancer but who are at great risk for it depending on their genetics or their family history [46].

1.4. Stress Biomarkers

Biologic marker (biomarker): a feature that is measured and assessed objectively as an indicator of normal biological processes, disease processes, and or pharmaceutical response to therapeutic intervention.

There are three different kinds of biomarkers: the first kind is biomarker disease used to detect the progression of a disease and diagnose it, second Biomarkers of medication efficacy used to monitor treatment protocol effectiveness or toxicity, third pharmacodynamics markers are used to measure pharmacological response [47].

1.4.1. Free Radicals

Free radicals can be described in atomic or molecular orbitals as molecules or molecular fragments which have one or more unpaired electrons. Examples: Nitric oxide ($\bullet\text{NO}$), hydroxyl radical ($\text{OH}\bullet$), superoxide (O_2^-), lipid peroxide radical ($\text{LOO}\bullet$). This unpaired electron(s) often gives the free radical a significant degree of reactivity oxygen-derived radicals constitute the most important class of radical species produced in living systems and the Oxidation of polyunsaturated fatty acids is one of the main effects of produce free radicals [48].

1.4.2. Reactive Oxygen Species:

Reactive oxygen species (ROS) is a general term for a class of oxidants that are either free radicals or molecular species which capable of producing free radicals. Reactive oxygen species (ROS) are by-products of normal cellular metabolism therefore also known as reactive

oxygen intermediates (ROIs) [49]. Low and moderate concentrations of ROS have positive health effects on various physiological processes including the destruction of invading pathogens, tissue repair processes, and wound healing. But at high concentrations, they cause undesirable changes in cellular components such as lipids (cell and organelle membranes), proteins (receptors and enzymes), and DNA so damages them, which is known to be a key cause of cancer [49, 50] and heart-related diseases, Alzheimer's disease [51].

ROS can be generated from either endogenous or exogenous sources. Endogenous sources including mitochondria, phagocytic cells, endoplasmic reticulum, peroxisomes, etc. And exogenous sources involved pollution, tobacco smoke, heavy metals, alcohol, transition metals, pesticides, industrial solvents, and certain drugs like paracetamol, halothane, and radiation. In endogenous metabolic reactions, cells generate ROS like oxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$), and organic peroxides that formed as biological products of molecular oxygen [52] and in Exogenous sources contributing to the total ROS exposure of our cells. Ionizing radiation may cause the breakdown intracellular H_2O covalent bonds, forming radicals of hydrogen ($H^\bullet$) and hydroxyl radical ($OH^\bullet$) these radicals can potentially release from the production place unless they are inactivated by antioxidants [53, 54].

1.4.3. Reactive nitrogen species (RNS)

Reactive nitrogen species (RNS) are an important class of radicals generated in living systems. That is either oxidizing agents or easily transformed into free radicals. Oxidants containing nitrogen, like nitric oxide (NO) peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), etc. Reactive nitrogen species are produced via nitric oxide (NO) and superoxide ($O_2^{\bullet -}$) when reacted together through the enzymatic activity of nitric oxide synthase 2 (NOS2) and NADPH oxidase. When the production of RNS exceeds on the antioxidant capacity of the cell is defined as nitrosative stress [48].

1.5. Antioxidant Defense System

The antioxidant is a molecule that can be synthetic or naturally occurring and it is stable to give an electron to the free radical that neutralizes it, thus reducing its ability for damage. Antioxidants inhibit or prevent cellular damage through free radical modification. Antioxidants and a variety of DNA repair enzymes control the balance between the construction and elimination of ROS [53]. The human body has a complex antioxidant defense system, which is based on an antioxidant diet along with endogenous antioxidant production. We can classify antioxidants into two main groups; that is, enzymatic and nonenzymatic antioxidants. Some of these antioxidants are produced in an endogenous way, including enzymes (superoxide

dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GRx)), low-molecular-weight molecules (ascorbic acid (vitamin C), tocopherols (vitamin E), glutathione (GSH), uric acid, selenium, and bilirubin) and cofactors of enzymes. A lot of nonenzymatic antioxidants come from dietary sources. Antioxidants in diet can be classified into different groups: That includes polyphenols which are the largest groups. Polyphenols are composed of phenolic acids and flavonoids, and other types of dietary antioxidants that are consist of vitamins, organosulfural compounds, carotenoids, and minerals [55].

1.6. Oxidative Stress

Oxidative stress refers to the imbalance between the oxidative system and the antioxidant system in the body and is considered to be a significant factor leading to aging and disease.

The production of reactive oxygen (ROS) species is nearly balanced against antioxidant systems in healthy aerobes [50]. Oxidative stress is described as pro-oxidant-antioxidant balance disturbance or serious unbalance amongst ROS production and antioxidant defenses. The cells contain more oxidants than antioxidants; however, cells have a strong stock of antioxidant enzymes and small antioxidant molecules but maybe these agents not enough to normalize the redox status during oxidative stress which leads to enormous damage. This damage is often referred to as 'oxidative damage', thus cause various conditions such as inflammation, cancer, neurodegeneration [56]. Such as **Figure 1.2**.

Increased oxidative stress that arises from either a number of factors such as (1) increased free radical production, (2) reduced levels of available antioxidants, (3) increased consumption, degradation, or destruction of antioxidants, (4) reduced protective capacity like antioxidant enzymes and (4) electron leakage from the impaired mitochondrial electron transport chain [57].

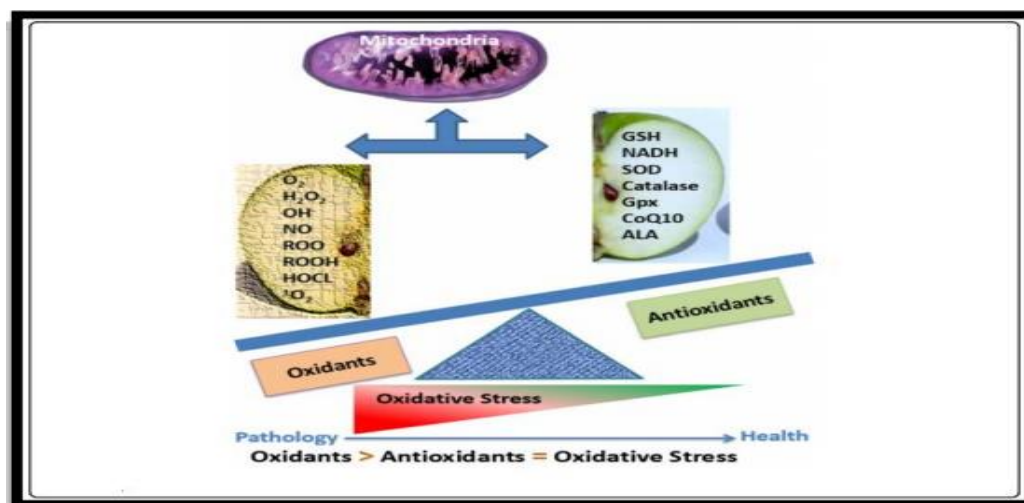


Figure 1.2.Types of oxidants and antioxidants that imbalance which cause oxidative stress [58]

A wide range of oxidation products is produced by lipid peroxidation or oxygen reaction with unsaturated lipids. Lipid peroxidation's (LOOH) are the main primary products. Malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) are a number of the various aldehydes that can form as secondary products during lipid peroxidation [32].

1.6.1. Oxidative stress with breast cancer

Oxidative stress plays an important role in initiating and preserving the progression of breast cancer. One way cellular ROS affects cell signals is by their localized aggregation.

ROS and RNS participate in various ways to promote carcinogenesis and malignancy in cancer cells. In fact, they are now recognized as a distinctive feature and hallmark of cancer. These structures contribute to genomic harm and genetic instability and engage in mitogenic and survival signals through growth factor receptors and adhesion molecules as intermediaries encourage and increase cell proliferation, cause inflammation/repair, and micro-environmental tumor angiogenesis [59].

1.6.2. Oxidative Stress Biomarker

The use of the ROS as biomarkers for oxidative stress is difficult as they are not stable enough to be observable. However, ROS fingerprint can be measured. ROS gives stable metabolites and targets oxidizing products, such as the end product of lipid peroxide and oxidized proteins [60]. The most frequently used biomarker for lipid peroxidation is the malondialdehyde (MDA), 4-hydroxynonenal(4-HNE) and the most commonly applied form of measuring protein oxidation is carbonyl group detection [5].

1.7. Lipid peroxidation

Lipid peroxidation can generally be defined as a process by which oxidants like free radicals or non-radical species attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) involving hydrogen extraction from carbon, with oxygen injection resulting in lipid peroxy radicals and hydroperoxides. The complete lipid peroxidation cycle consists of three stages: initiation, propagation, and termination [61]. A wide number of oxidation products are produced by lipid peroxidation or oxygen reaction with unsaturated lipids. The major lipid peroxidation results are the lipid hydroperoxides. Esterbauer and his collaborators studied extensively [62], among the many different aldehydes that can be produced as secondary products during lipid peroxidation is malondialdehyde (MDA), hexanal, propanal, and 4-hydroxynonenal (4-HNE), and the MDA seems the most mutagenic lipid peroxidation product [5, 63].

1.7.1. Malondialdehyde (MDA)

Malondialdehyde (MDA) is the principal form of aldehyde resulting from tissue lipid peroxidation of (omega-3 and omega-6) fatty acids and commonly used in the biomarker of oxidative stress as an indicator of lipid peroxidation) [64, 65]. MDA is a low-molecular-weight, three-carbon aldehyde with chemical formula $\text{CH}_2(\text{CHO})_2$ produced by different mechanisms and the chemical structure like **Figure 1.3**.

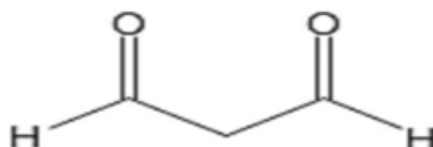


Figure 1.3.Chemical structure of Malondialdehyde.

MDA is low chemical reactivity in its physiological condition at normal pH. However, it can react in DNA with deoxyadenosine and deoxyguanosine, to form a different adduct. This adduct has a blocked area of the mutagenic Watson-Crick base pairing region so that is considered mutagenic [66]. MDA can be easily measured by thiobarbituric acid (TBA) reaction because it is the easy reaction with TBA to produce an intensely colored chromogen red fluorescent adduct and also can be used as an indication of cell membrane injuries [67]. MDA can bind with proteins or nucleic acids very rapidly so it is highly toxic [66, 68].

It is also can interact with the protein, like collagen. Furthermore, the MDAs can lead to cross-linkages between membrane elements by influencing the ion exchange from cell membranes [54] thus allowing for a change in the permeability of ions and the activity of enzymes. Thus, through its ability to react with molecules such as DNA and proteins, MDA can impair many physiological mechanisms of the human body [5]. All of these potentially genotoxic MDA activities may cause mutations and afterward to cancer. Therefore, it is important to consider the molecule as more than a lipid peroxidation product [69].

1.7.2. 4-Hydroxynonenal (4-HNE)

4-Hydroxynonenal abbreviated 4-HNE or HNE, molecular formula $\text{C}_9\text{H}_{16}\text{O}_2$, its formation mechanism, and biochemical characteristics were first reported in 1991 [62]. 4-HNE is an alpha, beta-unsaturated aldehyde that's derived from the lipid peroxidation process of omega-6 polyunsaturated fatty acids such as linoleic acid, linolenic acid, and arachidonic acid. 4-hydroxynonenal, **Figure 1.4** shows the basic structure of 4-HNE

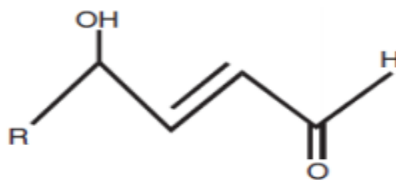
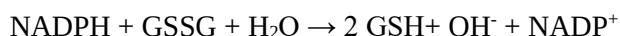
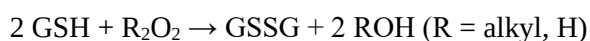


Figure 1.4.The basic structure of 4-hydroxynonenal

4-HNE is an extraordinarily reactive compound consisting of three functional groups involving a hybrid system of a C = C double bond and a C = O carbonyl group hydroxyl group (-OH) so supplying a partial positive charge to carbon 3, These three functional groups can cooperate in many cases to explain their high level of reactivity [70]. 4-HNE is widely distributed. Bio-phospholipid membranes are rich in omega-6 PUFAs, and 4-HNE is an amphoteric molecule with both hydrophilicity and lipophilicity, so it is distributed in cells and tissues of organisms. In addition, foods containing omega-6 PUFAs may produce trace amounts of 4-HNE during processing and storage [71]. 4-HNE can act as bioactive molecules in physiological and/or pathological conditions since it can primarily rate signaling processes by forming covalent adducts in proteins, nucleic acids and membrane fats with nucleophilic functional groups. The formation of these adducts (mostly irreversible) can lead to multiple adverse events such as inhibition of DNA, RNA, and protein synthesis [70]. In addition to biochemical modifications, 4-HNE can also cause physical changes in proteins and phospholipid membranes, such as altering cell membrane fluidity [72].

1.8. Glutathione

Glutathione is a non-enzymatic antioxidant in mammalian cells which prevents damage to important cellular components due to ROS. Glutathione is a tripeptide of glutamate, cysteine, and glycine so-known γ -glutamyl cysteinyl glycine, Found in virtually all mammalian tissue, most plants, and microorganisms. Eukaryotic cells have three main storage of GSH that is cytosol, mitochondria, and a small amount in the endoplasmic reticulum [6, 73]. Glutathione's functionality is derived from the sulfhydryl (SH) group of the cysteinyl moiety. Glutathione occurs in two forms, the Thiol-reduced (GSH) and disulfide oxidized (GSSG). GSSG synthesizes via the linkage of two GSH molecules by oxidation of the -SH groups in cysteine to create a disulfide bridge (-S-S-) due to an electrophilic substance (for example free radicals and reactive oxygen/nitrogen species) however, GSH can be reduced back by using NADPH as an electron donor through glutathione reductase so this cycle is defined to glutathione redox cycle [74].



The ratio between reduced form and the oxidized form of glutathione (GSH/GSSG) within cells is generally used as a measure of cellular damage or represents a primary antioxidant defense, therefore the glutathione system acts as a significant homeostatic redox buffer. Glutathione involved through the redox glutathione cycle in detoxifying reactive oxygen species (ROS) so it plays a role in the removal of xenobiotic and carcinogenic agents [6] and is also associated with heavy metal detoxification, and protects proteins as well from oxidation by a method of glutathionylation [75]. However it is participating in DNA repair and synthesis therefore glutathione is a real key redox signaling regulator that adjusts gene expression and supports the survival of cells [7, 76].

1.9. Amino Acids

Amino acids are the primary unit of peptides and protein production, which contains over 20 amino acids in nature and it consists of two functional groups (carboxyl- and amino group) linked to the same carbon atom that attached to a hydrogen atom and another variable (R) group which represents the characteristic properties of the various amino acids such as this chemical structure illustrated in **Figure 1.5**.

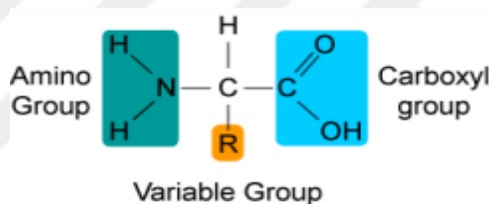


Figure 1.5. Basic amino acids structure.

All amino acids in the form of D or L contain asymmetric carbon atoms (except for glycine). All the amino acids which are found naturally in the tissues are generally produced as L form [11] amino acids which type produced into (D-amino acids) by non-genetically driven processes exist predominantly in lower organisms such as bacteria. There are 20 varieties of amino acids that are the most known in nature. In plants can be generated all 20 types of amino acids. By transformation, humans and animals are able to produce 12 of the 20 amino acids. The 8 remaining amino acids are conveniently obtained from the outside so the amino acids are arranged into two basic groups: essential amino acids (AA) which are obtained through food by the living organism, consisting of Lysine (Lys), Isoleucine (Ile), Leucine (Leu), Threonine (Thr), Methionine (Met), Valine (Val), Phenylalanine (Phe), and tryptophan (Try). Mammals need the complicated and long interaction path required for their synthesis. And second amino acids group is a nonessential amino acid that is synthesized through simple pathways of reaction by living organisms from pyruvates, oxaloacetates, alpha-ketoglutarates, and glycerate 3-phosphates with the exception of tyrosine (Tyr) which is derived from phenylalanine's amino

acid (Phe). Non-essential amino acids are amino acids that do not need to be obtained and recharged through supplemented with daily meals which include Alanine (Ala), Arginine (Arg), Histidine (His), Aspartate(Asp), Glycine (Gly), Cysteine (Cys), Proline (Pro), Serine (Ser), Glutamine (Glu) and the Tyrosine (Tyr). Generally, the essential amino acids are important for growth while non-essential amino acids are needed to preserve the human body [77].

Amino acids play a role both in the protein synthesis process and in the intermediate processes. They are a fundamental source of atoms of nitrogen which play a role in the synthetic reaction pathways. Besides their role as precursors in interaction pathways, the carbon build of the amino acids are the source of energy. Because Amino acids are the key unit of protein synthesis and Proteins play an important role in various life activities and are an important part of cells, tissues and organs the amino acids known as “basic particles of life”[11].

Protein produced from amino acids via a condensation reaction that amino acids linked together by peptide bonds in which one amino acid carboxy-terminus reacts with the amino-terminus of another amino acid to form peptide and water. If peptide bonds bind more amino acids to gather, the molecule is commonly called a polypeptide, Proteins are formed in a biologically functional way when several polypeptides are bound together. Small changes in the amino acid sequence may lead to drastic variations in protein structure and function [78, 79].

Amino acids are strongly regulated in healthy populations to maintain a neutral net protein balance, in spite of the continuous cycling of amino acids for protein synthesis and degradation. In addition amino acids used to building cells and repairing tissue and also it is the main building material for antibodies to resist invader bacteria and viruses, however it is a basic part of the hormone and enzyme system, Although amino acids have an important role to play in transporting oxygen to various parts of the body, the represent an integral part of the activity of muscles and Amino acids play a role in certain hormones as neurotransmitters and raw materials or as an energy source [80]. When there's an imbalance or lacking in the proportion of amino acid ingestion, it leads to irregular physiological functions and diseases. The profiling of amino acids has demonstrated important differences of amino acid levels between cancer patients and healthy controls [81, 82]. In cancer patients, the degree of change in the amino acid pattern depends on several factors such as the specific type of cancer, the stage and extension of the disease, as well as the probable correlation of protein-calorie deficiency [83].

1.9.1. Amino acid classification

Amino acids, according to their side chains (positively or negatively charged), aromatic chains, and aliphatic chains, can be categorized as follows

1) Nonpolar aliphatic amino acids of the R group: they have side chains with aliphatic chains however they have a feature hydrophobic. This group includes glycine, valine, alanine, isoleucine and Leucine.

2) Those with aromatic R group: Amino acids having this group have the ability to absorb light. Tryptophan, Tyrosine, and Phenylalanine are members of this group

3) Those with positive charge R group: Arginine and lysine can be given as examples to this group which have basic characteristics.

4) Those with R group with negative charge: They are negatively charged at physiological pH. Aspartic and glutamic acid are in this group.

5) Those with uncharged R groups: These are functional groups that can make hydrogen bonds with water. (Cysteine, methionine (-SH)), (Threonine, serine (-OH group)) and (glutamine-asparagine (carrying amide group)) can be counted in this group[84].

1.9.2. Amino acid metabolism

Amino acids are very important essential units of protein in the gene expression process, they play a wide role, including the regulation of protein functions that promote translation of messenger RNA (mRNA). In nature, there are more than 700 varieties of amino acids found. They are also exclusively α -amino acids. There are plants, fungi, bacteria, and algae. Twenty relevant amino acids, known as the building blocks of all life in the universe, are essential to the content of peptides and proteins. For the preservation of the human body's health, amino acids are important. It usually encourages hormone development, muscle building, nervous system, operating vital organs, cell functioning [82, 84].

1.10. Vitamins

Generally, vitamins are an organic compound class, which is an essential component of a sufficient diet. Which are important for different biological functions in very small amounts. Their derivatives act as coenzymes, cellular antioxidants, and gene expression regulators. In human nutrition, 14 vitamins are recognized that are divided into two groups based on their relative solubility in water and fat which are a water-soluble vitamins and fat-soluble vitamins [85]. Deficiencies or excesses in consumption leading to an alteration in protein, carbohydrate, nucleic acid, fat, and/or mineral metabolism. Thus, the entire physiological system, such as those relevant to detoxification, cellular repair, immune processes, and endocrine activity and neural, depends on the host's nutritional and vitamin status. For these factors, the adequacy of the supply of vitamins to cells and tissues may be anticipated to influence the growth, advancement, and outcome of cancers [9].

1.10.1. Water-soluble vitamins

The complex Vitamin B (thiamin (vitamin B₁), riboflavin (vitamin B₂), niacin (vitamin B₃), pantothenic acid, vitamin B₆, folate and folic acid, vitamin B₁₂), along with vitamin C, form the water-soluble vitamins group. Various water-soluble vitamins are precursors of several coenzymes that are needed for the metabolism catalyzed by several enzymes [86]. Common syndromes caused by a water-soluble vitamin deficiency, such as scurvy (vitamin C) and beriberi (thiamine), are very uncommon in Western societies, although there is a considerable study as to whether inadequate intakes of such vitamins are related to the risk of significant chronic diseases such as cancer and heart disease [87, 88]. Water-soluble vitamins play an important role in breast cancer-related biological processes, especially those vitamins which are a necessary component of antioxidants. Antioxidant vitamins are suspected to have chemopreventive effects. Antioxidants of water-soluble vitamins which include ascorbic acid (vitamin C) are believed to reduce the risk of cancer through avoiding tissue damage, via capturing organic free radicals, or by deactivating excited molecules of oxygen [89]. It has been shown that water-soluble vitamin plays an important role in breast cancer biology; however, our understanding of the relationship between vitamin functions and the initiation, progression, and inhibition of carcinogenic processes in breast cancer is still gaping [90-92]. Therefore, in the serum of humans with or without cancer, there is a need for water-soluble vitamin analysis that can reveal the relationship between breast cancer and these vitamins.

1.10.2. Fat-soluble vitamins

fat-soluble vitamins such as vitamin A and its precursor (beta-carotene, etc) retinol and vitamin E. Classical vitamin deficiencies can clinically manifest as night blindness (vitamin A), increased oxidative cell stress (vitamin E) [93]. For a broad range of physiological functions and their catalytic roles in anabolic and catabolic pathways, the presence of fat-soluble vitamins (A, (retinol beta-carotene), E) in humans is of vital importance. These vitamins have a common absorption mechanism from the intestine along with fat, due to their solubility in fat [94].

In recent decades, vitamin deficiencies have been linked to increased cancer risk and a variety of immune system disorders. In addition, there is considerable evidence of interactions, especially among vitamins A and E [93, 95]. Experimental studies have shown that these vitamins neutralize reactive oxygen species (free radicals) and thus inhibit cellular damage caused by the reaction of protein and nucleic acid with these species. It is also believed that fat-soluble vitamins play a very important role in cancer prevention. For example, vitamin A may prevent tumor growth [96], carotenoids, and vitamins E may be protective because of antioxidant effects [97]. By quenching singlet oxygen, vitamin A (retinol) has a powerful antioxidant effect. It helps to division and differentiation of cells, regulates the immune system,

inhibits the existence or impedes the growth of cancer cells, as well as offers human anti-mutagenic, anti-tumoral, and degenerative properties [98]. The most prevalent source of vitamin A in foods is retinol, although many carotenoids commonly available in nature also have a pro-vitamin A effect, β -carotene is the most prevalent form. β -carotene can also prevent the risk of cancer due to its conversion to retinol, as retinol via its function as an antioxidant and scavenger of free radicals is involved in cell differentiation regulation [99].

Vitamin E (α -tocopherol) can prevent carcinogens from forming precursor compounds. [100]. Vitamin E by binding to free radicals and neutralizing its unpaired electrons has antioxidant effects, it scavenges lipid proxy radicals both in vivo and in vitro systems and also helps in wound healing processes. Vitamin E is protective both against the activation of tumors and against promoters. It also improves the immune response of the body and can control the expression of genes in mammalian cells [101]. Vitamin E and β -carotene have antioxidant activity, therefore, provide cellular defense against DNA-damaging by reactive oxygen species [102].

In order to determine their status, fat absorption disorders, or toxic levels, nutritionists and clinicians are increasingly important in evaluating fat-soluble vitamins in human plasma; their concentrations are considered as useful markers, especially in clinical and metabolomics studies.

1.11. Aims and objectives

- To evaluate the status of stress bio marker (MDA and 4-HNE, glutathione, oxidized glutathione disulfide (GSSG) and the ratio of GSH/GSSG) in women with breast cancer compared with the healthy control group. However, to the evaluation of vitamins and amino acids among studied groups.

1.11.1. Objectives of the study (should be in points)

O Define and match the levels of serum oxidative stress and antioxidant parameters (MDA and 4-HNE, glutathione, and GSH/GSSG) in breast cancer patients and healthy control groups

O Make a decision about whether there is the relationship of these antioxidants (glutathione, oxidized glutathione disulfide (GSSG) and the ratio of GSH/GSSG) and oxidative stress (MDA, 4-HNE) with clinical parameters or not.

O Evaluation of water-soluble vitamin with the fat-soluble vitamin in the serum sample

O Knowing the effecting of the vitamin in breast cancer patients

O Investigate amino acids in patients with breast cancer and healthy control group

O Determine the effecting of breast cancer on amino acids and vice versa

O Measure of the quantity of amino acids between breast cancer patient and healthy control groups

O To evaluate the effect of breastfeeding and take treatments on study parameters, then on breast cancer patients

1.11.2. Hypothesize

- Oxidative stress leads to the development of breast cancer, and biomarkers of oxidative stress (MDA, 4-HNE) will increase significantly in serum patients with breast cancer.
- Glutathione (GSH) contribute to preventing oxidative stress however GSH deficiency, or a decrease in the GSH/glutathione disulfide (GSSG) ratio, leads to an increased susceptibility to oxidative stress implicated in the progression of breast cancer
- Quantity of glutathione decreases in the serum of breast cancer patients compared in the control healthy group apposite the value of GSSG is an increase in patient groups.
- Glutathione is an inverse relationship with MDA, 4-HNE, and GSSG.
- Vitamins have a protective effect on breast cancers
- Plasma amino acid levels in breast cancer patients vary from the healthy comparison group.
- Breast feeding among have protective effect against breast cancer.
- Treatments may be alter the biochemical parameter (vitamins, oxidative stress parameter and amino acids).

2. MATERIALS AND METHOD

We describe the study method and materials in this chapter, also the instruments that we used in this study, we explain below, as well as the software program we used to explain the statistical parameter.

2.1. Materials

Instruments and equipment used in this study are listed as follows:

- High Performed Liquid Chromatography (HPLC) PDA detector
- Vortex mixer
- Centrifuge (KOKUSAN, H-19F)
- Water bath (Mettler, WNB 7, Germany)
- pH-meter (JENWAY-3505)
- Sensitive balance (DENVER IN)
- Micro tube centrifuge
- Oven
- Micro-pipette
- Plastic tube
- Glass tube
- HPLC vial
- Deep freezer for sample storage

Chemicals and standards

Chemicals and standards used in this study which supplied them from Sigma Chemical Company (Istanbul, Turkey) were listed below:

- HCL
- 1,1,3,3-tetra ethoxy propane
- Standard glutathione (GSH)
- Standard oxidized glutathione (GSSG)
- Perchloric acid (HClO_4)
- Sodium perchlorate (NaClO_4)
- Ethanol
- n-hexane
- Phosphoric acid (H_3PO_4)
- Acetonitrile (CH_3CN)
- Methanol

- Standard HNE
- Standard of vitamin C and B₁, B₂, B₃, B₅, B₆, B₉, B₁₂
- Standard of vitamin A, E, β -carotene
- Standard of amino acids
- Twice Distilled water

2.1.1. Subjects

77 of serum sample which takes in this study distributed as 45 with breast cancer and 32 as healthy controls group and among the patient groups 32 subjects is pretreatment that means didn't take any drug and 13 of the patient is post-treatment which treatment include drug supplement and chemotherapy besides 15 of the patient in a lifetime have breastfeeding and other 30 patients didn't have breastfeeding. all participants of this study age ranged between 20-65 years were selected in this study. Those women visited the Hiwa Cancer Hospital during the period from "September 2019 till October 2019"; Specialty doctors diagnosed the disease of breast cancer. All patients were subject to a personal interview using a specially designed questionnaire form of full history with accurate information. (Appendix-2).

2.1.1.1 Eligibility

Inclusion criteria

These criteria include Women with breast cancer and healthy women that their age ranges between 20-65.

Exclusion criteria

These criteria include Patients with diabetes mellitus, pre-diabetes, chronic liver or renal disease, and malignancy other than breast cancer and smoking, and alcohol consumption.

2.2. Method

We describe the study sample collection with a method of the study in this portion.

2.2.1. Samples collection

The samples were collected in Hiwa Cancer Hospital during the period from "September 2019 till October 2019"

The study was conducted in volunteers whose written consent was obtained prior to the study, with the approval of the study protocol by the institutional ethical committee (2019, issue no. 1) (Appendix-3) Document date and number 355284.

Venous Blood samples are withdrawn by using 10 mL syringes with steel needles. Six mL of the blood sample was taken from each patient. The blood was put into a plain tube. These samples

were allowed to stand for 10 minutes to clot at room temperature, then the tube was centrifuged at 3000 rpm for 10 minutes. Then the serum was transferred to the Eppendorf tube and stored at -20°C until the analysis day at Firat University laboratories in Turkey and hemolysis samples were discarded.

All samples, classified as below in two groups:

Group 1: the healthy control group comprises 32 healthy women in the range of age (20-65) years with no previous diseases that may interfere with the analyzed parameters.

Group 2: The breast cancer group comprised 45 patient women with the range of age (20-65) years and all patients were diagnosed by specialty doctors and the study was confirmed by the Ethical Committee of the Center and subjected to a personal interview with detailed information, using a specially designed questionnaire form to the full history.

2.2.2. Determination of Body Mass Index (BMI)

Measured height to nearest 0.5 cm and weight to nearest 0.1 kg. Body mass index is determined by dividing the weight (Kg) of subjects by their height (meter²). BMI is counted as:

$$BMI = \frac{\text{Mass(kg)}}{[\text{Height m}^2]}$$

BMI (< 16-18.5) is indicated to have underweight (18.5-24.9) is an indication of normal weight. A person with BMI (25-29.9) is overweight, while a person with BMI > 30-35 is moderately obese, > 35-40 is obese severity [103].

2.2.3. Sample analysis

Determination of the level of MDA, 4-HNE, GSH, GSSG, vitamins and amino acids in Serum by Using the method of High-Performance Liquid Chromatography (HPLC). This method is high Sensitivity and low cost

2.2.3.1 HPLC Method

HPLC is a method through which a compound of mixtures is separated into ingredients compounds by flowing mobile phase through a stationary phase

Samples were analyzed by HPLC system which HPLC was done with the model of Shimadzu prominence-İ LC-2030C 3D by PDA detector. equipped with column heater, autosampler, variable wavelength detector and control software for data acquisition, solvent equipped with binary delivery pump sample injected in sample injector. The Eluted peaks were monitored by UV absorbance detector. Standards of the suspected compounds were run for quantities and qualitative study under the same conditions applied to the samples.

2.2.4. Determination of water-soluble vitamins

First of all prepared standard vitamins (B vitamins and vitamin C) stock solution then the stock solution was diluted in different concentrations to know the peak area of standard vitamins that were used for the calibration graph. (Appendix-1)

Take 0.3 mL of serum and add 0.3 mL of 0.5 M perchloric acid (HClO_4), to precipitate proteins. After adding 2.4 mL of distilled water so the final volume of the solution is got 3ml after the solution was mixed by vortex for a few seconds then centrifuged for 10 min by the speed of 3000 rpm afterward supernatant is ready to analyses by HPLC.

The mobile phase was prepared through 0.38 g heptane sulfonic acid was dissolved in 125 mL of methanol, and add 375 mL of distilled water with 0.38 mL of triethylamine (TEA), the pH of the mixture was used by adjusting it to 2.8 with phosphoric acid (H_3PO_4). For determination of water soluble vitamins, we used the C18-DB column (15 cm length x 4.6 mm inner diameter x 5 μm particle size) The flow rate was 1.5 mL min^{-1} . Chromatograms were monitored at 260 nm wavelength for thiamin, nicotinamide, and riboflavin, 245nm for vitamin C while pyridoxine and folic acid are absorbance's at 290 nm and cobalamin occurred absorbance at 500 nm of wavelengths. and the injection volume was 20 μL [104, 105]. the retention time with the calibration curve of water soluble vitamins are presented in Appendix-1.

2.2.5. Determination of Fat-Soluble Vitamins and 4-HNE

Firstly, prepared stock solution of standard fat-soluble vitamins (vitamin A and, E and β -carotene) and 4-HNE then the stock solution was diluted in various amounts to knowing the peak area of standard vitamins and 4-HNE then used for the calibration graph. (Appendix-1)

For sample preparation, a 0.3 mL of the serum was taken and add 3 mL of ethanol with 1mL n-hexane then centrifuged for 10 minutes at 5000 rpm so the mixture is divided into two portions thus, fat-soluble vitamin and 4-HNE were extracted into the n-hexane phase so separated at the top then put the top portion to glass tube then this extraction process was repeated twice, again add 1 mL n-hexane and centrifuged at 5000 rpm for 10 minutes this for correctly dissolved fat-soluble vitamin and 4-HNE to n-hexane, after taking a top portion to a glass tube, must be evaporated n-hexane by vacuum oven after that add 1ml methanol and mixed by vortex then put 1mL to HPLC vial to an analysis by HPLC, the separation was performed using a Supelcosil LC-18 column (25.0 cm $\times$ 4.6 mm $\times$ 5.0 μm). The mobile phase being used is methanol-acetonitrile-water (63%, 33%, 4%, v/v) respectively at a flow rate of 1.0 mL min^{-1} . The wavelength for identification of individual analytic vitamin A, β -carotene, and vitamin E was 326 nm, 470 nm, and 296 nm respectively and 4-HNE at 223 nm [106, 107]. The working graph and equations used to calculate the concentration of samples with the retention time of the samples are given in Appendix-1.

2.2.6. Determination of MDA, GSH, and GSSG.

- **Preparation of (MDA) Standard Solution:**

Prepare 2.92 ppm of stock solution, by dissolving 10 μ L of 1,1,3,3-tetra ethoxy propane (TEP) in 10 mL of 0.1 M HCl then closed the test tube with screw caps and placed in the water bath to boiling for 10 minutes after quickly cooled with tap water thus the stock solution is ready to prepare a series solution through dilution hence made 6 different concentrations of standard MDA solution, that were used for the calibration graph [108]. (Appendix-1)

- **Preparation of GSH and GSSG Standard Solution:**

Prepared standard solutions for each of GSH and GSSG that are in different concentrations of working standard solution, which were used for the calibration curve. (Appendix-1)

- **Principle and Preparation of Samples to determination MDA, GSH, and GSSG level:**

For sample preparation, take 0.3 mL of serum after completed thawing and add 0.3 mL of 0.5 M perchloric acid (HClO_4) for precipitate proteins and release the MDA, GSH, and GSSG attached to the amino compound. And then completed to 3.0 mL by distilled water and the solution was mixed by vortex for 20 seconds then centrifuged at 3000 rpm for 10 minutes after we take 1mL of the supernatant to the HPLC vial then ready for HPLC analysis.

The chromatographic conditions in terms of peak area, column efficiency, retention time, resolution, and sensitivity were selected. Column Utisil-XB-C-8 (25 cm, 4.6 mm ID, 5 μ m) column in HPLC with PDA detector was used for separation and determination of MDA, GSH, and GSSG. The mobile phase was prepared by dissolving 3.6 g sodium perchlorate ($\text{NaClO}_4 \cdot \text{H}_2\text{O}$) to 375 mL of double distilled water with 0.34 mL of phosphoric acid (H_3PO_4) to adjusted PH =4, and HPLC chromatograms were monitored at 254 nm, 210 nm, and 210 nm for MDA, GSH and GSSG respectively[109]. The calibration curve and equations used to calculate the concentration and retention time of the samples are fixed in Appendix-1.

2.2.7. Determination of amino acids

- **Preparation of (amino acids) standard solution:**

The standard amino acids were dissolved and diluted with 0.1 M HCl aqueous solution prepared with double distilled water to the required concentration Appendix-1

- **Preparation of hydrolyses serum sample**

Take 0.5 mL serum into glass tube then add 5 mL of 6.0 N HCl after mixing by vortex for 10 second then samples by vacuum hydrolyzer are hydrolyzed for 24 hours at 110°C after that we filtration all sample to reduce the big particles then sample completed to 10 mL with double distilled water on the volumetric flask and then keep the sample in the refrigerator until analyses

- **Principle and Preparation of Samples to determination amino acids level:**

We take 100 μ L of Both standard and hydrolyzed samples then drying at 60°C in a vacuum oven then neutralized samples by adding 100 μ L of the mixture of methanol: water: TEA 2:2:1(v / v) respectively, and mixed vigorously with a vortex stirrer, then they were dried under a vacuum oven at 60°C. Derivatization was done through adding 100 μ L ethanol-water–TEA–PITC (7:1:1:1, v/v/v/v) respectively, and mixing well with a vortex stirrer for 10 seconds. The phenyl isothiocyanate (PITC) derivatization of the amino acids to produce phenylthiocarbamyl (PTC) amino acids was performed at room temperature for 30 minutes, after that samples were dried completely by vacuum oven at 40°C. The drying process is repeated by adding 100 μ L of methanol, For each sample and standards, phenylthiocarbamyl amino acids were dissolved from the dried matrix The derivatization procedure was a modification of the method of Kwanyuen, P. and Burton, J.W [110].

The chromatographic analysis of the amino acid derivatives was performed by using Supelcosil LC-18-DB column (25 cm x 2.1 mm ID, 3 μ m particle size). The column temperature was maintained at 40 °C. The wavelength was set at 254 nm and recorded, and the analysis time was 30 min.

A gradient resulting from mixing eluents (mobile phase) A and B have separated and eluted the PTC amino acids, that mobile phase A included 0.07 M NaCH₃COO, (pH 6.4 by H₃PO₄). Mobile phase B included 6:4 acetonitrile: water (v/v). the flow rate was 1.25 mL/min. and the gradient consisted of the profiles is listed in **Hata! Başvuru kaynağı bulunamadı.** [110, 111]. The retention time with a calibration curve of amino acids are given in Appendix-1

Table 2.1.Gradient program used for the separation of Phenylthiocarbamyl-amino acids

Time (minute)	Flow rate (mL/min)	%mobile phase A	% mobile phase B
0.01	0.8	90	10
12.00	0.8	70	30
16.00	0.8	65	35
16.01	0.8	50	50
25.00	0.8	100	0
30.00	0.8	50	50
30.01	0.8	10	90
35.00	0.8	10	90

2.2.8. Statistical Analysis Made:

The data were evaluated using SPSS 22.0 program compatible with Windows. Comparison of categorical variables between the groups was done with Descriptive analysis. Comparison of continuous variables between the multiple groups was done with the One-Way ANOVA test.

The homogeneity between patients with breast cancer and healthy controls in terms of general characteristics were examined in Pearson Chi-squared tests.

Correlation analysis of variables that were evaluated by Pearson analysis and Regression analysis. Results were expressed as mean $\pm$ standard deviation $p < 0.05$ and $0 < 0.001$ with were considered statistically significant.



3. RESULT

3.1. Patient Descriptive Data

In this section we describe all feature of participant that effect this study

3.1.1. Distribution of Groups

The present study included 77 volunteers divided into two groups; the first group is the volunteer patients with breast cancer 45 subjects, the second group is the 32 apparently healthy individuals as a control group, both shown below in **Figure 3.1** with their percentage.

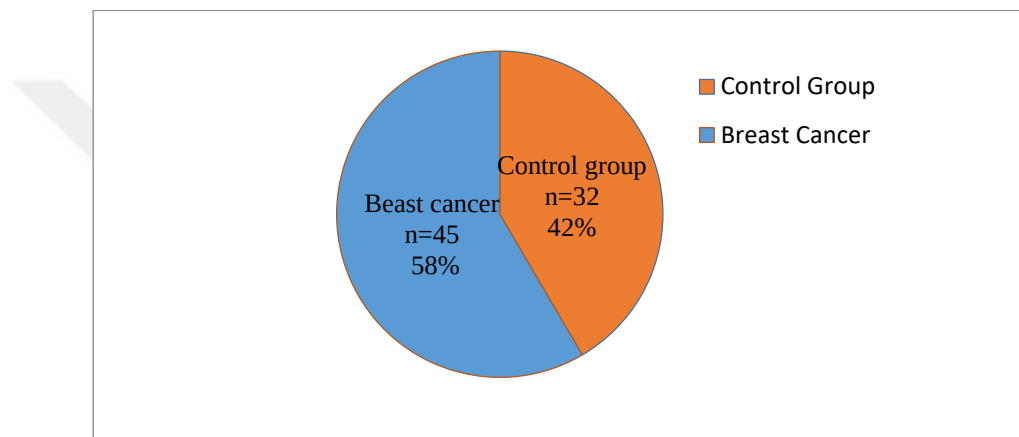


Figure 3.1. Distribution of studying group.

3.1.2. General characteristic of groups

The general characteristics of the groups in the underlying study shown in **Table 3.1** include; Age, body mass index, participant family history, Marital Status, parity, and breastfeeding.

The study included a total of 45 female breast cancer patients who were recruited from the same demographic area. As shown in Table 3.1. and Figure 3.1, the general characteristics of 45 breast cancer subjects and 32 healthy control groups were compared. The mean age of women with breast cancer was 47.26 ± 11.41 years. The youngest patient with breast cancer was a 23-year-old and the distribution of age groups is represented in Table 3.1 and Figure 3.2 which indicates that the lowest percentage (7%) of breast cancer patients was <30 years, while the highest percentage (40%) was at ≥ 50 years. There was a significant increase of body max index among breast cancer patients 29.62 ± 5.43 kg/m² than control groups 25.43 ± 5.47 kg/m² in addition a significant association of obesity 44% with breast cancer was observed ($p < 0.05$) as shown in table 3.1 and figure 3.3. A family history of breast cancer was observed in 44% of patients. Regarding the marital status, parity, and breastfeeding, it was shown that most of the breast cancer patients were found in

married women 80% than single 20%. Parity among breast cancer patient was found 60% having a child, Breastfeeding was observed in 33% of women among the patient groups.

Table 3.1. General characteristic data for all studied groups.

Characteristics	Study groups		P-Value
	Breast cancer (n=45)	Control (n=32)	
Age (yrs.) Mean±SD	47.26±11.41	38.81±11.61	0.002 ^a
Age distribution No. (%)			0.113 ^b
<30 yrs.	3 (7.0%)	8 (25.0%)	
30-39	8 (15.0%)	7 (22.0%)	
40-49	16 (38.0%)	8 (25%)	
≥50	18(40.0%)	9 (28.0%)	
BMI (kg/m ²) Mean±SD	29.62±5.43	25.43±5.47	0.001 ^a
BMI distribution No. (%)			0.016 ^b
Normal weight	11 (25.0%)	18 (56.0%)	
Overweight	14 (31.0%)	7 (22.0%)	
Obese	20(44.0%)	7(22.0%)	
Family history of BC No. (%)			0.027 ^b
Yes	20 (44.0%)	6 (19.0%)	
No	25 (56.0%)	26 (81.0%)	
Marital Status No. (%)			0.024 ^b
Married	36 (80.0%)	17(53.0%)	
single	9 (20.0%)	15 (47.0%)	
Parity No.(%)			0.353 ^b
Parent	27 (60.0%)	15(47.0%)	
Nulliparity	18(40.0%)	17(53.0%)	
Breastfeeding No.(%)			0.244 ^b
yes	15(33.0%)	14(44.0%)	
no	30(67.0%)	18(56.0%)	

^a one way anova test, and ^b Pearson Chi-squared test were performed for statistical analyses.

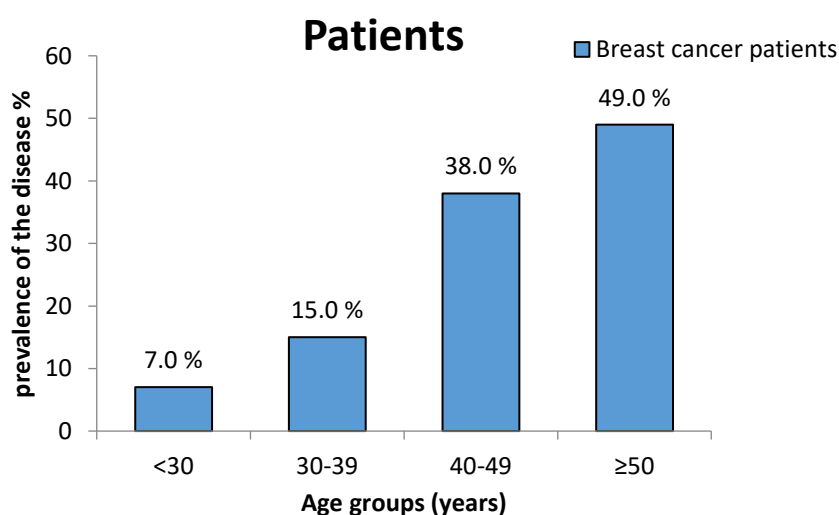


Figure 3.2. Prevalence of breast cancer classified by age groups.

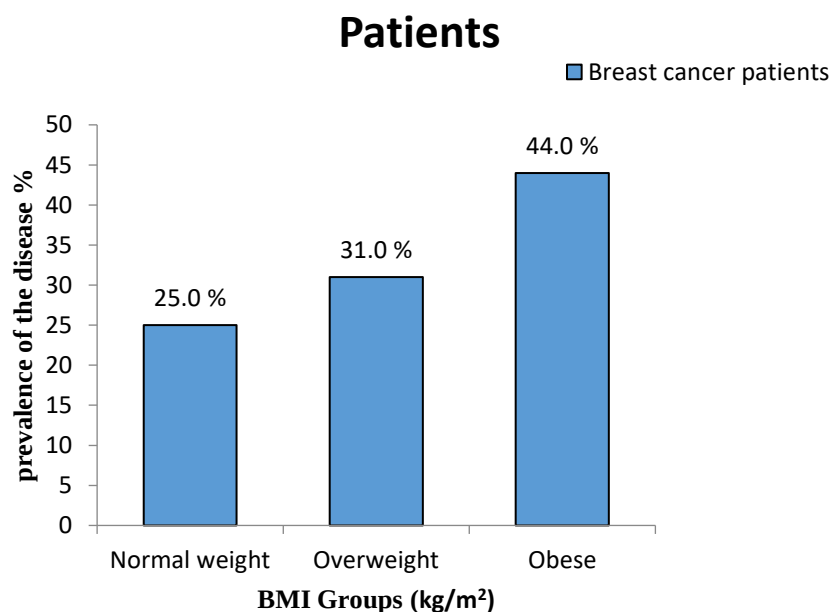


Figure 3.3. Prevalence of breast cancer classified by body mass index (BMI).

3.2. Vitamins Levels

Table 3.2.and Figure 3.4. Show a significant difference between the concentration of water and fat soluble vitamins in breast cancer patients and healthy control groups. Most of the water soluble vitamins studied include thiamine (B₁), Riboflavin (B₂), Nicotinamide (B₃), folic acid (B₉), and Pyridoxine (B₆) were found significantly decreased in the breast cancer group compared to healthy groups with ($p < 0.05$) except vitamin C, pentatonic acid (B₅), and cobalamin (B₁₂) levels had non-significant($p > 0.05$) difference between breast cancer and healthy groups.

However, for vitamin A strong significantly $p < 0.001$ decreased in breast cancer patients 0.485 ± 0.121 $\mu\text{g/mL}$ compared with healthy control subjects 1.891 ± 0.651 $\mu\text{g/mL}$. The results of vitamin E showed there were a highly significant difference ($p < 0.001$) between healthy control subjects 9.177 ± 0.099 $\mu\text{g/mL}$ and breast cancer subjects 4.269 ± 0.043 $\mu\text{g/mL}$. Serum β -carotene levels were decreased high significantly in breast cancer 0.542 ± 0.174 $\mu\text{g/mL}$ compared with healthy control group 0.89 ± 0.196 $\mu\text{g/mL}$ with $p < 0.001$.

Table 3.2. The concentration of vitamins in healthy control and breast cancer subjects.

Biochemical parameters	Study groups (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Breast cancer (n=45)	Control (n=32)	
Vitamin C	16.645 ± 4.154	17.865 ± 4.679	0.235 N.S.
Vitamin B ₁	0.966 ± 0.253	1.1594 ± 0.335	0.007*

Vitamin.B ₂	0.671± 0.241	0.891 ± 0.272	0.001**
Vitamin.B ₃	2.326± 0.534	2.63±0.469	0.014*
Vitamin B ₅	0.809±0.215	0.810±0.163	0.983 N.S.
Vitamin B ₆	0.1465± 0.047	0.179±0.060	0.013*
Vitamin B ₉	0.461± 0.176	0.583±0.244	0.017*
Vitamin B ₁₂	102.55±70.831	153.419±162.470	0.050 N.S.
Vitamin A	0.485± 0.121	1.891±0 .651	0.000**
Vitamin E	4.269±0.043	9.177±0.099	0.000**
Beta Caroene	0.542±0.174	0.89±0.196	0.000**

one way ANOVA test, were performed for statistical analyses. *p<0.05 was significant and **p<0.001 was highly significant and N.S was Non-Significant

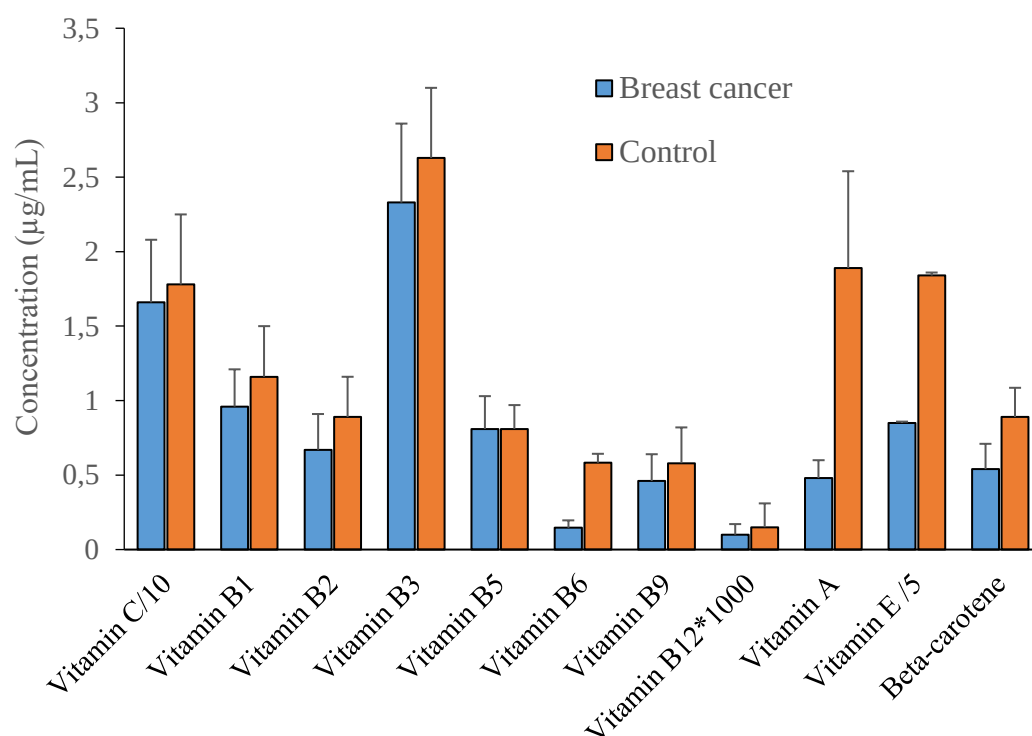


Figure 3.4. Mean Concentration of vitamins for studied groups (for clarity and to bring into scale; Vitamin B12 values multiplied by 1000, vitamin E values divided by 5, vitamin C divided by 10).

3.2.1. The effect of taking treatments on the level of vitamins in breast cancer patients

Table 3.3 and figure 3.5 demonstrate non-significant differences in serum levels of vitamins across the intervals of taking drug treatments such as chemotherapy and drug supplements with pre-treatment which didn't take any drug between women with breast cancer ($p > 0.05$).

Table 3.3. Mean concentration of vitamins parameters in breast cancer women considering take treatments

Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Pre treatment (n=31)	Underline treatments (n=14)	
Vitamin C	16.145 $\pm$ 4.405	17.839 $\pm$ 3.337	0.221 N.S.
Vitamin B ₁	0.94 $\pm$ 0.263	1.025 $\pm$ 0.225	0.320 N.S
Vitamin B ₂	0.659 $\pm$ 0.266	0.695 $\pm$ 0.18644	0.662 N.S
Vitamin B ₃	2.388 $\pm$ 0.601	2.195 $\pm$ 0.340	0.289 N.S
Vitamin B ₅	0.747 $\pm$ 0.215	0.937 $\pm$ 0.156	0.007 N.S.
Vitamin B ₆	0.147 $\pm$ 0.046	0.144 $\pm$ 0.051	0.865 N.S
Vitamin B ₉	0.432 $\pm$ 0.136	0.521 $\pm$ 0.233	0.135 N.S
Vitamin B ₁₂	94.814 $\pm$ 71.06	118.61 $\pm$ 70.36	0.326 N.S.
Vitamin A	0.450 $\pm$ 0.104	0.499 $\pm$ 0.126	0.218 N.S
Vitamin E	3.921 $\pm$ 0.518	4.414 $\pm$ 0.173	0.154 N.S
Beta Carotene	0.525 $\pm$ 0.141	0.549 $\pm$ 0.188	0.678 N.S

one way ANOVA test, were performed for statistical analyses. *p<0.05 was significant and **p<0.001 was highly significant and N.S was Non-Significant

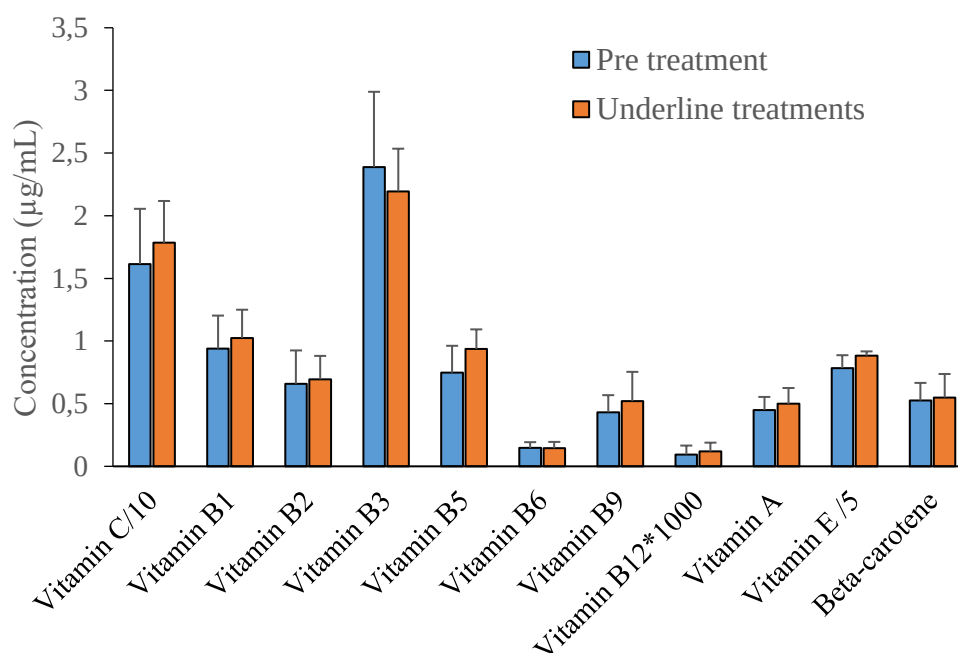


Figure 3.5. Concentration of vitamins parameters in breast cancer women considering take treatments.

3.2.2. Effect of breastfeeding on vitamins level among breast cancer patients

Vitamins level, which classified according to who have breastfeeding and who didn't breastfeed between breast cancer women that are illustrated in Table 3.4 and figure 3.6. The differences between the mean values of all vitamin parameters remained statistically non-significant $p>0.05$.

Table 3.4. Mean concentration of vitamins parameters in breast cancer women considering breastfeeding

Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Have breastfeeding (n=15)	Didn't have breastfeeding (n=30)	
Vitamin C	16.728 $\pm$ 4.358	16.598 $\pm$ 4.114	0.922 N.S.
Vitamin B ₁	0.89 $\pm$ 0.26	1.006 $\pm$ 0.244	0.172 N.S
Vitamin.B ₂	0.592 $\pm$ 0.249	0.715 $\pm$ 0.23	0.118 N.S
Vitamin.B ₃	2.10 $\pm$ 0.42	2.44 $\pm$ 0.557	0.052 N.S
Vitamin B ₅	0.804 $\pm$ 0.161	0.811 $\pm$ 0.242	0.921 N.S.
Vitamin B ₆	0.132 $\pm$ 0.04	0.153 $\pm$ 0.05	0.188 N.S
Vitamin B ₉	0.476 $\pm$ 0.136	0.453 $\pm$ 0.196	0.695 N.S
Vitamin B ₁₂	105.92 $\pm$ 55.54	100.73 $\pm$ 78.81	0.828 N.S.
Vitamin A	0.47 $\pm$ 0.13	0.492 $\pm$ 0.117	0.610 N.S
Vitamin E	4.205 $\pm$ 0.651	4.305 $\pm$ 0.221	0.764 N.S
Beta Caroene	0.552 $\pm$ 0.179	0.537 $\pm$ 0.174	0.791 N.S
one-way ANOVA test, were performed for statistical analyses. * $p<0.05$ was significant and ** $p<0.001$ was highly significant and N.S was Non-Significant			

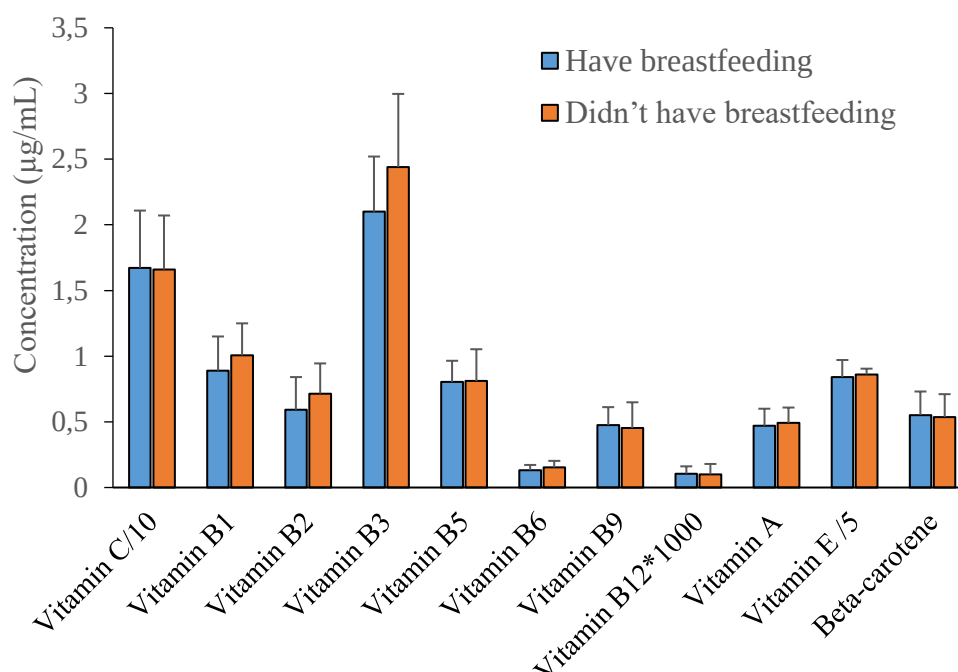


Figure 3.6. Concentration of vitamins parameters in breast cancer women considering breastfeeding.

3.3. MDA and 4-HNE levels

Serum MDA and 4-HNE levels were estimated in participants as oxidative stress markers. The mean serum MDA level in the BC patients group was 5.023 ± 1.107 $\mu\text{g/mL}$ the value was a highly significant ($p < 0.001$) increase compared with that of the control groups 0.329 ± 0.062 $\mu\text{g/mL}$ furthermore the mean serum 4-HNE levels showed a significant increase ($p < 0.001$) in breast cancer patients 9.053 ± 2.673 $\mu\text{g/mL}$ compared with the control group 4.752 ± 1.911 $\mu\text{g/mL}$ as shown in Table 3.5 and figure 3.7.

Table 3.5. Values of MDA between Studied Groups (mean $\pm$ SD).

Biochemical parameters	Study groups (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Breast cancer (n=45)	Control (n=32)	
MDA	5.023 ± 1.107	0.329 ± 0.062	0.000**
4-HNE	9.053 ± 2.673	4.752 ± 1.911	0.000**

one-way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and

** $p < 0.001$ was highly significant and N.S was Non-Significant

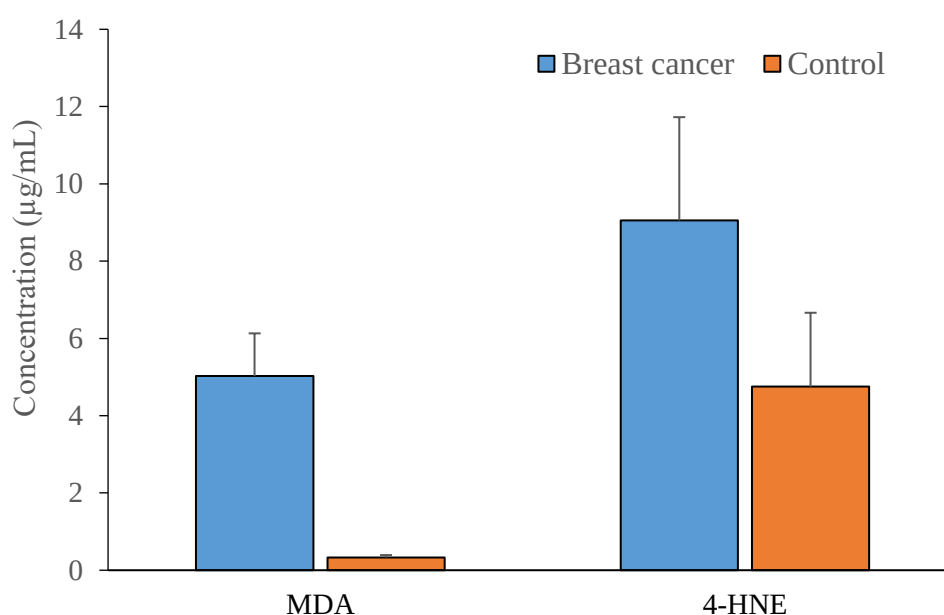


Figure 3.7. Mean Concentration of Oxidative Marker MDA and 4-HNE for Studied Groups.

3.3.1. The effect of taking treatments on MDA and 4-HNE levels in breast cancer patients

Biochemical parameters of oxidative stress (MDA, 4-HNE) regarding drug treatments classified into two groups who underline treatments and pretreatments who didn't take any treatments in breast cancer patients are illustrated in Tables 3.6 and figure 3.8. The differences between the mean values of the two parameters remained statistically non-significant in groups ($p > 0.05$).

Table 3.6. Mean concentration of MDA and 4-HNE in breast cancer women considering take treatments

Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Pre treatment (n=31)	Underline treatments (n=14)	
MDA	5.194 $\pm$ 1.133	4.629 $\pm$ 0.97	0.126N.S.
4-HNE	9.440 $\pm$ 2.701	8.160 $\pm$ 2.478	0.152N.S

A one-way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and ** $p < 0.001$ was highly significant and N.S was Non-Significant

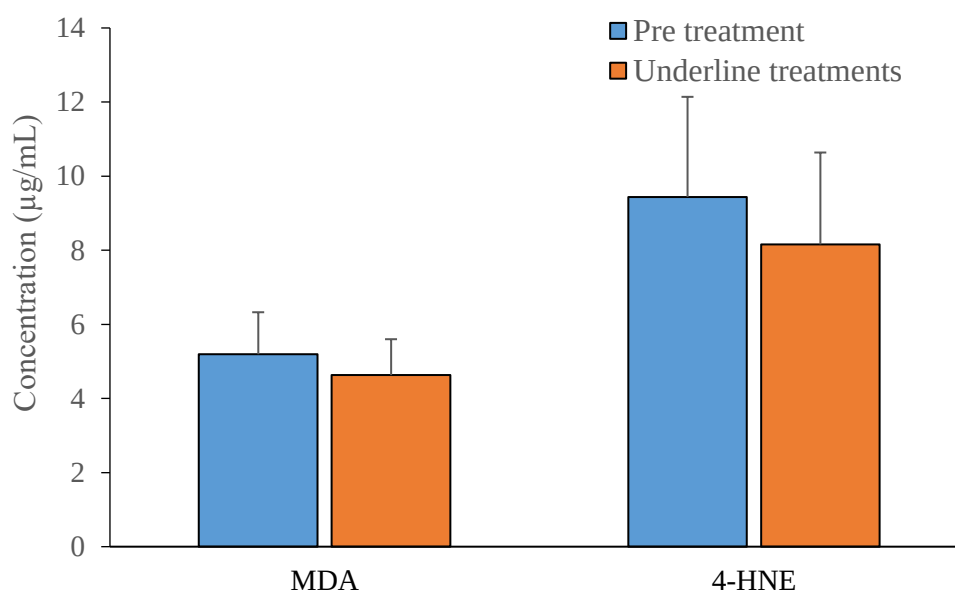


Figure 3.8. Concentration of MDA and 4-HNE in breast cancer women considering take treatments

3.3.2. Effect of breastfeeding on MDA and 4-HNE level among breast cancer patients

Regarding breastfeeding, the mean concentration of MDA and 4-HNE of the breast cancer groups are illustrated in the Table 3.7 and Figure 3.9 there were non-significant differences in serum MDA and 4-HNE levels between those have breastfeeding with didn't breastfeed groups ($p > 0.05$).

Table 3.7. Mean concentration of MDA and 4-HNE level in breast cancer women considering breastfeeding

Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Have breastfeeding (n=15)	Didn't have breastfeeding (n=30)	
MDA	4.891 $\pm$ 0.120	5.101 $\pm$ 0.112	0.554 N.S.
4-HNE	8.783 $\pm$ 0.765	9.213 $\pm$ 0.657	0.616 N.S

one-way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and

** $p < 0.001$ was highly significant and N.S was Non-Significant

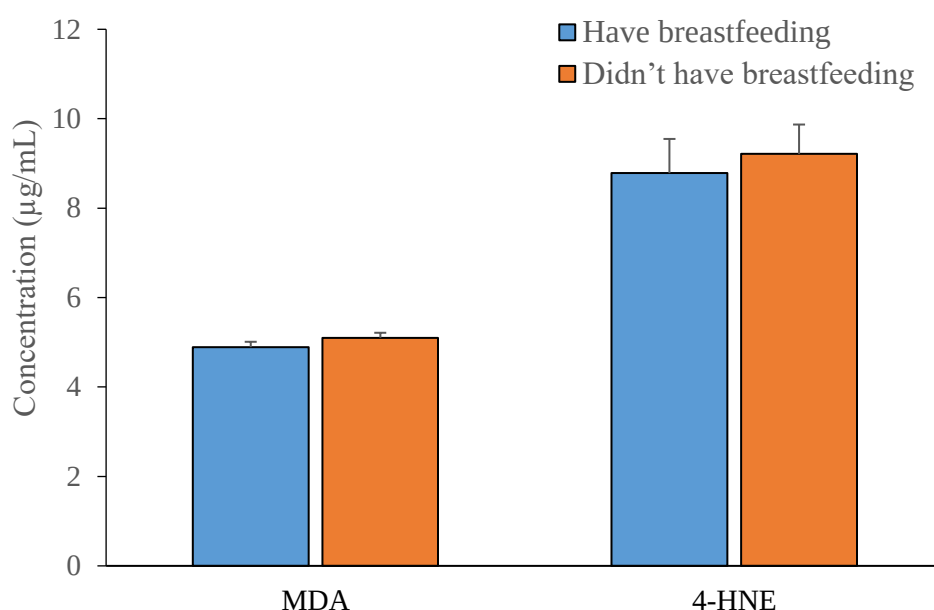


Figure 3.9. Concentration of MDA and 4-HNE level in breast cancer women considering breastfeeding

3.4. Glutathione GSH levels

In breast cancer patients the serum level of the antioxidant glutathione (GSH) decreased significantly ($p < 0.001$) compared with healthy control groups as **Table 3.8** and Figure 3.10. When comparing the GSSG values of the groups as shown, the GSSG level of individuals with breast cancer was found to be significantly ($p < 0.001$) increased than those of the control groups. The relative change in concentrations of GSH and GSSG is measured as the GSH / GSSG ratio that is considered one of the symptoms of oxidative stress. In this analysis, we also found that the mean GSH/GSSG ratio in breast cancer patients decreased significantly ($p < 0.001$) compared to healthy control groups as in **Table 3.8**. this reduction in the GSH/GSSG ratio shows an exposure of patients with breast cancer to oxidative stress.

Table 3.8. Values of GSH, GSSG, and ratio of GSH/GSSG between Studied Groups (mean $\pm$ SD).

Biochemical parameters	Study groups (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Breast cancer (n=45)	Control (n=32)	
GSH	139.483 $\pm$ 60.981	265.219 $\pm$ 55.356	0.000**
GSSG	56.786 $\pm$ 36.651	30.083 $\pm$ 7.562	0.000**
GSH/GSSG	3.7156 $\pm$ 2.365	8.9547 $\pm$ 1.166	0.000**

one way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and

** $p < 0.001$ was highly significant and N.S was Non-Significant

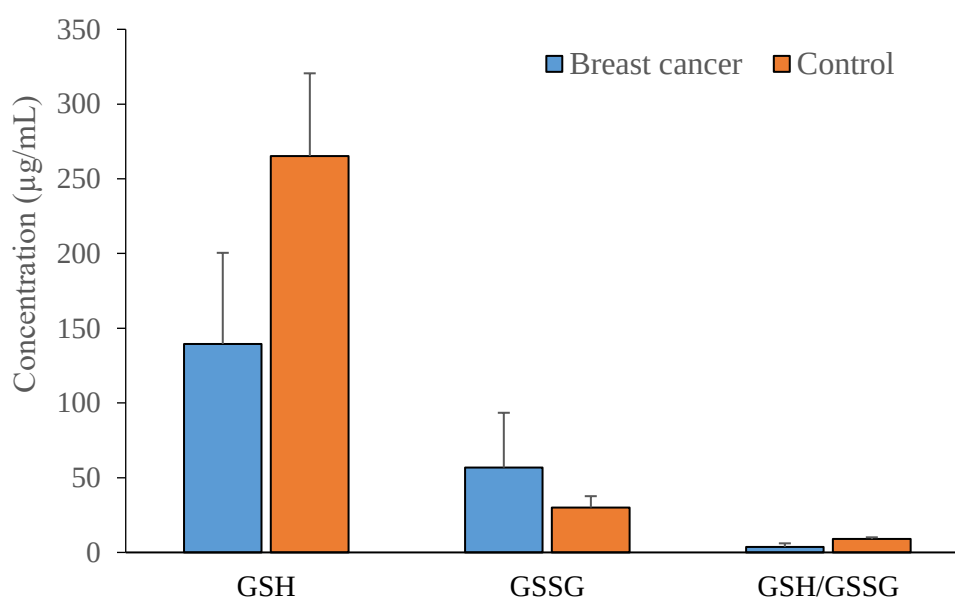


Figure 3.10. Mean Concentration (µg/mL) of (GSH, GSSG) for studied groups.

3.4.1. The effect of taking treatments on GSH, GSSG, and GSH/GSSG levels in breast cancer patients

From the study of the taking drug treatments on GSH, GSSG, and ratio GSH/GSSG in breast cancer patients as dedicated in Table 3.9 and Figure 3.11 results revealed a significant increase in the GSH and ratio GSH/GSSG and non-significant decreased in GSSG ($p > 0.05$) in groups that taking drug treatments than who pretreatment which didn't take any drug ($p < 0.05$) in breast cancer patients group.

Table 3.9. Mean concentration of GSH, GSSG, and ratio GSH/GSSG in breast cancer women considering take drug treatments

Biochemical parameters	Breast cancer patients group (Mean µg/mL ± SD)		P-Value
	pre treatment (n=31)	underline treatments (n=14)	
GSH	119.83±52.07	180.29±59.48	0.002*
GSSG	60.72±38.501	48.60±32.35	0.334N.S
GSH/GSSG	3.21±2.293	4.804±2.22	0.043*

one way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and ** $p < 0.001$ was highly significant and N.S was Non-Significant

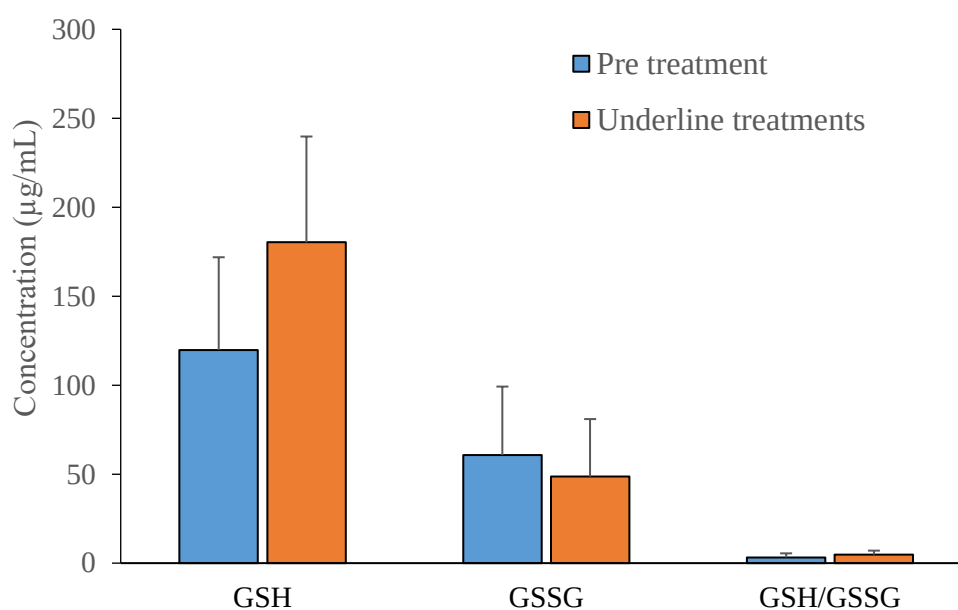


Figure 3.11. Concentration of GSH, GSSG, and ratio GSH/GSSG in breast cancer women considering take a treatments

3.4.2. Effect of breastfeeding on GSH, GSSG, and ratio GSH/GSSG level among breast cancer patients

According to breastfeeding in breast cancer patients, as shown in Table 3.10 and Figure 3.12 there were an increased significantly in mean values of serum GSH and GSH/GSSG 169.57 ± 63.06 $\mu\text{g/mL}$ and 5.089 ± 2.227 $\mu\text{g/mL}$ respectively with ($p < 0.05$), while serum GSSG 40.297 ± 21.63 $\mu\text{g/mL}$ were decreased statistically significant among those patients who breastfeeding compared with those who didn't breastfeed in their lifetime.

Table 3.10. Mean concentration of GSH, GSSG, and ratio GSH/GSSG level in breast cancer women considering breastfeeding

Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
	Have breastfeeding (n=15)	Didn't have breastfeeding (n=30)	
GSH	169.57 $\pm$ 63.06	123.281 $\pm$ 54.34	0.020*
GSSG	40.297 $\pm$ 21.63	65.665 $\pm$ 40.22	0.035*
GSH/GSSG	5.089 $\pm$ 2.227	2.923 $\pm$ 2.095	0.003*

one way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and ** $p < 0.001$ was highly significant and N.S was Non-Significant

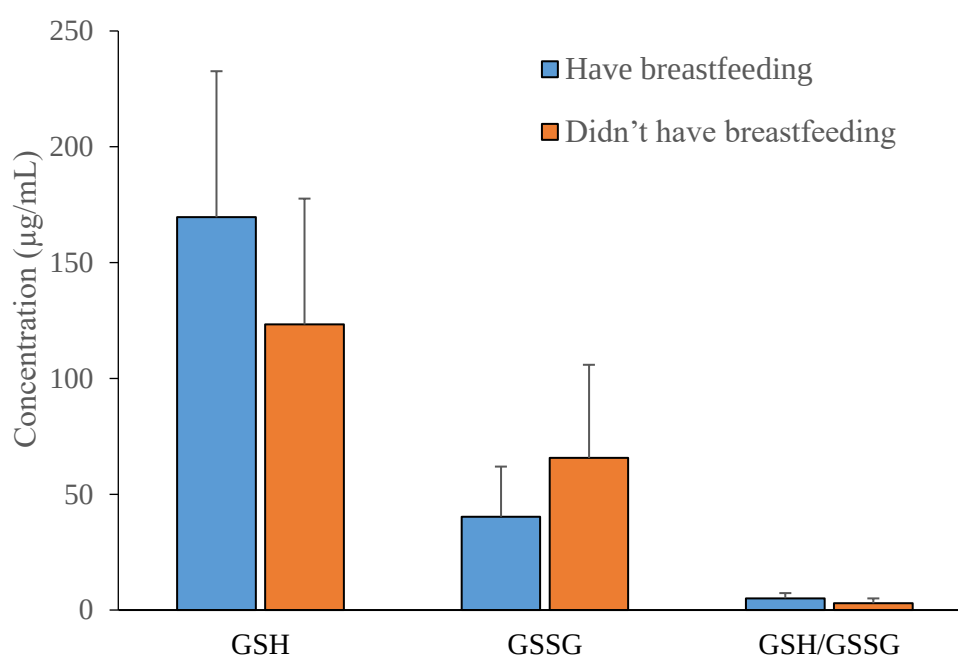


Figure 3.12. Concentration of GSH, GSSG, and ratio GSH/GSSG level in breast cancer women considering breastfeeding

3.5. Correlation analysis Pearson Correlation (r) between oxidant and antioxidant parameter

According to Pearson correlation coefficient (r), as illustrated in Table 3.11. and Figure 3.13. Our findings in the whole study participants showed that serum MDA level had a positively significant correlation with HNE level $r = -0.836$, $p < 0.001$, and also had a positively significant correlation with GSSG $r = 0.500$, $p < 0.001$, while had a negative high significant relation with GSH $r = -0.744$, $p < 0.001$, and with a ratio of GSH/GSSG

According to GSH observed in table 3.11 and figure 3.14. a negatively significant correlation with HNE and GSSG ($r = -0.612$, $p < 0.001$), ($r = -0.422$, $p < 0.001$) respectively put had positive correlation with ratio GSH/GSSG $r = 0.777$, $p < 0.001$. About the concentration of GSSG level had a positively significant correlation with HNE ($r = 0.516$, $p < 0.001$), and a negatively high significant correlation with ratio GSH/GSSG ($r = -0.759$, $p < 0.001$), however HNE have negative correlation with ratio of GSH/GSSG ($r = -0.738$, $p < 0.001$).

Table 3.11. Correlation analysis (r) of oxidant and antioxidant parameter between study groups

		Pearson Correlations				
	Variable	MDA	HNE	GSH	GSSG	GSH/GSSG
MDA	Pearson Correlation	1	0.836**	-0.744**	0.500**	-0.829**
	Sig. (2-tailed)		0.000	0.000	0.000	0.000
HNE	Pearson Correlation	0.836**	1	-0.612**	0.516**	-0.738**
	Sig. (2-tailed)	0.000		0.000	0.000	0.000
GSH	Pearson Correlation	-0.744**	-0.612**	1	-0.422**	0.777**
	Sig. (2-tailed)	0.000	0.000		0.000	0.000
GSSG	Pearson Correlation	0.500**	0.516**	-0.422**	1	-0.759**
	Sig. (2-tailed)	0.000	0.000	0.000		0.000
GSH/GS SG	Pearson Correlation	-0.829**	-0.738**	0.777**	-0.759**	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	

**. Correlation is significant at the 0.01 level (2-tailed).

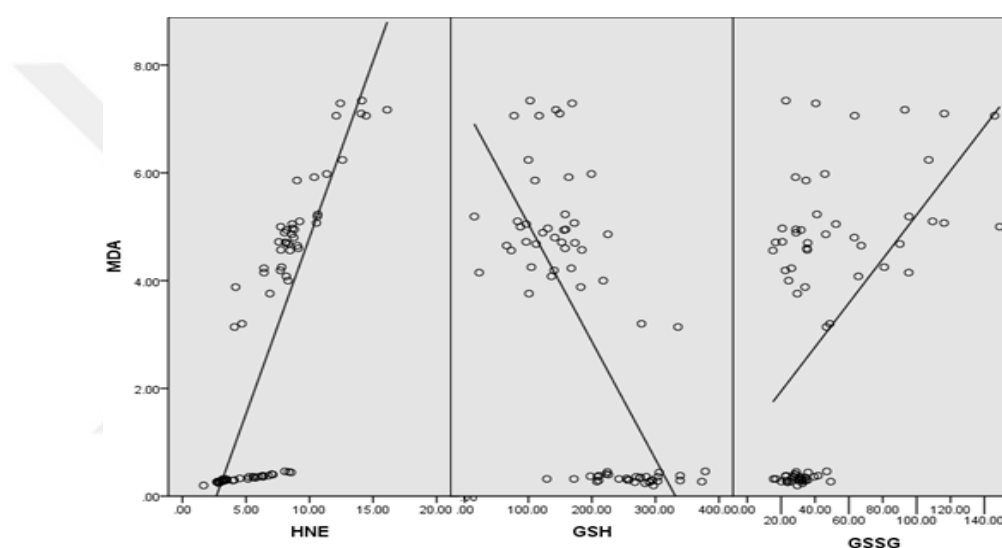


Figure 3.13. correlation analysis (r) MDA with HNE, GSH, and GSSG among study groups

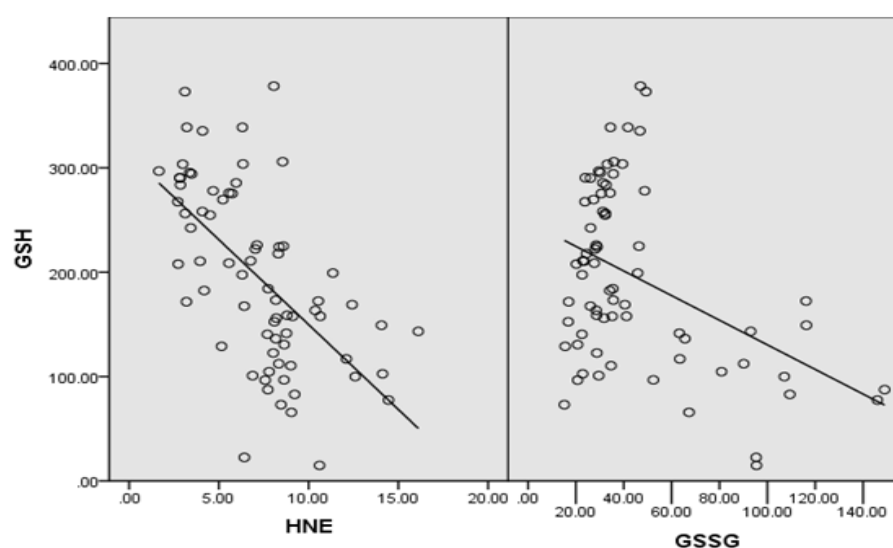


Figure 3.14. Correlation analysis (r) GSH with HNE and GSSG among study groups.

3.5.1. Correlation analysis Pearson Correlation (r) between oxidant and GSH with vitamin parameter

Our results in the whole study population, according to Pearson correlation's shown in Table 3.12, showed that serum MDA level had a negative strong significant correlation $p=0.000$ with vitamin A, E, and β -carotene ($r=-0.605$), ($r=-0.626$), and ($r=-0.792$), also have significant negative correlation with each of vitamin B₁, B₂, B₃, B₆, and folic acid ($r=-0.321$), ($r=-0.374$), ($r=-0.283$), ($r=-0.315$), and ($r=-0.286$) respectively, with $p<0.05$, however HNE have also significant $P<0.05$ negative correlation for each of vitamin B₁, B₂, B₃, B₆, folic acid, A, E, and β -carotene ($r=-0.250$; -0.172; -0.212; -0.241; -0.230; -0.142; -0.488, -0.252 and -0.379) respectively.

conversely, we found that GSH had a positive significant correlation with vitamin C, B₁, B₂, folic acid, A, E, and β -carotene ($r=0.313$; 0.293; 0.213; 0.349; 0.537; 0.314 and 0.342) respectively. Eventually, GSSG have significant correlation with vitamin A, E, β -carotene with ($r=-0.384$, -0.270, -0.334) with $p<0.05$ and furthermore there is no correlation between GSSG with the other vitamins.

Table 3.12. Pearson Correlation (r) between oxidant and GSH with vitamin parameter

Correlations												
VITAMIN		C	B1	B2	B3	B6	Folic acid	B12	E	β-carotene	A	B5
MDA	Pearson Correlation	-.152	-.321*	-.374	-.283*	-.315*	-.286*	-.164	-.605**	-.626**	-.792**	.066
	Sig. (2-tailed)	.194	.006	.001	.017	.008	.016	.171	.000	.000	.000	.582
HNE	Pearson Correlation	-.039	-.250*	-.172	-.212	-.241*	-.230	-.142	-.252	-.379	-.488**	.100
	Sig. (2-tailed)	.741	.033	.149	.076	.043	.054	.236	.030	.001	.000	.407
GSH	Pearson Correlation	.313*	.293*	.213	.128	.220	.349	.139	.314	.342*	.537**	.037
	Sig. (2-tailed)	.007	.013	.075	.287	.065	.003	.247	.008	.003	.000	.760
GSSG	Pearson Correlation	.119	-.096	-.062	.290*	.043	-.178	-.202	-.270*	-.334*	-.384**	.173
	Sig. (2-tailed)	.320	.425	.606	.014	.720	.138	.091	.023	.004	.001	.148

**, Correlation is significant at the 0.01 level (2-tailed).

*, Correlation is significant at the 0.05 level (2-tailed).

3.6. Amino acids level

In the Table 3.13 and Figure 3.15 serum Amino-acid concentration analysis illustrated a statistically high significant decreased ($p<0.001$) in the total value of valine 10.865 ± 2.137 $\mu\text{g/mL}$, glycine 36.548 ± 6.682 $\mu\text{g/mL}$, histidine 9.255 ± 1.492 $\mu\text{g/mL}$, threonine 17.961 ± 3.302 $\mu\text{g/mL}$, asparagine 17.584 ± 18.970 $\mu\text{g/mL}$, proline 19.0104 ± 3.61369 $\mu\text{g/mL}$, cysteine 3.709 ± 0.947 $\mu\text{g/mL}$, and tryptophan 12.945 ± 1.60 $\mu\text{g/mL}$ in breast cancer patients versus healthy control subjects in

opposite other amino acids, including the lysine $29.798 \pm 5.448 \mu\text{g/mL}$, phenylalanine $34.295 \pm 6.140 \mu\text{g/mL}$, isoleucine $21.737 \pm 3.514 \mu\text{g/mL}$, leucine $39.130 \pm 5.812 \mu\text{g/mL}$, methionine $12.015 \pm 3.129 \mu\text{g/mL}$, tyrosine $11.173 \pm 1.628 \mu\text{g/mL}$, alanine $94.9910 \pm 11.58051 \mu\text{g/mL}$, arginine $34.878 \pm 5.572 \mu\text{g/mL}$, glutamine $58.404 \pm 6.492 \mu\text{g/mL}$, serine $46.260 \pm 7.590 \mu\text{g/mL}$, glutamic acid $18.421 \pm 4.1059 \mu\text{g/mL}$ and aspartic acid $12.607 \pm 2.155 \mu\text{g/mL}$ higher statistically significant $p < 0.001$ were increased among patient with breast cancer when compared to healthy control groups and gated all concentration of amino acid by $\mu\text{g/mL}$ units.

Table 3.13. Mean concentration of Amino acids in healthy control and breast cancer subjects.

Catagory	Biochemical parameters	Study groups (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
		Breast cancer (n=45)	Control (n=32)	
Essential amino acids	Valine	10.865 ± 2.137	18.594 ± 3.715	0.000**
	Leucine	39.130 ± 5.812	26.909 ± 4.021	0.000**
	Isoleucine	21.737 ± 3.514	11.589 ± 1.789	0.000**
	Tryptophan	12.945 ± 1.60	16.929 ± 2.407	0.000**
	Glycine	36.548 ± 6.682	49.748 ± 6.015	0.000**
	Phenylalanine	34.295 ± 6.140	18.944 ± 3.605	0.000**
	Histidine	9.255 ± 1.492	13.552 ± 2.810	0.000**
	Methionine	12.015 ± 3.129	7.175 ± 1.771	0.000**
	Lysine	29.798 ± 5.448	17.80 ± 2.783	0.000**
Non-essential amino acids	Thronin	17.961 ± 3.302	22.327 ± 4.570	0.000**
	Alanine	94.9910 ± 11.58051	59.1300 ± 7.987	0.000**
	Proline	19.0104 ± 3.61369	24.8263 ± 3.373	0.000**
	Tyrosine	11.173 ± 1.628	5.476 ± 3.065	0.000**
	GlutamicAcid	18.421 ± 4.1059	13.847 ± 3.114	0.000**
	Cysteine	3.709 ± 0.947	5.807 ± 1.125	0.000**
	AsparticAcid	12.607 ± 2.155	9.201 ± 1.558	0.000**
	Arginine	34.87 ± 5.572	24.790 ± 4.1636	0.000**
	Glutamine	58.404 ± 6.492	39.229 ± 5.802	0.000**
	Serine	46.260 ± 7.590	31.849 ± 4.819	0.000**
	Asparagine	17.584 ± 18.970	23.927 ± 13.341	0.000**

one way ANOVA test, were performed for statistical analyses. * $p < 0.05$ was significant and ** $p < 0.001$ was highly significant and N.S was Non-Significant

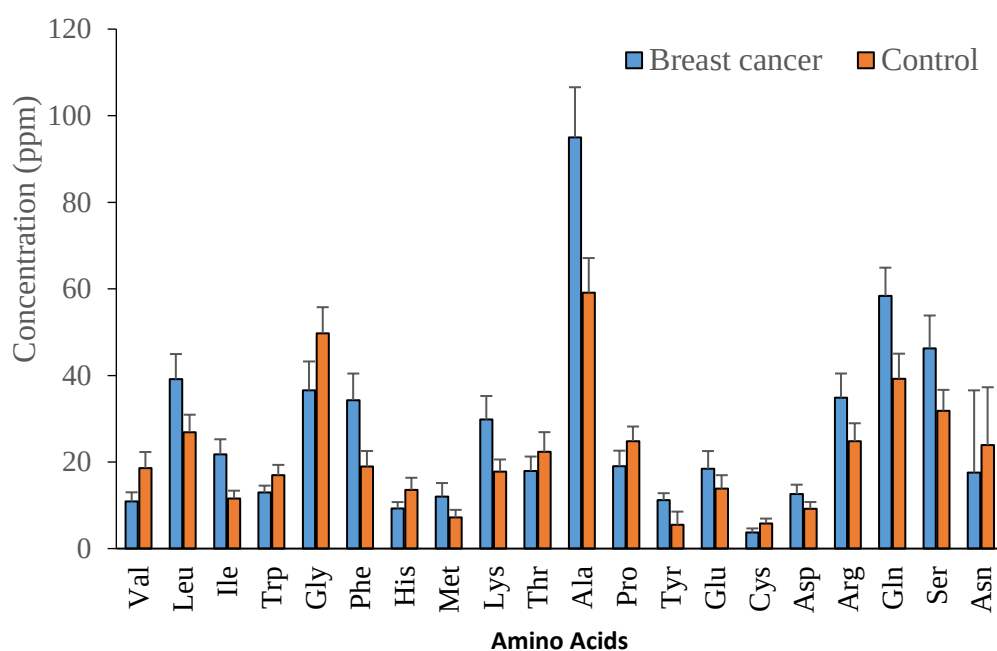


Figure 3.15. Mean Concentration ($\mu\text{g/mL}$) of Amino Acids for studied groups.

3.6.1. The effect of treatments on amino acids level in breast cancer patients

According to a comparison between mean values of amino acids categorized by taking the drug treatments was found to decrease significantly ($p < 0.05$) in the lysine, tyrosine, and aspartic acid level between patients that underline-treatments $25.943 \pm 2.427 \mu\text{g/mL}$, $10.487 \pm 1.387 \mu\text{g/mL}$, and $11.244 \pm 1.764 \mu\text{g/mL}$ respectively compared who didn't take any drug. However, no significant variation revealed among groups regarding other amino acids as illustrated in Table 3.14 and Figure 3.16.

Table 3.14. Mean concentration of amino acids in breast cancer women considering take treatments

Category	Biochemical parameters	Breast cancer patients group (Mean $\mu\text{g/mL} \pm \text{SD}$)		P-Value
		Pre treatment (n=31)	Underline-treatments (n=14)	
Essential amino acids	Valine	11.044 $\pm$ 2.230	10.422 $\pm$ 2.02	0.317 N.S
	Leucine	39.461 $\pm$ 5.999	39.190 $\pm$ 5.399	0.870 N.S
	Isoleucine	21.811 $\pm$ 3.684	22.120 $\pm$ 3.089	0.759 N.S
	Tryptophan	13.062 $\pm$ 1.665	13.038 $\pm$ 1.217	0.957 N.S
	Glycine	36.842 $\pm$ 7.5404	36.115 $\pm$ 3.90	0.704 N.S
	Phenylalanine	34.714 $\pm$ 6.345	34.082 $\pm$ 5.681	0.719 N.S
	Histidine	9.392 $\pm$ 1.567	9.019 $\pm$ 1.333	0.386 N.S
	Methionine	12.014 $\pm$ 3.213	12.425 $\pm$ 3.068	0.649 N.S

	Lysine	31.159±5.611	25.943±2.427	0.000**
Non-essential amino acids	Threonine	18.070±3.352	17.520±2.961	0.552 N.S
	Alanine	95.596±11.543	95.615±11.654	0.995 N.S
	Proline	19.098±3.771	19.0329±3.487	0.950 N.S
	Tyrosine	11.429±1.651	10.487±1.387	0.040*
	GlutamicAcid	18.799±4.439	17.412±3.114	0.241 N.S
	Cysteine	3.722±0.914	3.684±0.667	0.879 N.S
	AsparticAcid	13.226±2.059	11.244±1.764	0.001**
	Arginine	35.876±5.081	33.40±6.175	0.109 N.S
	Glutamine	58.647±7.167	58.431±4.679	0.909 N.S
	Serine	47.587±7.988	43.581±5.957	0.064 N.S
	Asparagine	16.471±18.767	20.811±20.051	0.425 N.S
one way ANOVA test, were performed for statistical analyses. *p<0.05was significant and **p<0.001was highly significant and N.S was Non-Significant				

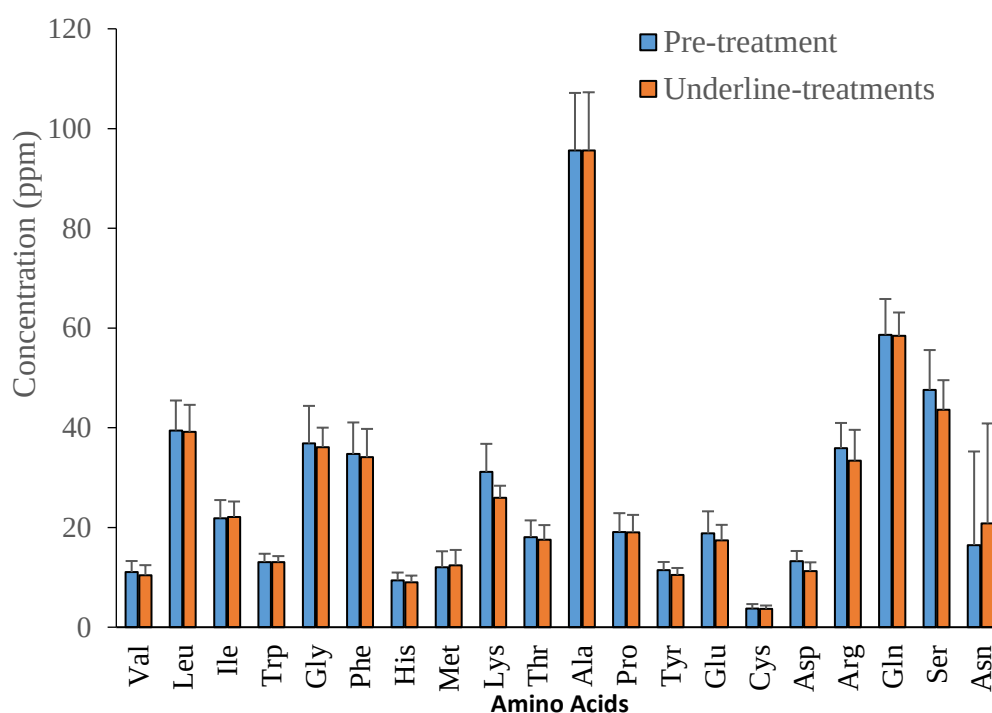


Figure 3.16. Concentration of amino acids in breast cancer women considering take treatments.

3.6.2. Effect of breastfeeding on amino acids level among breast cancer patients

Based on the comparison between the mean levels of amino acids classified according to breastfeeding, as shown in the Table 3.15 and Figure 3.17 the levels of aspartic acid and arginine among patients who have breastfeeding decreased significantly ($p<0.05$) 13.076 ± 2.073 $\mu\text{g/mL}$,

36.277±4.794 µg/mL respectively compared that they didn't breastfeed in their lifetime. While regarding other amino acids, non-significant differences were observed between the groups.

Table 3.15.Mean concentration of amino acids level in breast cancer women considering breastfeeding

Catagory	Biochemical parameters	Breast cancer patients group (Mean µg/mL± SD)		P-Value
		Have breastfeeding (n=15)	Didn't have breastfeeding (n=30)	
Essential amino acids	Valine	10.790±2.327	10.904±2.145	0.846 N.S
	Leucine	39.180±6.148	39.187±5.385	0.996 N.S
	Isoleucine	21.63±3.883	21.857±3.197	0.810 N.S
	Tryptophan	12.914±1.234	13.088±1.70	0.674 N.S
	Glycine	37.019±5.875	36.190±7.087	0.641 N.S
	Phenylalanine	33.882±6.281	34.767±6.128	0.590 N.S
	Histidine	9.207±1.551	9.262±1.434	0.890 N.S
	Methionine	11.971±3.283	12.101±3.093	0.877 N.S
	Lysine	29.084±4.605	30.270±5.893	0.417 N.S
Non-essential amino acids	Threonine	17.972±3.883	17.918±2.930	0.951 N.S
	Alanine	93.509±12.237	96.279±10.851	0.359 N.S
	Proline	18.915±3.893	18.991±3.450	0.936 N.S
	Tyrosine	10.806±1.753	11.309±1.507	0.238 N.S
	Glutamic Acid	17.256±3.784	19.074±4.293	0.101 N.S
	Cysteine	3.747±0.904	3.692±0.842	0.810 N.S
	Aspartic Acid	11.829±2.019	13.076±2.073	0.025*
	Arginine	33.171±6.226	36.277±4.794	0.031*
	Glutamine	57.823±7.105	58.840±6.314	0.562 N.S
	Serine	44.775±7.374	46.876±6.920	0.266 N.S
	Asparagine	16.684±18.053	18.427±19.893	0.733 N.S

one way ANOVA test, were performed for statistical analyses. *p<0.05 was significant and **p<0.001 was highly significant and N.S was Non-Significant

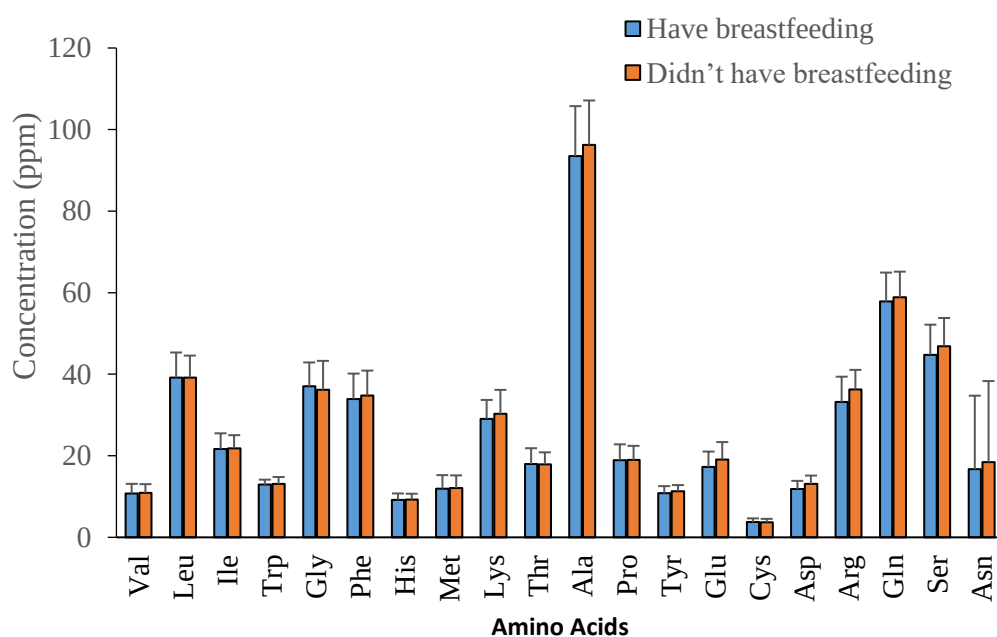


Figure 3.17. Concentration of amino acids level in breast cancer women considering breastfeeding.

3.6.3. Correlation analysis Pearson Correlation (r) between Non-essential amino acids parameter

Pearson's correlation analysis of non-essential amino acids together revealed the following expressions. as dedicated in Table 3.16 That serum Aspartic acid level had a positively high significant $P < 0.0001$ correlation with glutamic acid, serine, glutamine, arginine, alanine and tyrosine level ($r=0.476$), ($r=0.709$), ($r=0.658$), ($r=0.692$) ($r=0.655$) and ($r=0.504$) also had negatively High significant correlation with each of glycine, prolin, and cysteine ($r=-0.291$), ($r=-0.417$), ($r=-0.36$) and ($r=-0.260$) respectively. And serum glutamic acid level has negatively High significant correlation with glycine, prolin, and cysteine ($r=-0.312$), ($r=-0.225$) and ($r=-0.269$) but with serine, glutamine, arginine, alanine and tyrosine we observed a positively significant correlation ($r=0.395$), ($r=0.473$), ($r=0.421$), ($r=0.438$) and ($r=0.446$) respectively. About the concentration of serine, we showed that had a negatively High significant correlation with glycine, prolin, and cysteine ($r=-0.298$), ($r=-0.130$), and ($r=-0.326$) respectively, and had a positive correlation with glutamine, arginine, alanine, and tyrosine ($r=0.802$), ($r=0.793$), ($r=0.856$), and ($r=0.631$).

Conversely, we found that glycine had a negatively High significant correlation with glutamine, arginine, alanine, and tyrosine with values ($r=-0.365$), ($r=-0.39$), ($r=-0.418$), and ($r=-0.46$), respectively and had a positive relation with prolin ($r=0.780$) and cysteine ($r=0.345$) however each of glutamine, arginine, and alanine had negatively correlation with prolin ($r=-0.28$), ($r=-0.143$), ($r=-0.245$), and cysteine ($r=-0.335$), ($r=-0.341$), ($r=-0.365$) and positive correlation with tyrosine ($r=0.693$), ($r=0.543$), ($r=0.689$), in addition prolin had negative correlation with prolin ($r=-$

0.41) and positive with cysteine ($r=0.270$), Finally there is no correlation coefficient between asparagine and other nonessential amino acids,

Table 3.16.Correlation analysis (r) of Non-essential amino acids parameter between study groups

Correlations											
Pearson Correlation	Asp	glut	Asn	Ser	Gly	Gln	Arg	Ala	Pro	Tyr	Cys
Asp (r)	1	.476**	.009	.709**	-.417**	.658**	.692**	.655**	-.36**	.504**	-.260**
P-value		.000	.924	.000	.000	.000	.000	.000	.000	.000	.007
glu	.476**	1	-.045	.395**	-.312**	.473**	.421**	.438**	-.225*	.446**	-.269**
P valu	.000		.647	.000	.001	.000	.000	.000	.020	.000	.005
Asn	.009	-.045	1	.010	.075	-.013	-.085	-.198*	.069	.052	-.290**
P value	.924	.647		.921	.441	.890	.385	.041	.477	.595	.002
Ser	.709**	.395**	.010	1	-.298**	.802**	.793**	.856**	-.130	.631**	-.326**
P value	.000	.000	.921		.002	.000	.000	.000	.181	.000	.001
Gly	-.417**	-.312**	.075	-.298**	1	-.365**	-.39**	-.418**	.780**	-.46**	.345**
P value	.000	.001	.441	.002		.000	.000	.000	.000	.000	.000
Gln	.658**	.473**	-.013	.802**	-.365**	1	.702**	.865**	-.28**	.693**	-.335**
P valu	.000	.000	.890	.000	.000		.000	.000	.004	.000	.000
Arg	.692**	.421**	-.085	.793**	-.388**	.702**	1	.845**	-.143	.543**	-.341**
P valu	.000	.000	.385	.000	.000	.000		.000	.143	.000	.000
Ala	.655**	.438**	-.198*	.856**	-.418**	.865**	.845**	1	-.245*	.689**	-.365**
P value	.000	.000	.041	.000	.000	.000	.000		.011	.000	.000
Pro	-.361**	-.225*	.069	-.130	.780**	-.275**	-.143	-.245*	1	-.41**	.270**
P value	.000	.020	.477	.181	.000	.004	.143	.011		.000	.005
Tyr	.504**	.446**	.052	.631**	-.459**	.693**	.543**	.689**	-.40**	1	-.761**
P value	.000	.000	.595	.000	.000	.000	.000	.000	.000		.000
Cys	-.260**	-.269**	-.29**	-.326**	.345**	-.335**	-.34**	-.365**	.270**	-.76**	1
P value	.007	.005	.002	.001	.000	.000	.000	.000	.005	.000	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4. DISCUSSION

Breast cancer is the world's second-most common cancer which represents the highest morbidity and death among women.

Forty-five breast cancer patients diagnosed at Hiwa cancer Hospital were included in the study. All of these BC patients were females among this patient groups 32 subjects are pretreatment that means didn't take any drug and 13 of the patient is post-treatment which treatment include drug supplement and chemotherapy, besides 15 of the patient in a lifetime have breastfeeding and other 30 patients didn't have breastfeeding however 32 healthy participants included in this study as the control group (Figure 3.1).

Differences in breast cancer risk factors are because of genetic and epidemiological risk factors such as age, family history, late marriage, childbirth, breastfeeding, and environmental factors.

The distribution of demographic characteristics of participants and the relation between identified and suspected factors in the development of breast cancer is presented in table 3.1 and in Figure 3.2. It reveals that 15% of the patient group were in the age group of 30-39 years, 38% were lies in 40-50 years, 40% of them belong to more than 50 years of age and only 7% of them were <30 years. This indicates that the majority 40% of breast cancer were in the 40-49 year age group. The reason is that due to the risk of occurrence of breast cancer increases in middle-aged (after 40 years) and elderly women compared to young women [112]. Human cancer is mainly an age-related disease that involves a person 50 years old or older as our cells change over time and are more susceptible to genetic damage and less able to deal with damage when it occurs. This problem is thought to be due largely to a reduction in our immune system's ability to detect and destroy abnormal cells as they occur; the decreased immune response gives those cells time to develop into potentially lethal cancer [113].

It is well known that breast cancer is associated with weight gain, especially in postmenopausal women. As depicted in Table 3.1 and Figure 3.3. Obesity is characterized by fat body excess. The most commonly used record is the body mass index (BMI) as weight in kilograms divided by squared height in meter. Subjects in both groups were observed to range from normal weight, overweight to obese regardless of their diagnosis. In our research, the distinguished of BMI done as following BMI (< 16-18.5) is indicated to have underweight (18.5-24.9) as normal weight. A person with BMI (25-29.9) is overweight, while a person with BMI > 30 is obese,

In the present study, the maximum number of BMI subjects 44% of breast cancer patients were present obesity in the BMI group and 31% are overweight, while the lowest 25% were in the normal BMI group. the result of this study is confirmed by a direct association between BMI and the risk of the disease developing [114] because obesity changes hormone levels in postmenopausal

women's, such as estrogen, because the extra fat cells make estrogen, which can cause hormone-receptor-positive breast cancers, these changes could raise the risk of breast cancer [115].

The distribution of marital status and family history of patients with breast cancer is illustrated in Table 3.1. This indicates that 80% were married and 20% were unmarried. Results indicated in Table 3.1 shown that 44% patients had positive family history, while the other patients 56% have no family history. The results showed that patients with a family history due to genetic risk factor getting cancer so had mutations in BRCA1 or BRCA2 genes. The risk factors for breast cancer are marital status and a positive family history of breast cancer, and never-married women have a greater risk of breast cancer.

Women, especially those who have children when they are younger or have more children, are less likely to be get breast cancer. In this study parity of breast cancer patients 60.0 % having a child and 40% of patient is nulliparous. Women who are single and nulliparous reported strong elevated risk of breast cancer in the most research relative to multi-parity women at the same age. Marital status itself is not a contributing factor for an increased or decreased risk of breast cancer, and the main protective effect is from pregnancy in the first full term [116].

Various factors have been reported to clarify the possible protective effect of pregnancy on breast cancer, such as reduced estrogen and progesterone, elevated sex hormone-binding globulin levels, and differentiation of breast tissue caused by pregnancy.

Observed breastfeeding among patients with breast cancer in Table 3.1. This shows that the percent of the patient that done breastfeeding was only 33% and who that don't breastfeeding was 67%. It is not clear how breastfeeding decreases the risk of breast cancer. The biological basis for an inverse relation among breastfeeding and breast cancer risk has not been adequately explained. Although various mechanisms have been observed, One assumption is that lactation causes long-term endogenous hormone changes, possibly decreased estrogen, and increased production of prolactin, which can reduce the cumulative exposure of a woman to estrogen, thus inhibiting the formation or growth of breast cancer cells [117].

Water soluble vitamins maybe have a complex role in protecting or inhibiting cancer. There are several concerns about the quantities required for their biological functions, about their beneficial and toxic effects on human health [118]. In this study as depicted in Table 3.2 and figure 3.4., serum thiamin (vitamin B₁) levels were significantly lower $p < 0.05$ in breast cancer patients $0.966 \pm 0.253 \mu\text{g/mL}$ than in healthy control groups $1.1594 \pm 0.335 \mu\text{g/mL}$ is similar which reported in some researches [119, 120]. Thiamine (B₁) is acts as a co-factor and the active form of thiamine is thiamine diphosphate, which is essential in the biosynthesis of reducing the equivalents being used oxidant stress defenses [121]. So thiamine decreased in patients has been associated with the promotion of neurodegeneration and increased oxidative stress [18]. Nevertheless, its effectiveness in the treatment of cancer diseases is still uncertain. However in our study riboflavin (vitamin B₂)

is strong significantly $p < 0.001$ decrease in breast cancer cases 0.671 ± 0.241 and $\mu\text{g/mL}$ as compared to healthy controls 0.891 ± 0.272 $\mu\text{g/mL}$, due to riboflavin is a cofactor in redox reactions which is converted from flavin mononucleotide to flavin adenine dinucleotide [122]. this finding was agreed with other studies which recorded similar results [123, 124]

These flavocoenzymes perform a crucial role in maintaining the stability of a variety of bioenergetics flavoproteins, DNA repair, and other physiologically relevant processes, therefore, the deficiency of riboflavin have been linked with numerous diseases including cancer and cardiovascular disorders, and there is some indication that treatment of riboflavin may be effective against oxidative stress-related diseases [77] such as breast cancer.

However, In the present study, serum Nicotinamide (B_3), Pyridoxine (B_6), levels were significantly decreased ($p < 0.05$) in patients with breast cancer 2.326 ± 0.534 , 0.1465 ± 0.047 $\mu\text{g/mL}$ compared with healthy control groups 2.63 ± 0.469 , and 0.179 ± 0.060 $\mu\text{g/mL}$ respectively. Nicotinamide (B_3), the amide form of niacin or nicotinic acid is used in redox reactions (as NAD^+ and NADP^+). It plays a major role in energy metabolism, signal transduction, cell injury, aging, and demonstrates significant inhibition of ROS-induced oxidative damage [125]. Pyridoxine (vitamin B_6) is a cofactor for many amino acid metabolism enzymes and functions as the coenzyme in two separate stages of this pathway: in 5,10-methylenetetrahydrofolate synthesis, which is essential for DNA synthesis, repair, and methylation and in the catabolism of homocysteine to glutathione, which detoxifies several carcinogenic compounds and protects cells against oxidative stress which damages DNA [126]. Several studies have concluded that vitamins B_3 and B_6 may be a protective factor against cancer and reported a lower level of this vitamin in cancer patient [9, 127, 128].

The serum concentration of folic acid in this study was significantly lower in breast cancer patients than in healthy controls, as known that folic acid are essential cofactors that have been converted to tetrahydrofolate and transfer one-carbon molecules both in the synthesis of nucleotides used for DNA synthesis with implications for improving gene expression, these functions could have a significant role in cancer and numerous disorders prevention. Two studies show that folic acid levels generally agree on the inverse relationship with breast cancer risk [129, 130]. While the mean levels of serum of vitamin C, B_5 , and B_{12} showed a non- significant decreased in breast cancer patients compared to healthy control groups. However (Vitamin C) is necessary for, wound healing, bone formation, immune system enhancement, and antioxidant action [122]. Because of its antioxidant action, the release of free radicals from advancing cancer is likely to lead to higher demand and consumption of vitamin C. One assessment is that, in this context, plasma vitamin C represents poorer health, determined by cancer activity, and dietary intake is a symptom of that poorer health and markers of the inflammatory response. This would likely result besides having little effect either on symptoms or on survival al [131]. In a wide range of metabolic functions

pantothenic acid (vitamin B₅) plays an important role. It is required to form coenzyme A, that is essential for the synthesis of fatty acids, amino acids, steroid hormones, and other significant compounds [132]. Vitamin B₁₂ is partnered in the synthesis of DNA with folic acid (folate). A deficit of either vitamin contributes to disordered DNA synthesis and, thus, to reduced red blood cell production. Vitamin B₁₂ also has a special biochemical function in the synthesis of fatty acids in the myelin sheath surrounding nerve cells [133]. This result supports the other study that these vitamins have lowered in the patient group [131, 134-136].

In this study, patients with breast cancer had highly significantly $p < 0.001$ lower serum levels of β -carotene, vitamin A, and vitamin E than healthy control subjects.

Few studies have investigated the serum levels of vitamins A and E, and β -carotene, in breast cancer patients. prospective studies have shown that low serum levels of vitamins A and E, β -carotene, and retinol precede the development of different cancer types [137-139]. Results of previous research on vitamin (A and E) concentrations in the serum with breast cancer have provided compelling support for the theory that vitamin A and its precursors prevent mammary carcinogenesis [137, 140]. Lower levels of plasma β -carotene have been found in the present study, which may be related to the ability of β -carotene to quench the radical singlet oxygen. β -carotene can be an intracellular redox agent that serves as a protection against free radicals [141] The acid form of vitamin A (retinoic acid, a highly active form of vitamin A that is not retained and does not circulate in large amounts) binds to nuclear receptors and controls the expression of genes [142]. Lower levels of vitamin A in breast cancer patients may be related to The release of free radicals from breast cancer is expected to lead to higher demand and use of vitamin A due to its antioxidant activity of retinol [96]. The lower vitamin E levels in patient groups may be concerning the ROS attack because preventing the oxidation of polyunsaturated fatty acid. Vitamin E is the main antioxidant breaking chain which is recycled from the radical α -tocopheroxyl to α -tocopherol.

In this study, we evaluated the effect of drug treatments and breastfeeding on vitamin among breast cancer patients that demonstrated this factor didn't influence the vitamin level because the differences in vitamins value between groups were statistically non-significant as shown in Table 3.3, Figure 3.5 and Table 3.4, Figure 3.6 respectively. that one study found the same result the anticancer treatment was indeed without effect on the vitamin, so maybe due to anti-cancer drug only effect to kill cancer cell so drug supplements neutralized the free radical-induced by chemotherapy [143] and about breast feeding only can effect on the regulation of hormone so didn't impact on vitamin as found in previous study [117].

Vitamins deficiency in the metabolism of patients with breast cancer can be explained in various ways; can be a cause or effect of cancer. It is reported that breast cancer is linked to women who consume fewer quantities of fruits and vegetables. Consequently, the reduced levels of vitamins found in breast cancer patients can also be due to reducing nutritional eating. As a result,

lower plasma of vitamin levels suggests higher oxidative stress caused by lipid peroxidation, DNA damage, and mutations that contribute to higher breast cancer risk. In a wide variety of diseases reduced amounts of individual serum vitamins are generally diagnosed [144]. The findings obtained suggest an association between vitamins and antioxidants and an inverse association with breast cancer. Our research confirms findings in previous studies that serum concentrations of selected vitamins and carotenoids in the serum of patients with other cancers are lowered compared with healthy control groups [144-146].

Different lines of research clearly show that cellular oxidative stress is involved in carcinogenic processes. In this study, oxidative stress serum biomarkers including malondialdehyde, 4-Hydroxynonenal as an indicator of lipid peroxidation, are evaluated to know relation of oxidative stress on breast cancer.

In the current study, in comparison to healthy control groups, serum MDA levels were significantly elevated in women with breast cancer this increase is highly significant ($p < 0.001$) Table 3.5 and Figure 3.7. This finding is similar to other studies that reporting high levels of MDA in patients with breast cancer compared with the control groups [147-149].

In addition, our results show a statistically significant ($p < 0.001$) increase in 4-HNE levels among breast cancer patients $9.053 \pm 2.673 \mu\text{g/mL}$ when compared to a healthy control group $4.752 \pm 1.911 \mu\text{g/mL}$. These results were agreed with the study which showed a significant elevation of 4-HNE in breast cancer patients [150]. These data indicate that 4-HNE and MDA are significant markers of oxidative stress that can lead to the production of lesions through its ability to alter proteins, cause cell dysfunction, and the formation of DNA adducts so it has been shown that increased intracellular ROS generation plays a significant role in various types of cancer such as breast cancer. thus this causes damage to the polyunsaturated fatty acid membrane resulting in lipid peroxidation generation lead to an elevation of MDA and 4-HNE in these patients [151]. These observations by other researchers show that oxidative stress can play a significant role in the etiology of breast cancer. The following studies are aligned with our research [72, 152]. Furthermore as shown in Figures 3.13, there was a significant positive correlation between MDA with HNE in the directly related study groups. The direct correlation between these parameters indicates a rise in oxidative stress at all levels in the cell in the other study also found a positive correlation between MDA and 4-HNE [153].

The amount of oxidative damage is not only dependent on ROS rates but also on the cellular antioxidant defense mechanisms; One of the cellular antioxidant defenses is glutathione that functions as the first line of defense against free radicals that antitumor molecules produced. In the present study, as illustrated in Table 3.8 and Figure 3.10 the GSH levels highly significantly ($p < 0.001$) decrease in patients with breast cancer $139.483 \pm 60.981 \mu\text{g/mL}$ compared to the healthy control groups $265.219 \pm 55.356 \mu\text{g/mL}$, while GSSG levels significantly ($p < 0.001$) increased in

patients 56.786 ± 36.651 $\mu\text{g/mL}$ compared to control groups 30.083 ± 7.562 $\mu\text{g/mL}$. These results were agreed with other research that reported a low level of GSH and increase GSSG levels in breast cancer patients than healthy control groups. The ratio of GSH/GSSG may be used as an oxidative stress marker, GSH/GSSG is maintained at a high ratio under physiological conditions, and GSH is oxidized to GSSG under oxidative stress, and the ratio of GSH/GSSG decreases so that the damage to lipid peroxidation can be evaluated. in the present study, we found that the ratio of GSH/GSSG which our results showed that the GSH/GSSG ratio in breast cancer patients (3.7156 ± 2.365 $\mu\text{g/mL}$) was significantly ($p < 0.001$) lower, compared to control groups. Such as some researches that finding lower rate of ratio GSH/GSSG in the patient with breast cancer [154, 155]. The result of this research indicated that patients with breast cancer have an imbalance of the antioxidant status or a greater than normal exposure to oxidative stress or both [156]. However as shown in Table 3.13 and Table 3.14 GSH level of serum among participants have a statistically significant negative correlation with each of MDA, HNE, and GSSG that is indicated GSH has an inverse relationship with oxidative stress due to antioxidant properties of GSH that is equalized free radical by oxidation to GSSG so high level of GSSG is presented to have high oxidative stress and in this study correlation between GSSG with MDA and 4-HNE was positive and these results agrees with this study [157].

In addition, we examined the effects of drug therapy and breastfeeding on oxidative stress parameters in a group of patients, as shown in Table 3.6, Figure 3.8 and Table 3.7, Figure 3.9, which showed that drug therapies and breastfeeding didn't affect MDA and 4-HNE levels In the case of cancer therapies may be due to that patient underline treatments give chemotherapy with antioxidant drug supplements together because such as revealed in other research that oxidative stress-induced lipid peroxidation produces many electrophilic aldehydes during cancer chemotherapy that can attack several cellular targets. These oxidative stress products may delay the development of cancer cells through the cell cycle then cause stopping checkpoint of the cell cycle, results that can conflict with the effectiveness of anticancer drugs to destroy cancer cells. The use of antioxidants during chemotherapy will boost treatment by reducing the production of aldehydes caused by oxidative stress [158].

While as demonstrated in Table 3.9, Figure 3.11 and Table 3.10, Figure 3.12 revealed that both underline treatments and have breastfeeding during a lifetime can increase the amount of GSH and ratio of GSH/GSSG that mean by increased GSH can reduce the free radical however breastfeeding can also decrease the value of GSSG so reduced GSSG level with the increased value of ratio GSH/GSSG mean to decreased oxidative stress after that is the impact to improve the patient of breast cancer as in other study detected the impact of drug and chemotherapy on increased antioxidants and reduced oxidative stress [143, 159].

Although there is an ongoing controversy about the effect of vitamin on protecting of breast cancer. In the current study, the correlation between lipid peroxidation parameter (MDA, 4-HNE) and (GSH, GSSG) parameters with the vitamin in the whole study population as illustrated in Table 3.12 showed that MDA and 4-HNE had a negative correlation with each of vitamins especially high significant negative association with vitamin A, E, β -carotene and inapposite GSH have a positive correlation with each of vitamin C, B₁, B₂, A, E, β -carotene, and GSSG had negative relationships with vitamin A, E, β -carotene.

The findings supported our hypothesis that, as seen the oxidants would have risk factor to malignancy disease, the vitamins have protective factor against oxidant and then on cancer disease especially breast cancer, and can have protective. The resulting of this study showed consistency with the earlier study results [160, 161].

High possible biomarkers for breast cancers and other cancer are well thought out serum amino acids since it was known that a tumor's presence resulted in increased metabolism of protein so their importance as presumed breast cancer biomarkers is highlighted in much scientific research [162]. Studies have been performed on variation in serum amino acid levels as probable measures of the tumor's impact on host proteins, and which amino acids should require a protein synthesis neoplasm.

The study which was presented verified that amino acids are a group of metabolic that have a high potential to be used as biomarkers for breast cancer and may enhance the screening of breast cancer due to the results obtained showed that breast cancer causes significant changes in serum amino acid levels (Tables 3.13, and Figure 3.15).

In this analysis, the factors leading to serum amino acid levels in breast cancer were analyzed, by focusing on the effect of breast cancer on the serum amino acid levels. In breast cancer patients, we observed significant $p < 0.001$ reduced serum, histidine, threonine, proline and asparagine levels compared to healthy controls as a reason of high-demand tumor metabolism associated with early symptoms of malnutrition for protein and DNA synthesis in cells during cancer development, have been translated as reduced levels of this amino acids in accordance with the study of Elizabeth et al [163].

However, the level of cysteine, valine, tryptophan, and glycine is a statistically significant decreased ($p < 0.001$) in breast cancer patients versus of control groups that are meaning these amino acids have an inverse relation with breast cancer, By regulating redox balancing, cysteine intake plays a significant role in cancer, and several studies have demonstrated the effectiveness of cysteine prevention on tumor development and found lower levels of cysteine in the cancer group [164]. In cancer immunotherapy, tryptophan catabolism has been in the focus because of Promoting a microenvironment of tumor immunology which is deficient in the detection and elimination of malignancy cells.

Valine and glycine are known as significant amino acids for the biosynthesis of protein and decreased in cancer groups due to glycine depletion can indicate higher demand of gluconeogenesis or glutathione, caused by oxidative stress in the cancer patient. this finding supports the previous study that done among colon cancer patient [165].

In the contrary the mean value of methionine, glutamine, lysine, and alanine were significant $p < 0.001$ increased in serum of patients with breast cancer compared in the healthy controls and our result agreed with the previous study [166] and elevated this amino acid may be due to Malignant tumor has been shown the ability to increase protein degradation in normal tissue or muscles to consume some portion of amino acids in gluconeogenesis A huge amount of energy is absorbed by tumor development. Glycolysis was established as the primary source of energy for partially differentiated cells because glycolysis could release a little adenosine triphosphate (ATP), so to obtain sufficient energy through gluconeogenesis. Most studies have shown that this amino acid can be involved in gluconeogenesis because it has higher serum amounts than other amino acids [167].

In addition, the concentration of serin, glutamic acid, aspartic acid, leucine, isoleucine were high significant $p < 0.001$ higher in cancer patient than in healthy groups, that is this condition can be described which Leucine with isoleucine has the greatest potential to activate the mTOR pathway and facilitate the synthesis of proteins in different tissues. Leucine may also encourage the production of insulin that facilitates the activation of the mTOR pathway. A substantial decrease in the proliferation potential of breast cancer cells when lacking of isoleucine and leucine. in leucine level in our study was a highly significant increase among breast cancer patient compared to control groups that are agreed with the previous study that showed that leucine can promote the proliferation and metastasis of tumor cells [168]. Tumors need amino acids to accommodate their growth, such as glutamine aspartic acid and glutamic acid for the synthesis of purine and pyrimidine and serine for synthesis lipid component membrane also serine acts as a key source for several forms of cancer cell survival and proliferation in the metabolic network., resulting in a selective asking for amino acids that is caused a slow loss of muscle mass to manage with protein turnover in tissues resulting in reduced amino acid supply so the amount the amino acids is increased. In breast cancer patients, we find elevated levels of phenylalanine and tyrosine as a consequence of enhanced proteolysis of muscles. In the catabolism of phenylalanine, phenylalanine is first metabolized by phenylalanine hydroxylase in the liver into tyrosine. In inflammatory conditions such as infection, and cancer, increased serum levels of phenylalanine and tyrosine have been described [169].

In our result also Arginine level is elevated among breast cancer patients because arginine is involved in nitric oxide metabolism, which is one type of free radical that takes part in nitrosative stress, which is a factor in the invasion, and metastasis in the inflammatory process and

carcinogenesis. The cause of increased NO has been estimated to be increased levels of arginine. so Arginine reduction may offer a potential method of treatment for breast cancer. this result is similar to other studies [82].

As shown in Table 3.14 and Figure 3.16 we investigated on effect of cancer treatments on amino acids between patients group that underline treatment such as chemotherapy and drug supplements can reduce the amount of lysine, tyrosine, and aspartic acid, which this amino acids were in patients with breast cancer significantly increased compared to control groups so this detected these amino acids have a line relationship with breast cancer and due to drug treatments this amino acids value are decreased may be due to destruction of malignancy cell by drug or by chemotherapy while for other amino acids differences between groups were non-significant so mean that treatments didn't effect on other amino acids so the previous study support our result that chemotherapy effect to reduce lysine and tyrosine with same amino acid so it is detected effect of chemotherapy treatment on amino acid [170], However, other research evaluating the impact of chemotherapy on amino acid that has demonstrated chemotherapy Doesn't Influence on amino acid such as our result that treatments didn't effect on amino acids except on lysine, tyrosine and aspartic acid which decreased value detected in underline treatment patients [171].

Furthermore when the evaluated impact of breastfeeding on amino acids level among patients with breast cancer as illustrated in Table 3.15 and Figure 3.17 we found significantly reduce level aspartic acid and arginine level among those patients that have breastfeeding during their lifetime than who didn't breastfeed, which this amino acid inverse relationship with breast cancer so breastfeeding can impact factor against breast cancer thought didn't statistically effect on other amino acid however other research support our result that breastfeeding is impact factor against breast cancer [172].

According to the Pearson correlation coefficient (r) among nonessential amino acids, our results in the whole participants are illustrated in Table 3.16. each of the aspartic acid, tyrosine, alanine, arginine, glutamine, serine, the glutamic acid level had a negatively high significant correlation with glycine proline and cysteine and had a significant positive correlation with each other, that is mean each of the proline, glycine, and cysteine have a protective effect against breast cancer due to all the aspartic acid, tyrosine, alanine, arginine, glutamine, serine, the glutamic acid have a high concentration between breast cancer patients and also had High significant negative correlation with proline, glycine, and cysteine which have decrease concentration among patients and our study agree with other studies that detect impact factor of proline, glycine, and cysteine on breast cancer as nonessential amino acid [173].

The essential role of amino acids in cancer metabolism in each of tumorigenic and tumor-prevention forms has been shown, that is contributed in pathways that are nutrient for cancer cells, and some amino acids are involved and afford building blocks for the development of cancer cells.

So the detection of amino acids concentration in breast cancer disease with such metabolite formation has provided clinicians a new viewpoint in terms of relative screening and the development of new therapeutic methods.



5. CONCLUSIONS:

Our findings indicate that serum levels (amino acids, oxidative stress biomarker, and water-soluble vitamin with fat-soluble vitamin) could be useful as a screening tool to diagnose individuals with breast cancer and predict outcomes

- There is a high degree of oxidative stress associated with a reduction in the level of the antioxidant system and a significant effect of lipid peroxidation in breast cancer patients.
- The reduction in serum GSH levels and the elevated GSSG levels in patients with breast cancer reflect an enhanced capacity of oxidative stress in cancer groups due to enhanced oxidation of GSH to GSSG. so expected for the treatment potential is used GSH antioxidant to reduce oxidative stress that is the marker of cancer.
- The negative association between the participants in the oxidative marker (4-HNE, MDA, GSSG) with antioxidant GSH was demonstrated, suggesting that elevated oxidants and a weak antioxidant protection mechanism may play an important role in the elevated oxidative stress reported among breast cancer patients.
- The low levels of water-soluble vitamins in breast cancer patients indicate that the demand for these B vitamins has been increased for these patients due to the accelerated energy metabolism and/or tumor growth. In order to re verify the change in parameters and determine whether breast cancer patients require supplementation with B vitamins, more large-scale research is required.
- In conclusion, we studied the serum levels of various fat-soluble vitamins (Vitamin A, E, and β -carotene) in patients with breast cancer and control subjects to check at the isolation of these vitamins in relation with breast cancer. Concentrations of vitamin A, E, and β -carotene were high significantly lower ($p < 0.001$) in breast cancer patients compared to control groups. Our analysis gives insights into the possible functions and effects of fat-soluble vitamins in breast cancer patients
- We have demonstrated that variations in serum amino acid levels are characteristic of breast cancer individuals. Our findings demonstrate that the sampling of serum amino acids may have significance as a serum-based screening test for breast cancer diagnosis and prognosis.

RECOMMENDATIONS

- . To analysis the role of environmental and genetic factors in the sensitivity and severity of patients with breast cancer
- This is a cross-sectional study so more investigations are needed with additional oxidant stress markers such as oxidatively affected DNA metabolites and major enzyme antioxidants in larger participants.
- Investigate the enzyme-antioxidant relationship in a breast cancer patient



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APPENDICES

APPENDIX- 1: CALIBRATION CURVE FOR THE CORRECT EQUATION OF ANALYSIS VARIABLES

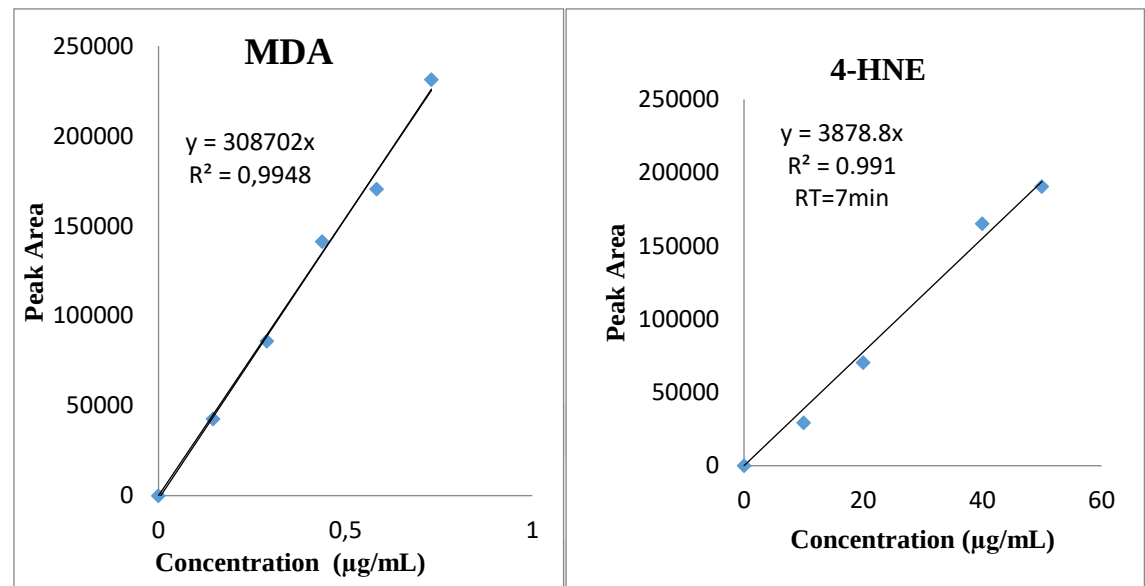


Figure 5.1.Calibration curve for the correct equation of MDA and 4-HNE

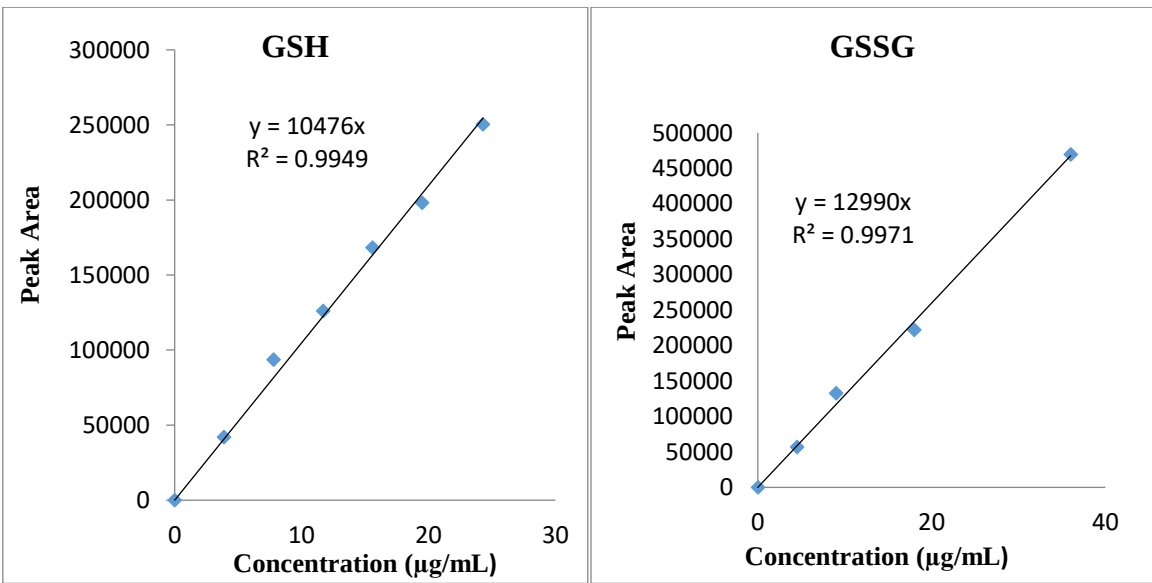


Figure 5.2.Calibration curve for the correct equation of GSH, GSSG

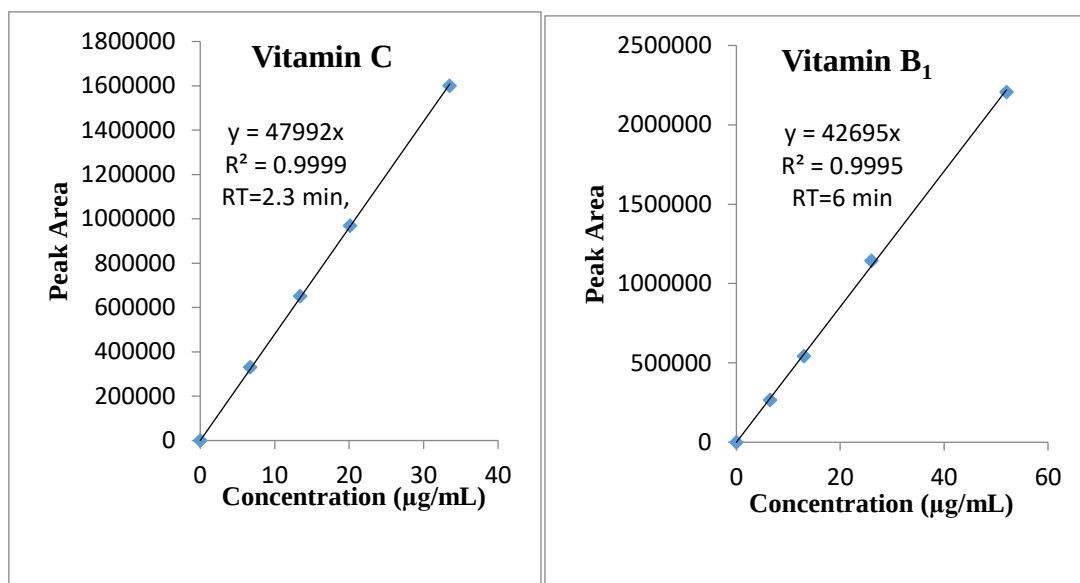


Figure 5.3.Calibration curve for the correct equation of Vitamin,C and B₁.

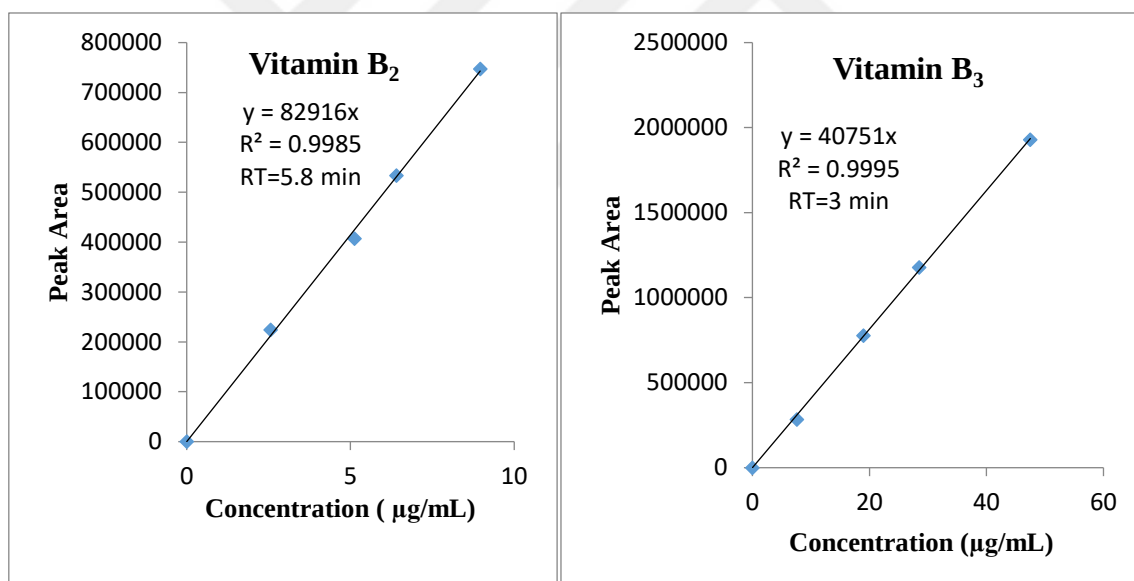


Figure 5.4.Calibration curve for the correct equation of Vitamin,B₂ and B₃.

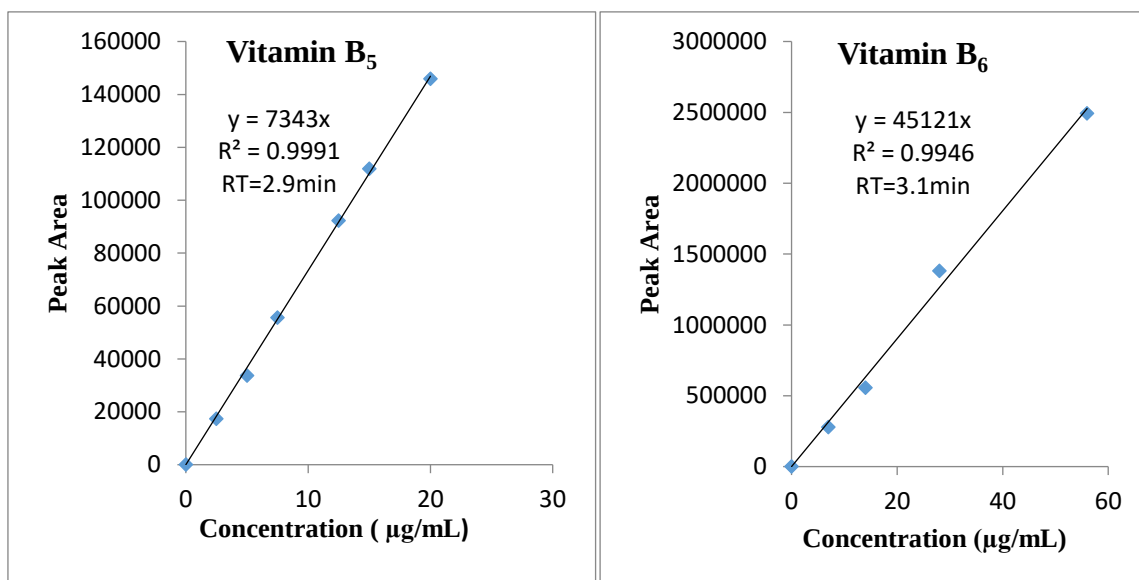


Figure 5.5. Calibration curve for the correct equation of Vitamin, B₅ and B₆.

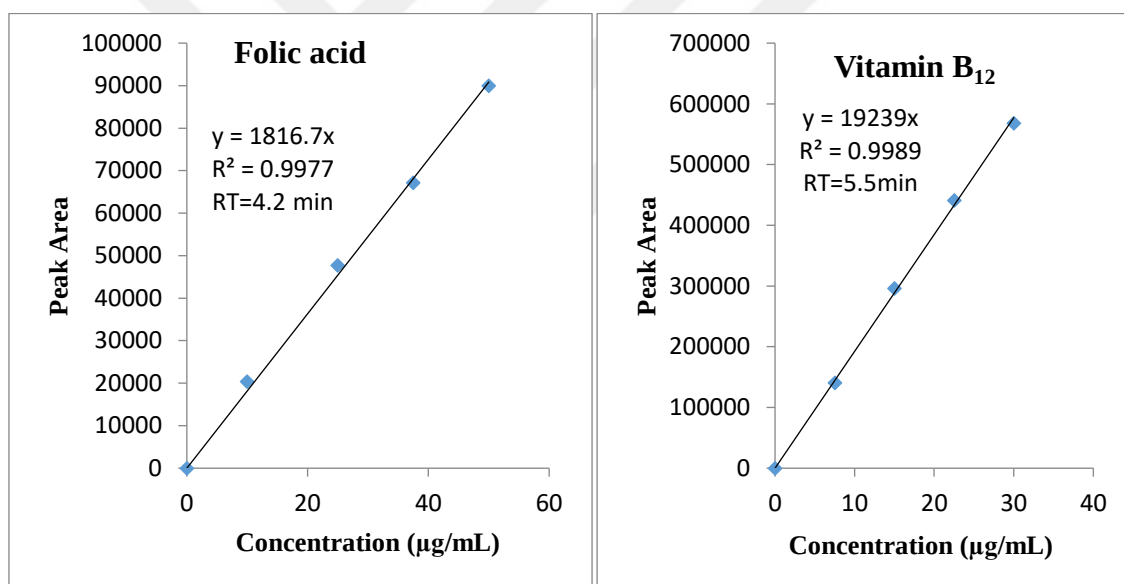


Figure 5.6. Calibration curve for the correct equation of Vitamin B₉ and B₁₂

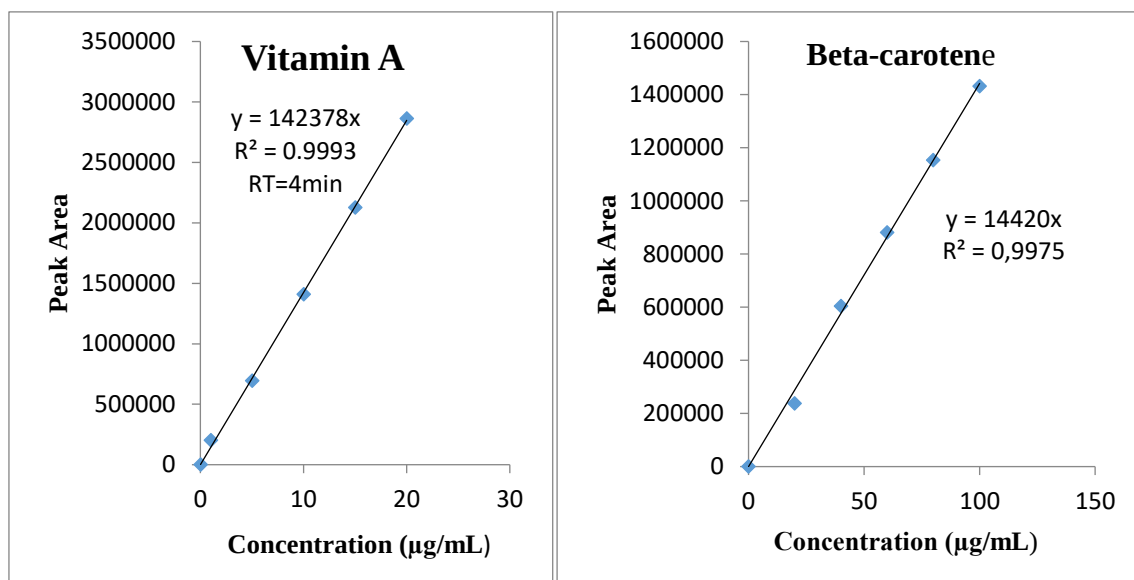


Figure 5.7. Calibration curve for the correct equation of Vitamin, A, and Beta Carotene

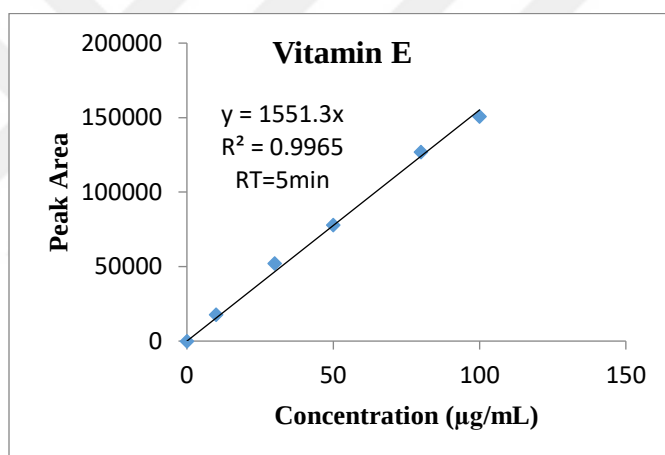


Figure 5.8. Calibration curve for the correct equation of Vitamin E

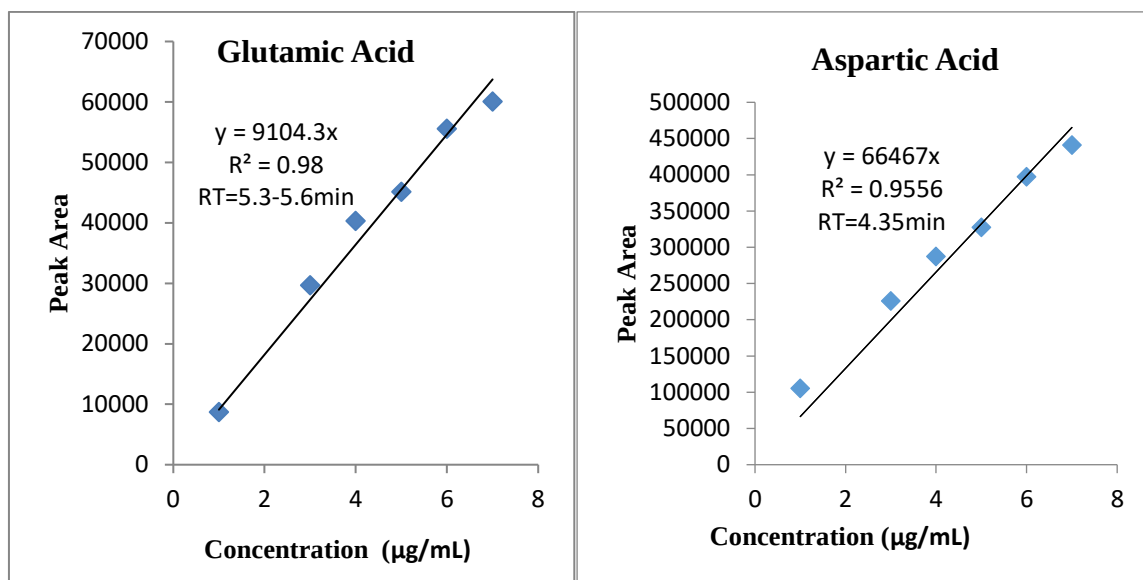


Figure 5.9.Calibration curve for the correct equation of Glutamic acid and Aspartic acid

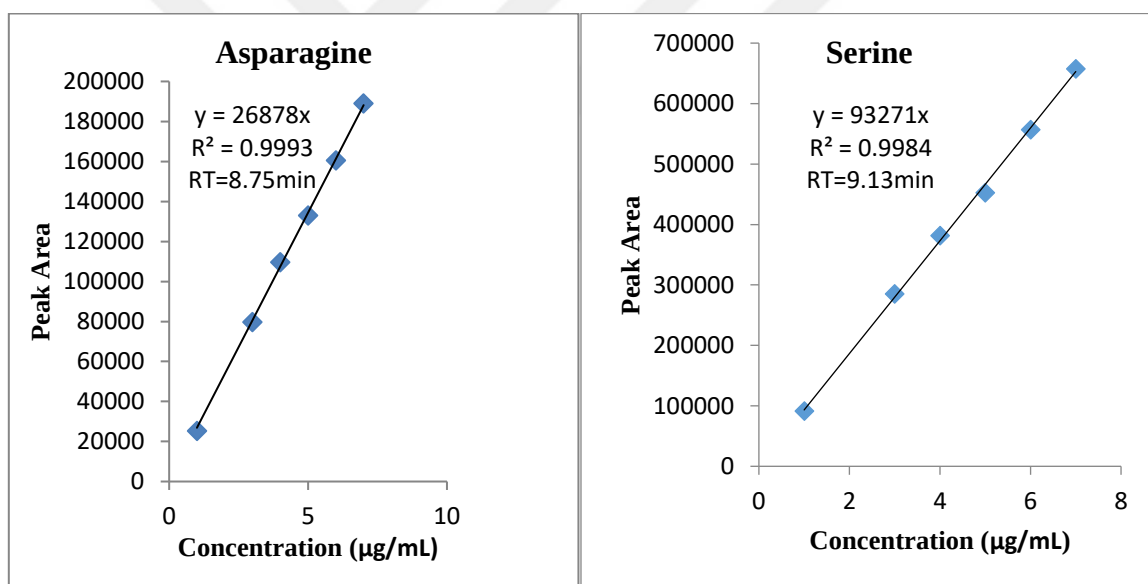


Figure 5.10.Calibration curve for the correct equation of Asparagine and serine

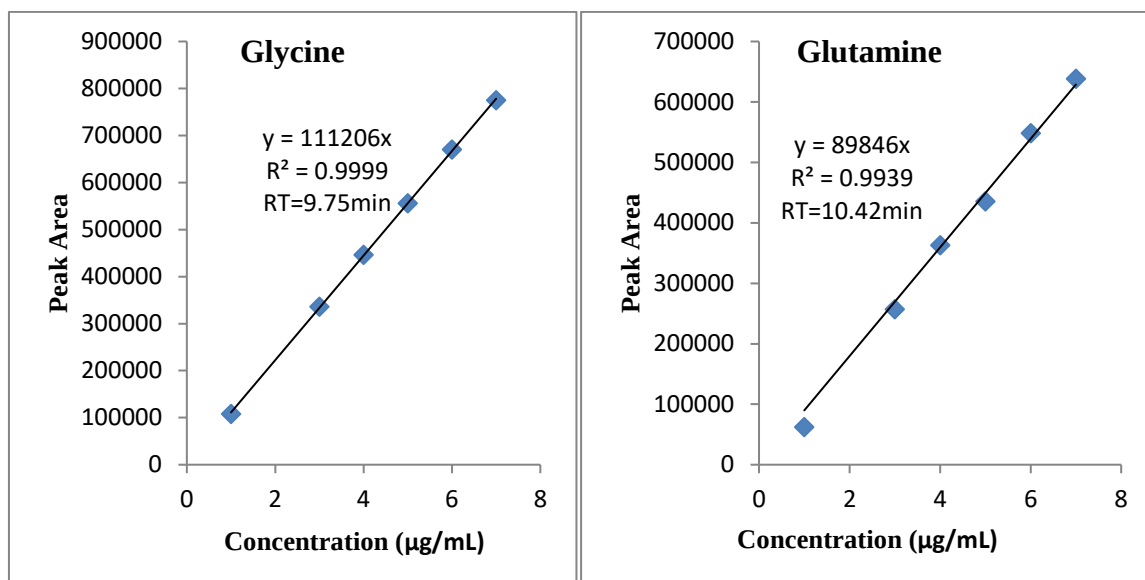


Figure 5.11. Calibration curve for the correct equation of glycine and glutamine.

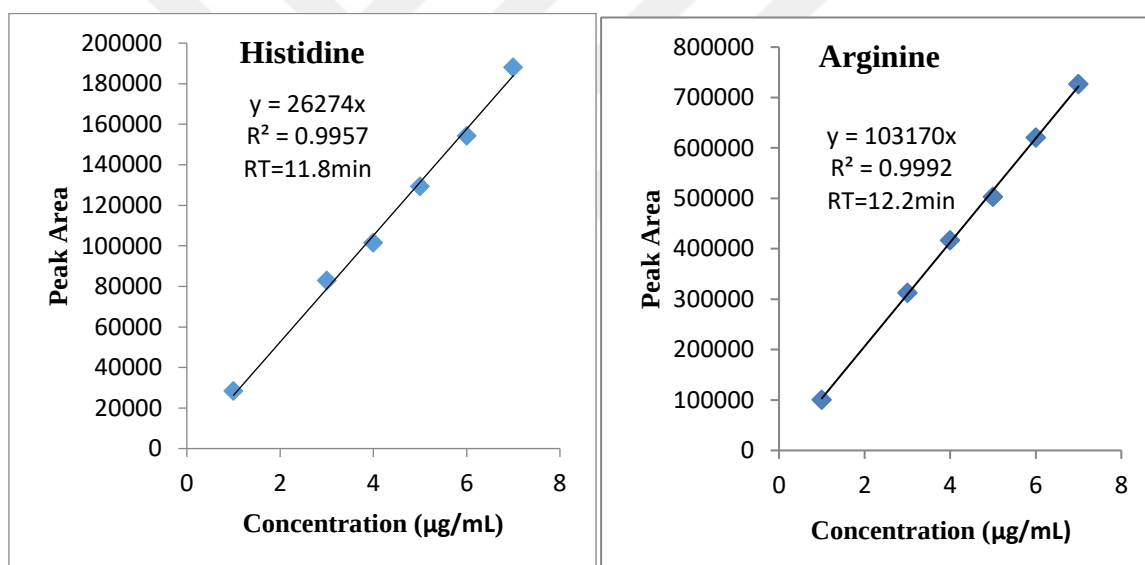


Figure 5.12. Calibration curve for the correct equation of Histidine and Arginine

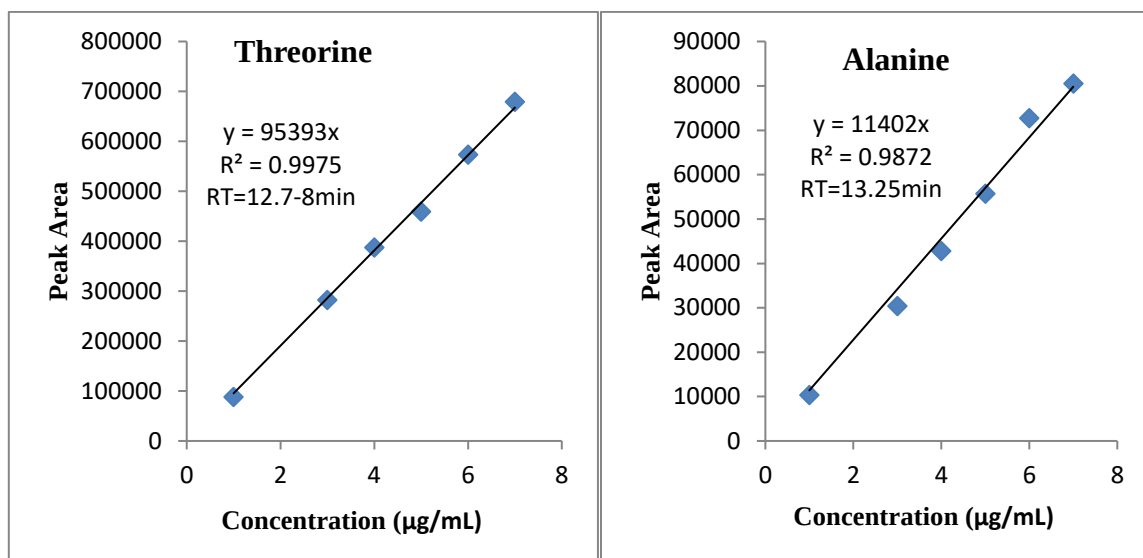


Figure 5.13.Calibration curve for the correct equation of Threonine and Alanine

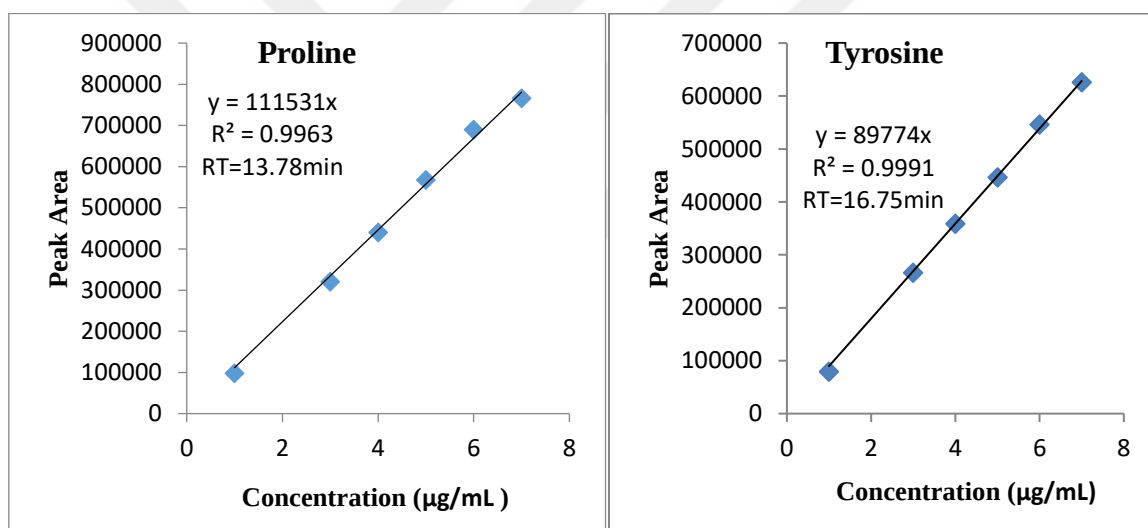


Figure 5.14. Calibration curve for the correct equation of proline and tyrosine.

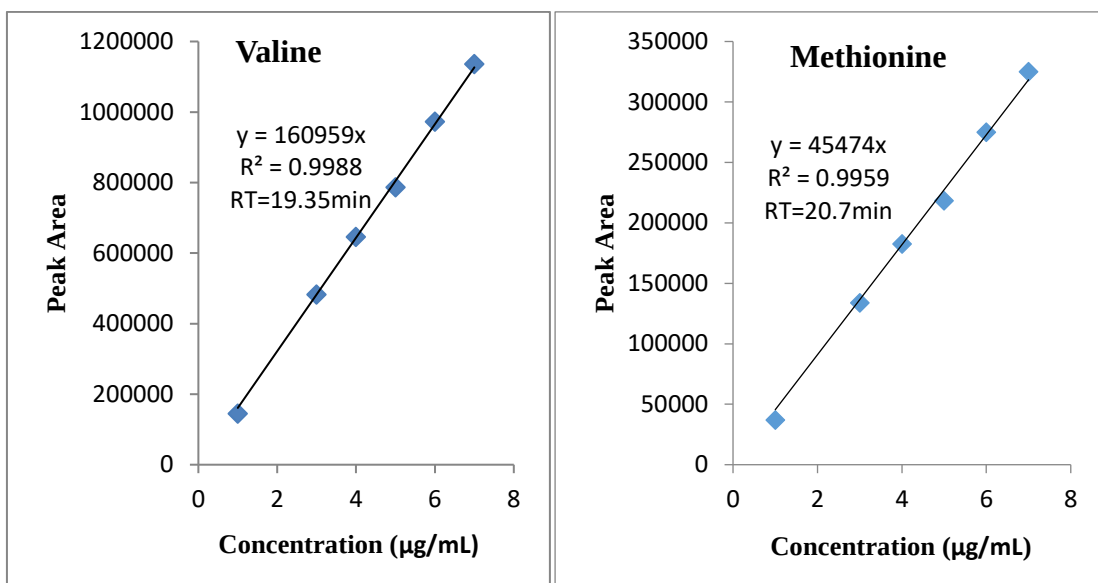


Figure 5.15.Calibration curve for the correct equation of valine, and methionine,

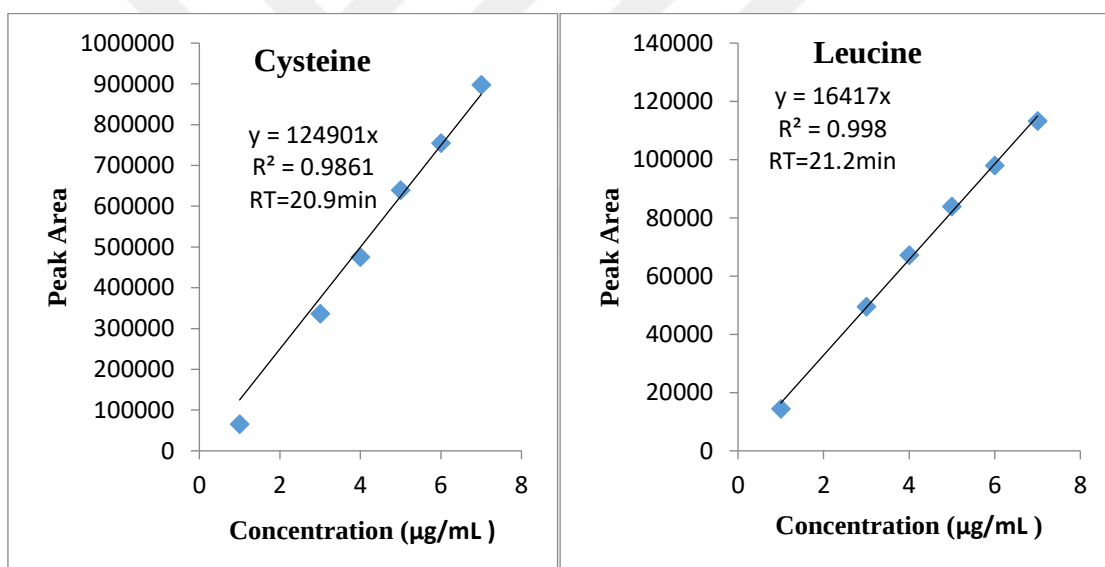


Figure 5.16.Calibration curve for the correct equation of, cysteine, and leucine.

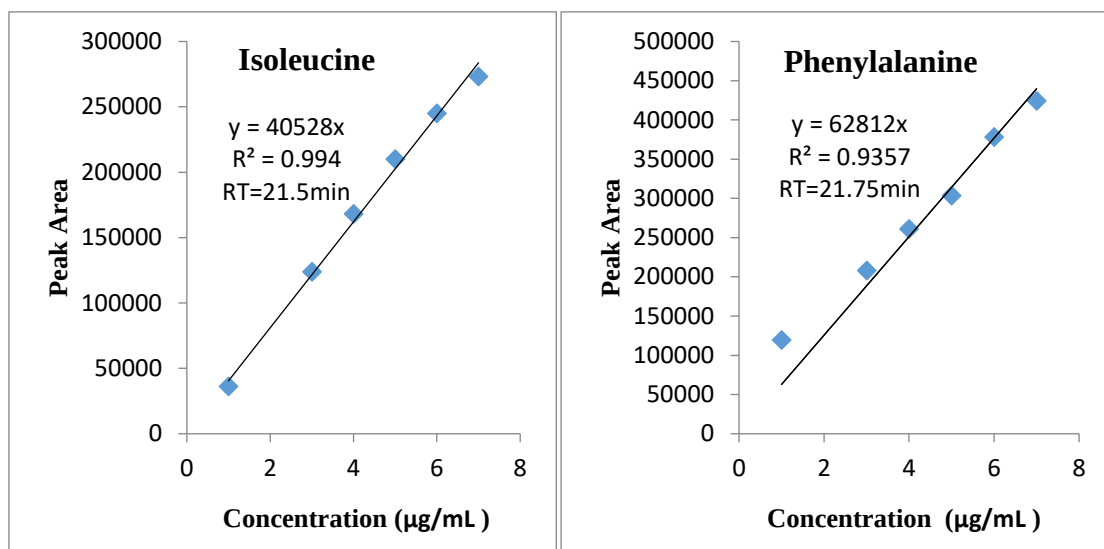


Figure 5.17. Calibration curve for the correct equation of isoleucine and Phenylalanine.

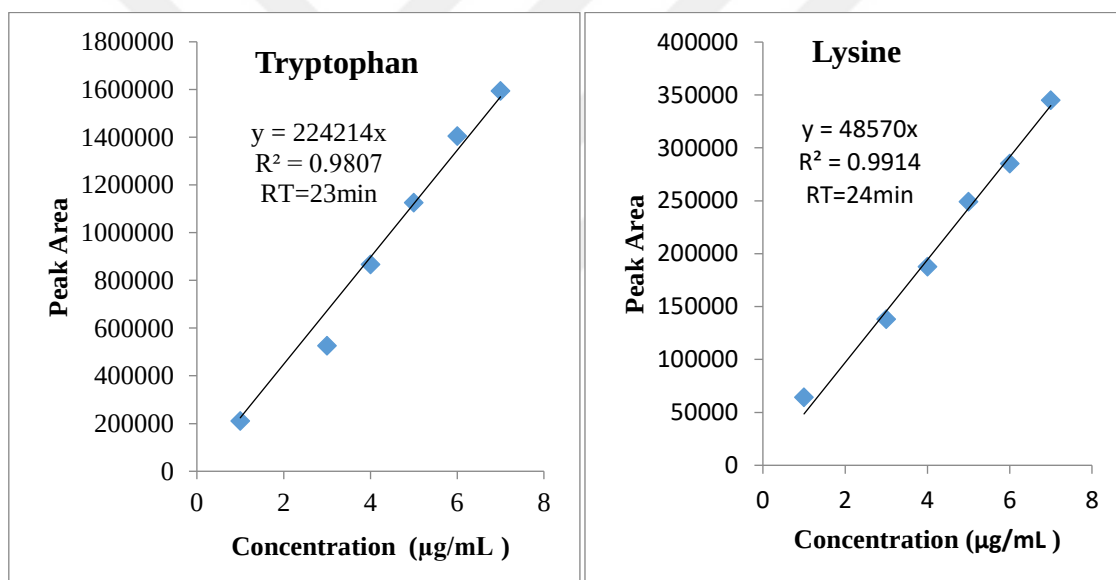


Figure 5.18. Calibration curve for the correct equation of Tryptophan and Lysine..

APPENDIX- 2: THE QUESTIONNAIRE FORM

Volunteer NO .

Date: / / 201

Phone no:

Name: ----- Age: -----(Y)

Address-----

Economic situation: -----

Marital status: married: ☐ single: ☐

Number of Children (gender and ages):

Age at first birth:

Birth weight (participant birth weight):

Time with a parent (early life):

Length of time participant breastfed her children:

Age at first menarche:

Date of diagnosis:

Stage at diagnosis:

Finding:

Breast cancer: ☐ Normal: ☐

Anthropometric measurement:

Height: -----cm, -----m

Weight: -----kg

BMI: -----kg/m2

Waist Measurement: -----cm

Treatment history:

Surgery: radiation therapy:

Antioxidant supplements: ☐ Hormone replacements: ☐

Disease history:

Liver disease :

Thalassemia:

Cancer:

Chronic inflammatory disease:

Kidney disease :

Gastrointestinal diseases :

diabetes :

Risk factors:

Family History :

Smoking :

Physical

Activity:

alcohol abuse:

APPENDIX- 3: APPROVAL OF RESEARCH ETHICS COMMITTEE

Kurdistan Regional Government Ministry of Health Slimani Directorate General of Health Department of Planning Scientific Research Division	
Approval of Research Ethics Committee	
Date: 29 September, 2019	
Reference number: 1	
Research title: Evaluation of amino acid and Oxidant-Antioxidant Status Among Women with breast cancer	
Research name (s): Awat Hamad Awla Braim	
Type of approval: Unconditional, taking into consideration the following mandates:	
1) Obtaining the approval of Hiwa Hospital center in slimani Governorate. 2) The participants don't responsible for participate in funding any procedure(s) and /or investigation(s). 3) Health facilities are not responsible for the cost of any procedure(s) and /or investigation(s). 4) The participants have the right to withdraw from the study. 5) Confidentiality of the data should be secured. 6) Notifying the participant about the results of the procedure(s) and investigation(s) performed if applicable. 7) Any change in the methods of the study needs prior approval of Research Ethics committee. 8) Grantee of this letter is given the right to publish the results of the study.	
	
Address: Room 10, Floor 4 Slimani Directorate General of Health Slimani, Kurdistan Region, Iraq	Contact: Email: researchdoh.sul@gmail.com

CURRICULUM VITAE

[REDACTED]

PERSONAL INFORMATION

[REDACTED] [REDACTED]
[REDACTED] [REDACTED]
[REDACTED] [REDACTED]
[REDACTED] [REDACTED]
[REDACTED] [REDACTED]
[REDACTED] [REDACTED]

[REDACTED]

[REDACTED] [REDACTED]
[REDACTED] [REDACTED]

EDUCATION

[REDACTED] [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]
[REDACTED]
[REDACTED] [REDACTED]

RESEARCH EXPERIENCES

- ✓ Laboratory Instruments you use, such as test systems etc. you know
- ✓ Computer Programming languages available (SPSS, MATLAB,.)
- ✓ Computer programs you can use (CAT/CAM, PROTEUS, CALCULUS, vb.)

WORK EXPERIENCE

2013 – 2014 Assist.of Chemistry in Salahaddine University
2014 – 2018: Assist of Chemistry in Raparin University
2018 start studying of master degree

ACADEMIC ACTIVITIES

Paper:

1.
2.

Proceedings:

1.
2.

Projects:

- 1.....
- 2.....