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Study of Antioxidants, Homocysteine, Cytokines in Different Grads of Breast Cancer Patients

A Theses

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بسم الله الرحمن الرحيم

يَرۡفَعِ ٱللَّهُ ٱلَّذِينَ ءَامَنُواْ مِنكُمۡ وَٱلَّذِينَ أُوتُواْ ٱلۡعِلۡمَ دَرَجَٰتٖۚ وَٱللَّهُ بِمَا تَعۡمَلُونَ خَبِيرٞ ﴿11﴾

صدق الله العظيم

سورة المجادلة

الأية رقم (11)

Supervisor Certification

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Dediction

To whom Allah made them a lifeboat for the people of the earth (Muhammed and The family of Muhammed) the beast of wilderness .

To the one who honored me to bearded his name my father God bless you .

To the light of my eyes, the joy of my life, my dear mother whose prayers and words were the companion of brilliance and excellence God bless her .

To the beast beautiful thing my eyes see and my heart feels, to those whom are my support and help in the life my brothers and sisters, God bless them.

To my lovely daughter, God bless her.

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Summary

Breast cancer is the most common cancer in women. It accounts for more than a third of all new cases of cancer in women. When an anomaly is discovered during a screening examination or a person presents symptoms, several examinations must be carried out. It is the anatomopathological examination of the tissues taken at the level of the anomaly which establishes the diagnosis of breast cancer. This removal at the level of the anomaly is most often carried out by micro or macro biopsies through the skin. Different types of treatments can be used to treat breast cancer: surgery, radiotherapy, hormone therapy, chemotherapy and targeted therapies.

Asprosin, a novel glycogenic cytokine, released by white adipose tissue during fasting and involved in the development and progression of various clinical conditions including diabetes Mellitus , obesity, cardiomyopathy, cancer

Spexin (SPX) is a novel cytokine related to many metabolic effects, such as gastrointestinal movements, insulin and glucose homeostasis, lipid metabolism and energy balance.

Midkine (MK) is a heparin-binding growth factor that can induce various effects in the targeted cells including nerve cells, neutrophils, macrophages, smooth muscle cells, fibroblasts, mammary gland cells and tumor cells.

Homocysteine (Hcy) is physiologically essential for processes such as cell cycle progression and maintenance of cellular homeostasis.

Malondialdehyde (MDA) It is the end product of lipid peroxidation. Because MDA is a product of lipid peroxidation , it is frequently used as a biomarker of oxidative stress during major health issues such as cancer.

The goal of the present study was to predict the progression of cancer by investigating a novel cytokines in serum of early detected cancer in patients. Subjects are 113 divided into 45 controls, 68 patient who have breast cancer. This study was designed as a case-control study. Breast cancer patient was divided into four groups: before treatment, after the first dose, after the second dose, and after third dose. The samples were collected from Babylon Oncology Center in Merjian Teaching Hospital in Hilla City during the period from August 2021-March 2022. The age range of all subjects female (35±10 years). Blood samples were withdrawn after nearly 12 hours of overnight fasting. Serum concentrations of lipid profile was measured by colorimetric methods. Serum concentrations of total antioxidant capacity (TAC), malondialdehyde (MDA), asprosin, sepxin, and midkine were measured by using Enzyme Linked Immune Sorbent Assay technique.

The results showed a significantly increased lipid peroxidation, measured as MDA, in the serum of breast cancer patients who were not taken any medication were higher than controls groups ($p<0.001$)were (23.22±12.27943) nmol/ml of MDA levels in breast cancer patient and (16.55± 4.995255) nmol/ml of MDA levels in healthy controls. In current study found that treatment by chemotherapy resulted a significant decrease in MDA levels when compare with MDA levels in patients who were not taken any medication. The mean plasma homocysteine level in breast cancer patients were elevated before treatment. In addition, it is found that the mean serum TAC levels in breast cancer patients were significantly less than the control group. Moreover, a positive correlation was observed between activity of chemotherapy and MDA levels. The same correlation between tHcy levels and TAC levels while a negative correlation was observed between TAC levels with MDA levels in the patient group. also in this study it is found that Spexin were lowered in breast cancer patients than the healthy group while the Spexin tend to

increase in breast cancer patients who receiving chemotherapy. Inversely, midkin and asprosin were higher in breast cancer patients than control but the Asprosin were tended to decrease in breast cancer patients who receiving chemotherapy compared with patient before chemotherapy.

It can be concluded that the measured cytokines are indicators for progression of tumor cancer in patient since the spexin decreased in patients while both midkin and Asprosin increased in the patient due to the most well-known metabolic abnormality in cancer cells. Also, monitoring of serum cytokines may be helpful for evaluation of chemotherapy effects. In addition the other parameters MDA, Hcy, and TAC levels have the important role in the progression of cancer after and before chemotherapy.

List of Contains

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List of Abbreviation

Terms	Symbol
1-Axillary Websyndrome	AWS
2-Adeno myoepithelioma	AME
3- Adenosine Tri Phosphate	ATP
4- American Cancer Society	ACS
5- Analysis of Variance	ANOVA
6- Body Mass Index	BMI
7-Breast Cancer	BC
8-Breast Cancer Related Lymph node Transfer	BCRLT
9- Breast Cancer type 1	BRCA1
10-Breast Tumor Micro Environment Cell	BTME
11- Total Cholesterol	TC
12-Ductal Carcinoma In situ	DCIS
13-Diatry Antioxidant Capacity	DAC
14-Extra Cellular Matrix	ECM
15-Epithelial Mesenchymal Transition	EMT
16-Fibrillin 1	FBN1
17- Enzyme Linked Immune Sorbent Assay	ELISA
18-Eosin Stain	ES
19-Glycerol Kinase	GK
20- Glycerol Phosphate Oxidase	GPO
21- High Density Lipoprotein	HDL
22-Homocysteine	Hcy
23-Hyper Homocysteine	HHcy
24-Hematoxylin Stain	H
25-Intra Ductal Papilloma	IDP
26-Invasive Ductal Carcinoma	IDC

27-Invasive Lobular Carcinoma	**ILC**
28-Invasive Ductal Carcinoma-No Specific Type	**IDC-NST**
29-Interlukin-1	**IL-1**
30- Low Density Lipoprotein	**LDL**
31- Least Significant Difference	**LSD**
32- Lipoprotein Esterase	**LPL**
33-Midkine	**MK**
34-Malonodialdyhyde	**MDA**
35-Optical Density	**OD**
36-Periodic acid –reactive Schiff	**PAS**
37- Peroxide	**POD**
38-Poly cystic ovarian syndrome	**PCOS**
39-Reactive Oxygen Species	**ROS**
40- Statistical package for social science version -24	**SPSS-24**
41- Standard Deviation	**SD**
42-Tumor Protein 53	**TP53**
43-Transforming growth factor-b	**TGF-b**
44- Triglycerides	**TG**
45-Range of motion	**ROM**
46-United States	**US**
47-Vascularized Lymph node	**VLNT**
48-Very Low Density Lipoprotein	**VLDL**
49-World Health Organization	**WHO**

50-White Adipose Tissue	**WAT**

Chapter one

Introduction and Literature Review

1.Introduction and Literature Review

Breast cancer is a significant reason of death among women all over the world and the most common type of cancer. Nearly 2 million cases of breast cancer were reported in 2018. In 2022, there will be an estimated 287,850 new cases of invasive breast cancer diagnosed in women; 2,710 cases diagnosed in men, and an additional 51,400 cases of ductal carcinoma in situ (DCIS) diagnosis in women. (ACS, 2022). Its prevalence is increasing on a global scale, apart from the United States, where its stability is relatively consistent (Thouvenot *et al*., 2018). Breast cancer is caused due to genetic, reproductive, environmental, and dietary and lifestyle related risk factors (Dhillon, 2009). The diagnosis of breast cancer is based on observations through the breast clinical examination and the use of imaging techniques. Imaging tests are useful for screening and giving the diagnostic of this kind of disease and other breast diseases (McDonald *et al*., 2016).

Tumor marker is a biomarker that is found in blood, urine or body tissues that can be elevated by the presence of one or more types of cancer. It is produced either by the tumor itself or by the host in the response to a tumor (Kilpatrick & Lind, 2009). The ideal tumor marker should be both specific and sensitive to detect small tumors to allow early diagnosis or help in (Amayo & Kuria, 2009). The American Society of Clinical Oncology (ASCO) has updated its recommendations for use of tumor markers in prevention, screening, treatment and surveillance of breast cancer. 13 categories of breast tumor markers were considered. The tumor markers that showed evidence of clinical utility and were recommended for use in practice include Cancer Antigen 15-3 (CA 15-3), Cancer Antigen 27.29 (CA 27.29), Carcinoembryonic antigen (CEA), Estrogen receptor (ER), Progesterone

receptor (PR), Human epidermal growth factor receptor 2 (HER2), urokinase plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1) and multi parameter assays for gene expression(Donepudi *et al*., 2014). There are other categories that would be studied for detection of breast cancer such as (MK, Spx, Asp, Hcy, MDA, and TAC).

Homocysteine (Hcy) impaired plasma Hcy level is closely related to malignant processes (Li *et al*., 2020) In summary, an elevated level of Hcy can contribute to cancer initiation, promotion, and progression. The specific polymorphisms in genes contributing to Hcy metabolism or diet defciency in folate, vitamin B6, or cobalamin directly correlate with the Hcy level's misbalance(Koklesova *et al*., 2021).

Malondialdehyde (MDA) is one of the final decomposition products of lipid peroxidation, is known to be present in human plasma and to possess biological properties that may be relevant to carcinogenesis(Lee *et al*., 2012). cmjoo

Spexin (SPX) it is extensively expressed in central and peripheral tissues (Kolodziejski *et al*., 2018).

Asprosin,(Asp) is secreted from white adipose tissue and metabolized in the liver, where it promotes rapid glucose release into the bloodstream (Romere *et al*., 2016).

Midkine (MK) is a soluble secreted cytokine, it can be readily quantitated in blood samples, thereby making it a minimally invasive biomarker for detecting, monitoring and managing illness(Cellmid, 2014).

1.1 Breast cancer

Breast cancer (BC) is the most prevalent type of cancer seen in women even though various awareness programs and modern diagnostics increased detection at an early stage(Stimpfel & Virant-Klun, 2016). Prevalence is increasing on a global scale, apart from the United States, where its stability is relatively consistent (Stimpfel & Virant-Klun, 2016) Moreover, genetic factors also contribute in disease development but only account for only 5–10% (Campeau *et al*., 2008) Mammary glands tissue provides the site for disease progression with an evolution time of 7 years, because cancer cells are doubled in 100–300 and 30 doublings are required for emersion of disease (Navarro, 2006). Worldwide statistics show that in 2018 approximately two million new cases were detected, with total BC accounting for 11.6% of all cancers (kim, 2019). The majority of BC cases are still detected in later stages as the early stages are often asymptomatic, patients often ignore telltale signs of the disease and avoid screening programs(Springfield *et al*., 2019). Depending upon the severity of cancer, treatment modalities include surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy (Springfield *et al*., 2019). Chemotherapeutic drugs are administered to patients before surgery (as neoadjuvant therapy) or post-surgery (as adjuvant therapy) and are primarily aimed at selective inhibiting the proliferation and activating apoptosis in cancer cells (Michele *et al*., 2017). However very often patients encounter moderate to severe side effects in addition to developing acquired resistance to the drug (which is a more adaptive response to the presence of drug) due to prolonged treatment (Michele *et al*., 2017). In addition to acquired drug resistance several other factors contribute to treatment resistance and variations in the pattern of resistance are observed between different cancers and anticancer drugs (Mansoori *et al*., 2017). In BC the response to chemotherapeutic drugs varies with the

different BC-subtypes which to a certain degree is attributed to their heterogeneity. Additionally specific genetic, epigenetic, and metabolic attributes of the subtypes confer an in-built resistance (intrinsic resistance due to gene mutations) against certain drugs (Calaf *et al*., 2015).

1.2 Anatomy of the Female Breast

The mature breast is located within the anterior thoracic wall, between the second and sixth intercostal cartilage. More specifically, the innermost portion of the breast lies atop the pectoralis fascia of the pectoralis major, serratus anterior, external oblique abdominal muscles, and the upper extent of the rectus sheath, it measures from 10 to 12 cm in diameter. Breasts are cone shaped structures that extend from each lateral border of the sternum to the anterior axillary line (Alex *et al*., 2020). During puberty the onset of the menstrual cycle and changes in hormones such as estrogen and progesterone in the body leads to the incomplete development of the breast. Only during pregnancy will the female breast mature to its full capacity. Most of the incompletely developed breast consists of adipose tissue, but also comprises fibro glandular parenchyma and connective tissue. The breast parenchyma contains 15−20 units called lobes. These lobes are made up of 20−40 lobules and each lobule consists of 10−100 hollow cavities known as alveoli that are a few millimeters in size (Alex *et al*., 2020). Cuboidal epithelium capable of synthesizing the protein and lipid components of breast milk, and myoepithelial cells capable of contracting epithelial cells compose each alveolus. The lobes of the breast drain into lactiferous ducts which broaden to form a sinus prior to converging with the nipple. Major ducts are made up of double layers of cuboidal epithelial cells and minor ducts are made up of a single layer of cuboidal cells

while the lactiferous sinus is lined by stratified squamous epithelial cells Figure 1-1 (Alex *et al*., 2020). The lactiferous sinus drains to the nipple-areola complex- the more pigmented circular area on the vertex of the breast (Quora *et al*., 2021). Underneath the areola, smooth muscle fibers lie in a circular pattern in the dense connective tissue and parallel to lactiferous ducts in order to erect nipples in response to appropriate stimuli. The areola also contains sweat, sebaceous and accessory glands called the Montgomery tubercles, which secrete oils (Quora *et al*., 2021).

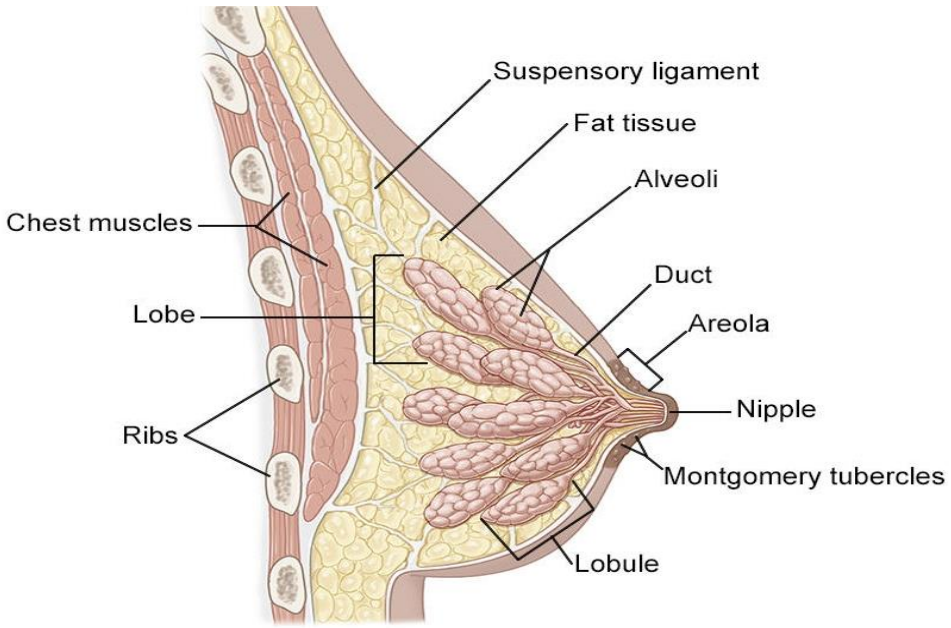


Fig. 1-1 The anatomy of the woman breast (Alex *et al*., 2020).

1.3 The Lymphatic System of the Breast

The lymphatic system includes lymph nodes and lymphatic vessels. The lymph fluid is made in the process of balancing hydrostatic and oncotic pressure in capillaries and tissues. It has several interesting and unique characteristics. The

lymphatic system, which begins with the blind tubes, passes through the lymph nodes and eventually connects to the heart through the veins, such as the subclavian vein (Jeon & Chang, 2020). Unlike the veins, the lymphatics have fewer bifurcations, and they show the clear direction towards axillary or inguinal lymph nodes in the extremities and moves in one direction through the smooth muscle action and valves in the lymphatic vessels. It is also intriguing that the connection between the superficial and deep lymphatic system is not significant (Jeon & Chang, 2020). Although there exist perforating lymphatic vessels and they connect the superficial and deep lymphatic vessels, some authors argue that they only parallel the arteries originating below the deep fascia and do not connect the two lymphatic systems definitely (Jeon & Chang, 2020). The lymphatic vessels can be largely divided into three categories including lymph capillaries, pre-collectors, and lymph-collecting vessels (Suami & Scaglioni, 2018). The lymph capillaries (20 to 70 μm) are located just beneath the epidermis and have blind endings. They are formed with the endothelial cells that are loosely overlapped with each other. Anchoring filaments connect the endothelial cells and the surrounding tissue. In the case of excess interstitial fluid being, the anchoring filaments are stretched and the gaps between the endothelial cells are open to absorb the fluid (Suami & Kato, 2018). The lymph capillaries don't have valves and are connected by the pre-collectors in the deep dermal layer. The pre-collectors (70 to 150 μm) have unidirectional valves helping lymph flow from the superficial to deep layers and become larger vessels through meeting together in the dermal layer (Suami, 2016). Via the efferent pre-collectors, they connect to the lymph-collecting vessels in the subcutaneous layers. The lymph-collecting vessels (150 to 500 μm) can be subdivided as superficial and deep ones according to their anatomical location to the deep fascia. They have three-layered structure, including the endothelial, smooth muscle, and collagen fiber with fibroblast layers (Greene & Goss, 2018) .

The smooth muscle layer performs a major role in rhythmic contraction for the directional flow of the lymph fluid. In the upper extremities, there may be an alternative pathway of the superficial lymph-collecting vessels that skips the axillary lymph nodes (sentry nodes) and goes to the supraclavicular lymph nodes through the deltopectoral nodes (interval lymph nodes). It can work as a detour for preventing upper-extremity lymphedema (Greene & Goss, 2018).

1.4 Etiology and Risk Factors of Breast Cancer

Breast cancer is common in the United States (US), with approximately one in eight women diagnosed during their lifetime . Incidence increases with age, but roughly 18% occur before the age of 50 years (DeSantic *et al*., 2019). Razavi suggests that there is etiologic heterogeneity of breast cancer associated with age at onset (young-onset vs. older-onset) (Razavi *et al*., 2020) . It was proposed that prenatal exposure to endogenous or exogenous hormones might influence the trajectory of breast cancer development later in life, implying that a critical window for carcinogenesis of the breast might begin during prenatal life. For example, intrauterine exposure to insulin-like growth factors has been proposed to have a role in breast cancer risk (Schernhammer, 2002). Other perinatal factors, such as birth weight and birth length, might reflect in utero exposure to growth hormones (Zheng *et al*., 2021). Most of the research studies evaluating the association between birth weight and risk among young women have reported a modest increase in breast cancer risk associated with high birth weight (Bothou *et al*., 2020).

1.5 Prevalence of Breast Cancer

Women in the Middle East face a significant risk of high mortality rate from BC, due to the delay in the diagnosis and the advanced stages of the disease at the time of diagnosis. In kingdom of Saudi Arabia, BC is usually diagnosed at late stage and more frequently in young, pre-menopausal women, under the age of 45, in comparison to western countries (Alshareef1 *et al*., 2019). In Iraq breast cancer is the most common type of malignancy among Iraqi women as shown in Figure 1-2 (Al-Rawaq et al., 2016). It accounts approximately one third of the registered female cancers according to the latest Iraqi Cancer Registry which shows a trend for the disease to be affected younger age groups(Alwan N, 2014). The morbidity and mortality of cancer in Iraq show a horrible results in increased incidence of breast cancer (4,542) cases in female annually, The mortality 9,000 deaths in female annually 1 in 6 women worldwide develop cancer during their lifetime, 1 in 11 women die from the disease (Ferlay *et al*., 2018). An additional 51,400 cases of ductal carcinoma in situ (DCIS) diagnosis in women. (ACS, 2022). The etiologies of the majority of BCs are unknown, with only about 25% to 40% of them attributed to well known risk factors (ACS, 2009). Awareness of BC risk factors (gender, age, family, personal history, racial factors, radiation exposure, breast changes, early menarche, late menopause, prolonged null parity, obesity, diet, alcohol consumption, tobacco smoking, excessive estrogen exposure, oral contraceptive use, stress, anxiety) and perception of personal risk, are important factors for motivation, prevention, and/or early detection of the disease (RB .,2010). Knowledge about the disease (including screening methods and warning signs) plays an important and effective role towards developing and employing screening programs in a community. This can effectively improve the chances of early detection of BC, should result in an improvement in survival rates and quality

of life (Elmore *et al*., 2005). Late diagnosis of BC is mainly due to lack of awareness in the population and barriers to access health services as shown in Figure 1-3 (WHO, 2018). Early detection of BC can be achieved through: Implementing effective screening programs and annual mammogram in the targeted population; Improving public awareness about signs and symptom of BC; and the encouragement of females to take a prompt action (Saud & Siddig, 2017).

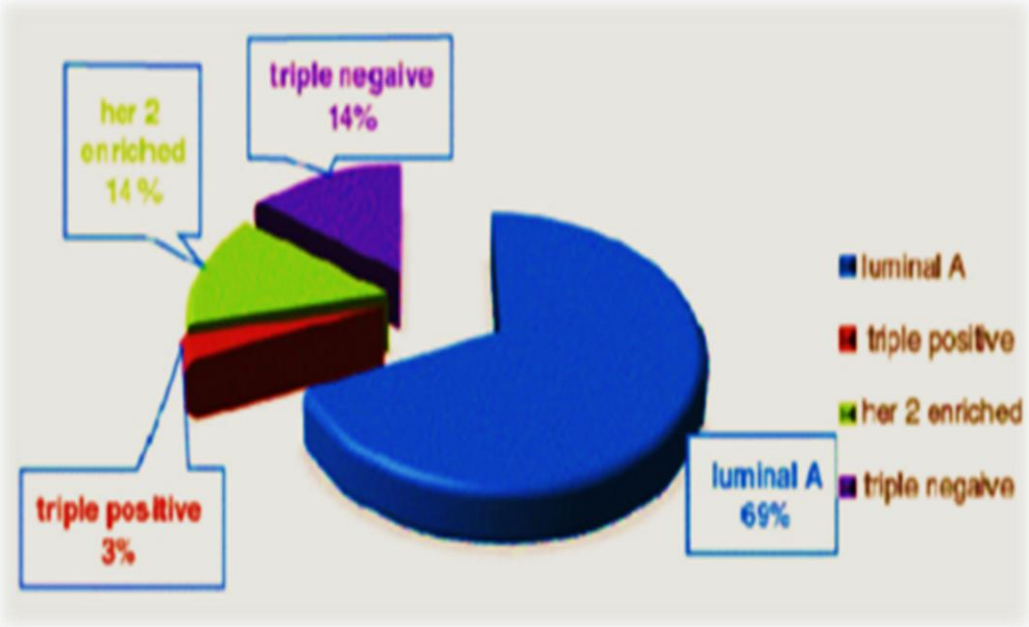


Fig 1-2 prevalence of molecular subtypes of breast cancer in Iraq (Al-Rawaq *et al*., 2016)

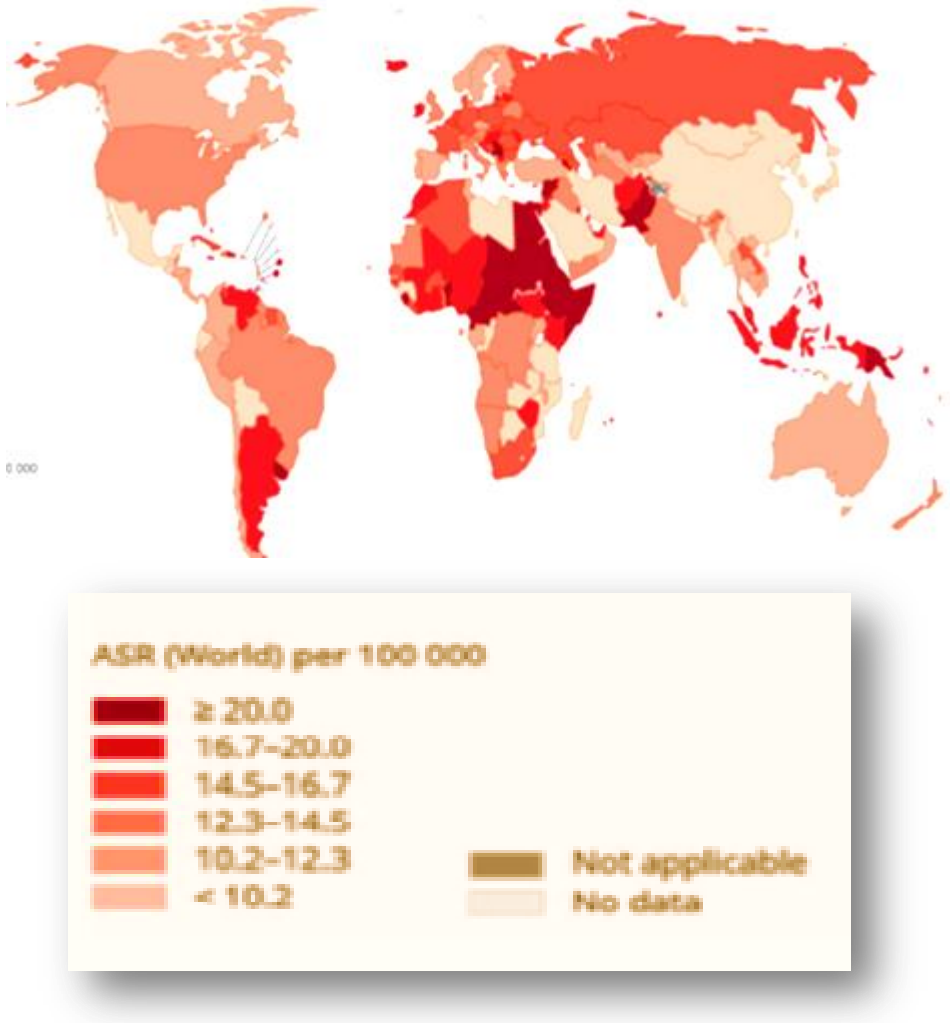


Fig 1-3 Prevalence of breast cancer according to (WHO and Globocan 2018).

1.6 Signs and symptoms

Some people have no symptoms and the cancer is found during a mammogram (a low-dose x-ray of the breast) through the National Breast Cancer Screening Program. If you do have symptoms, they could include (Cardoso *et al*., 2019) :

• A lump, lumpiness or thickening, especially if it is in only one breast.

• A change in the size or shape of the breast

• A change to the nipple, such as a change in shape, crusting, sores or ulcers, redness, a clear or bloody discharge, or a nipple that turns in (inverted) when it used to stick out

• A change in the skin of the breast, such as dimpling or indentation, a rash, a scaly appearance, unusual redness or other color changes

• Swelling or discomfort in the armpit

• Ongoing, unusual pain that is not related to your normal monthly menstrual cycle, remains after your period and occurs in one breast only (Cardoso *et al*., 2019).

1.7 Histological classification of breast tumors

According to the English version of histological classification of breast tumors in the General Rules 18th ed. prepared by the General Rule Committee of the Japanese Breast Cancer Society as shown in Figure 1-4 (Tsuda, 2019).

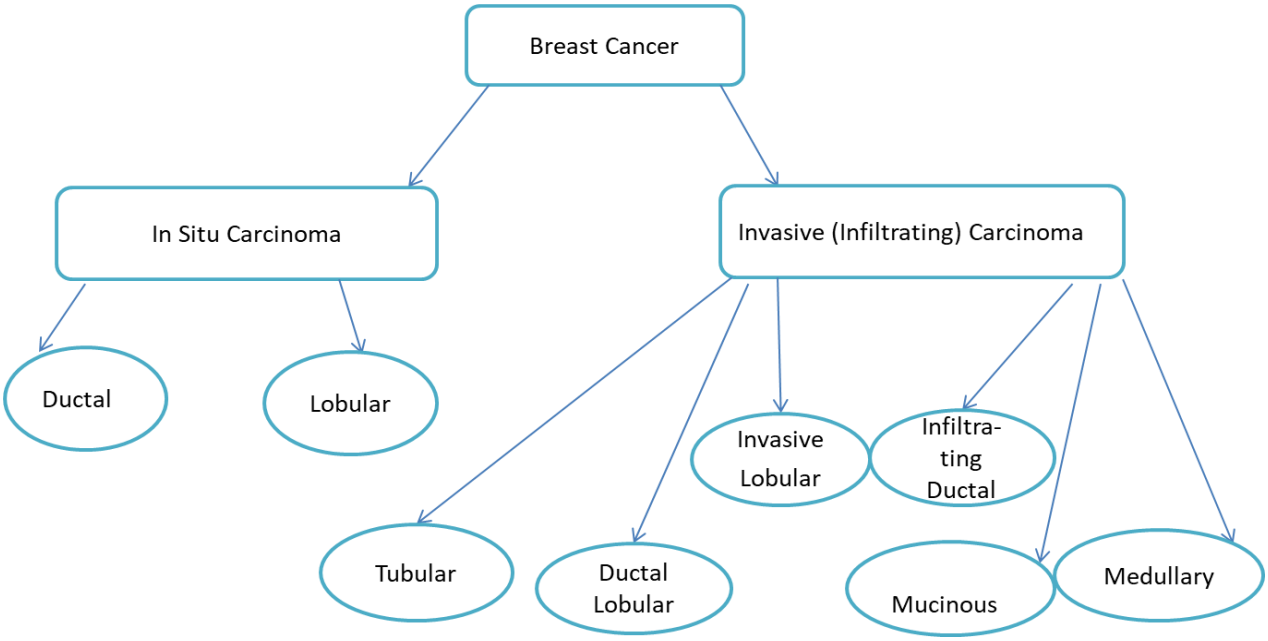


Fig 1-4 Histopathological classification of breast cancer

1.7.1 Benign Tumors

Adenomyoepithelioma (AME) is composed of both duct epithelial cells and myoepithelial cells, wherein the proliferation of the latter predominates. This tumor shows the coalescence of well circumscribed multiple nodules surrounded by fibrous connective tissue as shown in Figure 1-5 (Aliȣ Talaat, 2020). In general AME is a benign tumor. It rarely shows malignant transformations in either or both of duct epithelial and myoepithelial components, and in that case, is classified as AME with carcinoma or malignant. AME in the category of malignant tumor. Pleomorphic adenoma, apocrine adenoma. These entities can be incidentally detected as solitary lesions or accompanied by other benign lesions. Their implications as risk indicators of invasive cancer occurrence and/or as precancerous lesions in themselves are under discussion. The classification of

benign tumors maintains its previous five categories: intra ductal papilloma, ductal adenoma, nipple adenoma, adenomas, and adeno mayo epithelioma (Tsuda, 2019).

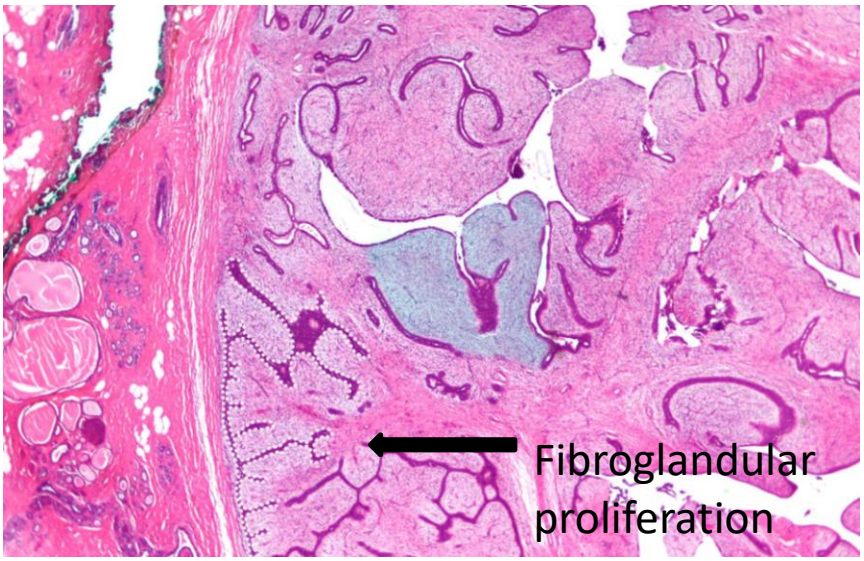


Fig. 1-5 Cell of benign tumor of breast (Ali & Talaat, 2020).

1. Intra ductal papilloma (IDP):- Is papillary tumor arising in the mammary duct. Clinically, discharge, sometimes bloody discharge, is common from the nipple. Some tumors are solitary, while others are multiple (Tsuda, 2019).

2. Ductal adenoma:- Comprises the proliferation of benign epithelial cells, and is often accompanied by central scar like fibrosis. This entity sometimes expands outside mammary ducts as pseudo-invasion and/or shows apocrine metaplasia with relatively conspicuous cellular atypia. Note: ductal adenoma is a synonym of sclerosing intra ductal papilloma.

3. Nipple adenoma:- Is an adenoma of papillary or solid structure arising within the nipple or a sub-areolar mammary duct. This tumor may be confused with carcinoma.

4. Adenoma is mainly composed by the proliferation of epithelial components with relatively scant stromal components. The adenoma is sub classified into tubular adenoma and lactating adenoma. The former entity has a tubular structure. The latter occurs during lactation and is composed of mammary gland tissue showing secretory changes (Tsuda, 2019).

5. Adenomyoepithelioma (AME) is composed of both duct epithelial cells and myoepithelial cells, wherein the proliferation of the latter predominates. In general, AME is a benign tumor. It rarely shows malignant transformations in either or both of duct epithelial and myoepithelial components and in that case is classified as AME with carcinoma or malignant (Masood, 2016).

1.7.2 Malignant Tumors

For the morphological study of breast cancer, first must understand whether the tumor is limited to the epithelial component of the breast or has invaded the surrounding stroma, and whether this tumor appeared in the mammary ducts or lobes (Vuong *et al*., 2014). However, in histopathological practice, cell type characteristics, number of cells, type and location of secretion, immune histochemical profile and architectural characteristics determine if the tumor is ductal or lobular, in addition to its sub-classifications, rather than its precise location in the mammary tissue (Makki, 2015). About 50% to 80% of newly diagnosed breast cancer cases are called invasive ductal carcinoma (IDC); the rest of the cases are classified as invasive lobular carcinoma (ILC) (Lynn Henry & Lisa, 2019). IDCs can be classified as “no specific type” because these tumors do not present sufficient morphological characteristics to be determined as a characteristic histological type; they can also be recognized as a “special type” if

they present sufficient distinctive characteristics, and particular cellular and molecular behavior (Masood, 2016).

1.7.2.1 Invasive Ductal Carcinoma

No specific type (IDC-NST) The histological subtype IDC-NST is the most common, constituting about 40% to 75% of all invasive breast carcinomas. Usually, it has a wide scope of morphological variation and clinical behavior (Masood, 2016). Tumor cells are pleomorphic with protruding nucleoli and numerous mitoses as shown in Figure 1-6.

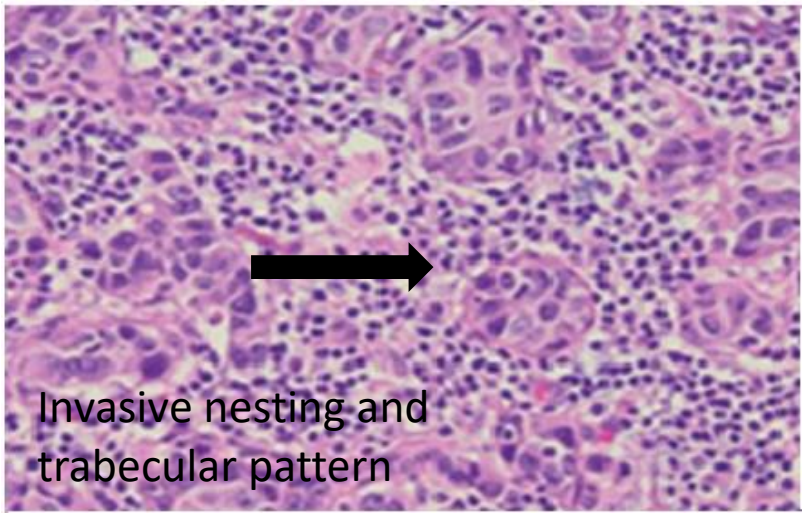


Fig 1-6 Cells of invasive ductal carcinoma (Masood, 2016).

1.7.2.2. Medullary carcinoma

Special subtype of invasive breast carcinoma, responsible for approximately 5% of all cases, and associated with better clinical results and lower rates of involvement in axillary lymph nodes (Akram *et al*., 2017) It usually affects

patients between 30 and 40 years old and is often associated with mutations in the BRCA1 germline (Breast cancer gene 1), as shown in Figure 1-7 (Masood, 2016).

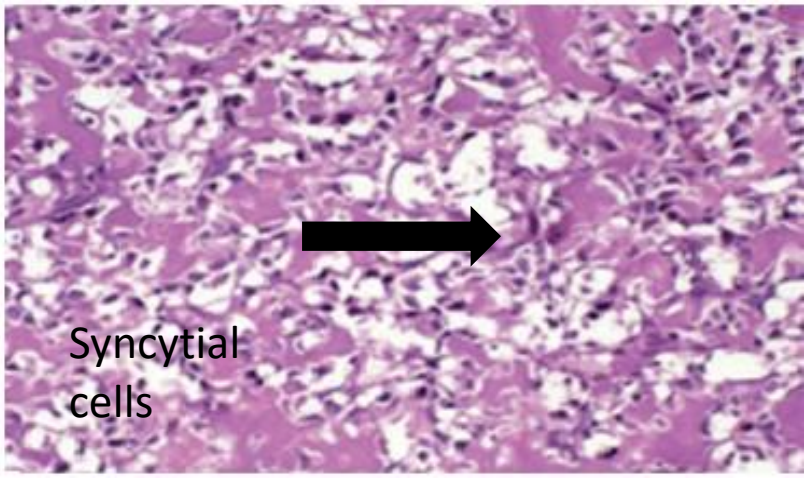


Fig. 1-7 Cells of medullary carcinoma (Masood, 2016).

C. Metaplastic carcinoma

Metaplastic carcinoma histological subtype is characterized by the dominant component of metaplastic differentiation, representing approximately 1% of all cases and affecting women, mainly in post-menopause (Nascimento & Oton, 2020). Morphologically it is a poorly differentiated heterogeneous tumor that contains ductal carcinoma cells mixed with other histological elements such as squamous cells, spindle cells as shown in Figure 1-8 (Papa, *et al*., 2021).

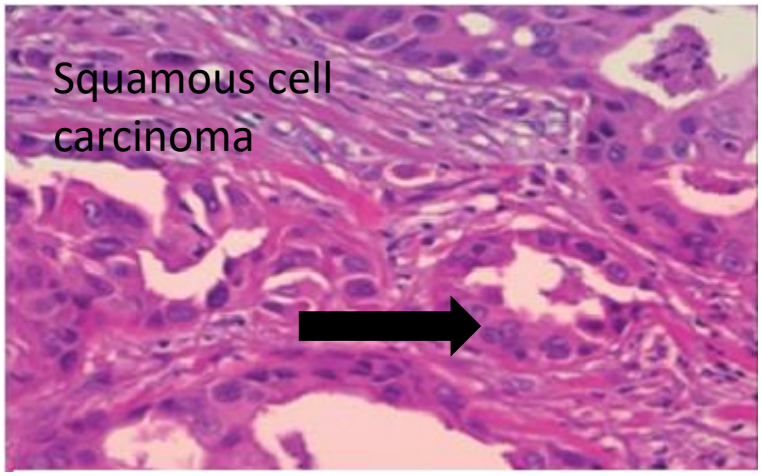


Fig 1-8 Cells of metaplastic carcinoma (Nascimento & Oton, 2020).

D. Apocrine carcinoma

Apocrine carcinoma constitutes about 1% to 4% of all cases, with prominent apocrine differentiation comprising at least 90% of tumor cells (Makki, 2015). This subtype is generally of high histological grade, it is more commonly seen in postmenopausal women (Vranic *et al*., 2013). Microscopically tumor cells are large with an abundant granular eosinophilic cytoplasm, positive for PAS (Periodic acid-reactive Schiff) staining and prominent nucleoli; in addition, bizarre tumor cells with multi lobulated nuclei can also be observed as shown in Figure 1-9 (Vranic *et al*., 2013).

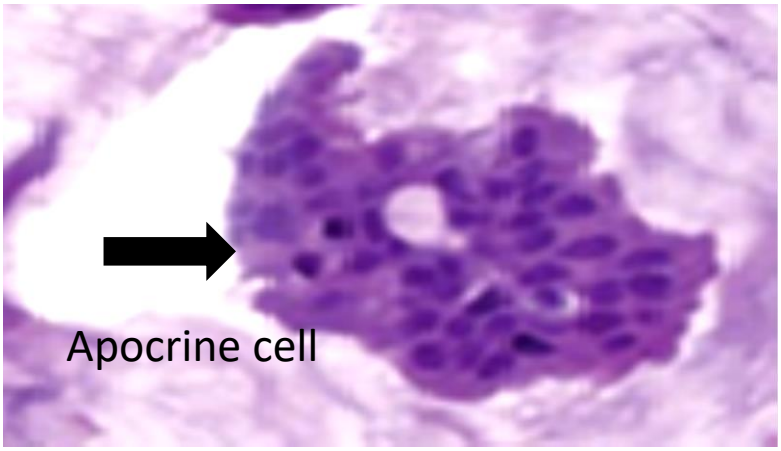


Fig 1-9 Cells of apocrine carcinoma (Vranic *et al*., 2013).

E. Mucinous carcinoma

Mucinous carcinoma is a special subtype of breast cancer, also known as colloid, gelatinous, mucous and mucoid carcinoma, responsible for 2% of all newly diagnosed cases (Akram *et al*., 2017). This subtype has been associated with a favorable prognosis and often affects women over 60 years of age (Marrazzo *et al*., 2020). Morphologically, these tumors have abundant amounts of extracellular mucin, surrounding small clusters of tumor cells with different growth patterns and with mild nuclear atypia as shown in Figure 1-10 (Zhang *et al*., 2018).

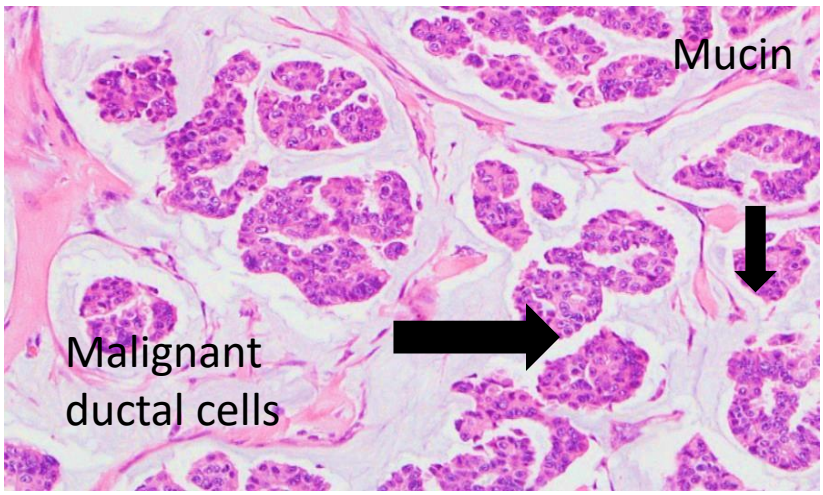


Fig 1-10 Cell of Mucinous carcinoma (Hu *et al*., 2021).

1.2.7.6. Cribriform carcinoma

Special subtype associated to a good prognosis, generally affecting patients who are approximately 50 years old and constituting about 1% to 3.5% of all breast cancer cases. Cribriform carcinoma has almost no evidence of regional or distant metastasis (Makki, 2015). Microscopically, this subtype presents islands of uniform tumor cells, with low-grade atypia as shown in Figure 1-11, cribriform appearance in 90% of the tumor and often associated with DCIS (Ductal carcinoma in situ) without well-defined stromal invasion (Cong *et al*., 2015).

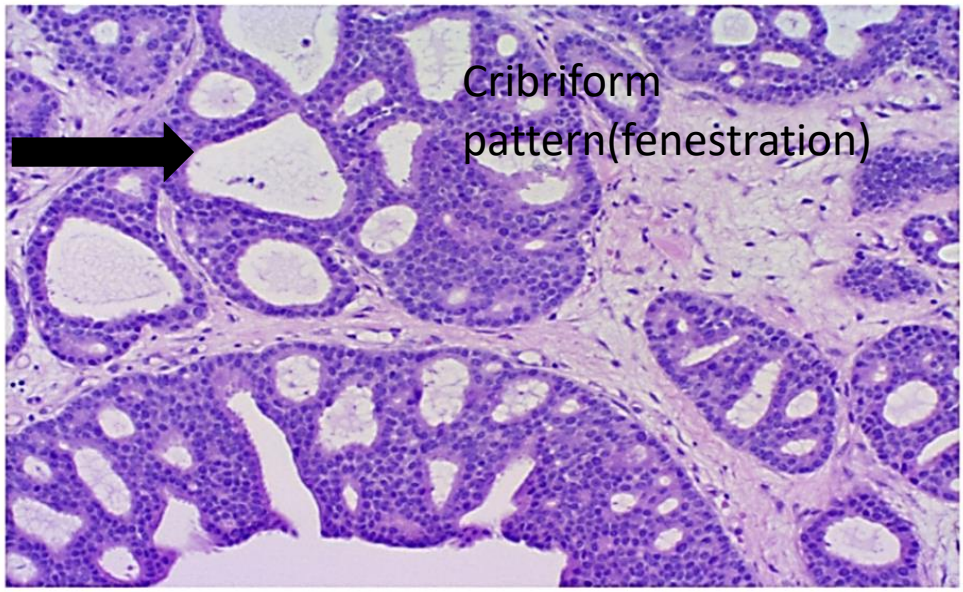


Fig 1-11 Cells of cribriform carcinoma (Mukend *et al*., 2018).

1.7.2.7. Tubular carcinoma

Well-differentiated subtype, occurring in women between 50 and 60 years of age and constituting about 2% of all newly diagnosed cases. Most tubular carcinomas are associated to a wide range of potentially premalignant proliferative lesions (Zhang *et al*., 2018). This subtype is characterized by the proliferation of

prominent tubules (>90%), which can be angled, oval or elongated, with a disorganized disposition and open lumen covered by a single layer of epithelium, usually without presentation of necrosis and mitosis as shown in Figure 1-12 (Zhang *et al*., 2018).

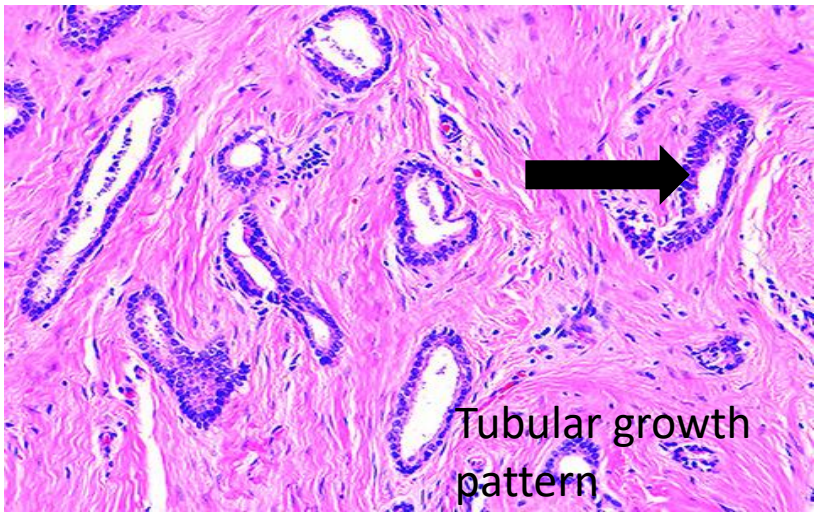


Fig 1-12 Cells of Tubular carcinoma (Masood, 2016).

H. Neuroendocrine carcinoma

Neuroendocrine carcinoma is constitutes about 0.5% to 5% of all cases of breast cancer and commonly occurs in older ages (Masood, 2016). This type of tumor has characteristics similar to neuroendocrine tumors of the gastrointestinal tract and lung, consistently expressing the markers chromogranin A and synaptophysin in more than 50% of neoplastic cells (Jurčić *et al*., 2016). Morphologically, there is an infiltrative growth pattern with solid aggregates of tumor cells arranged in alveolar, trabecular or rosette patterns, and peripheral palisades can also be observed as shown in Figure 1-13. Neoplastic cells can be of different sizes and generally have fine eosinophilic granular cytoplasm (Li *et al*., 2017).

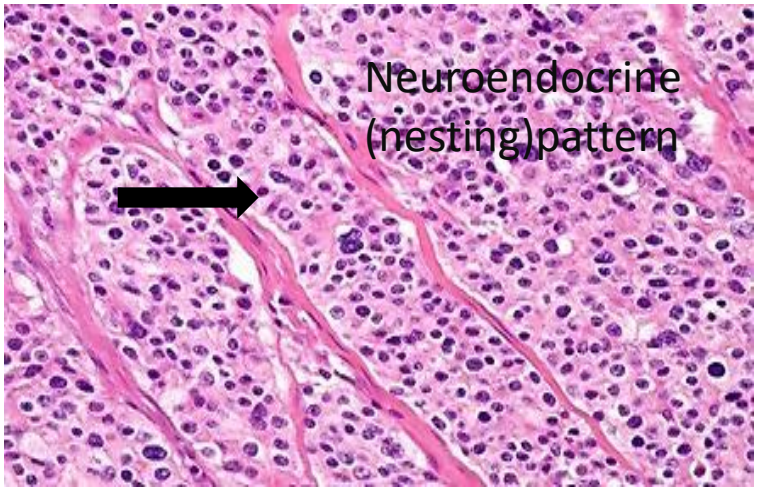


Fig 1-13 Cells of neuroendocrine carcinoma (Li *et al*., 2017).

1.7.2.9. Invasive lobular carcinoma

Invasive lobular carcinoma is the second largest biologically distinct carcinoma, representing about 5% to 15% of all newly diagnosed cases and generally affecting women of advanced age. The classic form of the ILC is characterized by the presence of small tumor cells with little atypia, uniformly distributed throughout the stroma in a concentric pattern as shown in Figure 1-14. Among pleomorphic ILC, tumor cells have a hyperchromatic and eccentric nucleus, prominent mitoses and apocrine. Histiocytic or signet ring cells can be observed and they are more likely to have TP53 mutations (Tumor protein 53) (Nascimento & Oton, 2020)

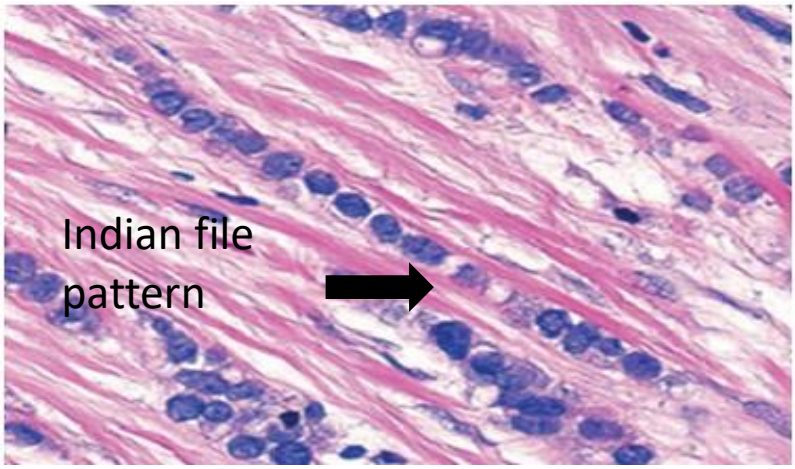


Fig 1-14 Cells of invasive lobular carcinoma (Nascimento & Oton, 2020).

1.8 Cancer Grading

Histological tumor grade is based on the degree of differentiation of the tumor tissue. In breast cancer, it refers to the semi-quantitative evaluation of morphological characteristics and is a relatively simple and low-cost method, requiring only adequately prepared hematoxylin-eosin-stained tumor tissue sections to be assessed by an appropriately trained pathologist using a standard protocol. NGS is based on the evaluation of three morphological features: (a) degree of tubule or gland formation, (b) nuclear pleomorphism, and (c) mitotic count (Pathology Reporting, 2005). Invasive carcinomas are morphologically subdivided according to their growth patterns and degree of differentiation, the latter of which reflects how closely they resemble normal breast epithelial cells. This subdivision is achieved by assessing histological type and histological grade, respectively. The Nottingham (Elston-Ellis) modification of the Scarff-Bloom-Richardson grading system, also known as the Nottingham Grading System (NGS) (Elston CW *et al*., 1991**)** , is the grading system recommended by various professional bodies internationally (World Health Organization [WHO], American Joint Committee on Cancer [AJCC], European Union [EU], and the Royal College of Pathologists (UK RCPath) (Pathology Reporting, 2005, and Tavassoli *et al*., 2003) .

1.9 Cancer staging

Breast cancer staging is based on the TNM system, defined by the American Joint Committee on Cancer, which takes into account tumor (T) size, the extent of regional lymph node (N) involvement, and the presence or absence of metastasis

(M) beyond the regional lymph nodes as shown in Table 1-1 and Table 1-2 . Using this system, whose criteria 3and details are outlined in Table 1, breast cancer is staged from 0 to IV. Stage 0 implies in situ cancer, while stages I to IV indicate invasive cancer, with IV implying metastatic spread to distant organs (Hammer *et al*., 2008).

Table 1-1 TNM staging system of breast cancer for histopathology (Kalli *et al*., 2018)

TNM Class	Criteria
T0	No evidence of primary tumor
T1a	Carcinoma in situ
T1	< or = 2 cm
T1m1c	Microinvasion .1 cm or less
T1a	>.1 to .5 cm
T1b	>.5 to 1 cm
T1c	>1 to 2 cm
T2	>2 to 5 cm
T3	>5 cm
T4	Any size tumor with direct extension to: a) Chest well or b) skin
T4a	Chest well , not including pectoralis muscle
T4b	Skin edema, ulceration, satellite skin nodule
T4c	4a and 4b
T4d	Inflammatory carcinoma
Nx	Regional lymph nodes cannot be removed
N0	No regional lymph node metastasis
N1	Metastasis to movable ipsilateral axillary lymph node

N2	Metastasis in ipsilateral axillary lymph nodes fixed of matted (N2a) or met. Only in clinically apparent ipsilateral mammary nodes without clinically evident axillary lymph nodes. (N2b)
N3	Metastasis in ipsilateral infraclavicular lymph nodes (N3a) or clinically apparent ipsilateral internal mammary lymph nodes (N3b) or ipsilateral supraclavicular lymph nodes (N3c)
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Table 1-2 Staging system of breast cancer for oncology (Amin *et al*., 2017)

Breast cancer stages	Classification criteria based on TNM
Stage 0	Tis, N0, M0
Stage IIA	T0, N1, M0 or T1, N1, M0 or T2, N0, M0
Stage IIB	T2, N1,M0 T3, N0, M0
Stage IIIA	T0, N2, M0 or T1, N2, M0 or T2, N2, M0 or T3, N1, M0 or T3, N2, M0

Stage IIIB	T4, N0, M0 or T4, N1, M0 or T4, N2, M0
Stage IIIC	any T, N3, M0
Stage IV	any T, any N, M1

1.9.1 Stage Zero

Stage zero means that breast cancer cells have developed, but they did not have time to spread to any surrounding tissues, or to the lymph nodes or other organs. Usually zero stage means the so-called ductal carcinoma in situ or lobular carcinoma in situ .At stage 0 survival rate during 5 years is almost 100% (Blanco *et al*., 2018).

1.9.2 The first stage of breast cancer

The first stage means that tumor size is less than 2 cm. And that indicates that has not yet spread to the lymph nodes or to other organs (i.e. not metastasized) (Stage 1A) or was able to form only micro metastases in 1-3 lymph nodes in the underarm area (Stage 1B) (Epstein *et al*., 2011). On the 1st stage, the survival rate during 5 years is almost 100% (ACS, 2016).

1.8.3 The second stage of breast cancer

1.9.3.1 Stage2A

It indicates that tumor size is less than 2 cm (or not detected) and has one or both of the following characteristics:

A -Already spread to no more than 3 lymph nodes under the arm, forming metastases more than 2 mm in diameter.

B-Small metastases were found in the lymph nodes in mammary glands. C- tumor is greater than 2 cm in diameter, but less than 5 cm and has not spread to the lymph nodes or other organs (Laaksonen & Mitsuk, 2016).

1.9.3.2 Stage2B

This stage indicates that tumor is greater than 2 cm in diameter, but less than 5 cm and has not spread to the lymph nodes or other organs or small cancer metastases were found in no more than three lymph nodes in the axilla and / or lymph nodes in mammary gland, but not found in the internal organs; or the tumor is larger than 5 cm in diameter, but has not grown into the chest wall or skin and did not form metastases in the lymph nodes or internal organs (Laaksonen & Mitsuk, 2016). At stage 2 the survival rate during 5 years is about 93% .

1.9.4 The third stage of breast cancer

1.9.4.1 Stage 3A

This stage indicates that tumor is not more than 5 cm across and has spread to no more than 9 lymph nodes in the axilla or formed metastases in the lymph nodes in mammary gland, but not to internal organs. Or the tumor is greater than 5 cm in diameter, has spread to no more than 9 of the lymph nodes under the arm and formed metastases in the lymph nodes in mammary glands, however, did not grow into the breast bone or skin and has no metastasis in internal organs (ACS, 2016).

1.9.4.2 Stage 3B

This stage means that the tumor had time to grow into the chest wall or skin, but did not form metastases in the internal organs and has one of the following characteristics found from :

A-No metastases in the lymph nodes.

B- It as only 1-3 micro metastases in lymph nodes or axillaries lymph nodes in mammary glands.

C- It does not spread to more than 9 lymph nodes in the axilla or form metastases in the lymph nodes inside the mammary gland.

1.9.4.3 Stage 3C

In this stage the tumor regardless of its size; it has one of the following characteristics, as said in :

A-Has metastases in more than 10 lymph nodes under the arm.

B-Has metastases in the lymph nodes under or above the collarbone. During the stage 3 survival rate during 5 years is approximately 72% (Burbage, 2020).

1.2.9 .5 The fourth stage of breast cancer

It is the stage where the tumor is regardless of its size, was able to form metastases in other organs. In most cases, breast cancer metastases are formed in bones, liver, brain or lungs (Li *et al*., 2017). Survival rate during 5 years at 4th stage is on average 22% (Burbage, 2020).

1.10 Breast cancer prevention

For many women and their health care providers, breast cancer prevention is synonymous with mammography screening. Regular breast cancer screening of asymptomatic women through mammography is considered a form of secondary prevention, because the early detection of breast cancer through screening can lead

to more effective treatment, better outcomes, and fewer deaths (Secretan *et al*., 2015). The decline in breast cancer mortality from 1975 to 2000 was attributed, in part, to mammography screening (Berry *et al*., 2007). although improvements in treatment likely drove most of that and subsequent declines (Narod *et al*., 2015). Nearly 90% of the women diagnosed with breast cancer in the United States will survive at least 5 years (Owens *et al*., 2019).

1.11 Treatment of breast cancer

Drugs used to treat breast cancer are considered systemic therapies because they can reach cancer cells almost anywhere in the body. Some can be given by mouth, injected into a muscle, or put directly into the bloodstream. Depending on the type of breast cancer, different types of drug treatment might be used, including:

l -Chemotherapy for Breast Cancer

2- Hormone Therapy for Breast Cancer

3- Targeted Drug Therapy for Breast Cancer

4- Immunotherapy for Breast Cancer

The ACS reach to recovery program connects people facing breast cancer – from diagnosis through survivorship – with trained volunteers who are breast cancer survivors. Clinical trials are carefully controlled research studies that are done to get a closer look at promising new treatments or procedures. Clinical trials are one way to get state-of the art cancer treatment. In some cases they may be the only way to get access to newer treatments. They are also the best way for doctors to

learn better methods to treat cancer. Still, they're not right for everyone. If you would like to learn more about clinical trials that might be right for you, start by asking your doctor if your clinic or hospital conducts clinical trials (ACS,. 2018).

1.11.I Surgery for Breast Cancer

Most women with breast cancer have some type of surgery as part of their treatment. There are different types of breast surgery, and they may be done for different reasons, depending on the situation. For example, surgery may be done to:

1-Remove as much of the cancer as possible (breast-conserving surgery or mastectomy).

2- Find out whether the cancer has spread to the lymph nodes under the arm (sentinel lymph node biopsy or axillary lymph node dissection)

3-. Restore the breast's shape after the cancer is removed (breast reconstruction)

4-. Relieve symptoms of advanced cancer.

There are two main types of surgery to remove breast cancer:

1-Breast-conserving surgery is surgery to remove the cancer as well as some surrounding normal tissue. Only the part of the breast containing the cancer is removed. How much breast is removed depends on where and how big the tumor is, as well as other factors. This surgery is also called a lumpectomy, quadrantectomy, partial mastectomy, or segmental mastectomy (Hudis *et al*., 2016).

2-Mastectomy is a surgery in which the entire breast is removed, including all of the breast tissue and sometimes other nearby tissues. There are several different

types of mastectomies. Some women may also have both breasts removed in a double mastectomy (Cruz *et al*., 2015).

1.11.II Chemotherapy

Chemotherapy is recommended in the vast majority of triple-negative, HER2-positive breast cancers and in high-risk luminal-like HER2-negative tumors . The absolute benefit of is more pronounced in ER-negative tumors (Berry *et al*., 2007). Chemotherapy regimens containing both anthracycline (eg, adriamycin) and taxane (such as adriamycin/cyclophosphamide followed by taxane) achieve the greatest risk reduction and remain the appropriate choice in high risk patients. Specifically, the use of anthracycline appears most important in patients with more lymph node involvement and with triple-negative disease (Alvaro *et al*., 2017). In patients who receive a complete course of neoadjuvant chemotherapy with anthracycline and taxane. Arecent trial suggested that capecitabine could lower recurrence rates and improve survival for patients with residual disease following neoadjuvant chemotherapy, (Masuda *et al*., 2017) .Some chemotherapeutic drugs used in Iraq for breast cancer treatment :

Gemzar (gemcitabine for injection, USP) is a nucleoside metabolic inhibitor that exhibits antitumor activity. Gemcitabine HCl is 2´-deoxy-2´,2´-difluorocytidine mono hydrochloride (β-isomer) (Celia, C., *et al*., 2021).

Cytoxan(Endoxan) (cyclophosphamide) is a common chemotherapy drug that stunts or halts cancer cell growth. It is used in combination with other medications to treat breast cancer and several other forms of cancer. When considering Cytoxan or any breast cancer treatments, (Caroline *et al*., 2019). Cytoxan is often combined with one other drug, Adriamycin, in a chemotherapy infusion to treat breast cancer. This combination is called AC (Abe, O., *et al*., 1982).

Taxanes are a class of diterpenes. They were originally identified from plants of the genus Taxus (yews), and feature a taxadiene core. Paclitaxel (Taxol) and docetaxel (Taxotere) are widely used as chemotherapy agents (Hagiwara & Sunada, 2004). Taxotere belongs to a class of chemotherapy drugs called plant alkaloids. Plant alkaloids are made from plants (Ottaggio *et al*., 2008).

Neupogen is a medication given to some people receiving chemotherapy to prevent or treat a condition called chemotherapy-induced neutropenia. Neutrophils are a type of white blood cell (WBC) that help prevent infection (Rigby & DeLeo, 2012). Neupogen works by stimulating the bone marrow to increase the production of white blood cells. After chemotherapy administration, the medication is given as a subcutaneous injection (in the tissue under the skin) (Lee, et al.,2015) .

Table (1-3) Types of common chemotherapy drugs used for breast cancer treatment(Sadeque *et al*., 2000)

CMF	Cyclophosophomide,Methotrexate,5-F4
AC	Doxorubicin, Cyclophosphamide
CAF	Cyclophosphamide,doxorubicin,5-F4
AC→T	Doxorubicin, cyclophosphamide, paclitaxel
CEF	Cyclophosphamide, epirubin,5-F4,vincristine,prednisone
AT	Doxorubicin, docetaxel (Taxoter)
AC→Taxoter	Doxorubicin, cyclophosphamide , docetaxel

1.11.III Radiation Therapy for Breast Cancer

Radiation therapy (also called Radiotherapy) improves breast cancer outcomes in women treated with lumpectomy and in some patients who receive mastectomy. It is often the last step of active treatment for breast cancer patients who had surgery and when indicated, chemotherapy. Radiation therapy causes biological effects in the cancer cells that cause them to die (Balaji *et al*., 2016). After surgery, radiation may be used to stop the growth of any cancer cells that remain. Radiation therapy dose is calculated ahead of time by a team of radiation oncologists, physicists, and dosimetrists. The therapy is based on an individual's specific case, as well as health and body type (Balaji *et al*., 2016). The dose is enough radiation to kill a cancer cell, while still allowing normal cells to recover before the next dose. In patients who are treated with chemotherapy first and then surgery, or surgery without chemotherapy, radiation typically begins 6 to 8 weeks after surgery. In patients who are treated with surgery first and then chemotherapy, radiation typically begins 4 to 6 weeks after the last cycle of chemotherapy (Balaji *et al*., 2016).

1.11.IV Target therapy

With a better understanding of the etiology of breast cancer, molecularly targeted drugs have been developed and are being testing for the treatment and prevention of breast cancer. Targeted drugs that inhibit the estrogen receptor (ER) or estrogen-activated pathways include the selective ER modulators (tamoxifen, raloxifene, and lasofoxifene) and aromatase inhibitors (AIs) (anastrozole, letrozole, and exemestane) have been tested in preclinical and clinical studies. Tamoxifen and raloxifene have been shown to reduce the risk of breast cancer and promising

results of AIs in breast cancer trials, suggest that AIs might be even more effective in the prevention of ER-positive breast cancer. However, these agents only prevent ER-positive breast cancer. Therefore, current research is focused on identifying preventive therapies for other forms of breast cancer such as human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer (TNBC, breast cancer that does express ER, progesterone receptor, or HER2). HER2-positive breast cancers are currently treated with anti-HER2 therapies including trastuzumab and lapatinib (Mohamed *et al*., 2013).

1.12 Oxidative stress

Oxidative stress caused by an imbalance in the redox status of the body. In such a state, increase of free radicals in the body can lead to tissue damage. Damage caused by oxidative stress is involved in many types of diseases, including neurological diseases (Alzheimer's and Parkinson's), diabetes, atherosclerosis, arthritis, inflammation, and, most importantly, broader types of cancer including breast cancer (Nourazarian *et al*., 2014) Oxidative stress is an imbalance in the ratio between oxidants (free radicals) and antioxidants, a condition in which the body's redox and oxidation reactions - resuscitation problems arise. Disorders are caused by the increasing of the imbalance between production and removal of free radicals and reactive species in the body (Samir *et al*., 2019). Many cellular processes, including cell metabolism, signaling pathways, pathways regulating gene expression, cell proliferation, and apoptosis (programmed cell death), are affected by oxidative stress (Poli *et al*., 2004). Increasing the free radicals changes the structure and functions of the main bimolecular body, including changes in proteins, lipids, and nucleic acids, and can lead to tissue damage. Products derived

from this injury are used as biomarkers of oxidative stress in the assessment and diagnosis of all cancers. Especially breast cancer (Abdal Dayem *et al*., 2010). Previous studies have demonstrated that oxidative stress is associated with carcinogenesis and the incidence of cancer. During the carcinogenesis process, the level of reactive oxygen species in cancer cells increases and levels of antioxidants dwindle. destruction caused by oxidative stress has an impressive role in the occurrence and progression of breast cancer. Studies suggest that in this disease oxidative stress is increased. Many mechanisms are effective in enhancing oxidative stress, including genetic variations in antioxidant enzymes, estrogen therapy, and excess reactive oxygen species (Badid *et al*., 2010). Tumor cells produce more free radicals compared with normal cells; thus, they are influenced by oxidative stress. Because of that, markers of oxidative stress have been identified in samples of breast carcinoma. Numerous factors through different mechanisms are involved in increasing reactive oxygen species of breast tumor cells (Mohseni *et al*., 2019).

1.13 Reactive oxygen species (ROS)

Reactive oxygen species (ROS) is a collective term referring to unstable, reactive, partially reduced oxygen derivatives that are created as a by-product of normal metabolic processes (In *et al*., 2017). including superoxide anion, hydrogen peroxide, hydroxyl radical, lipid peroxides, protein peroxides and peroxides formed in nucleic acids (Lushchak, 2014). They are maintained in a dynamic balance by a series of reduction-oxidation (redox) reactions in biological systems and act as signaling molecules to drive cellular regulatory pathways (DmitryZorov *et al*., 2014). Excessive oxidative stress derived from ROS accumulation

deregulates the anti oxidative defense system, which is closely associated with various diseases (Stowe *et al*., 2017). especially cancers. Though emerging studies have demonstrated the primary ligand stimulants, the enzymatic generation mechanisms as well as the putative downstream targets , the major mechanisms by which ROS participates in cancer development in concentration dependent, spatially dependent and temporally dependent manners remain insufficiently understood (Reczek & Chande, 2018).

1.14 Reactive Oxygen Species and Breast Cancer

Beginning in the 1990s, studies set the stage for the concept that ROS are a driving factor for tumor genesis. One important characteristic of cancer cells is their increased ROS levels compared to those in their counterpart cells and the subsequently elevated levels of antioxidants to detoxify the accumulated ROS to reinstitute a redox balance. ROS are thought to play oncogenic roles by contributing to activation of proto-oncogenes and inactivation of tumor suppressor genes and by acting as signaling molecules to induce abnormal cell growth and metastasis(In *et al*., 2017). ROS accumulation in normal cells is one of the initiating factors in the early stage of the neoplastic process. Therefore, an appropriate application of antioxidants can decrease the oxidative stress burden, consequently preventing normal cells from sliding into the abyss of malignant transformation. Numerous epidemiologic data and preclinical/ clinical studies have suggested that keeping an anti oxidative dietary or pharmaceutical application of anti oxidative phytochemicals can effectively prevent tumorigenesis. Breast tumor microenvironment (TME) cells is a perplexing niche that comprises diverse cell types and is supported by an extracellular matrix (ECM) and soluble factors (Deepak *et al*., 2020). BC cells instigate and promote the progression of tumors by establishing tumor-associated stroma from adjoining normal fibroblasts, immune

cells as well as endothelial cells. The TME is characterized by hypoxia, metabolic reprogramming, nutrient deprivation, by enhancing epithelial mesenchymal transition(EMT) and switching to glycolytic metabolism. They originate largely from the mitochondria, transverse to the cytosol, and mediate cellular signaling. ROS are associated with tumorigenesis by affecting multiple biological activities including regulation of cell proliferation, mediation of genomic instability, induction of inflammation, development of resistance to apoptosis, initiation of metabolic reprogramming, and promotion of metastasis (Kalyanaraman *et al*., 2018). Extensive research over the years established the involvement of ROS in BC initiation and progression. In contrast the overproduction of ROS is detrimental to the survival of BC. Chemotherapy, radiotherapy, and photodynamic therapy trigger cell death by inducing ROS, which are also implicated in the activation of apoptotic signaling pathways and the tumor suppressor genes p53 (Averill-Bates *et al*., 2016). However the functional role of ROS in survival or cell death is different among the subtypes of BCs.

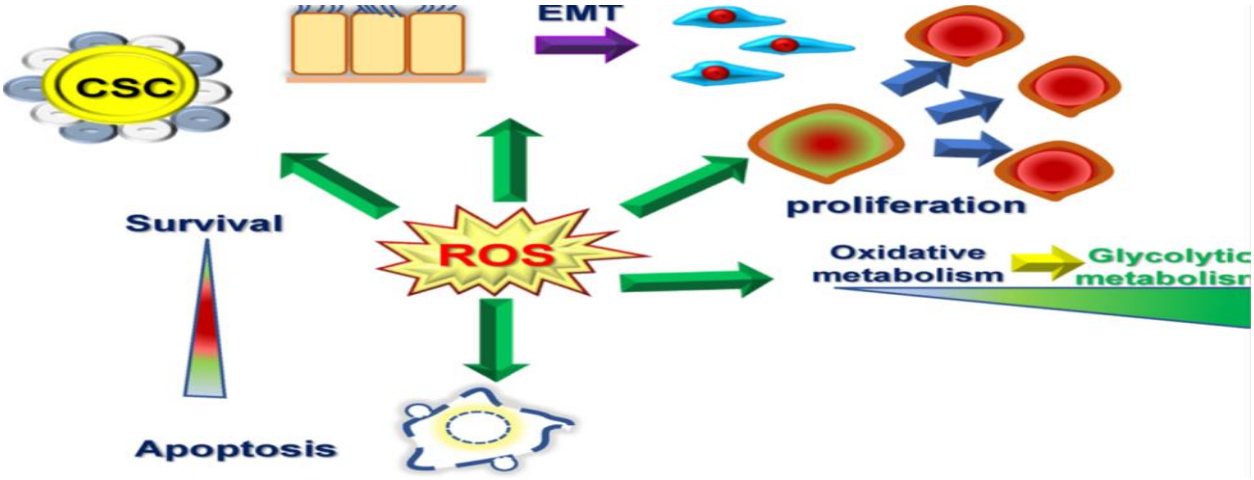


Fig. 1-15 Pathophysiological functions of ROS in breast cancer. (Kalyanaraman *et al*., 2018).

1.15 Lipid Profile and Breast Cancer

lipids are the major cell membrane components essential for various biological functions including cell growth and division of normal and malignant tissues. Usefulness of variations in tissue/blood cholesterol levels in diagnosis and treatment of various diseases has been studied by several workers. Plasma lipids level reflects dietary lipid intake in individuals. There are several reports of elevated plasma lipid level such as total lipids, phospholipids, triglycerides (TG), total-cholesterol (T-CHOL), low density lipoprotein-cholesterol (LDL-C) and free fatty acids in pre and post-menopausal breast cancer patients (Bani, *et al*., 1986). It has been postulated that changes in the concentration of serum lipids in the breast cancer patients could result in an increase production of tumor necrosis factor and inhibit adipose lipoprotein lipase activity by the action of insulin(Knapp, *et al*., 1991). Human mammary tissue metabolizes lipids from plasma affected by female gonadal hormones. Malignant proliferation of breast tissue in women has been associated with changes in plasma lipid and lipoproteins levels (Lane, *et al*., 1995). Low LDL-C in malignancy might be explained by an increased demand of cholesterol from neoplastic cells, resulting in increased LDL removal through the enhancement of LDL receptor activity (Vitols, *et al*., 1985,ß Goldstein, *et al*., 1977). This pattern of lipid abnormalities is very similar to that observed during the acute-phase response in a variety of acute and chronic diseases (Veneracion, *et al*., 1989), and might be due to the release of proinflammatory cytokines (Hardardóttir, *et al*., 1994). It is possible that lipid abnormalities in cancer patients might represent an acute-phase response due to cytokines delivery by inflammatory cells around the tumor or by the tumor cell itself (Blackman, *et al*., 1993).

1.16 Malondialdehyde (MDA)

Malondialdehyde is one of the results of lipid peroxidation is known to be present in human plasma and to possess biological properties that may be relevant to carcinogenesis (Gönenç *et al*., 2001). Lipid peroxides and their products can cause damage to membrane bound enzymes and other macromolecules, including DNA, and have been implicated in several disease processes, including cancer. Numerous studies have examined the possibility of a connection between lipid peroxidation and cancer (Raffa & Tallarida, 2010) and in the last few years, it has come to be considered the main indicator of lipoperoxidative processes (We *et al*., 2021). Thus justifying the studies concerning its role as an indicator of oxidative stress (Hassanpour *et al*., 2021) and a precursor of endothelial disorder(Polidori *et al*., 2002). Some studies showed that lower total antioxidant capacity and higher MDA levels increased oxidative stress and could be related to breast cancer (Go¨nenc *et al*., 2007).

1.17 Homocysteine

Homocysteine (Hcy), a sulfhydryl-containing non-proteinogenic amino acid, is a metabolic intermediate produced by the demethylation of methionine (Met) in the body and is physiologically essential for processes such as cell cycle progression and maintenance of cellular homeostasis (Koklesova *et al*., 2021). In turn, Hcy metabolism contributes to (1) the folate-dependent/independent re methylation to form Met and (2) the trans sulfuration pathway (via cystathionine) to form cysteine as shown in Figure 1-16(Judd *et al*., 2018). Hcy is commonly found in blood plasma in four different forms: circulates as free thiol (1%), remains disulfide-bound to plasma proteins such as albumin (70–80%), and

combines with other Hcy to form the dimer Hcy or combines with other thiols (20–30%) (Hankey, 1999). Elevated Hcy is related to various pathologies, while many of them are also associated with oxidative stress (Sibrian-Vazquez *et al*., 2010) , or inflammation (Elsherbiny *et al*., 2020). Redox state disbalance and oxidative stress are suggested as primary mechanisms associated with pathogenesis related to HHcy. ROS generation occurs during oxidation of the free thiol group of Hcy during its binding either with plasma proteins (such as albumin) or with other low-molecular plasma thiols or another Hcy molecule. Some of the proposed mechanisms of oxidative stress induced by Hcy include auto-oxidation of Hcy (Lehotský *et al*., 2016). Impaired plasma Hcy level is closely related to malignant processes (Hasan et al., 2019). Recent evidence revealed an elevated level of plasma Hcy in a cohort of patients with different cancer types, including breast (Yamashita et al., 2013), colorectal (Ferroni *et al*., 2009), ovarian (Saorin *et al*., 2020), or lung (Xu *et al*., 2019). A high level of Hcy is associated with venous thromboembolism, one of the leading causes of death in cancer patients (Yang *et al*., 2018). Patients with an advanced stage of cancer manifest both HHcy and venous thromboembolism. On the other hand, patients with early-stage cancer have a low plasma Hcy, and venous thromboembolism is absent (He *et al*., 2021). In general, HHcy is a risk factor for venous thromboembolism after chemotherapy or surgery treatment (Pruner *et al*., 2019).

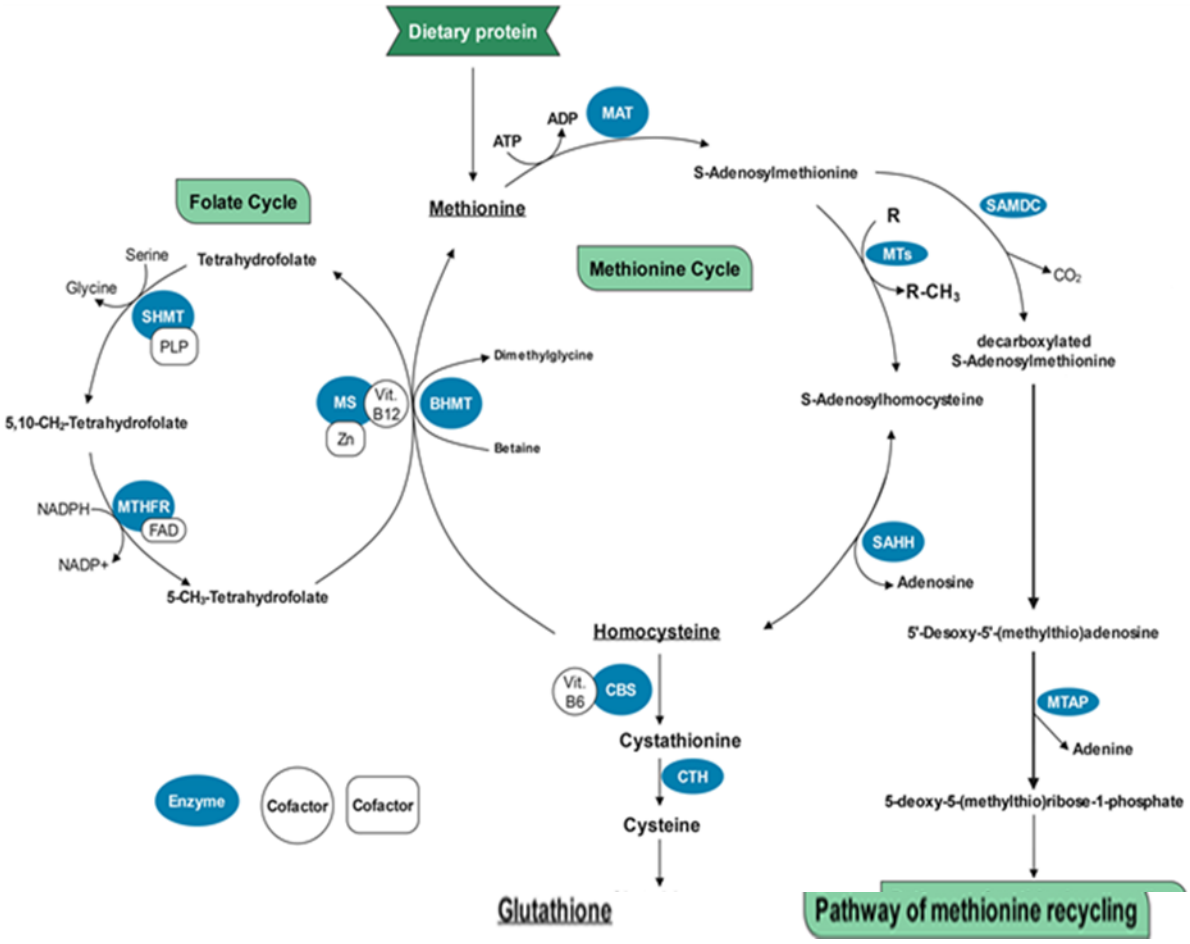


Fig 1-16 Methionine-homocysteine cycle and polyamines pathways. (Yaméogo, N**.** *et al*., 2022).

1.18 Antioxidants

Antioxidants are compounds that inhibit the oxidation of other molecules by neutralizing ROS and other reactive species (Kurutas, 2016), which can be enzymatic, such as superoxide dismutase or catalase, or nutrient-delivered molecules, consumed with foods or by dietary supplement intake (Gulcin, 2020) Research has shown that oral antioxidant supplementation may reduce side effects of chemo and radiotherapy, leading to better quality of life (Singh *et al*., 2017). However, there is also a controversy around the positive effect of antioxidant

supplementation on cancer treatment, since it was associated with lower therapeutic efficacy, and cancer recurrence (Ambrosone *et al*., 2019). Previous studies have shown that a higher content of dietary antioxidants, obtained through food, was associated with lower oxidative stress biomarkers in healthy adults (Bacchetti *et al*., 2018). In this context, the Dietary Antioxidant Capacity (DAC) is a useful tool for evaluating the dietary antioxidant content, since it considers all type of food antioxidants present in the diet, and the synergistic effects between them (Mozaffari *et al*., 2019). It has been shown that DAC is inversely associated with breast cancer risk , but there is lack of evidence regarding the influence of DAC on oxidative stress in women undergoing adjuvant treatment for breast cancer (Sasanfar *et al*., 2020).

1.19 Antioxidant and Breast cancer.

The application of antioxidants in cancer prevention ROS accumulation in normal cells is one of the initiating factors in the early stage of the neoplastic process. Therefore, an appropriate application of antioxidants can decrease the oxidative stress burden, consequently preventing normal cells from sliding into the abyss of malignant transformation. Numerous epidemiologic data and preclinical/ clinical studies have suggested that keeping an antioxidative dietary or pharmaceutical application of antioxidative phytochemicals can effectively prevent tumorigenesis Breast tumor microenvironment (TME) cells is a perplexing niche that comprises diverse cell types and is supported by an extracellular matrix (ECM) and soluble factors (Deepak *et al*., 2020). BC cells instigate and promote the progression of tumors by establishing tumor-associated stroma from adjoining normal fibroblasts, immune cells as well as endothelial cells. The TME is

characterized by hypoxia, metabolic reprogramming, nutrient deprivation, , by enhancing EMT and switching to glycolytic metabolism. They originate largely from the mitochondria, transverse to the cytosol, and mediate cellular signaling. ROS are associated with tumorigenesis by affecting multiple biological activities including regulation of cell proliferation, mediation of genomic instability, induction of inflammation, development of resistance to apoptosis, initiation of metabolic reprogramming, and promotion of metastasis (Kalyanaraman *et al*., 2018). Extensive research over the years established the involvement of ROS in BC initiation and progression. In contrast, the overproduction of ROS is detrimental to the survival of BC. Chemotherapy, radiotherapy, and photodynamic therapy trigger cell death by inducing ROS, which are also implicated in the activation of apoptotic signaling pathways and the tumor suppressor genes p53 (Kalyanaraman *et al*., 2018). However, the functional role of ROS in survival or cell death is different among the subtypes of BCs.

1.23 Cytokines and Breast Cancer

Cytokines are highly inducible, secretory proteins that mediate intercellular communication in the immune system. They are grouped into several protein families that are referred to as tumor necrosis factors, interleukins, interferons, and colony-stimulating factors. In recent years, it has become clear that some of these proteins as well as their receptors are produced in the organisms under physiological and pathological conditions. The exact initiation process of breast cancer is unknown, although several hypotheses have emerged. Inflammation has been proposed as an important player in tumor initiation, promotion, angiogenesis, and metastasis, all phenomena in which cytokines are prominent players. Several

cytokines regulate the inflammatory tumor microenvironment. Interleukin (IL)-1, IL-6, IL-11, and transforming growth factor-b (TGF-b) stimulate cancer cell proliferation and invasion (Nicolini *et al*., 2006) and cytokine receptor activation and intracellular signaling by NF-kB accelerate tumor progression (Ndez-Garcı´a *et al*., 2019; Taniguchi & Karin, 2018).

1.21 Spexin

Spexin is a cytokine (peptide) that was identified through modern bioinformatics search strategies (Ma *et al*., 2018). In humans, spexin is a product of the Ch12orf39 gene. The prepro peptide is composed of 116 amino acid residues . Mature spexin contains 14 amino acids. Rat spexin differs from the human and mouse peptide sequences by one amino acid within the C-terminal portion, ending with the sequence GHR instead of GRR (Wang *et al*., 2020). Previous studies have revealed the widespread expression of spexin mRNA and protein in rat tissues including the hypothalamus, cerebral cortex, hippocampus, pons, retina, esophagus, stomach, kidney, ovary, and adrenal gland . Its location in endocrine cells and its ability to be secreted suggest its possible role as an endocrine factor. Moreover, an important role of spexin in the functioning of epithelial cells has also been suggested . However, the expression of spexin mRNA and protein in human tissues is unknown (Kolodziejski *et al*., 2018).

1.22 Asprosin

Asprosin is a recently discovered cytokine (hormone) released by white adipose tissue (WAT) that is typically significantly elevated in obese adults.

Consequently, the adverse effects of increasing WAT in obesity during breast cancer (BC) development and progression have attracted interest of researchers and clinical practitioners. Asprosin, encoded by the fibrillin 1 (FBN1) gene, is secreted from white adipose tissue and metabolized in the liver, where it promotes rapid glucose release into the bloodstream (Romere *et al*., 2016). Empirical evidence further indicates that its levels are increased in obese women, as well as those affected by polycystic ovarian syndrome (PCOS), type 2 diabetes, and metabolic syndrome, whereby this adverse effect is linked to insulin resistance (Li et al., 2018). Although the relationship between asprosin and cancer is insufficiently studied, extant findings indicate that asprosin immunoreactivity is increased in malignant mesothelioma (Kocaman & Artaş, 2020), suggesting that it may play a role as a glucogenic peptide in ovarian cancer (OZCAN *et al*., 2021).

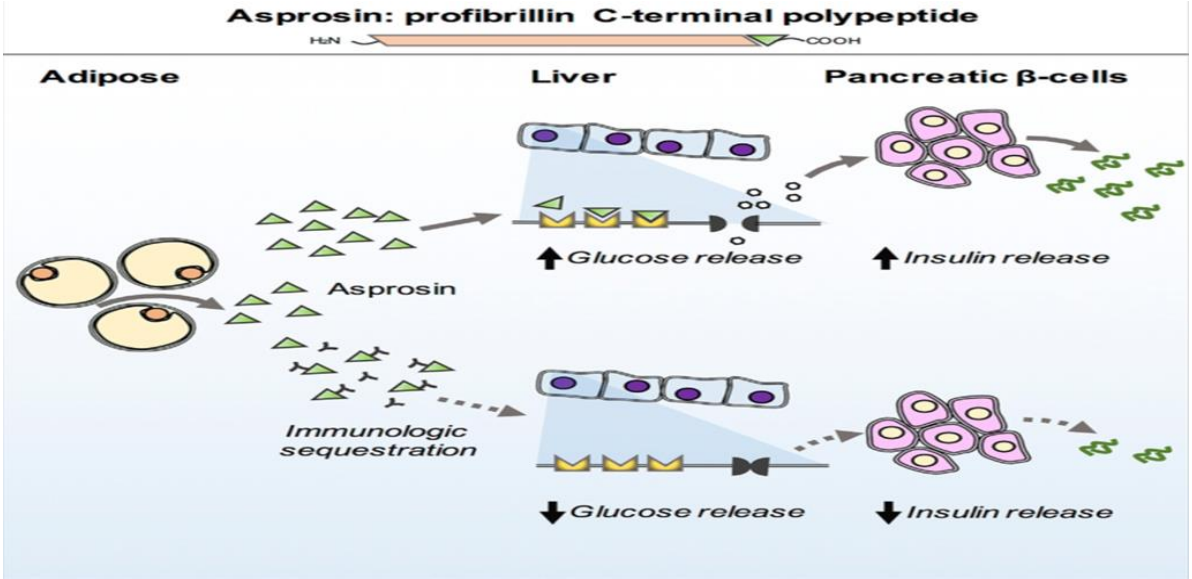


Fig 1-17 Asprosin :profibrllin C-terminal polypeptide (Romere *et al*., 2016)

1.23 Midkine

Midikine is heparin-binding growth factor, is up-regulated in many types of cancer. Multiple growth factors have been identified that are up-regulated in

malignant tumors, and these factors play crucial roles in tumorigenesis and tumor progression, including tumor survival, growth, invasion, angiogenesis, and metastasis Midkine, was originally reported to be a product of a retinoic acid-responsive gene in embryonal carcinoma cells (Pürnak *et al*., 2020). The human midkine gene is located on chromosome 11q11, (Ross-Munro *et al*., 2020) and encodes a 13-kDa protein rich ina basic amino acids and cysteine (Garapati *et al*., 2010), Midkine is plays an important role in the development of tooth (Alvis *et al*., 2019). Lung (Toriyama *et al*., 1996) kidney,(Sakurai *et al*., 2001), bone (Dreyfus et al., 1998) and nerve tissue (Kadomatsu & Muramatsu, 2004). MK is widely conserved from Drosophila to human . Human MK sequences are extremely highly conserved: 87% of amino acids are identical and all amino acid changes are conservative except for an insertion (Cai *et al*., 2020).

Aim of Study

The present study was planned to evaluate the levels of cytokines and antioxidants in association with different grade of breast cancer.

To complete this aim the following objective were done:-

1. Estimation of serum cytokines (Midkine , Aspirosin, and spexin) in breast cancer patients before and after chemotherapy.
2. Estimation of serum total antioxidant capacity (TAC), malondialdehyde (MDA), and homocysteine (Hcy) in breast cancer patients before and after chemotherapy.
3. Estimation of serum lipid profile in breast cancer patients before and after chemotherapy.
4. Study the relationship between cytokines, TAC, MDA, Hcy, and lipid profile with grades of breast cancer .
5. Investigation new biomarker for progression of breast cancer in breast cancer patients before and after chemotherapy by estimation the level of cytokines and compare cytokine with Hcy and MDA in all patients.

Chapter two Materials and Methods

2. Materials and Methods

2.1 Chemicals

Below are the chemicals used in the current study.

Table 2-1 Chemicals used in the study

Chemicals	Symbol	Origin ﻉ company
Alcohol 70%,80%,95%	Alcohol	Clenzium, UK
Antioxidant ELISA kit (cat. No. E2199 Hu) Bioassay laboratory	TAC	Zhejiong, china
Asprosine ELISA kit (Cat. No.E4095 Hu) Bioassay laboratory	Asp	Zhejiong, china
Cholesterol kit	TC	DIRUI, China
Eosin stain	Eosin	ACS,USA
Formalin 10%	Formalin	USA
High density lipoprotein cholesterol kit	HDL-C	DIRUI, china
Homocysteine ELISA kit (Cat. No. E3292Hu) Bioassay laboratory	Hcy	Zhejiong, china
Hematoxylin stain	Hematoxylin	ACS,USA
Malonodialdehyde ELISA kit (Cat. No. E1371 Hu) Bioassay laboratory	MDA	Zhejiong, china
Midkin ELISA kit (Cat. No. E1633 Hu) Bioassay	MK	Zhejiong, china

laboratory		
paraffin	Paraffin	Paraplast, USA
Spexin ELISA kit(Cat. No. E3507 Hu)Bioassay laboratory	Spx	Zhejiong, china
Triglyceride reagent kit	TG-C	DIRUI, china
Xylene	xylene	Burlington, Canada

2.2 Instruments

There are several tools and techniques are used in current study they are listed in Table 2-2 .

Table 2-2 Instruments and tools used in the study

Instruments	Company	Origin
Bloating paper	MP	China
Biochemistry full automated-analyzer	DRIUI	China
Centrifuge	Kubota	Germany
Disposable syringe	Almateen	China
Eppendorf tube (0.5 ,1.5)	Bioplastic	Netherlands
ELISA	CHRO MATE	USA
Microscope	Novel	Germany
Micropipettes	Watson Nexty	Japan
Water path	Memmert	Germany

2.3 Ethical Considerations

Ethical issues achieve base on:

A) Agreement of College of Science for Women (University of Babylon, Iraq and Department of Chemistry at the same institution).
B) Scientific committee agreement of Oncology Center in Merjan Teaching Hospital in Babylon province in Hilla City.
C) Oral agreement of all patient and healthy control people before taking blood samples from them.
D) The goals and methods of this research have been clarified to all members in the present research in order to obtain their oral approval.

2.3.1 Patients group

This study was designed to test population comprised of 68 females histopathelogically diagnosed as breast cancer either familial. The patients age ranged between 35 ± 10 year (mean ± SD). They were collected from the Oncology Unit in Babylon Oncology Center in Marjan Medical City Teaching hospital in Al-Hilla city when they attended to the hospital for treatment or for check up and some of them admitted in the hospital. All patient were participated after surgery. They were all undergoing chemotherapy treatment according the following protocol for treatment (Gemzar, Taxol, ACT, Nepogen, Endoxan) . They are from City of Hilla in the middle region of Iraq. Any subject suffered from the following health problems were excluded from the current study.

Exclusion Criteria:

• Diabetes mellitus.

• Cardiac diseases

• Hypertension

• Primary renal dysfunction.

• Other types of malignancies not related to the metastasis of breast cancer.

2.3.2 Controls group

The control group included 45 apparently healthy age 35±10years (mean ± SD). They were selected from general population, women attending to the hospital who were patients relatives, visitors, medical staff and their relatives and friends. Only individuals free from signs and symptoms of any chronic diseases such as DM, cardiac diseases, hypertension, renal diseases or others were selected to participate in this study.

2.3.3 Study diagram

The diagram of work steps of current study is shown in Figure 2-1.

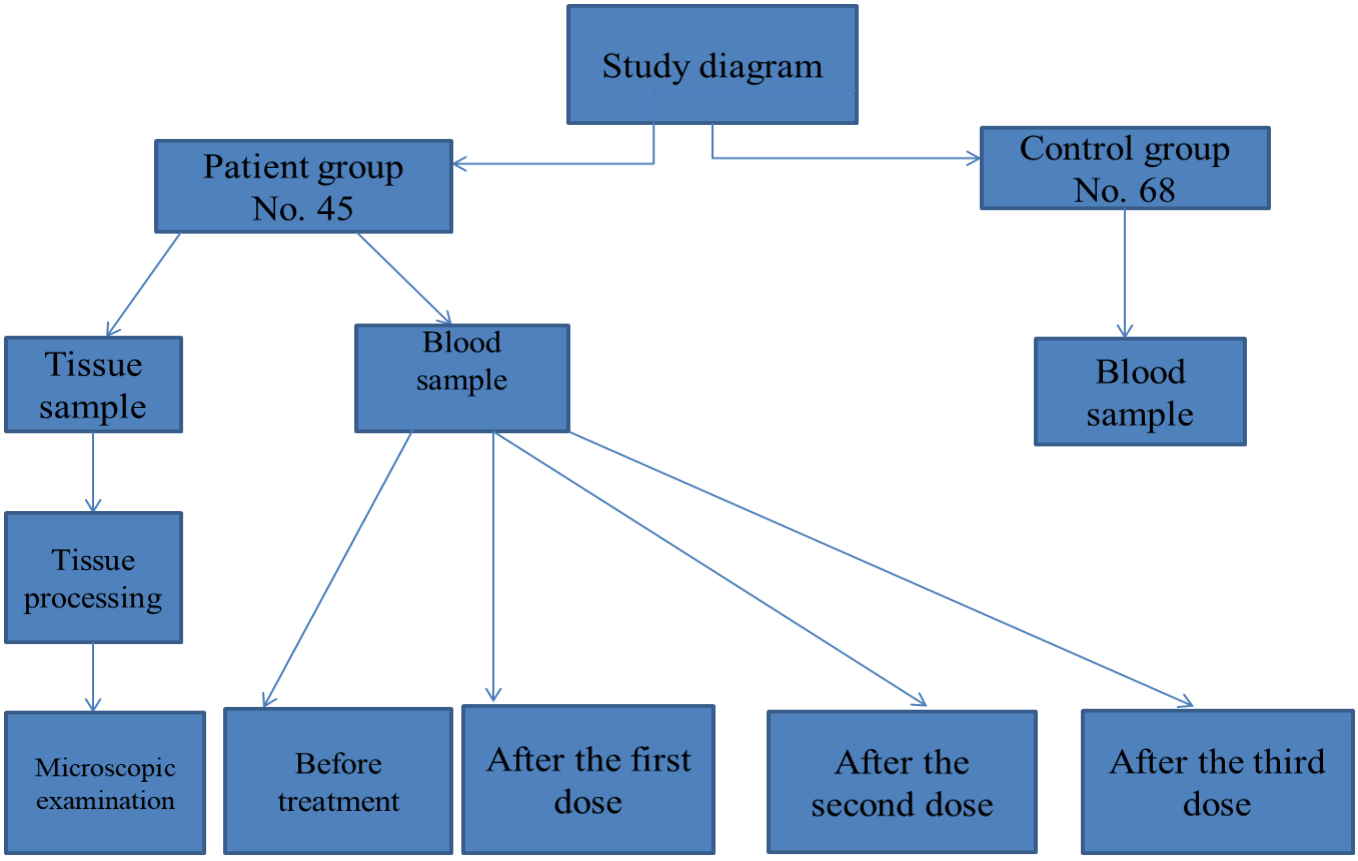


Fig 2-1 Study subject for present study

2.4 Tissue Experimental design

2.4.1 Tissue Preparing and Microscopic Examination

All tissue samples that were examined histologically were taken from the Surgical excision after surgery of removal breast mass or masstectomy and lymph node. Below the steps of tissue processing in histological laboratory:-

•Tissue processing

A procedure which need to take place after gross examination between tissue fixation, embedding and then sectioning of paraffin blocks is called tissue processing.

•There are 2 main steps in preparation of the slide:

1. Sectioning of tissue

2.Staining of tissue

Routine stain

Hematoxylin and Eosin stain (H and E stain)

2.4.2 Paraffin Sectioning

Tissues from the body taken for diagnosis of disease processes must be processed in histopathology laboratory to produce microscopic slides that are viewed under microscope by pathologist

Paraffin sectioning can be done by 2 ways:

1.Automated tissue processioning (specimen numbers high)

2.Manual tissue processioning (specimen numbers low or high)

2.4.3 Tissues that come to the histological laboratory are:-

a. Biopsy specimen like endoscopy (colonoscopy, endocervical biopsy. .etc)

b. Resected disease organ like gall bladder, uterus, breast, intestine, bone, cartilage, soft tissue etc.

c. Resected organ or tissue after chemotherapy or radiotherapy.

d. Forming cell block from FNAC.

e. Autopsy

f. Animal like mouse

2.4.4 Specimen Accessioning

Tissue specimen received in the surgical pathology have a request form, that list contain the patient information and history along with a description of the site of origin. The specimen were accessioned by giving them a number that will identify each specimen for each patient and maintaining a registrar book.

2.4.5 Gross Examination

Tissue removed from the body for diagnosis arrive in pathology department and they were examined by a pathologist ,pathology assistant. The specimen must be placed in a fixative on arrival.

2.4.6 Fixation

Fixative is a chemical substance which preserve the architecture of tissue near normal by killing cells before enzymatic degradation by cellular enzymes. Fixation done in a fixative (formalin 10%) for 12 hours.

Factors that affecting fixative

a. Buffering

b. Penetration

c. Volume

d. Temperature

e. Concentration

f. Time interval

2.4.7 Manual Tissue Processioning

a. Dehydration

First the water from the tissue must be removed by dehydration. This is usually done by 90 minutes each series of alcohol from 70% to 80% to 95%.

b. Clearing

Consist of removal of dehydrant with a substance that will miscible with the embedding media. The commonest clearing agent is Xylene. Tissue was placed in Xylene 1 and Xylene 2 each for 30 minutes.

c. Infiltrating

Tissue was then infiltrating with embedding agent like molten paraffin wax in a Becker placed in a hot air woven for 6-8 hours which replaces toluene.

D. Embedding

After tissue have been dehydrated, cleared and infiltrating with embedding material like paraffin which is then hardened. This is achieved by placed tissue in a metallic angle and then was cooled .

E. Sectioning

The blocks then was chilled in a try of ice because the cold wax makes a clean cut compared to paraffin wax cut at room temperature. The paraffin block was cut by using a rotatory microtome with 4-5 micro-miter thickness.

F. Labeling

For indefinite storage of block, an identification number was written by Indian ink on a small piece of paper and attached this in one side of block.

G. Water Bathing

After sectioning the sectioned tissue was placed in warm water bath to floated that help to remove the wrinckles.

H. Making Slide and Numbering

Sections tissue were picked up on a glass microscopic slides from water bath and placed in a Hot air oven for 15 minutes to help the sections add here to the slides. The slide was then numbered by non- removable ink.

I. Staining

Clearing rinsed in xylol for 10 dips to remove wax. Then rinsed in graded alcohol (70%, 80%, and 95%) for 5dips each to remove xylol.

J. Drying

After staining, slides were drying by bloating paper.

K. Cover-Slipping

The stained section on the slide was covered by a thin piece glass with DPX to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for years to come.

L. Drying

Then the slide was dried in hot air oven for 5 minutes.

M. Microscopic examination

Stained slide then was examined by pathologist. Pathologist was wrote the report in a register book.

2.5 Blood Sampling

Three milliliters of blood was drawn by vein puncture from all individuals participated in this study. The blood placed in gel tube. It was left thirteen minutes at room temperature for coagulation. Blood was centrifuged for10-15 minutes at 3000 Xg. Serum was collected and divided into three aliquots then stored at -20˚C till analyses for measuring the level of homocysteine, midkine, malonodialdehyde, antioxidant, spexin, asprosin and concentration of lipid profile.

2.6 Enzyme Linked Immuno Sorbent Assay (ELISA)Technique

Specific kit for measuring human levels of (MDA level, TAC level, Hcy level, Spx level, Asp level, MK) in serum was supplied by Bioassay technology laboratory .

2.7 Methodology

2.7.1 Estimation of serum (MDA, TAC and Hcy) levels.

Principle

The kit used a double-antibody sandwich enzyme-linked immune sorbent one-step process to assay (MDA, TAC and Hcy) in human serum.

Assay Procedure

1. All reagents, standard solutions and samples were used as instructed. All reagents were brought to room temperature before use. The assay was performed

at room temperature.

2. The number of strips required for the assay were determined. the strips were inserted in the frames for use. The unused strips were stored at 2-8°C.

3. Fifty microliters of standard was add to standard well. Antibody dose not add to standard well because the standard solution contains biotinylated antibody.

4.Forty microliters of sample was add to sample wells and then 10µl from (MDA ,or TAC, or Hcy) anti-body was add to sample wells, then 50µl streptavidin-HRP was add to sample wells and standard wells (Not blank control well). They were mixed well. The plate was covered with a sealer. It was incubated 60 minutes at 37°C.

5. The sealer was removed and the plate were washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, all wells were aspirated and washed 5 times with wash buffer, The wells were over filled with wash buffer. The plate was blotted onto paper towels.

6. Fifty microliters of substrate solution A was add to each well and then 50µl substrate solution B was add to each well. The plate were incubated covered with a new sealer for 10 minutes at 37°C in the dark.

7. Fifty microliters from Stop Solution was add to each well, the blue color was changed into yellow immediately.

8. The optical density (OD value) of each well was immediately used a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Reagent Preparation for MDA

Standard was reconsidiuted by adding the 120µl of the standard (80nmol/ml) with 120µl of standard diluent to generate a 40nmol/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (40nmol/ml) 1:2 with standard diluent to produce 20nmol/ml, 10nmol/ml, 5nmol/ml and 2.5nmol/ml solutions.

Reagent Preparation for Hcy

Standard was reconsidiuted by adding the 120µl of the standard (64nmol/ml) with 120µl of standard diluent to generate a 32nmol/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (32nmol/ml) 1:2 with standard diluent to produce 16nmol/ml, 8nmol/ml, 4nmol/ml and 2nmol/ml solutions.

Reagent Preparation for TAC

Standard was reconsidiuted by adding the 120µl of the standard (96U/ml) with 120µl of standard diluent to generate a 48U/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (40U/ml) 1:2 with standard diluent to produce 20U/ml, 10U/ml, 5U/ml and 2.5U/ml solutions.

Wash Buffer

A 20ml of Wash Buffer Concentrate 25x was diluted into distilled water which yield 500 ml of 1x Wash Buffer. It was mixed gently until the crystals have

completely dissolved.

Calculation of Results: Results were obtained by plotting absorbance at Y-axis and concentration at X-axis.

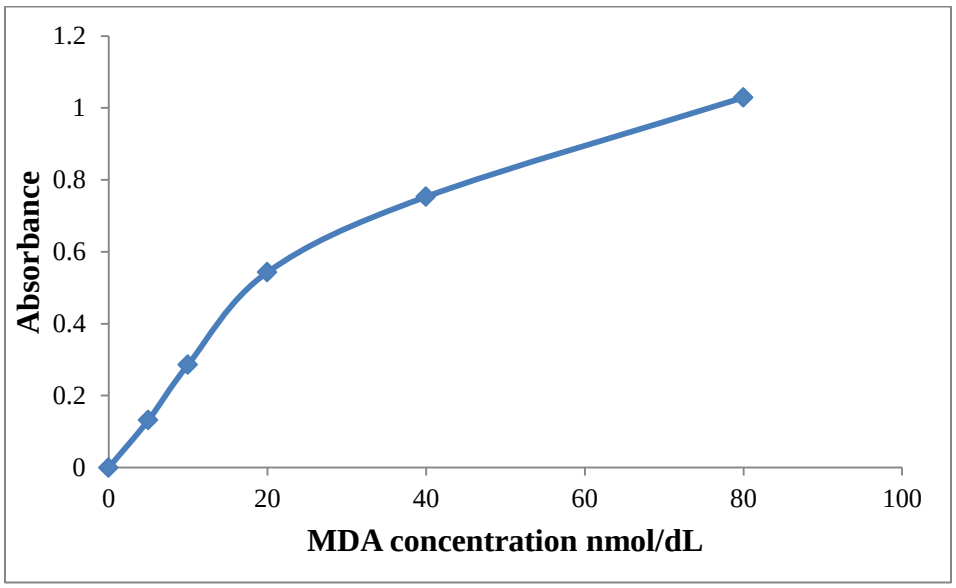


Fig. 2-2 Standard curve for MDA

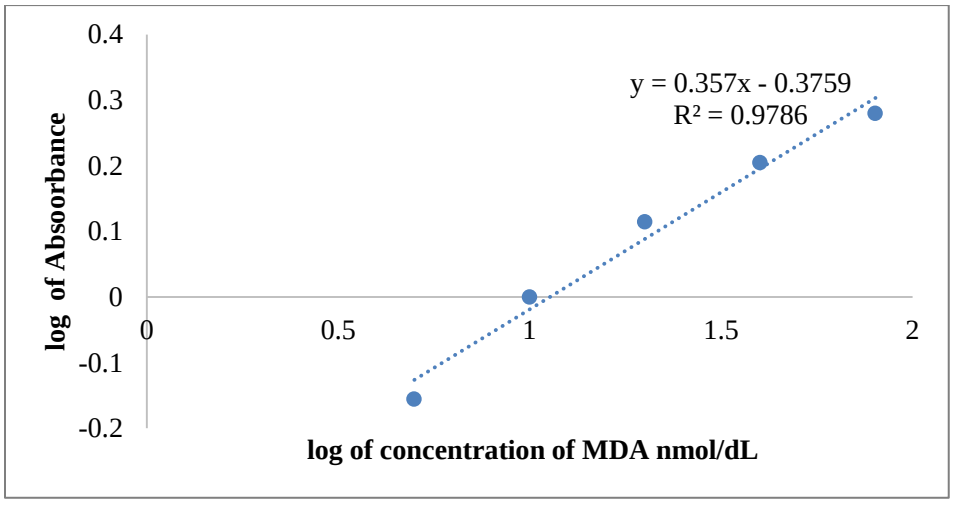


Fig. 2-3 log-log calibration curve of MDA

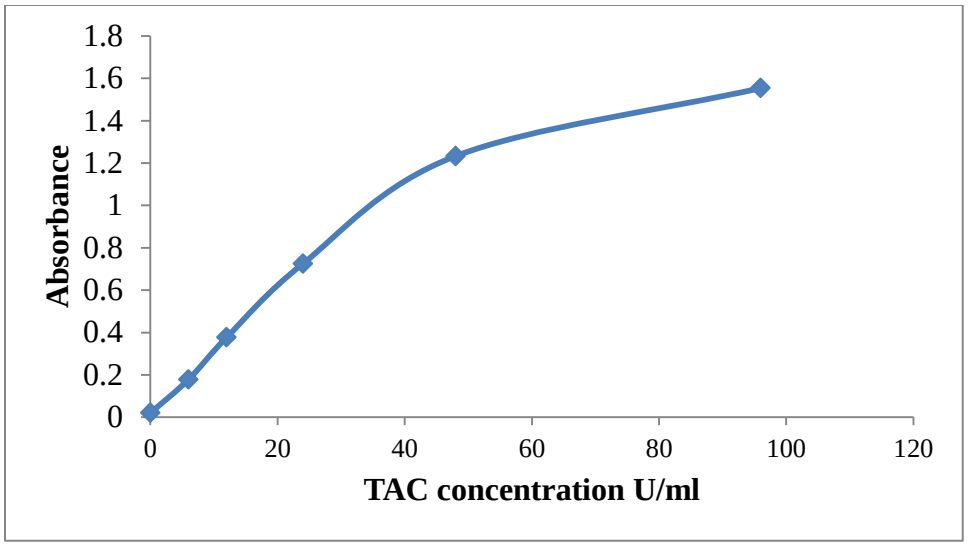


Fig. 2-4 Standard curve of TAC

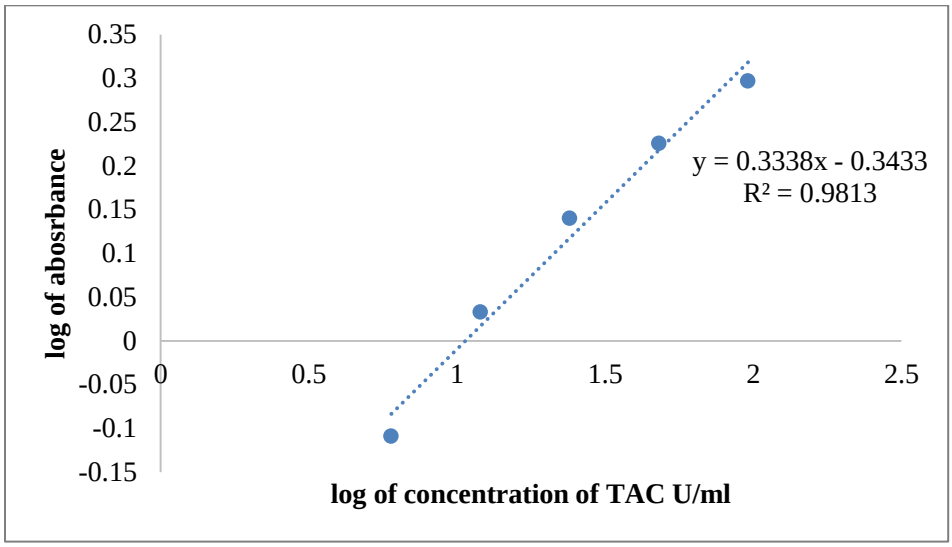


Fig 2-5 log-log calibration curve of TAC

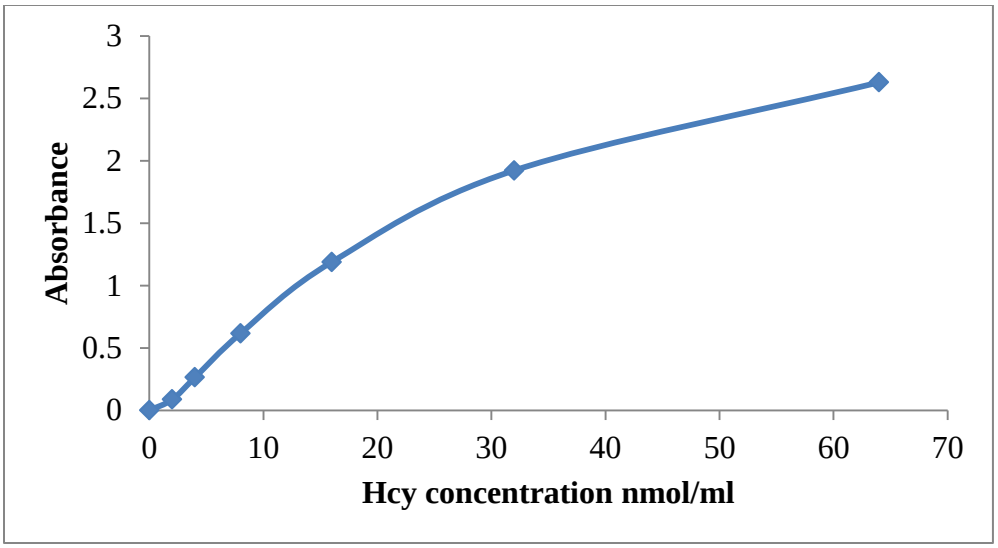


Fig. 2-6 Standard curve for Hcy

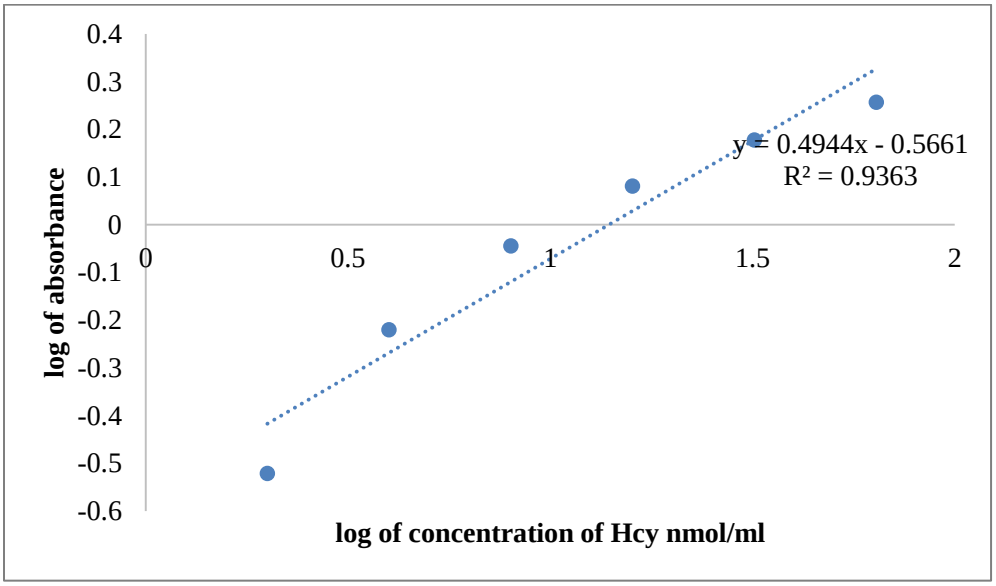


Fig 2-7 log-log calibration curve of Hcy

2.7.2 Estimation of serum (Asp, Spx and MK) level

Principle

The kit used a double-antibody sandwich enzyme-linked immune sorbent one-step process to assay(Asp, Spx, and MK) level in Human serum.

Assay Procedure

1. All reagents, standard solutions and samples were as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.

2. The number of strips required for the assay were determined. the strips were inserted in the frames for use. The unused strips were stored at 2-8°C.

3. Fifty microliters from standard reagent was add to standard well. Antibody dose not add to standard well because the standard solution contains biotinylated antibody.

4.Forty microliters from sample was add to sample wells and then 10µl from (Asp, or Spx, or MK) anti-body was add to sample wells, then 50µl streptavidin-HRP was add to sample wells and standard wells (Not blank control well). They were mixed well. The plate was covered with a sealer. It was incubated 60 minutes at 37°C.

5. The sealer was removed and the plate were washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, all wells were aspirated and washed 5 times with wash buffer, The wells were over filled with wash buffer. The plate was blotted onto paper towels.

6. Fifty microliter from substrate solution A was add to each well and then 50µl substrate solution B was add to each well. The plate were incubated covered with a new sealer for 10 minutes at 37°C in the dark.

7. Fifty microliters from Stop Solution was add to each well, the blue color was changed into yellow immediately.

8. The optical density (OD value) of each well was immediately used a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Reagent Preparation for Spx

Standard Reconstitute the 120µl of the standard (4800ng/L) with 120µl of standard diluent to generate a (2400ng/L) standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (2400ng/L) 1:2 with standard diluent to produce 1200ng/L, 600ng/L, 300ng/L and 150ng/L solutions.

Reagent Preparation for Asp

Standard Reconstitute the 120µl of the standard (128ng/ml) with 120µl of standard diluent to generate a 64ng/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (64ng/ml) 1:2 with standard diluent to produce 32ng/ml, 16ng/ml, 8ng/ml and 4ng/ml solutions.

Reagent Preparation for MK

Standard Reconstitute the 120µl of the standard (2400pg/ml) with 120µl of standard diluent to generate a 1200pg/ml standard stock solution. The standard was allowed to sit for 15 mins with gentle agitation prior to making dilutions. Duplicate standard points was prepared by serially diluting the standard stock solution (1200pg/ml) 1:2 with standard diluent to produce 600pgml, 300pg/ml, 150pg/ml and 75pg/ml solutions.

Wash Buffer

A 20ml of Wash Buffer Concentrate 25x was diluted into distilled water which yield 500 ml of 1x Wash Buffer. It was mixed gently until the crystals have completely dissolved.

Calculation of Results:

Results were obtained by plotting absorbance at Y-axis and concentration at X-axis.

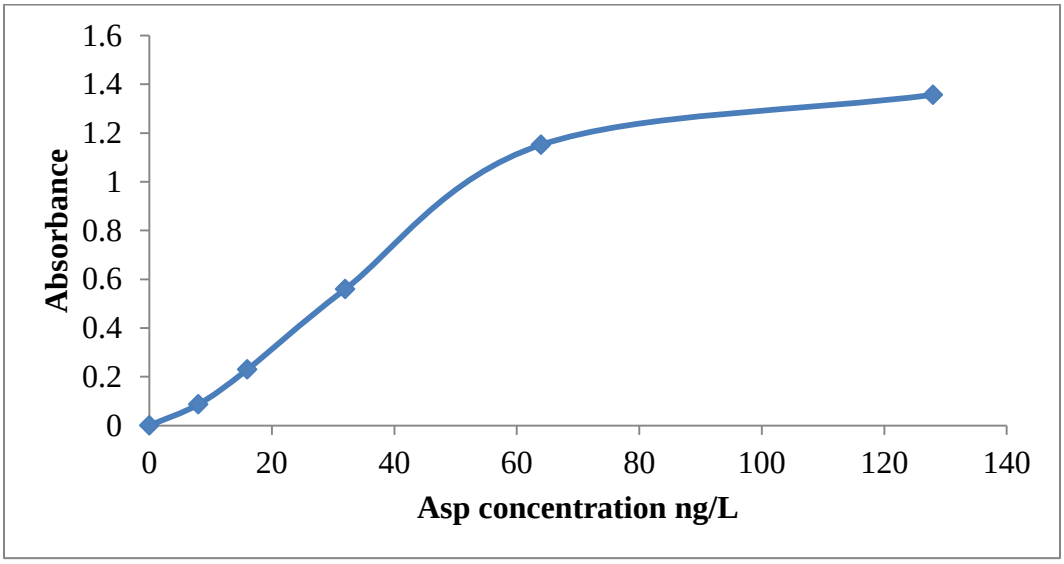


Fig. 2-8 Standard curve for Asp

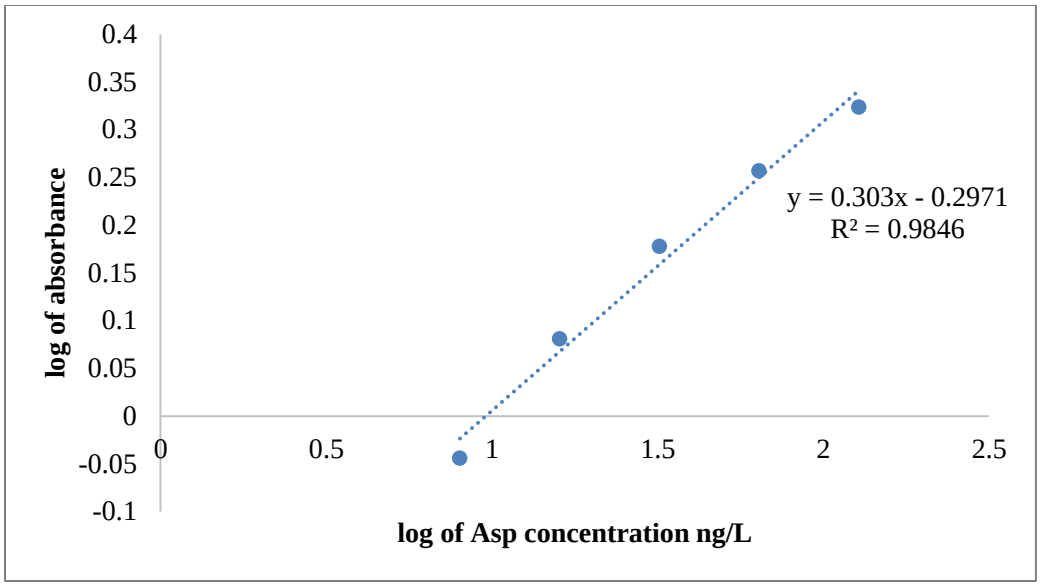
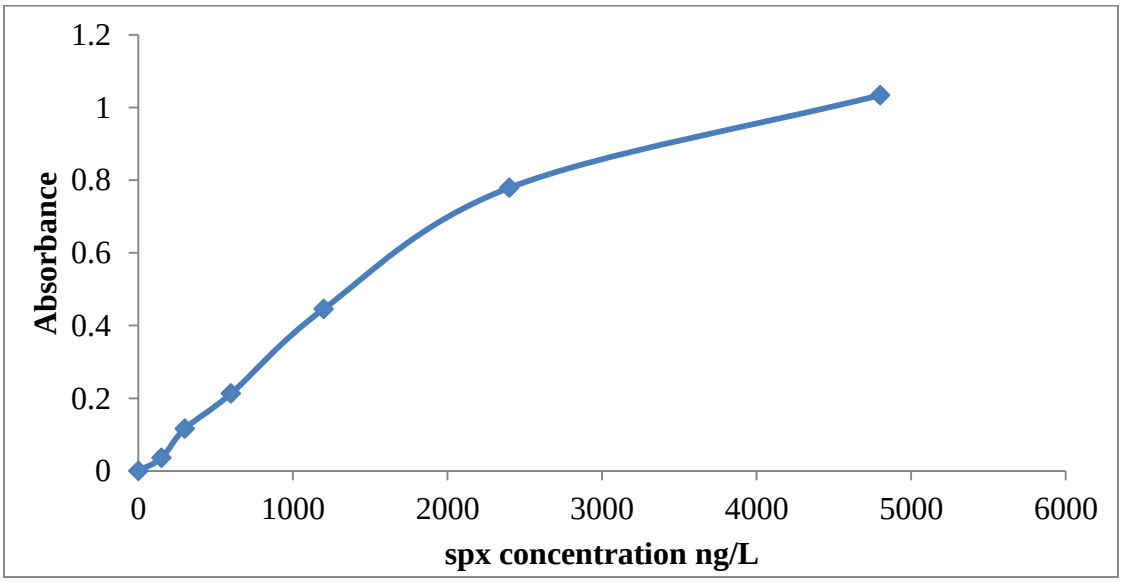


Fig 2-9 log-log calibration curve of Asp



. Fig. 2-10 standard curve for Spx

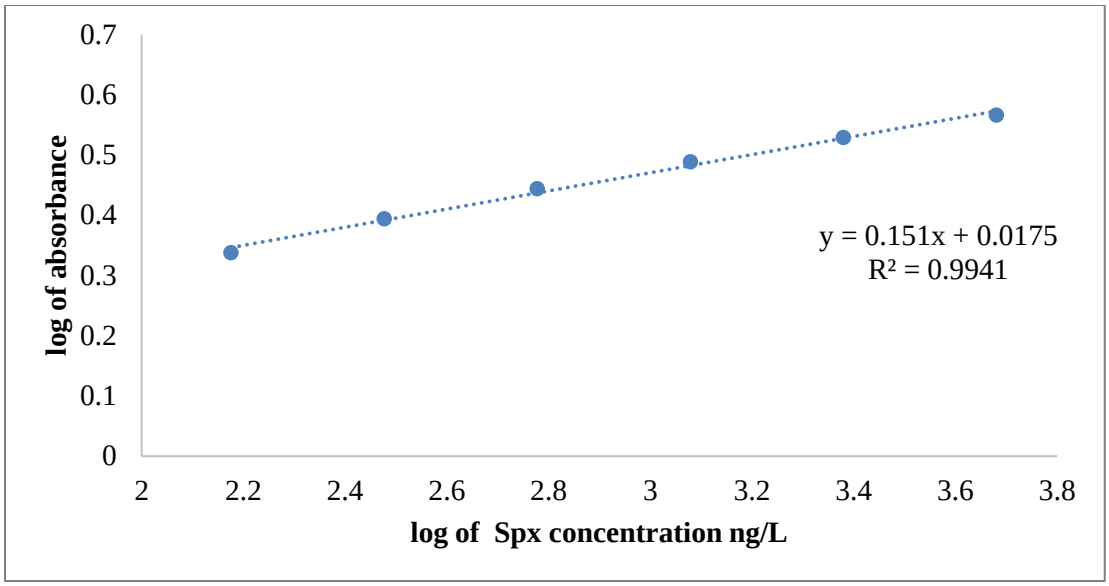


Fig 2-11 log-log calibration curve for Spx

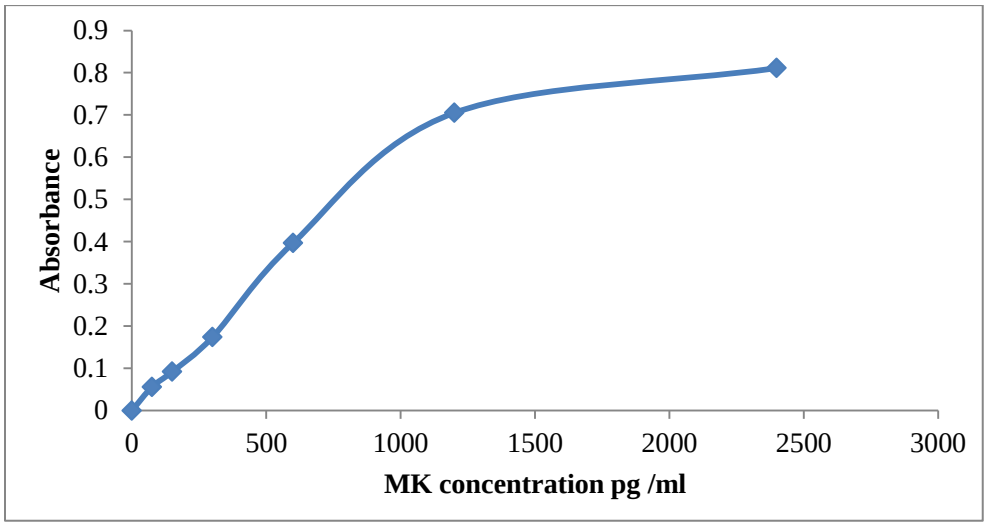


Fig. 2-12 Standard curve for MK

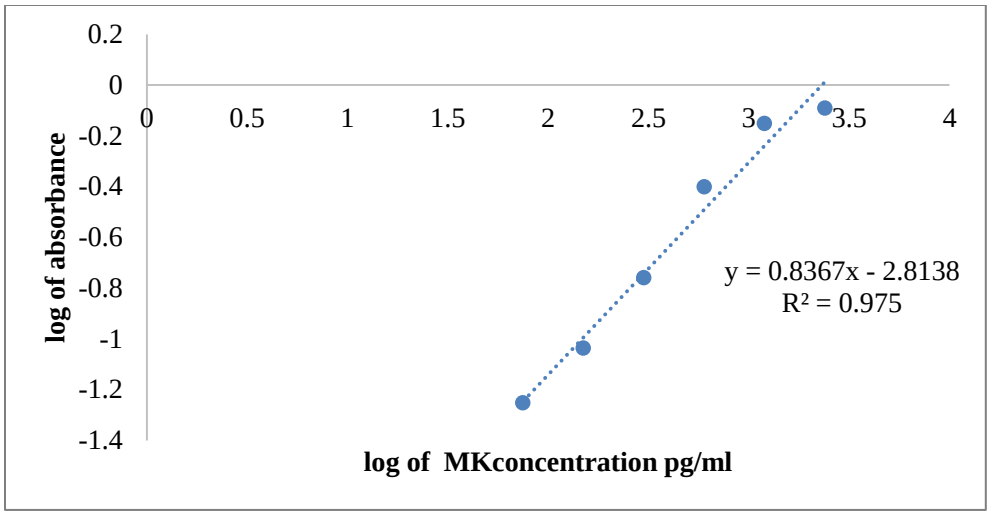


Fig 2-13 log-log calibration curve for MK

2.8 Estimation of body mass index

Body Mass Index (BMI) defining anthropometric characteristics in mass / height2 Adults and groups for classification (categorization) the common interpretation is that it represents an index of the fatness of a person. Body mass index (BMI) was determined by the following equation (Misra & Dhurandhar,2019).

$$BMI=\frac{weight(Kg)}{Height\ (meter)}$$

2.9 Estimation of Lipid Profile

2.9.1 Estimation of Serum Total Cholesterol(TC)

The total Serum TC is determined by the enzyme method for the quantitative in vitro diagnostic measurement using a kit.

Principle

Cholesterol ester in the sample, under the existence of lipoprotein esterase, hydrolyzed into cholesterol and free fatty acid. Total cholesterol oxidized by cholesterol oxidase to generate cholest-4-ene-3-ketone and hydrogen peroxide. The generated hydrogen peroxide, under the existence of peroxide , react with hydroxyl benzoic acid and 4-amino–antipyrine to produce H_2O and quinone imine pigments. The generated volume of quinone imine pigments is proportional to total cholesterol volume in the sample, by measuring the generated pigment volume at specific wavelength, total cholesterol concentration can be calculated.

Cholesterol ester + H_2O $\xrightarrow{LPL}$ Cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholesteric -4-en -3- keto + H_2O_2

H_2O_2 + 4-Aminoantipyrine + para hydroxyl benzoic acid $\xrightarrow{POD}$ Quinone imine Pigments + H_2O

Procedure

1-Reagent 1 and Reagent 2 are liquid and ready for used .

2-Test condition

Temperature	37^{o}C	Reagent 1 volume	240µl
Main wavelength	500~520	Sample volume	3µL
Absorbance range	0~2A	Reaction time	300-600s
Optical path	1.0 cm	Test mode	2point end assay
Sub –wavelength	660nm	Reagent 2 volume	60µL

3- The solutions were mixed at 37^{o} for 5 minutes at least .

4- The absorbance of sample and standard were measured at 500nm (Perović & Dolčić, 2019).

Calculation :

Sample concentration (mmol/L)

$$=\frac{sample\ tube\ Ao}{calibration\ tube\ absorbance} \times calibrator\ concentration(\frac{mmol}{L})$$

Reference range

Optimal range :<5.2 mmol /L

Brim rise :5.2 mmol /L ~ 5.71 mmol /L

Rise :≥ 5.72 mmol /L.

2.9.2 Estimation of Serum Triglyceride(TG)

Serum TG were determined by the oxidase method for the quantitative in vitro diagnostic measurement using a kit

Principle

Triglycerides in the sample catalyzed by lipoprotein lipase (LPL)and hydrolyzed into glycerol and free fatty acid, under the existence of glycerol kinase (GK) and adenosine triphosphate (ATP) generate glycerol, the glycerol is phosphorylated to form 3-glcerophosphate. Under the existence of glycerol phosphate oxidase (GPO), it reacts with oxygen to produce hydrogen peroxide and dihydroxyacetone phosphate. Catalyzed by peroxidase (POD), hydrogen peroxide react with 4- amino –antipyrine to produce H_2O and quinone imine pigment, the volume of quinone imine pigment is proportional to triglyceride content in the

sample. By measuring the generated pigment volume at specific wavelength, the triglyceride concentration of the sample can be calculated .

Triglyceride + H_2O $\xrightarrow{\text{LPL}}$ glycerol + free fatty acid

Glycerol + ATP $\xrightarrow{\text{GK+ } Mg^{+2}}$ 3- phosphoglyceride + ADP

3-phosphoglyceride + O_2 $\xrightarrow{\text{GPO}}$ dihydroxyacetone phosphate + H_2O_2

H_2O_2 +4-aminoantipyrine $\xrightarrow{\text{POD}}$ Quinone imine pigment + H_2O .

Procedure

1-Reagent 1 and Reagent 2 are liquid and ready for used .

2-Test condition

Temperature	37^{o}C	Reagent 1 volume	240µl
Main wavelength	500~520	Sample volume	3µL
Absorbance range	0~2A	Reaction time	300-600s
Optical path	1.0 cm	Test mode	2point end assy
Sub –wavelength	660nm	Reagent 2 volume	60µL

3- The solutions were mixed at 37^{o} for 5 minutes at least .

4- The absorbance of sample and standard were measured at 500nm(Perović & Dolčić, 2019).

Calculation :

Sample concentration (mmol/L)

$$= \frac{sample\ tube\ Ao}{calibration\ tube\ absorbance} \times calibrator\ concentration(\frac{mmol}{L})$$

Reference range

Male :0.45 mmol /L ~ 1.81 mmol / L 40 mg /dl ~ 160 mg /dl

Female : 0.40 mmol /L ~ 1.53 mmol /L 35 mg/dl ~135 mg /dl

2.9.3 Estimation of Serum High. Density Lipoprotein Cholesterol

Serum HDL.C was determined by the direct method for the quantitative in vitro diagnostic measurement using a kit.

Principle

High – density lipoprotein cholesterol in the sample, under the existence of surfactant in the reagent, is selectively catalyzed and hydrolyzed by cholesterol esterase into cholesterol and free fatty acid. The generated cholesterol oxidized by cholesterol oxidase to produce cholest -4-ene -3- ketone and hydrogen peroxide. Under the existence of peroxidase, hydrogen peroxide react with aniline color of the original material and 4-amino antipyrine to produce H_2O and quinone imine pigment, the generated quinone imine pigment volume generated is proportional to high density lipoprotine cholesterol content in the sample, by masuring the final pigment volume at specific wavelength, high density lipoprotein cholesterol concentration in the sample can be calculated .

High –density lipoprotein cholesterol + H_2O Cholesterol esterase → Cholesterol + free fatty acid.

Cholesterol + O_2 Cholesterol oxidase → cholest -4-ene-3-ketone + H_2O_2 .

H_2O_2 + 4- Amino anti pyrine + Aniline color for the origin al material $\xrightarrow{\text{Peroxidase}}$

Quinone imine pigment + H_2O .

Procedure

1-Reagent 1 and 2 are liquid and ready for used

2- Test condition

Temperature	37°C	Reagent 1 volume	300µL
Main wavelength	540~570	Sample volume	4µl
Absorbance range	0~2A	Reaction time	300s
Optical path	1.0 cm	Test mode	2point end assy
Sub –wavelength	660nm	Reagent 2 volume	100µL

3- The solutions were mixed at 37° for 5 minutes at least .

4- The absorbance of sample and standard were measured at 546 -660 nm (Perović & Dolčić, 2019).

Calculation :

Sample concentration (mmol/L)

$$=\frac{sample\ tube\ Ao}{calibration\ tube\ absorbance} \times calibrator\ concentration(\frac{mmol}{L})$$

Reference range

Male 48.762 mg/dl ~ 66.951 mg/dl (1.70 mmol/L~ 1.73 mmol /L)

Female 41.409 mg /dl ~ 73.143 mg /dl (1.26 mmol /L ~ 1.89 mmol/ L)

2.9.4 Estimation of Serum Low. Density Lipoprotein Cholesterol and Very –Low Density Lipoprotein Cholesterol

The estimated LDL-c and VLDL-c serum level was determined by the Fried wald equation (Scharnagl *et al*., 2005). The levels for LDL-c were calculated by the Fried wald formula. In 1972 Fried wald had proposed a formula for LDL-c value calculation based upon the assumption that VLDL-c is present in serum at a concentration equal to one-fifth of the concentration of triglyceride (Perović & Dolčić, 2019) .

Therefore: LDL-c= TC- [HDL-c +VLDL-c]

VLDL-c= TC-[HDL-c+ TG/5]

Reference Values Normal value of LDL cholesterol less than 100 mg/dL

Suspicious: 150 mg/dL

Elevated: 190 mg/dL

Calculations of Atherogenic indices:

1. Castelli's Risk Index (CRI): Castelli‟s Risk Index is based on three important lipid profile parameters, i.e., TC, LDLc and HDLc, and it is categorized into two indices;

CIR-1 and CIR-11 (Castelli et al., 1983).

Mathematically, it is estimated as follows;

CRI-I= TC/HDLc

CRI-II= LDLc / HDLc.

2. Atherogenic Index of Plasma (AIP) = Atherogenic Index of Plasma is a logarithmically transformed molar ratio of TG to HDLc (Dobiásová et al., 2011):

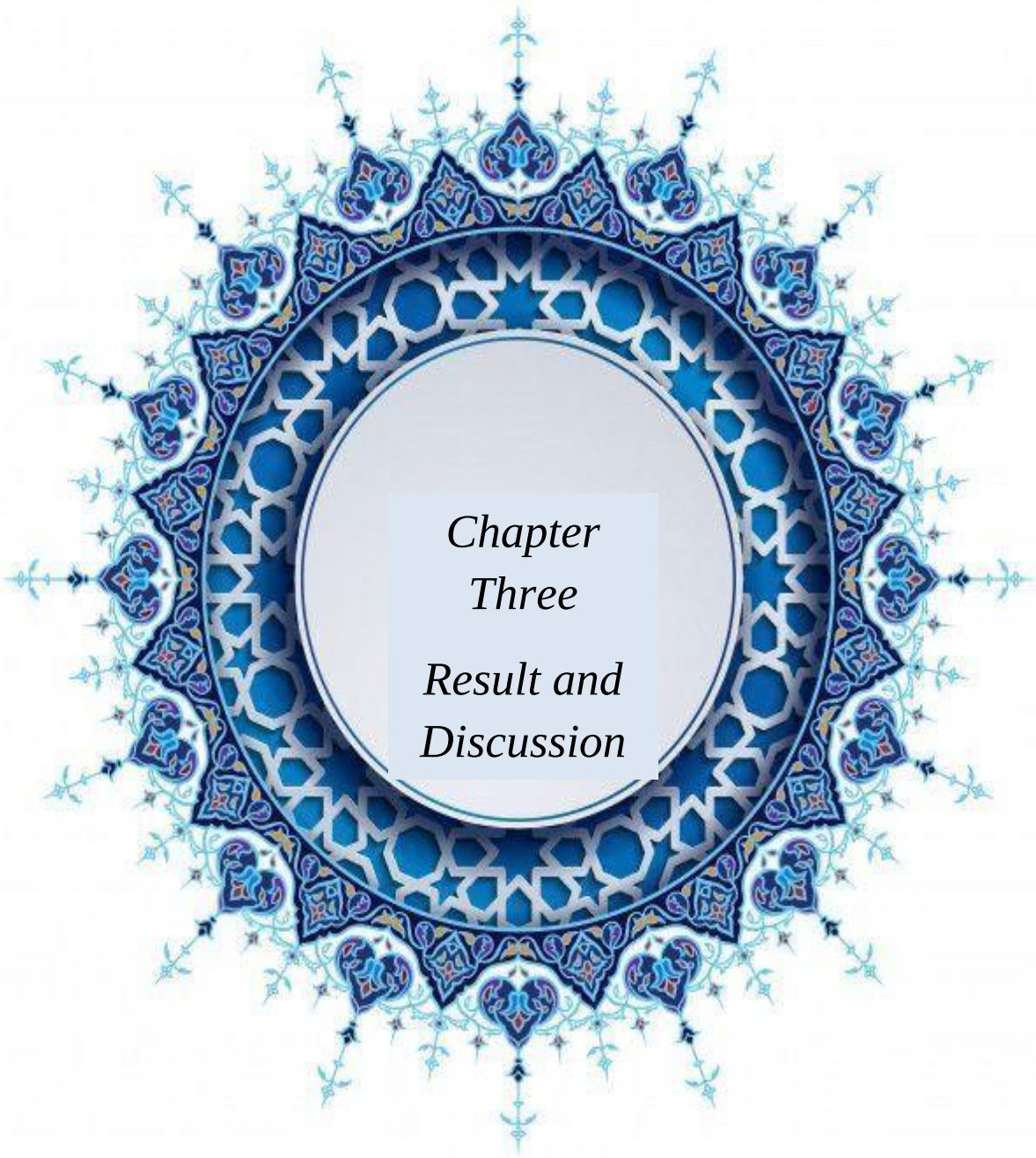
AIP= Log_{10} (TG/HDLc) ratio

3. Atherogenic Coefficient (AC) = Atherogenic coefficient is an indirect measure of cholesterol in VLDLc, IDLc, and LDLc fractions in reference to the HDLc fraction. Mathematically, it is expressed as;

AC= (TC- HDLc)/HDLc or AC=(Non-HDLc)/HDL (Bhardwaj et al., 2013).

2.10 Biostatistical Analysis

The results were subjected to statistical analysis and analyzed using computer facility of Microsoft Excel 2013 and SPSS-24 (statistical package for social science-version 24). The results were expressed as numbers and mean ±SD (Standard deviation) and median for cytokines . The one-way ANOVA (Analysis of variance) and Fishers Least Significant Difference (LSD) were applied to compare the differences among the studied groups.The correlation of parameters was determined using Pearson‟s correlation coefficient, taking $p \leq 0.05$ lowest limit of significance.



Chapter Three

Result and Discussion

3.Results and Discussions

3.1 Histopathological study of breast mass tissue:

After diagnosed of breast mass tissues by specialist (Histopathologisit) using routine test for histopathology. The results were revealed the following :-

3.1.1 Histopathology and Grades

The primary diagnosis of breast cancer was depend on different criteria, one of the most important one is histopathological study as well as immunohistochemistry in regarding to specific tumor markers.

The microscopic examination by histipathologisit showing that the following results

1.Normal breast tissue

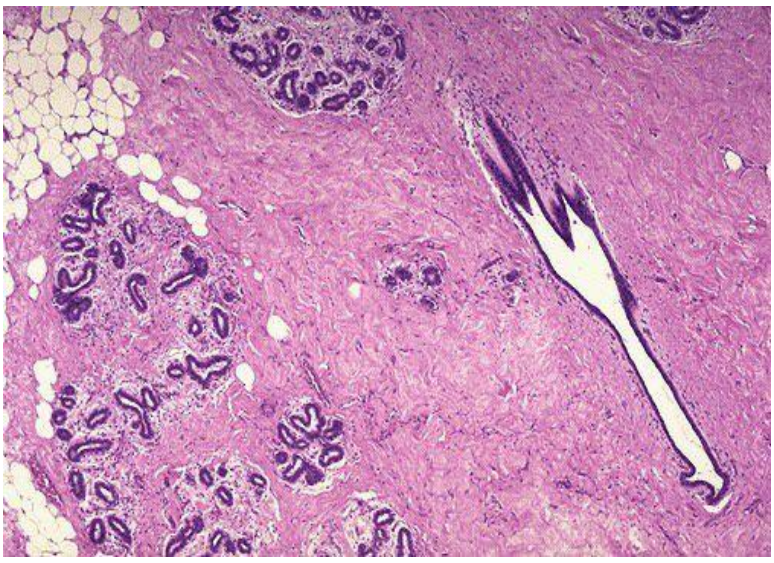
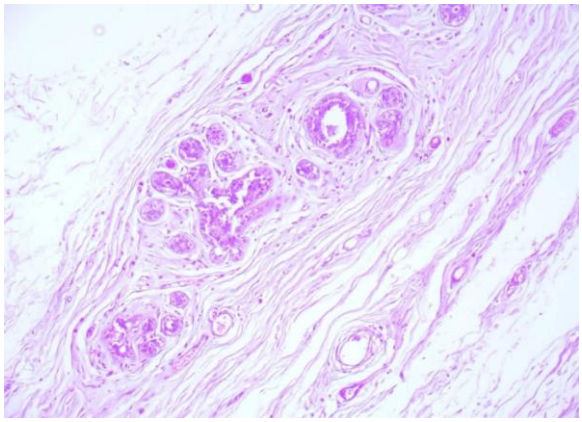
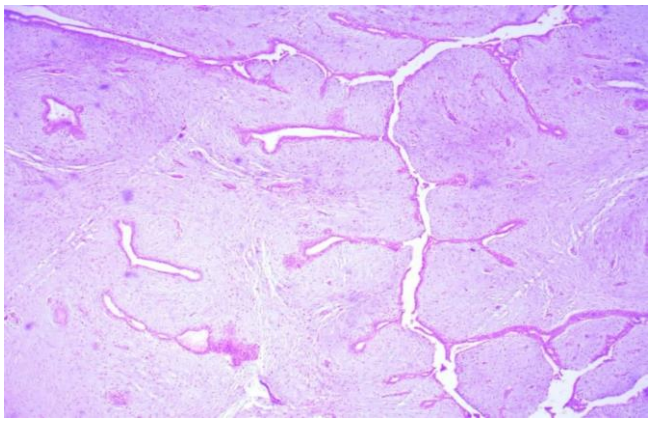


Fig 3-1 Normal breast tissue

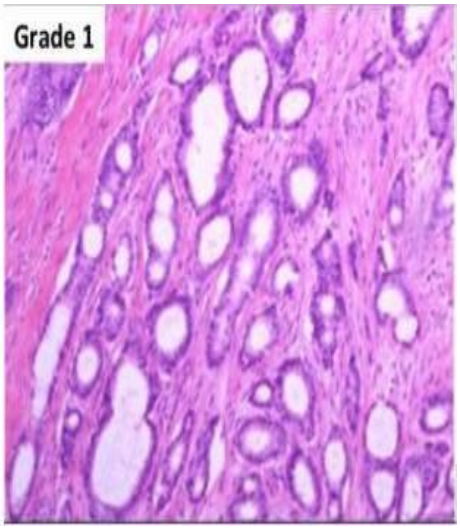
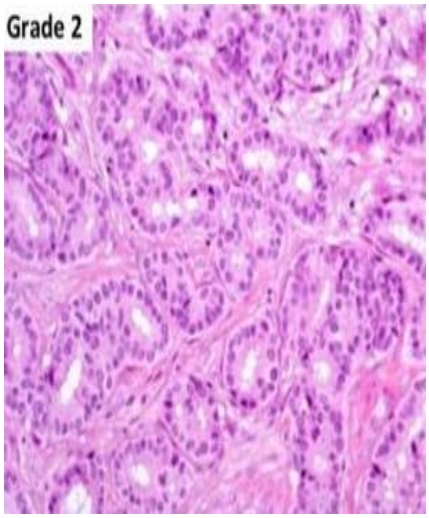
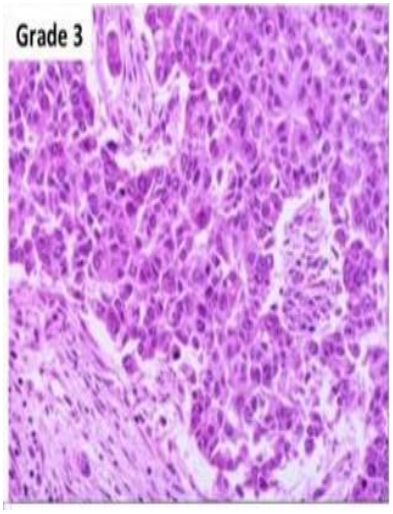
2.Benign tumor of breast.

❁A.	❁ B.
	

(❁)A. Benign looking breast lobule , B. Benign looking glandular components within fibromyxoid stroma

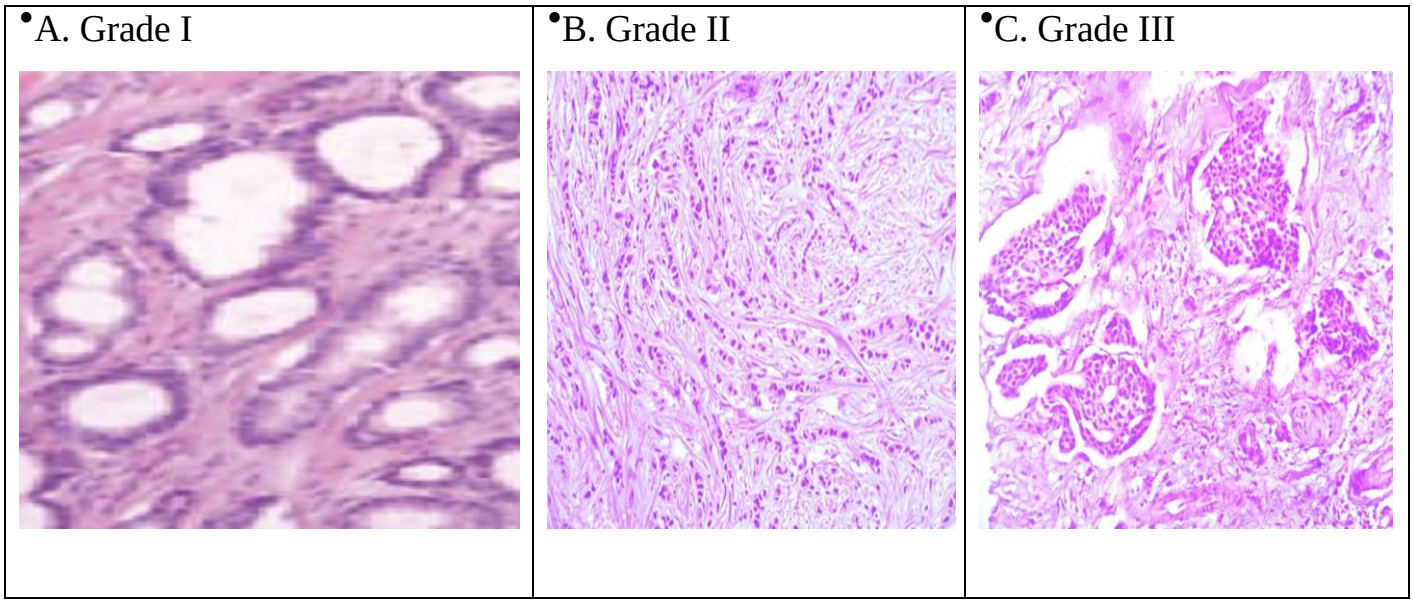
Fig 3-2 Benign tumor of breast tissue

3.Malignant tumor of breast .

*A. Grade I	*B. Grade II	*C. Grade III
		

(*) A. well –differentiated (appear normal, growing slowly , not aggressive) B. Cords of hyperchromatic cells with little pleomorphism within lakes of mucin (Grade II) C. syncytial growth pattern of pleomorphic hyperchromatic cells with frequent mitoses (Grade III).

Fig 3-3 Malignant grade of breast tissue by IHC.



(●)A. well – differentiated (appear normal, growing slowly, not aggressive) B. Indian file pattern of small hyperchromatic cells dissecting the markedly desmoplastic storma (Grade II) C. Clusters of pleomorphic and hyperchromatic cells with abundant cytoplasm infiltrating fibroblastic storma (Grade III)

Fig 3-4 Malignant grade of breast tissue according to IHC

3.2 Comparative study of all studied parameters in patients and control.

Sixty eight breast cancer patients with (45) healthy population as a control for studied parameters (Hcy, Asp, Spx, MDA, MK, TAC, and lipid profile). The results were shown as the following :-

3.2.1 Measurements of MDA levels in patient and control

The results of the current study found the a brightly illuminated location that the mean of serum MDA levels are a significantly increase in patient with breast cancer ($p < 0.005$) as compared with control group, at mean ± SD (23.22 ± 12.27 , 16.55 ± 4.99) respectively, as like as in Figure 3-5.

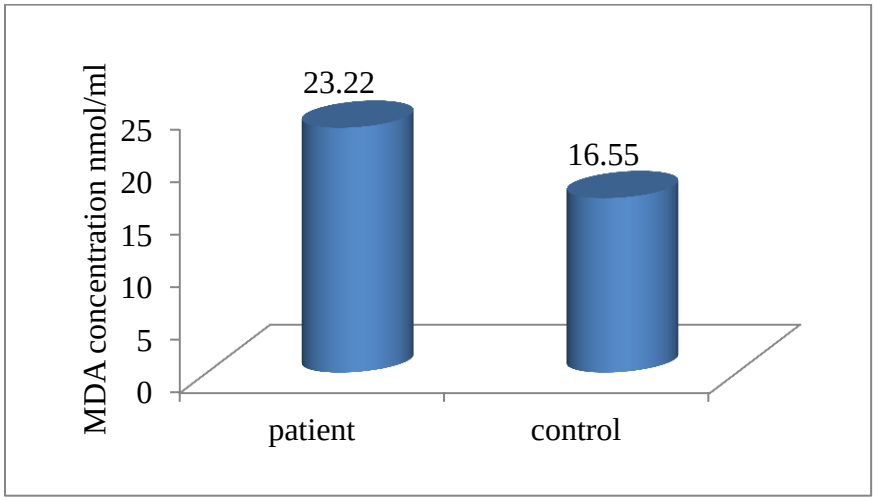


Fig 3- 5 MDA concentration nmol/ml

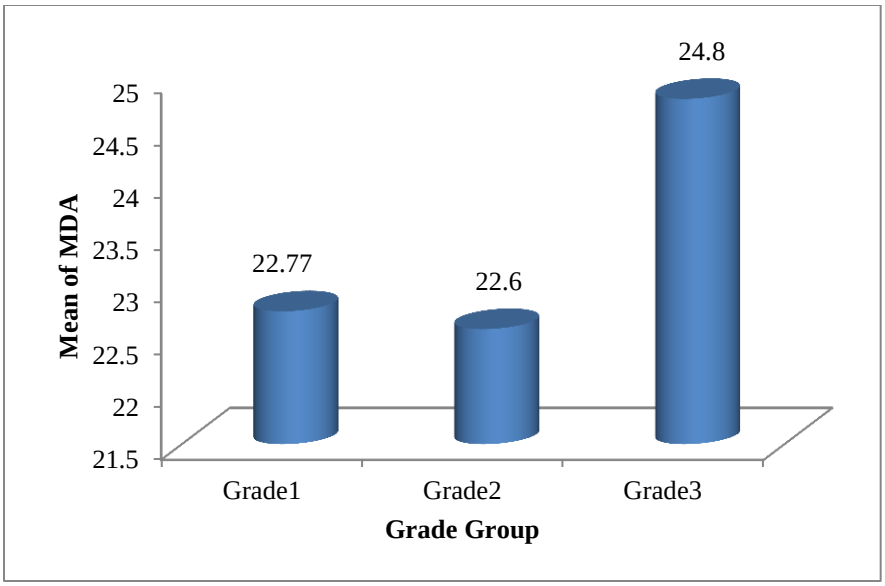


Fig. 3-6 MDA levels at different Grade in patient

The results of the current study found lighted area that the mean of serum MDA levels are a significantly decrease in patient with breast cancer during different doses, (before treatment, first, second, and third doses) respectively , as like as in Figure 3-7.

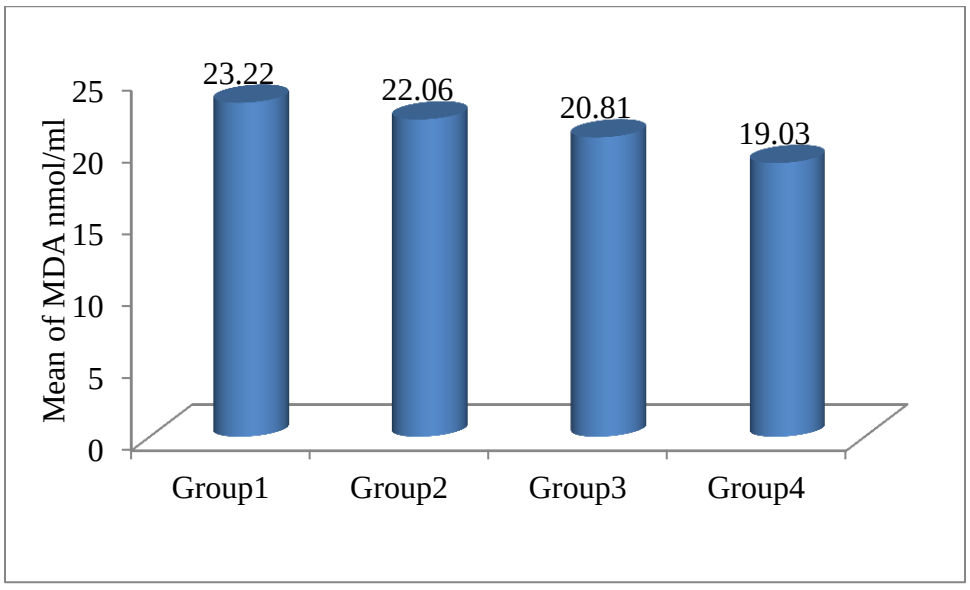


Fig 3-7 MDA concentration according to doses in patient group

Group1 and Group2 p = 0.66, Group1 and Group3 p= 0.648

Group1 and Group4 p= 0.704, Group2 and Group3 p= 0.006

Group2 and Group4 p= 0.000, Group3 and Group4 p= 0.125

Studies that are agreement with our conclusion are; (Gupta *et al*., 2012) showed increase in MDA level in breast cancer as compared to controls, and thus suggesting increased lipid peroxidation in breast cancer patients, also(Gönenç *et al*., 2001) MDA levels in total cancer patients and in both breast and lung cancer patients were significantly higher than those in healthy subjects ($P < 0.001$ in all three comparisons) . There was no significantly effect of BMI, age or menopausal status on plasma MDA in either breast cancer patients or controls, while (Go¨nenc *et al*., 2007) found that in patients, MDA levels were significantly higher than those in controls ($p<0.05$), also (Gönenç *et al*., 2006) was found that tissue MDA levels in benign breast disease were higher than those of breast cancer patients ($p < 0.05$), and (Saad *et al*., 2020) showed that the serum levels of MDA as a marker of oxidative stress-induced lipid peroxidation, were significantly lower in the healthy controls compared to the BC patients ($p < 0.001$). Therefore we proposed excess of LPO which cause increase a MDA product that transport in circulation system, hens elevated MDA levels in carcinoma patients.

3.2.2 Measurements of TAC levels in patient and control

The results of the current study found the a brightly lit area that the mean of serum TAC levels are a significantly decrease in patient with breast cancer ($p< 0.005$) as compared with control group, at mean ± SD (4.74 ± 4.31, 7.89 ± 5.70) respectively, as like as in Figure 3-8.

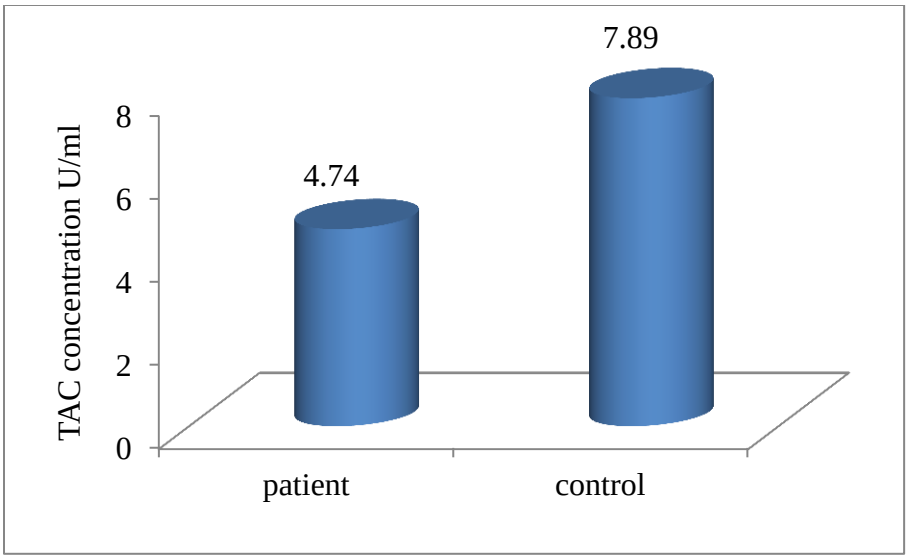


Fig 3-8 TAC concentration U/ml

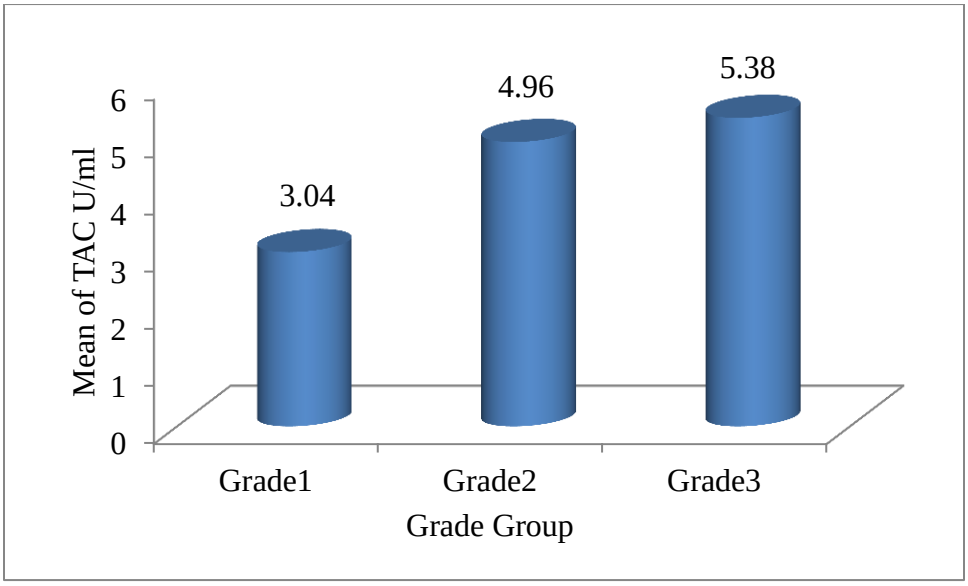


Fig. 3-9 TAC level at different Grade in patient

The results of the current study found the expanded location that the mean of serum TAC levels are a significantly increase in patient with breast cancer during different doses, (before treatment, first, second, and third doses) respectively, as like as in Figure 3-10.

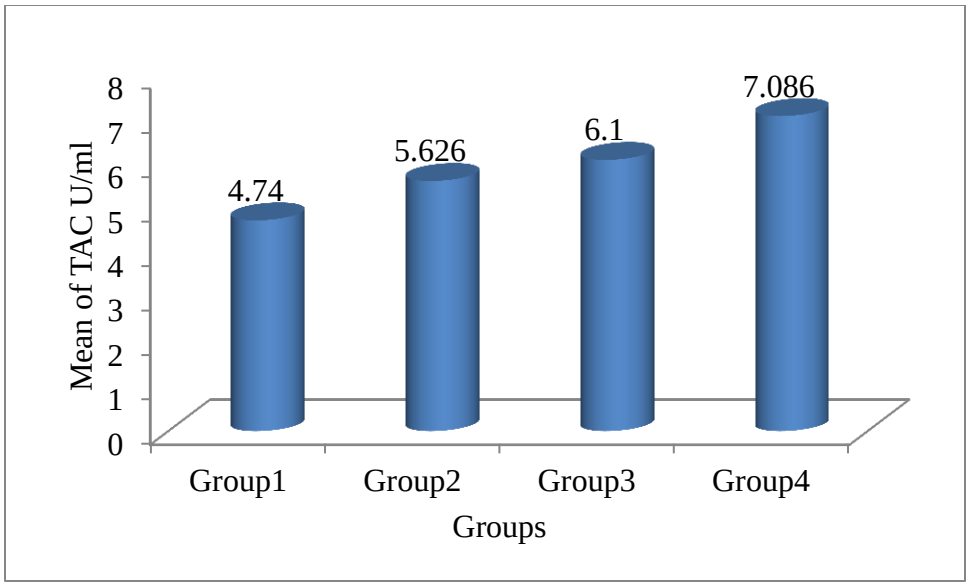


Fig 3-10 TAC concentration according to doses in patient group

Group1 and Group2 p= 0.368, Group1and Group3 p=. 0.000

Group1 and Group 4 p= 0.000, Group2 and Group3 p= 0.219

Group2 and Group4 p= 0.188, Group3 and Group4 p= 0.000

The studies that agreement with our finding, (Gupta et al., 2012) found that the level of TAC and activity of both SOD and GSH-Px decreased in breast cancer patient as compared to the healthy control, while (Hoseini *et al*., 2021) said that there were lower TAC levels in cases compared to controls and was statistically significant. The results suggest that increased serum MDA and decreased TCA activity may be due to oxidative stress, which may play an important role in BC formation, according to (Saad *et al*., 2022) the increase in serum lipid peroxidation in breast cancer was associated with enhanced antioxidant capacities. Increased generation of oxygen free radicals can induce TAC but not SOD, the activity of TAC is significantly increased with the increase of tumor stage and tumor size,

while (Saad *et al*., 2020) said that compared with the BC patients, a notable decrease in the serum levels of TOS and increase in serum levels of TAC are shown in the healthy controls ($P < 0.001$), also (Gönenç *et al*., 2006) demonstrated that both nitrate and nitrite and total antioxidant capacity in the benign group were lower than in the malign group ($p < 0.05$). and (Go¨nenc *et al*., 2007) suggested that serum levels of TAC measured in patients were significantly lower than those in controls ($p<0.05$) . In addition in patients with cancer, there is an increase in vascular permeability and hence increase in the albumin flux across the capillary wall towards the extravascular compartment. This is due to the release of tumor necrosis factor, which may increase microvascular permeability, leading to hypoalbuminemia (Nazha B, *et al*., 2015). Since the albumin is known as a component of TAC because of more than 70% of the free radical-trapping activity of serum was due to human serum albumin (HSA). Therefore the oxidative stress increase in cancer due to low activity of antioxidant against free radical.

3.2.3 Measurements of Hcy levels in patient and control

The results of the current study found the high lighting spots that the mean of serum Hcy levels are a significantly increase in patient with breast cancer ($p< 0.005$) as compared with control group, at mean ± SD (4.211± 5.04, 2.856± 1.58) respectively, as like as in Figure 3-11

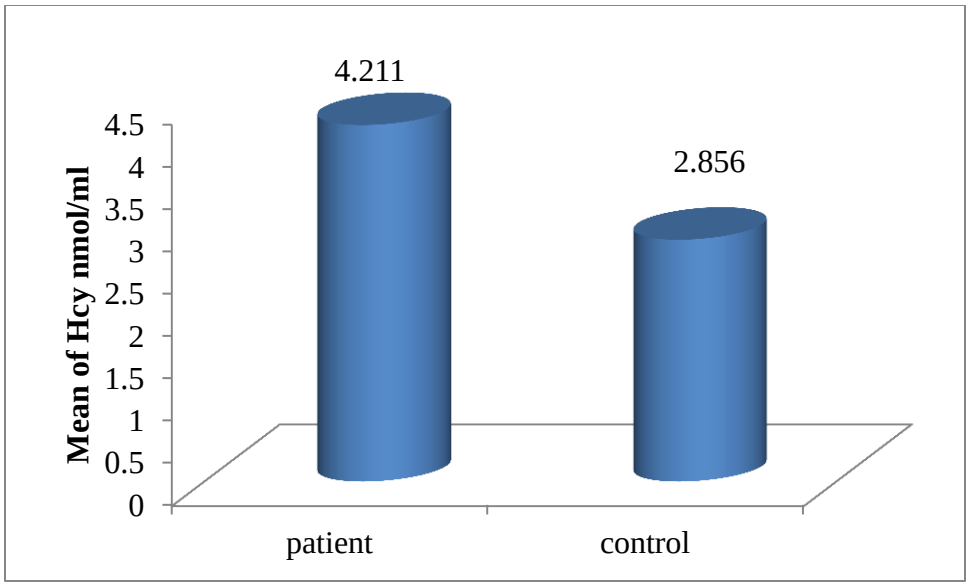


Fig 3-11 Hcy concentration

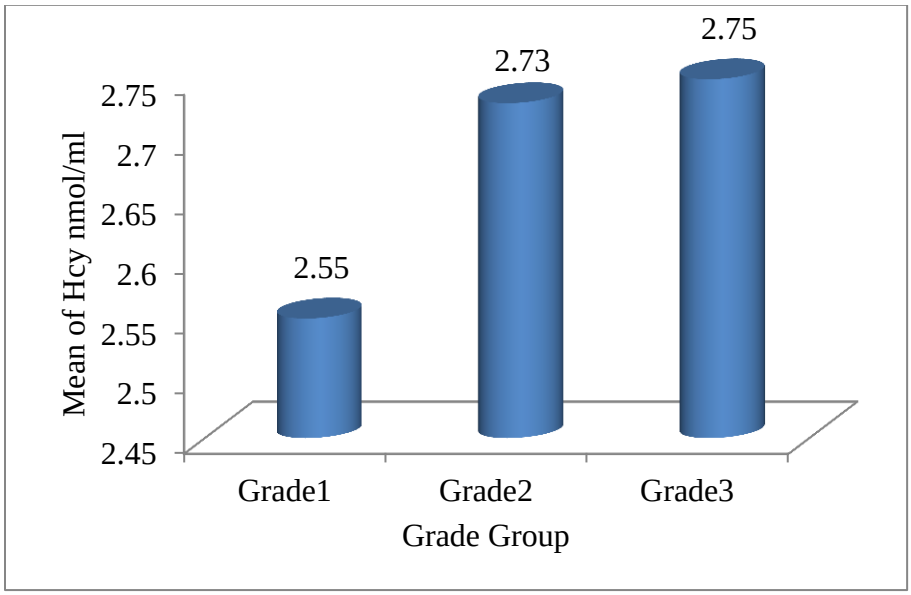


Fig 3-12 Hcy concentration in different Grade

The results of the current study found the bright point that the mean of serum Hcy levels are a significantly decrease in patient with breast cancer during different doses, (before treatment, first, second, and third doses) respectively, as

like as in Figure 3-13.

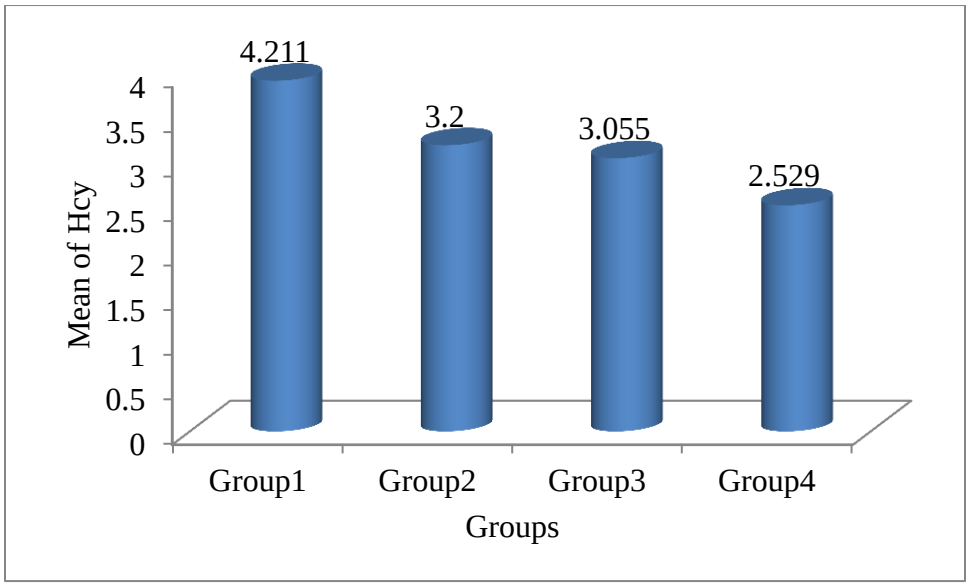


Figure 3-13 Hcy concentration according to doses in patient group

Group1 and Group2 p= 0.074, Group1 and Group3 p= 0.0001

Group1 and Group4 p= 0.0001, Group2 and Group3 p= 0.080

Group2 and Group4 p= 0.021, Group3 and Group4 p= 0.0001

The current study was in agreement with (Lin, *et al*,. 2010) higher Hcy levels may be associated with an increased risk for breast cancer among women with low folate status, also (Yamashita, *et al*., 2013) despite the fact homocysteine levels were normal at the diagnosis, we verified the increase of this amino acid during chemotherapy treatment, (Berger *et al*., 2000) pointed out that the highest estrogen level is associated with the decrease in serum concentration of total Hcy, while (Friso *et al*., 2006) showed that estrogen hormone may reduce homocysteine levels in postmenopausal women, and *(*Abdollahi ⅋, Omranipour,.2014) showed a significantly higher serum concentration of Homocysteine in patients with

malignant tumor before chemotherapy compared with benign tumor patients. Normal cells can cover their Met consumption from Hcy remethylation. Malignant cells in organs as lung, kidney, breast, colon and bladder are methionine-dependent, because they cannot convert Hcy to Met resulting to Hcy accumulation. An increased level of Hcy is also related to folate concentration. Folate cofactors act as essential intermediates in Hcy remethylation to Met, in SAM synthesis and in the production of nitrogenous bases for DNA/RNA synthesis (Almadori, *et al*,. 2005). Methionine-dependent cells have lower SAM/SAH ratio comparing with methionine independent cells (Warnecke, *et al*., 2000). Higher level of SAH increased Hcy level as long as Hcy is not converted to Cys by trans sulfuration pathways. Several studies observed higher Hcy level and unchanged plasma level of Cys in patients with cancer Since, homocysteine had played the role in a function in copper transport in the body, reducing cupric to cuprous, leads to higher cell damage (Sun, *et al*., 2002).

3.2.4 Measurement of Spx levels in patient and control

The results of the current study found a brilliantly lit environment that the mean of serum Spexin levels are a significantly decrease in patient with breast cancer ($p < 0.005$)as compared with control group, at median ± SD (680.45± 165.77 , 2818.65± 1702.085) respectively, as like as in Figure 3-14.

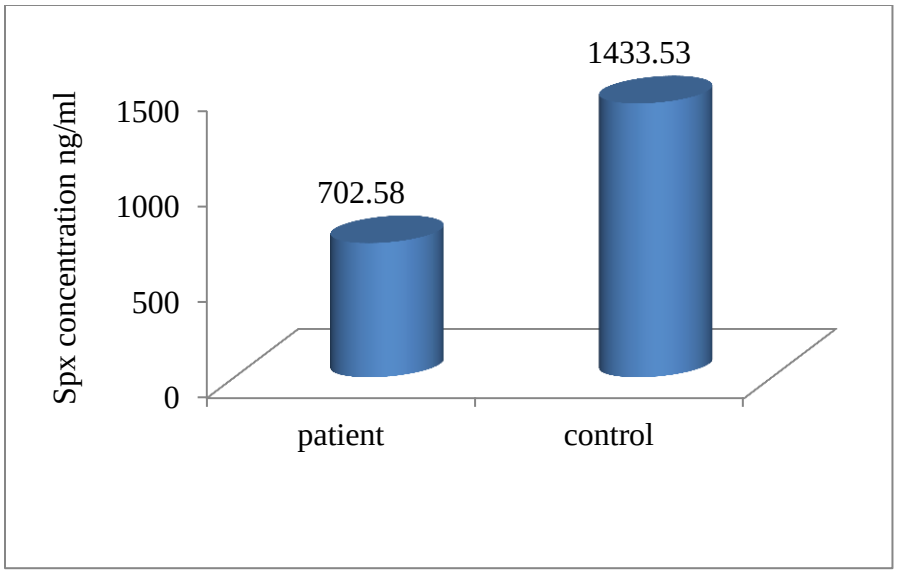


Fig 3-14 Spx concentration pg /ml

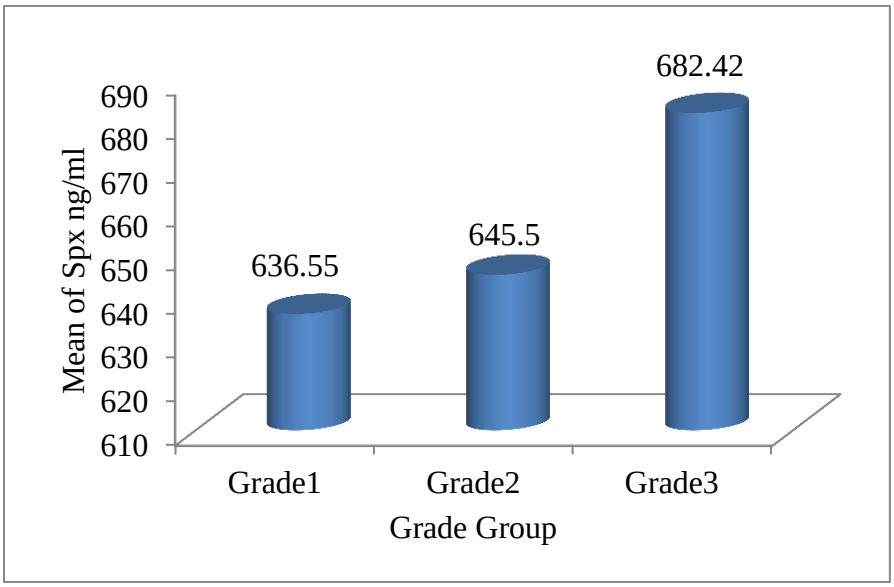


Fig 3-15 Spx concentration in different Grade

The results of the current study found the brightly spot that the mean of serum Spx levels are a significantly increase in patient with breast cancer ($p < 0.05$) during different doses (before treatment, first, second, and third doses) respectively in Figure 3-16.

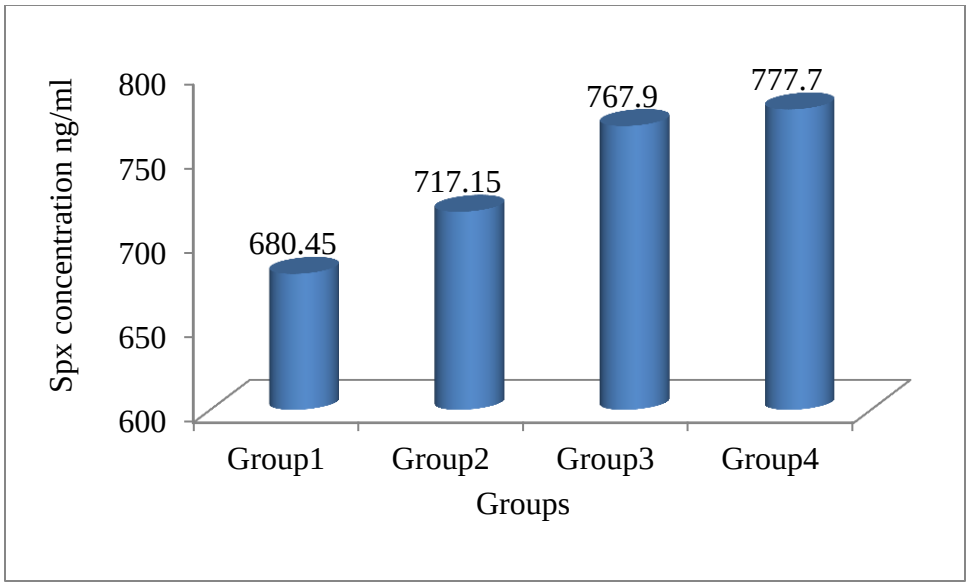


Fig 3-16 Spx concentration according to doses in patient group

Group1 and Group2 p= 0.011, Group1 and Group3 p= 0.0001

Group1 and Group4 p= 0.000, Group2 and Group3 p= 0.008

Group2 and Group4 p= 0.005, Group3 and Group4 p= 0.0001

Several studies demonstrated Spexin role : (Mamoor, 2021) found that Spx is expressed at significantly lower levels in breast tumors as compared to the breast. Spx was expressed at lower levels in tumors of the breast as compared to normal breast tissue. Decreased expression of Spx in primary breast tumors was statistically significant ($p<0.001$), where (Gu *et al*., 2015) demonstrated that spexin is highly expressed among endocrine and epithelial tissues, which suggests the tissue-specific functional involvement. Circulating spexin appears to be inversely related to blood glucose and lipids in T2DM, which may represent an adaptation to the rise of glucose and lipid associated with T2DM. Spx was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly. Diabetic women have a 27 percent greater chance of developing cancer than healthy women . therefore , the Spexin is

a novel peptide which has a potential role as a biomarker of insulin resistance.

3.2.5 Measurement of Asp levels in patient and control

The results of the current study found a bright point that the mean of serum Asp levels are a significantly increase in patient with breast cancer ($p < 0.005$) as compared with control group, at median ± SD (14.91±6.56, 13.11± 17.82) respectively, as like as in Figure 3-17.

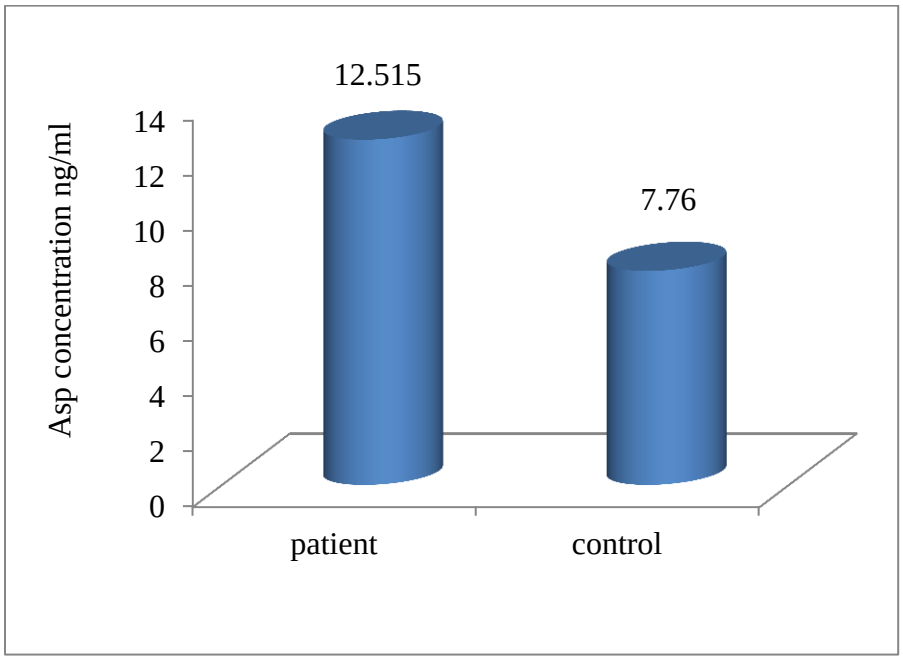


Fig 3-17 Asp concentration ng /ml

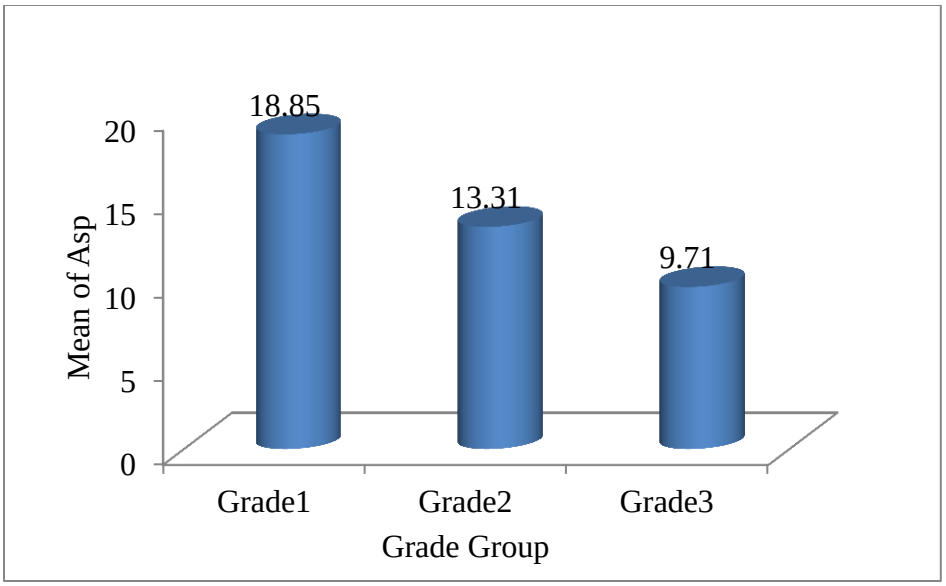


Fig 3-18 Asp concentration in different Grade

The results of the current study found the a highly lighting scene that the mean of serum Asp levels are a significantly decrease in patient with breast cancer (p >0.005) during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-19.

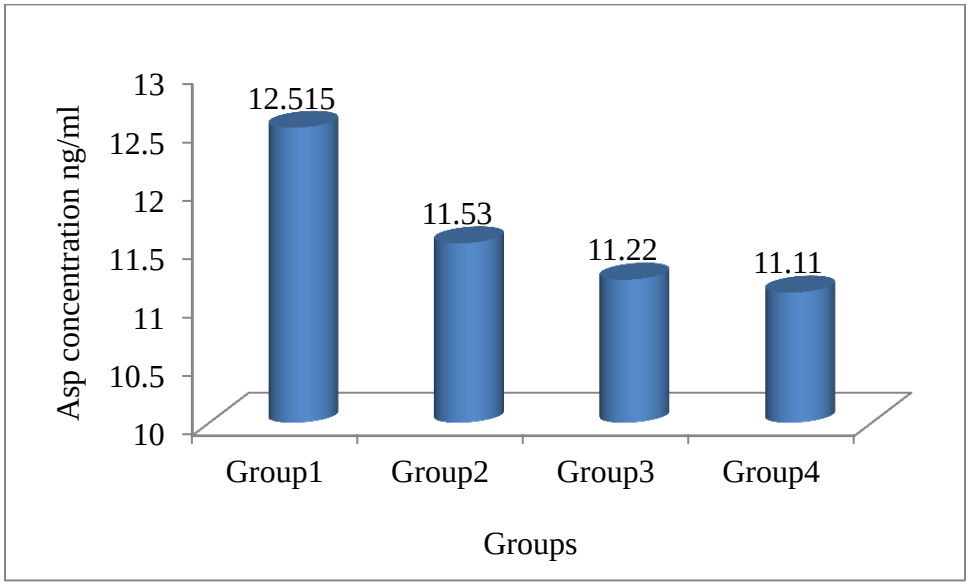


Fig 3-19 Asp concentration according to doses in patient group

Group1 and Group2 p= 0.184, Group1 and Group3 p= 0.283

Group1 and Group4 p= 0.157, Group2 and Group3 p= 0.861

Group2 and Group4 p= 0.648, Group3 and Group4 p= 0.014

Studies that reinforce with our finding is (Ozcan *et al*., 2021) indicate that asprosin levels in BC patients are significantly higher than in healthy women therefore, elevated asprosin values can be an indicator of an increased BC risk, as well as (Kocaman & Artaş, 2020) said that available evidence indicates that asprosin immunoreactivity is considerably raised in malignant mesothelioma, and can thus serve as its possible diagnostic marker, according to (Romere *et al*., 2016) asprosin dysfunction is caused by immunological or genetic factors, and results in a significant decrease in glucose and insulin concentrations. asprosin can reach pathological levels in BC patients, also (Leonard *et al*., 2021) showed that, an association between fasting plasma asprosin levels in healthy women and menstrual cycle, oral contraceptive use, and physical activity levels has been established. As glucose uptake is elevated in tumor cells, asprosin (as a novel glucogenic adipokine) may play a role in tumor development and progression. Available evidence indicates that asprosin immunoreactivity is considerably raised in malignant mesothelioma, and can thus serve as its possible diagnostic marker (Kocaman, *et al* ., 2020).

3.2.6 Measurement of MK levels in patient and control

The results of the current study found a bright spots that the mean of serum MK levels are a significantly increase in patient with breast cancer ($p < 0.005$) as compared with control group, at median ± SD (691.59 ± 420.28, 537.18 ± 414.59) respectively , as like as in figure (3-20).

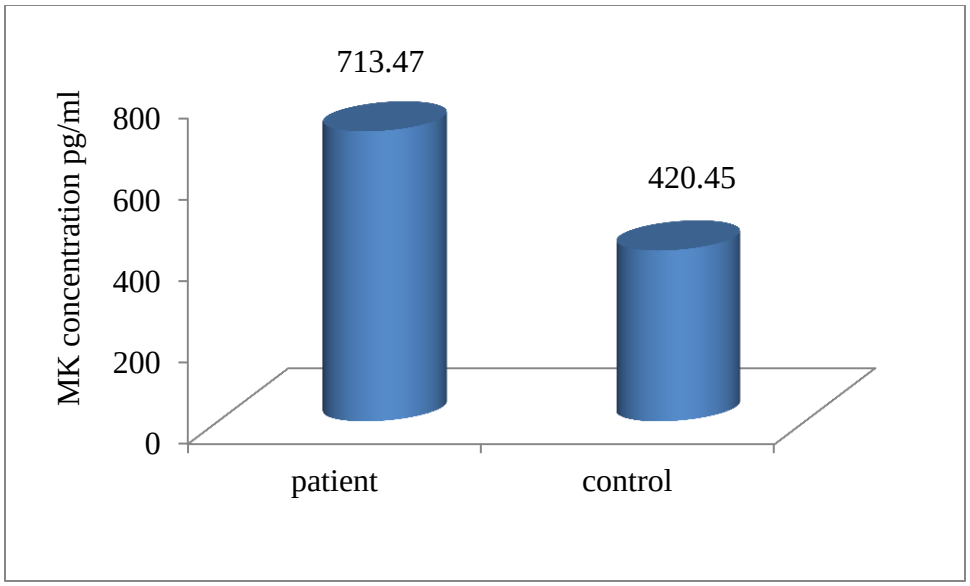


Fig 3-20 MK concentration pg/ml

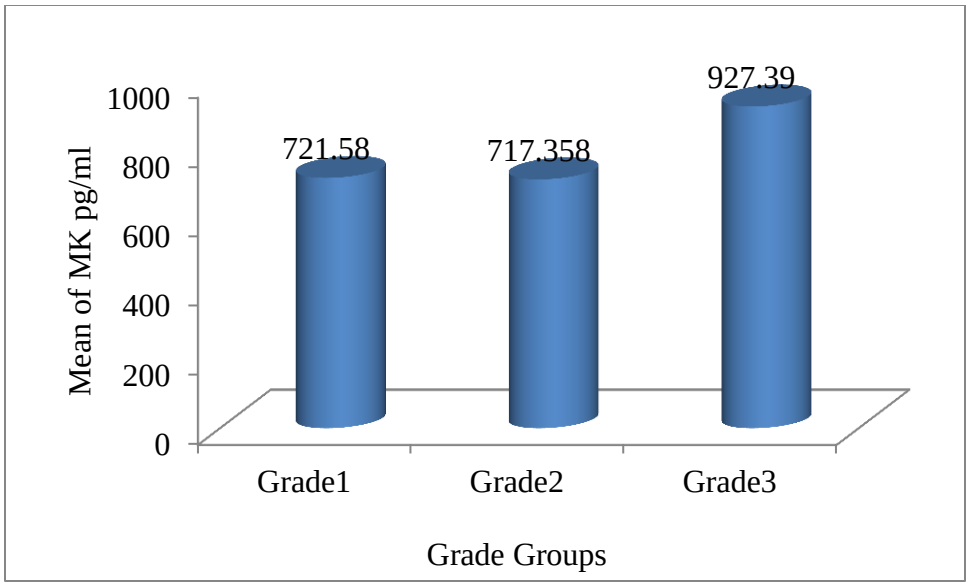


Fig 3-21 MK concentration in different Grade

The results of the current study found some few bright points that the mean of serum MK levels are a significantly decrease in patient with breast cancer ($p < 0.05$) during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-22.

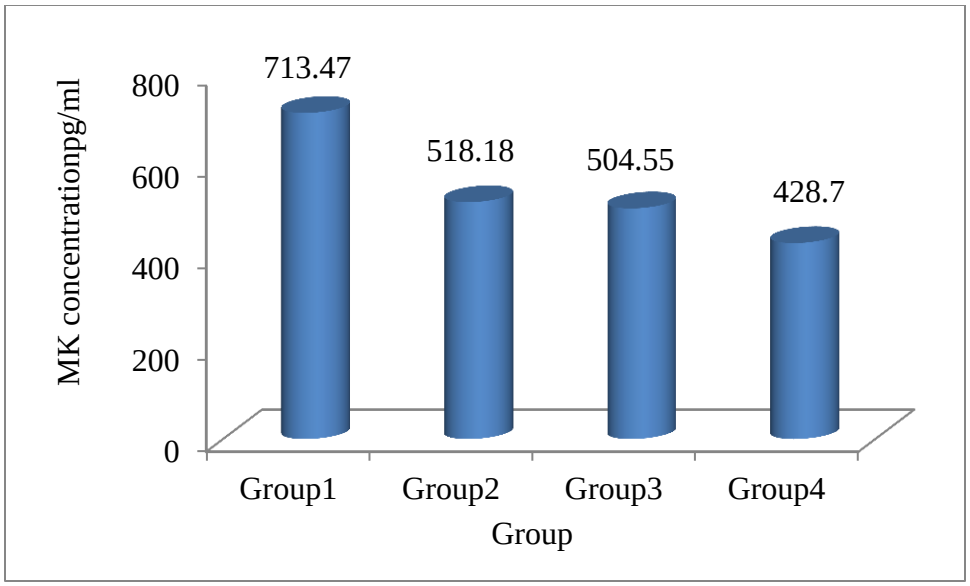


Fig 3-22 MK concentration according to doses in patient group

Group1 and Group2 p= 0.559, Group1 and Group3 p= 0.939

Group1 and Group4 p= 0.383, Group2 and Group3 p= 0.907

Group2 and Group4 p= 0.719, Group3 and Group4 p= 0.000

Our finding were agreement with previous studies (Hadida *et al*., 2019) showed that MK levels were significantly elevated in BC patients compared with healthy control samples matched in age and geographical distribution. Moreover, serum MK level positively correlated with clinical staging of breast cancer, in BC patients. The high sensitivity of MK suggest that it may be a novel marker for early breast cancer detection, also (Sorkun *et al*., 2016) demonstrated that there is an increase in MK expression in the presence of tumors. The fact that this expression increases at the serum level even at early stages and that there is a decrease in the serum MK level after the tumor has been removed shows that MK can be used as a cancer marker, while (Li *et al*., 2015) Found that MK was overexpressed in breast

cancer tissues compared with normal mammary tissues. Meanwhile, they found that MK protein overexpression was significantly correlated with clinical stages T classification (tumor size), N classification (lymphnode metastasis), and M classification (distant metastasis) in breast cancer patients, and (Ibusuki *et al*., 2009) showed that plasma MK levels were abnormally elevated in breast cancer patients, especially in metastatic cases. Measurement of plasma MK levels may be useful for detection of primary invasive cancer and metastatic cancer.

3.2.7 Measurement of Lipid profile levels in patient and control

The results of the current study found the places with a lot of light that the mean of serum (TC, TG, HDL-c, LDL-c, and VLDL-c) levels were a significantly increase in patient with breast cancer ($p < 0.005$)as compared with control group, at mean ± SD (227.67± 46.52, 190.7 ± 44.008, 204.99± 82.41, 173.41 ±69.71, 55.68±12.65, 56.16±8.55, 131.43± 43.72, 99.87±37.83, 43.21±17.72, 32.27± 9.008) respectively , as like as in Figure 3-23.

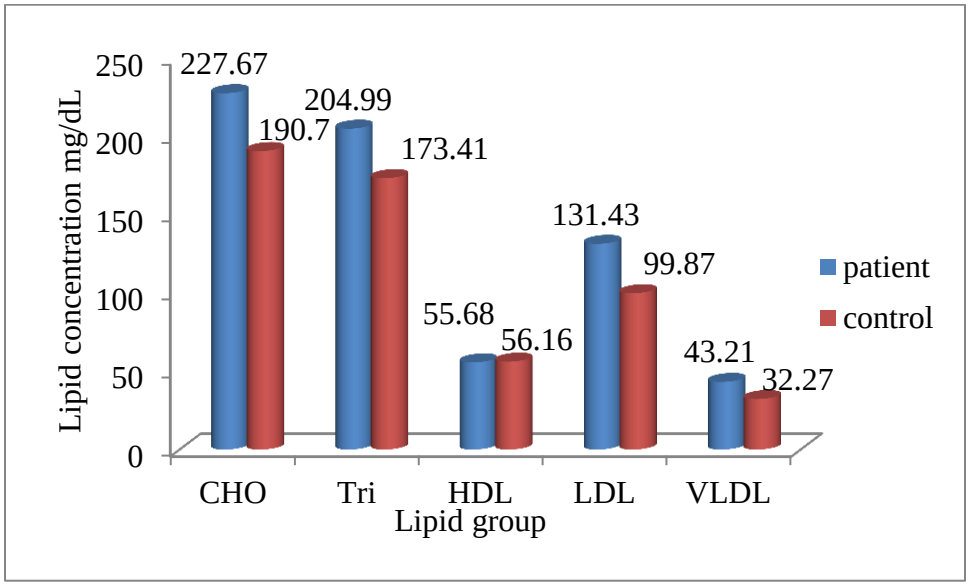


Fig 3-23 Lipid profile concentration

The results of the current study found spots with only a lot of light that the mean of serum TC levels are a significantly decrease in patient with breast cancer during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-24.

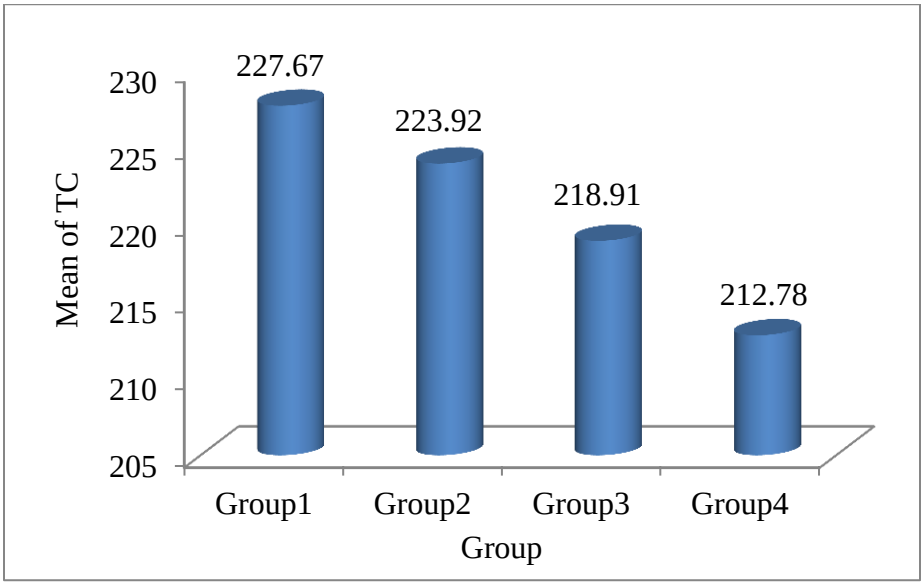


Fig 3-24 TC concentration according to doses in patient group

Group1 and Group2 p= 0.797, Group2 and Group3 p= 0.786

Group3 and Group4 p= 0.231, Group2 and Group4 p= 0.461

The results of the current study found the expanded location that the mean of serum TG levels are a significantly decrease in patient with breast cancer during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-25.

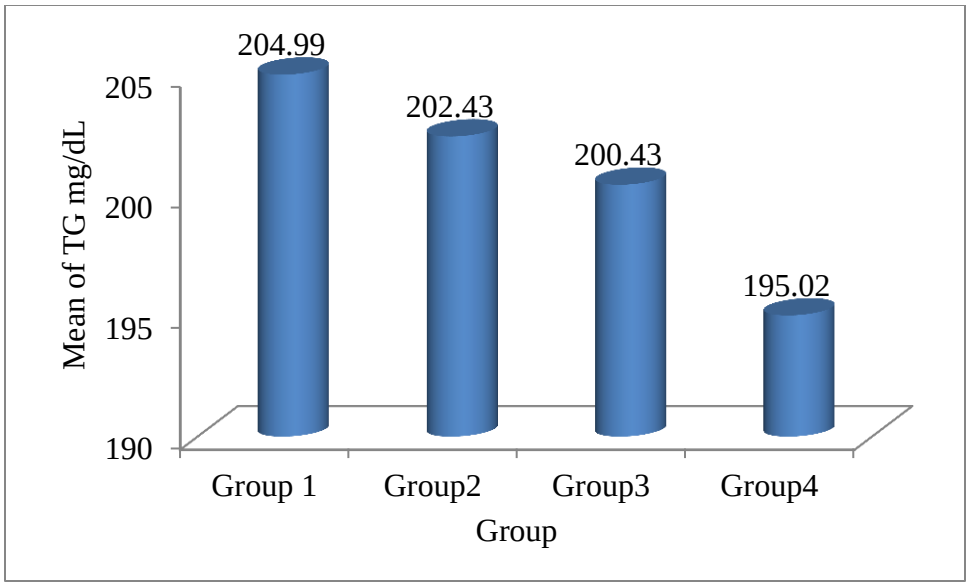


Fig 3-25 TG concentration according to doses in patient group

Group1 and Group2 P= 0.224, Group2 and Group3 p= 0.393

Group3 and Group4 P= 0.898, Group2 and Group4 p= 0.377

The results of the current study found a brightly spot that the mean of serum HDL-c levels are a significantly increase in patient with breast cancer during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-26.

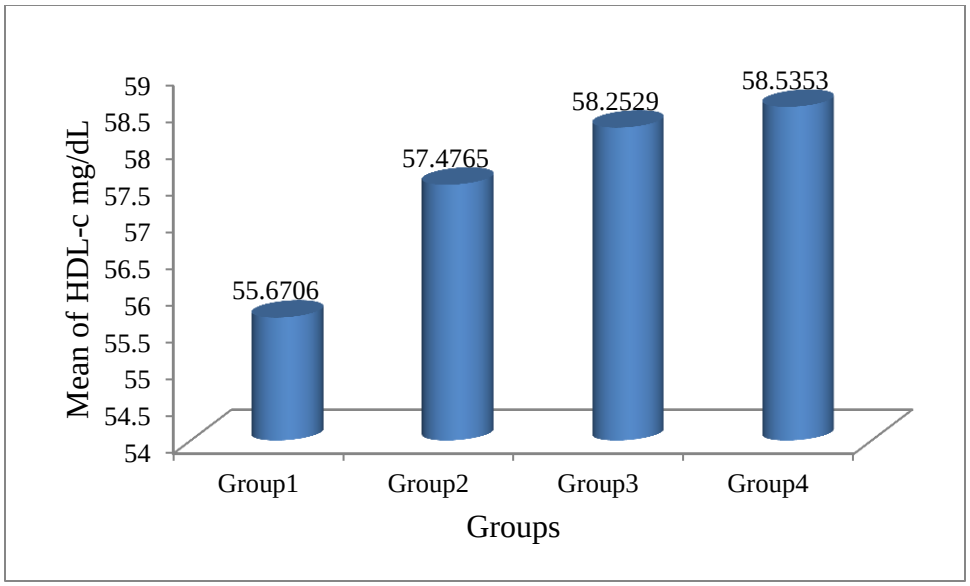


Fig 3-26 HDL-c concentration according to doses in patient group

Group1 and Group2 P= 0.596, Group2 and Group3 P= 0.713

Group3 and Group4 P=. 0.920, Group2 and Group4 P= 0.655

The results of the current study found a high lighting area that the mean of serum LDL-c levels are a significantly decrease in patient with breast cancer during different doses (before treatment , first, second, and third doses) respectively, as like as in Figure 3-27.

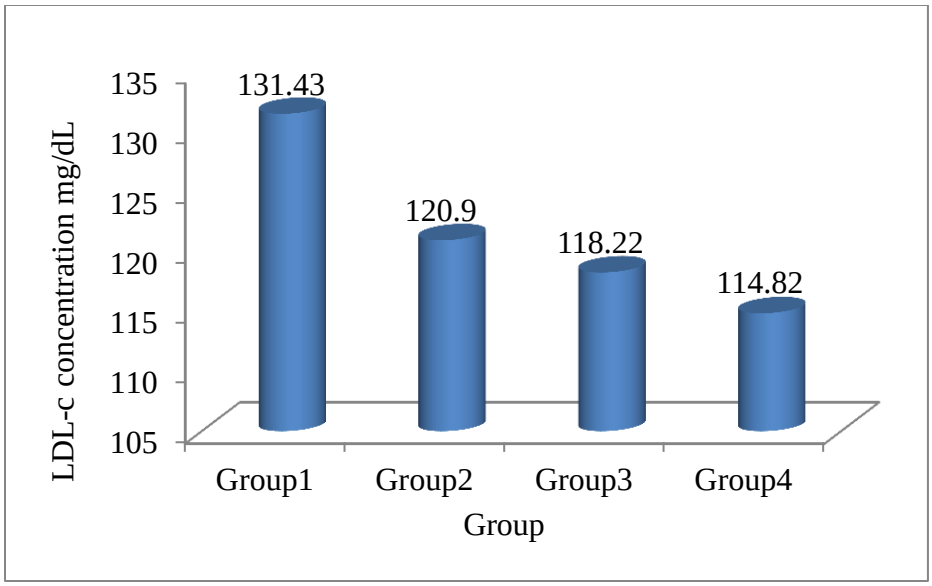


Fig 3-27 LDL-c concentration according to doses in patient group

Group1 and Group2 P=. 0.336, Group2 and Group3 P= 0.972

Group3 and Group4 P= 0.646, Group2 and Group P= 0.674

The results of the current study found an expanded location that the mean of serum VLDL-c levels are a significantly decrease in patient with breast cancer during different doses (before treatment, first, second, and third doses) respectively, as like as in Figure 3-28.

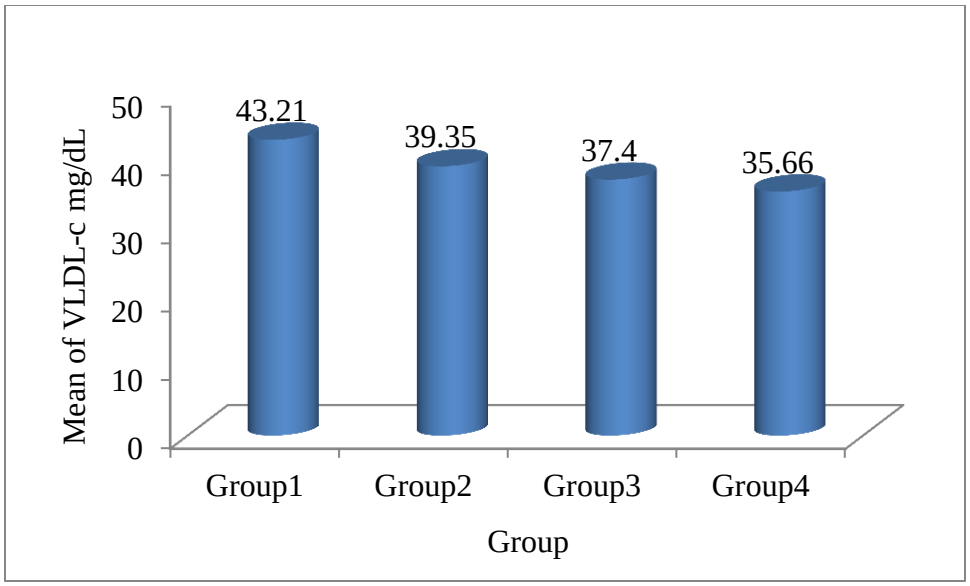


Fig 3-28 VLDL-c concentration according to doses

Group1 and Group2 P= 0.442, Group2 and Group3 P= 0.351

Group3 and Group4 P= 0.800, Group2 and Group4 P= 0.485

Several studies that was agreement with our result : (Shah *et al*., 2008) Find that , plasma TC as well as HDL levels were significantly lower and TG and VLDL levels were higher in breast cancer patients as compared with the controls. Plasma LDL levels were lower in breast cancer patients as compared with the controls, while (Abdelsalam *et al*., 2012) were found that values of TG, LDL, and TG/ HDL ratio were significantly increased in all the four stages of breast cancer (P <0.05), while the values of HDL and VLDL were not significantly changed , also (Rakesh *et al*,.2012) demonstrated that , when compared with breast cancer patients, total serum cholesterol and triglyceride levels were increased in cases; whereas HDL, though being in the normal range, was found to be decreased in cases as compared to controls. However, LDL level did not differ between the two groups, and (Xin Li *et al*,. 2018) The levels of TG and LDL in breast cancer

group were significantly lower than those in control group ($P < 0.001$). With age stratification, the levels of TG and LDL in breast cancer group were still significantly lower than those in control group ($P < 0.001$). Significant statistical difference was observed in the TG level of breast cancer group compared to normal group ($P < 0.05$) There was a significant statistical difference in HDL level among breast cancer group compared to control group ($P < 0.001$), respectively. In tumor cells present higher levels of reactive oxygen species (ROS) than not tumor cells, which allow them to activate prosurvival and epithelial-to-mesenchymal transition programs to support cancer progression and dissemination. Nevertheless, excessive ROS may promote apoptotic cell death. It has been demonstrated that enzymes implicated in the mobilization of intracellular neutral lipids provide metabolic flexibility to increase the levels of FAs for oxidation at mitochondria. In the FAO pathway, acyl-Co As are cyclically dehydrogenated, hydrated, and decarboxylated, resulting in the progressive shortening of the FA. Enhanced mitochondrial β-oxidation of FAs has been described in pancreatic cancer (Khasawneh J, *et al*. 2009, and Freigang S, *et al*., 2013) and in metastatic breast cancer (Li S, *et al*., 2014). Therefore, the cancer has Increased FAO augments by the metabolic oxidative stress.

3.3 Atherogenic Indices for Patient and Control

Table 3- 1 Comparison between atherogenic indices for patient and control

Atherogenic indices	Patient	Control
CRI-I	4.088	3.395
CRI-II	2.360	1.77
AIP	0.565	0.489
AC	3.088	2.395

Table 3-8 Receiver operating characteristic- area under curve (AUC) analysis of the measured biomarkers for the diagnosis of breast cancer

Variables	Cut-off Conc.	Sensitivity %	Specificity %	AUC	P- value	CI 95%
Hcy pg/ml	3.5150	70.6%	66.7%	0.786	0.003	0.614-0.958
MK pg/ml	512.5000	64.7%	57.1%	0.613	0.234	0.434-0.793
ASP pg/ml	9.5850	76.5%	76.2%	0.745	0.010	0.583-0.907
SPX pg/ml	750.6000	35.3%	23.8%	0.246	0.008	0.088-0.405
MDA pg/ml	17.9450	76.5%	71.4%	0.746	0.010	0.577-0.916
Antioxidant Pg/ml	3.4550	17.6%	14.3%	0.083	0.000	0.000-0.195

The results in Table 3-8 and Figure 3-28 showed that Hcy, Asp, MDA (followed by pg/ml) have the top three sensitives (70.6%, 76.5%, 76.5%) respectively for differentiation between breast cancer patient and healthy control. While the (MK, TAC, Spx) have no significant diagnostic ability ($p > 0.05$). Therefor subjects with an Asp concentration higher than 9.5 pg/ml have significant potential ($p<0.001$) to be diagnosed as breast cancer patients with sensitivity of 76.5% and specificity of 76.2%. these result reflect the impact of high Asp levels on the appearance of breast cancer symptoms. ROC curve analysis indicated the highest sensitivity and specificity for predicting breast cancer before metastasis or growing in tumor size .

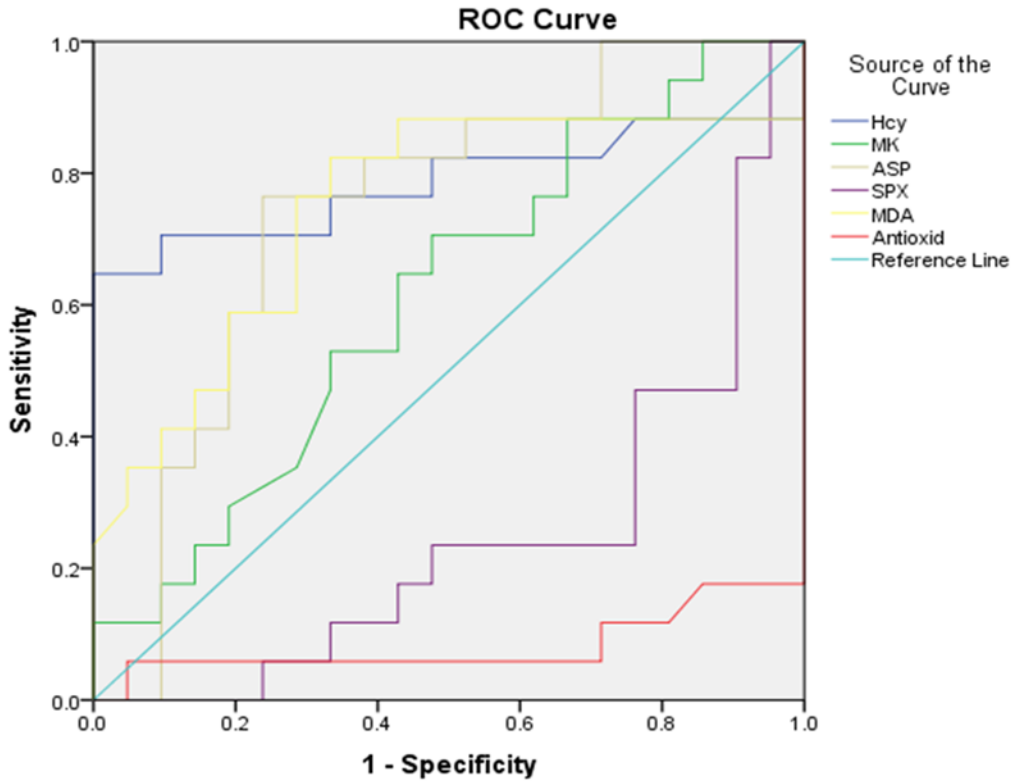


Fig. 3-29 Receiver operating characteristic curve of all cytokines which can used for diagnosis of breast cancer against healthy control.

3.4 Correlation Study

The result of correlation of studied parameters in condition that related to the therapeutic doses, in the Table 3-2 the result of patients groups before treatment uses, revealed that a significant correlation between TAC1 with Hcy1 ($p< 0.01$) and Spx1 ($p< 0.01$), also highly positive significant correlation between Hcy1 and Spx1 ($p< 0.01$), and weakly positive correlation between Asp1 with MK1 ($p< 0.01$) and MK1 with MDA1 ($p< 0.05$).

Table 3-2 Correlation of studied parameters before chemotherapy treatment.

Correlation		MDA1	MK1	Asp1	Spx1	Hcy1
MK1	Person correlation	0.579*				
	Sig (2-talid)	0.015				
Asp1	Person correlation	0.679**	0.393			
	Sig (2-talid)	0.003	0.118			
Spx1	Person correlation	-0.456-	-0.254-	-0.400-		
	Sig (2-taild)	0.066	0.324	0.112		
Hcy1	Person correlation	-0.441-	-0.267-	-0.383-	0.989**	
	Sig (2-taild)	0.076	-0.200-	0.129	0.0001	
TAC1	Person correlation	-0.359-	-0.200-	-0.369-	0.861**	0.903**
	Sig (2-taild)	0.157	0.441	0.145	0.0001	0.0001

* Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

In Table 3-3 the correlation result of breast cancer patients after receiving the first dose of chemotherapy, there is a significant direct positive correlation between Asp2 and MK2 ($p< 0.01$), also direct correlation between Hcy2 and Spx2 ($p< 0.01$) and between Spx2and MDA2 ($p< 0.01$) , also TAC2 has positive correlation with Spx2 ($p< 0.01$) and Hcy2 ($p< 0.01$).

Table 3-3 Correlation of studied parameters after the first dose of chemotherapy treatment.

Correlation		MDA2	MK2	Asp2	Spx2	Hcy2
MK2	Person correlation	0.375				
	Sig (2-talid)	0.138				
Asp2	Person correlation	0.339	0.054			
	Sig (2-talid)	0.183	0.838			
Spx2	Person correlation	0.551*	0.179	0.181		
	Sig (2-taild)	0.022	0.492	0.486		
Hcy2	Person correlation	0.411	0.235	-0.129-	0.854**	
	Sig (2-taild)	0.101	0.364	0.622	0.0001	
TAC2	Person correlation	0.200	0.195	-0.315-	0.664**	0.744**
	Sig (2-taild)	0.440	0.453	0.218	0.004	0.001

* Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

In Table 3-4 the correlation result of breast cancer patients after receiving the second dose of chemotherapy after21 day form last dose, there is a significant direct positive correlation between MK3 with MDA3 ($p< 0.05$), Asp3 with MK3 ($p< 0.05$) and MK3 ($p< 0.05$), also Hcy3 with Spx3 ($p< 0.01$), and TAC3 with HCY3 ($p< 0.01$), Spx3 ($p< 0.01$).

Table 3-4 Correlation of studied parameters after the second dose of chemotherapy treatment.

Correlation		MDA3	MK3	Asp3	Spx3	Hcy3
MK3	Person correlation	0.103				
	Sig (2-talid)	0.694				
Asp3	Person correlation	0.431	0.070			
	Sig (2-talid)	0.084	0.791			
Spx3	Person correlation	-0.433-	-0.332-	-0.188-		
	Sig (2-taild)	0.083	0.193	0.471		
Hcy3	Person correlation	-0.496-*	-0.337-	-0.246-	0.928**	
	Sig (2-taild)	0.043	0.186	0.340	0.0001	
TAC3	Person correlation	-0.574-*	-0.506-*	-0.318-	0.946**	0.924**
	Sig (2-taild)	0.016	0.038	0.214	0.0001	0.0001

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

In Table 3-5 The correlation result of breast cancer patients after receiving the third dose of chemotherapy after21 day form last dose, there is a significant direct correlation between Hcy4 with (MDA4, MK4, Asp4, and Spx4) ($p< 0.01$), and direct correlation between TAC4 with (MDA4, Asp4, and Spx4) ($p< 0.01$).

Table 3-5 Correlation of all studied parameter after the third doses of chemotherapy treatment

Correlation		MDA4	MK4	Asp4	Spx4	Hcy4
MK4	Person correlation	0.275				
	Sig (2-talid)	0.285				
Asp4	Person correlation	0.456	0.433			
	Sig (2-talid)	0.066	0.082			
Spx4	Person correlation	-0.108-	-0.128-	-0.104-		
	Sig (2-taild)	0.681	0.625	0.691		
Hcy4	Person correlation	-0.041-	0.028	-0.079-	0.966**	
	Sig (2-taild)	0.877	0.916	0.762	0.0001	
TAC4	Person correlation	-0.002-	0.087	-0.082-	0.900**	0.945**
	Sig (2-taild)	0.995	0.038	0.738	0.0001	0.0001

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

Table 3-6 Correlation between lipid profile, Cytokines and age group

		age group of patients	cholesterol	TG	HDL	LDL	VLDL
MK	Pearson Correlation	-0.080-	0.174	-0.058	0.188	0.088	0.066
	Sig. (2-tailed)	0.480	0.121	0.610	0.093	0.436	0.556
Asp	Pearson Correlation	0.0017	-0.035-	0.096	0.127	-0.116-	0.090
	Sig. (2-tailed)	0.884	0.755	0.393	0.259	0.304	0.426
Spx	Pearson Correlation	-0.091-	-0.039-	-0.250-	-0.001-	0.091	-0.252-*
	Sig. (2-tailed)	0.420	0.729	0.024	0.996	0.418	0.023
MDA	Pearson Correlation	0.012	0.034	0.013	-0.036-	0.015	0.010
	Sig. (2-tailed)	0.917	0.765	0.911	0.753	0.896	0.930
Hcy	Pearson Correlation	-0.129-	-0.148-	-0.165-	-0.092-	-0.063-	-0.165-
	Sig. (2-tailed)	0.252	0.188	0.141	0.416	0.579	0.140
TAC	Pearson Correlation	0.134	-0.007-	-0.083-	-0.004-	0.044	-0.086-
	Sig. (2-tailed)	0.234	0.948	0.459	0.971	0.693	0.447
age group of patients	Pearson Correlation		0.086	0.314**	-0.144-	0.001	0.333**
	Sig. (2-tailed)		0.428	0.003	0.180	0.992	0.002

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

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

Table 3-7 Correlation between lipid profile during different doses of chemotherapy

Paired Samples Correlations		Correlation	Sig.
Pair 1	TC before & TC1	0.199	0.445
Pair 2	TC1 & TC2	0.364	0.151
Pair 3	TC2 & TC3	0.670	0.003
Pair 4	TC1 & TC3	0.667	0.003
Pair 5	TG before & TG1	0.739	0.001
Pair 6	TG1 & TG2	0.679	0.003
Pair 7	TG2 & TG3	0.931	0.000
Pair 8	TG1 & TG3	0.635	0.006
Pair 9	HDL-c before & HDL-c1	0.149	0.569
Pair 10	HDL-c1 & HDL-c2	0.401	0.110
Pair 11	HDL-c2 & HDL-c3	0.142	0.587
Pair 12	HDL1 & HDL3	0.371	0.143
Pair 13	LDL-c before & LDL-c1	0.426	0.088
Pair 14	LDL-c1 & LDL-c2	0.253	0.326
Pair 15	LDL-c2 & LDL-c3	0.313	0.222
Pair 16	LDL-c1 & LDL-c3	0.466	0.059
Pair 17	VLDL-c before & VLDL-c1	0.718	0.001
Pair 18	VLDL-c1 & VLDL-c2	0.674	0.003
Pair 19	VLDL-c2 & VLDL-c3	0.811	0.000
Pair 20	VLDL-c1 & VLDL-c3	0.613	0.009

3.4 Analysis of Variance (ANOVA) Statics

Table 3-8 ANOVA Statics for all parameters

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
midkine	Between Groups	369365.806	2	184682.903	0.510	0.602
	Within Groups	28234847.015	78	361985.218		
	Total	28604212.822	80			
asprosin	Between Groups	218.520	2	109.260	0.213	0.808
	Within Groups	39942.853	78	512.088		
	Total	40161.373	80			
spexin	Between Groups	16310512.288	2	8155256.144	1.217	0.302
	Within Groups	522657046.712	78	6700731.368		
	Total	538967559.000	80			
malondialchehyche	Between Groups	484.167	2	242.083	1.190	0.310
	Within Groups	15872.576	78	203.495		
	Total	16356.742	80			
homocystein	Between Groups	1655.539	2	827.770	1.013	0.368
	Within Groups	63722.824	78	816.959		
	Total	65378.363	80			
anti oxidant	Between Groups	722.535	2	361.267	1.473	0.236
	Within Groups	19129.902	78	245.255		
	Total	19852.437	80			

Table 3-9 LSD Statics for lipid profil

LSD						
				95% Confidence Interval		
Dependent Variable	(I) age group of pateints	(J) age group of pateints	Mean Difference (I-J)	Sig.	Lower	Upper
cholesterol	<45	46-50	1.28433	.915	-22.4856-	25.0542
		>51	-10.25559-	.407	-34.7445-	14.2333
	46-50	<45	-1.28433-	.915	-25.0542-	22.4856
		>51	-11.53992-	.366	-36.7621-	13.6823
	>51	<45	10.25559	.407	-14.2333-	34.7445
		46-50	11.53992	.366	-13.6823-	36.7621
TG	<45	46-50	-64.32341-*	.010	-113.1014-	-15.5455-
		>51	-75.52261-*	.004	-125.7761-	-25.2691-
	46-50	<45	64.32341*	.010	15.5455	113.1014
		>51	-11.19920-	.668	-62.9574-	40.5590
	>51	<45	75.52261*	.004	25.2691	125.7761
		46-50	11.19920	.668	-40.5590-	62.9574
HDL	<45	46-50	1.38370	.545	-3.1482-	5.9156
		>51	3.16049	.182	-1.5085-	7.8294
	46-50	<45	-1.38370-	.545	-5.9156-	3.1482
		>51	1.77679	.465	-3.0320-	6.5855
	>51	<45	-3.16049-	.182	-7.8294-	1.5085
		46-50	-1.77679-	.465	-6.5855-	3.0320
LDL	<45	46-50	9.18171	.353	-10.3557-	28.7191
		>51	-.86166-	.932	-20.9901-	19.2668
	46-50	<45	-9.18171-	.353	-28.7191-	10.3557
		>51	-10.04337-	.338	-30.7745-	10.6878
	>51	<45	.86166	.932	-19.2668-	20.9901
		46-50	10.04337	.338	-10.6878-	30.7745
VLDL	<45	46-50	-12.81956-*	.012	-22.7773-	-2.8618-
		>51	-16.51049-*	.002	-26.7695-	-6.2515-
	46-50	<45	12.81956*	.012	2.8618	22.7773
		>51	-3.69093-	.489	-14.2571-	6.8752
	>51	<45	16.51049*	.002	6.2515	26.7695
		46-50	3.69093	.489	-6.8752-	14.2571

Table 3-10 ANOVA Statics for all parameters

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
cholesterol	Between Groups	18877.025	1	18877.025	9.503	0.003
	Within Groups	170825.538	86	1986.343		
	Total	189702.563	87			
TG	Between Groups	22952.974	1	22952.974	2.273	0.135
	Within Groups	868380.963	86	10097.453		
	Total	891333.937	87			
HDL	Between Groups	.722	1	.722	.009	0.925
	Within Groups	6960.717	86	80.939		
	Total	6961.439	87			
LDL	Between Groups	7794.200	1	7794.200	5.554	0.021
	Within Groups	120678.724	86	1403.241		
	Total	128472.924	87			
VLDL	Between Groups	1095.982	1	1095.982	2.591	0.111
	Within Groups	36374.502	86	422.959		
	Total	37470.484	87			

Chapter four
Conclusions and Recommendations

4.1 Conclusions :-

1. The bright points that the mean of serum MK, MDA, and Asperosin levels are more indicated to the patients response to treatment program of breast cancer.
2. Reduced the mean levels of serum Spx, Hcy, and TAC have be used as indicators of patient status before and after treatment.
3. The Serum MK level might be associated with disease activity and grade maturation .
4. The serum levels of MDA can be a marker of breast cancer as well as cytokines (MK, Asp, Spx) can be considered as marker for the grade of the breast tumor.

4.2 Recommendations :-

1- Study the apoH level and correlated with albumin in breast cancer patients before and after chemotherapy

2-Study the effects of S−Adenosyl Methionine (SAMe) level and S−Adenosyl Homocysteine (SAH) correlated with homocysteine levels in breast cancer patients before and after chemotherapy

3- Study of Midkine and Protein tyrosine phosphatase receptor type Z1 as marker for progression of cancer in all grade

4- Estimated of pleiotrophin levels in patients and relationship with Midkine in breast cancer patients before and after chemotherapy

5- Effects of obesity, insulin resistance and correlated with cytokine in breast cancer patients before and after chemotherapy

References

References

Alex, A., Bhandary, E., & McGuire, K. P. (2020). Anatomy and Physiology of the Breast during Pregnancy and Lactation. *Diseases of the Breast during Pregnancy and Lactation*, 3-7.

Abdollahi A, Omranipour R. Is Increase of Homocysteine, Anti-Cardiolipin, Anti-Phospholipid Antibodies associated with Breast Tumors?. Acta Med Iran. 2015;53(11):681-685.

Aung, T., & Tulsidas, H. (2021). Pursuing Investigations Establish the Challenging Diagnosis of Primary Aldosteronism. *Cureus*, *13*, (11).

Abdal Dayem, A., Choi, H. Y., Kim, J. H., & Cho, S. G. (2010). Role of oxidative stress in stem, cancer, and cancer stem cells. In *Cancers* 2, (2): 859–884. https://doi.org/10.3390/cancers2020859

Abdelsalam, K. E. A., Hassan, I. K., & Sadig, I. A. (2012). The role of developing breast cancer in alteration of serum lipid profile. *Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences*, *17* (6): 562.

Akram, M., Iqbal, M., Daniyal, M., & Khan, A. U. (2017). Awareness and current knowledge of breast cancer. *Biological research*, *50* (1):1-23.

Almadori, G., Bussu, F., Galli, J., Cadoni, G., Zappacosta, B., Persichilli, S., ... & Maurizi, M. (2005). Serum levels of folate, homocysteine, and vitamin B12 in head and neck squamous cell carcinoma and in laryngeal leukoplakia. *Cancer*, *103*(2), 284-292.

Al-Dujaili, M. N., Al-Khafaji, K. H. A., & Dujaili, A. N. (2018). Relationship Between Chordin Like-1 Protein Level and Patients with Pulmonary Arterial Hypertension Disease. *International Journal of Pharmaceutical Quality Assurance*, *9*(3): 308-316.

Al-Rawaq, K. J., Al-Naqqash, M. A., & Jassim, M. K. (2016). Molecular Classification of Iraqi Breast Cancer Patients and Its Correlation with Patients' Profile. *Journal of the Faculty of Medicine Baghdad*, *58*(3):197-201.

Ali, E. A., & Talaat, S. (2020). Ultrasound Lexicon in diagnosis and management of breast fibroadenoma: when to follow up and when to biopsy. In *Egyptian Journal of Radiology and Nuclear Medicine* 51(1). https://doi.org/10.1186/s43055-019-0125-5

References

Alshareef, B., Yaseen, W., Jawa, W., Barnawe, Y., Alshehry, W., Alqethami, H., ... & Alqumaili, O. (2020). Breast cancer awareness among female school teachers in Saudi Arabia: A population based survey. *Asian Pacific journal of cancer prevention: APJCP*, *21*(2), :337.

Alwan, N. A., Tawfeeq, F. N., & Mallah, N. (2019). Demographic and clinical profiles of female patients diagnosed with breast cancer in Iraq. *J Contemp Med Sci*, 5(1), :14-19.

Amayo, A. A., & Kuria, J. G. (2009). Clinical application of tumour markers: a review. In *East African medical journal* 86(12). https://doi.org/10.4314/eamj.v86i12.62909

Ambrosone, C. B., Zirpoli, G. R., Hutson, A. D., McCann, W. E., McCann, S. E., Barlow, W. E., ... & Albain, K. S. (2020). Dietary supplement use during chemotherapy and survival outcomes of patients with breast cancer enrolled in a cooperative group clinical trial (SWOG S0221). Journal of Clinical Oncology, 38(8),:804.

Amin, M. B., Greene, F. L., Edge, S. B., Compton, C. C., Gershenwald, J. E., Brookland, R. K., ... & Winchester, D. P. (2017). The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: a cancer journal for clinicians*, 67(2), pp 93-99.

Avila-Castro, I. A., Hernández-Martínez, A. R., Estevez, M., Cruz, M., Esparza, R., Pérez, R., & Rodríguez, A. L. (2017). Thorax thermographic simulator for breast pathologies. In *Journal of Applied Research and Technology* 15(2): 143–151 https://doi.org/10.1016/j.jart.2017.01.008

Averill-Bates, D., Grondin, M., & Ouellet, F. (2018). Activation of apoptosis signaling pathways by reactive oxygen species. *Cryobiology*, 80(170).

Al Zahrani, A. M., Alalawi, Y., Yagoub, U., Saud, N., & Siddig, K. (2019). A cross-sectional assessment of quality of life of breast cancer patients in Saudi Arabia. *Public Health*, l27:199-208.

Alvi, S., Hande, A., Chaudhary, M., Gawande, M., Patil, S., & Sharma, P. (2019). The assessment of expression of midkine in epithelial dysplasia and oral squamous cell carcinoma. *Journal of Datta Meghe Institute of Medical Sciences University*, *14*(4):378.

Bacchetti, T., Turco, I., Urbano, A., Morresi, C., & Ferretti, G. (2019).

Relationship of fruit and vegetable intake to dietary antioxidant capacity and markers of oxidative stress: A sex-related study. *Nutrition*, 61:64-172.

Badid, N., Baba Ahmed, F. Z., Merzouk, H., Belbraouet, S., Mokhtari, N., Merzouk, S. A., Benhabib, R., Hamzaoui, D., & Narce, M. (2010). Oxidant/antioxidant status, lipids and hormonal profile in overweight women with breast cancer. In *Pathology and Oncology Research* 16(2):159–167. https://doi.org/10.1007/s12253-009-9199-0

Balaji, K., Subramanian, B., Yadav, P., Radha, C. A., & Ramasubramanian, V. (2016). Radiation therapy for breast cancer: Literature review. *Medical Dosimetry*, 41(3),:253-257.

Barbe, M. D., Ross, J. A., & Fearon, K. C. H. (2009). *Changes in Nutritional, Functional, and Inflammatory Markers in Advanced Pancreatic Cancer*. 35 (2):106-110 https://doi.org/10.1207/S15327914NC352.

BaniIA, Williams CM, Boulter PS, Dickerson JW. Plasma lipids and prolactin in patients with breast cancer. Br J Cancer 1986;54:439-46

Berger, P. B., Herrmann, R. R., & Dumesic, D. A. (2000, January). The effect of estrogen replacement therapy on total plasma homocysteine in healthy postmenopausal women. In *Mayo Clinic Proceedings* 75(1):18-23. Elsevier.

Bitarafan, V., Esteghamati, A., Azam, K., Yosaee, S., & Djafarian, K. (2019). Comparing serum concentration of spexin among patients with metabolic syndrome, healthy overweight/obese, and normal-weight individuals. *Medical Journal of the Islamic Republic of Iran* 93 (*33*,).

Bothou, A., Zervoudis, S., Tsatsaris, G., Iatrakis, G., Deuteraiou, D., Anthoulaki, X., ... & Tsikouras, P. (2020). Maternal and paternal age at birth and the risk of breast cancer in greek women: a case-control study. *J BUON, 25* (2),:662-665.

Burbage, D., Duffy, N., Johnson, E. J., & Schneider, S. M. (2020). Cancer Survivorship Program: Implementation for individuals with recurrent breast cancer. *Clinical Journal of Oncology Nursing*,24(1).

Bezerra, L. A., Oliveira, M. M., Rolim, T. L., Conci, A., Santos, F. G. S., Lyra, P. R., & Lima, R. C. (2013). Estimation of breast tumor thermal properties using infrared images. *Signal Processing*, 93 (10), :2851-2863.

Berry D., K.Cronin, S.Plevritis, D.Fryback, L.Clarke, M.Zelen, J.Mandelblatt, A.Yakovlev, D.Habbema, & E.Feuer. (2007). Effect of Screening and Adjuvant Therapy on Mortality From Breast Cancer. In *Breast Diseases: A*

Year Book Quarterly 17(4,:)344). https://doi.org/10.1016/s1043-321x 80(07):120-2.

Blackman JD, Cabana VG, Mazzone T. The acute-phase response and associated lipoprotein abnormalities accompanying lymphoma. J Internal Med. 1993;233(2):201.

Cai, Y. qin, Lv, Y., Mo, Z. cheng, Lei, J., Zhu, J. ling, & Zhong, Q. qing. (2020). Multiple pathophysiological roles of midkine in human disease. In *Cytokine* 135. https://doi.org/10.1016/j.cyto.2020.155242

Calaf, G. M., Zepeda, A. B., Castillo, R. L., Figueroa, C. A., Arias, C., Figueroa, E., & Farías, J. G. (2015). Molecular aspects of breast cancer resistance to drugs (Review). In *International Journal of Oncology* 47,(2):437–445. https://doi.org/10.3892/ijo.2015.3055

Campeau, P. M., Foulkes, W. D., & Tischkowitz, M. D. (2008). Hereditary breast cancer: New genetic developments, new therapeutic avenues. In *Human Genetics* 124(1):31–42). https://doi.org/10.1007/s00439-008-0529-1

Cardoso, F., Kyriakides, S., Ohno, S., Penault-Llorca, F., Poortmans, P., Rubio, I. T., Zackrisson, S., & Senkus, E. (2019). Early Breast Cancer. pdf. (ESMO 2019). In *Annals of Oncology* 30(8):1194–1220. https://doi.org/10.1093/annonc/mdz173

Caroline de S. Araujo1, L. D. B., Tarifa2, M. O., Silva2, Da, N. J. F., S., K., Rodrigues3, Cavalcante4, M., D. G. S., Gomes4, S., A., Zocoler3, A., M., Yoshihara5, EidiMarjori, Camparoto2, L., Job4, Aldo, E.Kerche, & E., L. (2019). *Gene Expression Profiling of Carcinoma Breast and its, Prognostic Signature: A Review* (p. 7). https://doi.org/dx.doi.org/10.21276/ajs.2019.2.1.9

Chapman, C. M. (2019). Troubleshooting in the histology laboratory. In *Journal of Histotechnology* 42(3):137–149). https://doi.org/10.1080/01478885.2019.1640923

Cong, Y., Qiao, G., Zou, H., Lin, J., Wang, X., Li, X., ... & Zhu, S. (2015). Invasive cribriform carcinoma of the breast: a report of nine cases and a review of the literature. *Oncology Letters*, *9*(4):1753-1758.

Celia, C., Cristiano, M. C., Froiio, F., Di Francesco, M., d'Avanzo, N., Di Marzio, L., & Fresta, M. (2021). Nanoliposomes as multidrug carrier of gemcitabine/paclitaxel for the effective treatment of metastatic breast cancer disease: a comparison with Gemzar and Taxol. *Advanced Therapeutics*, 4, (1),

2000121.

CASTELLI, W. P., ABBOTT, R. D. & MCNAMARA, P. M. 1983. Summary estimates of cholesterol used to predict coronary heart disease. Circulation, 67, 730-734.

Deepak, K. G. K., Vempati, R., Nagaraju, G. P., Dasari, V. R., Nagini, S., Rao, D. N., & Malla, R. R. (2020). Tumor microenvironment: Challenges and opportunities in targeting metastasis of triple negative breast cancer. In *Pharmacological Research* 153. https://doi.org/10.1016/j.phrs.2020.104683

DeSantis, C. E., Ma, J., Gaudet, M. M., Newman, L. A., Miller, K. D., Goding Sauer, A., ... & Siegel, R. L. (2019). Breast cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69,(6),:438-451.

DOBIáSOVá, M., FROHLICH, J., SEDOVá, M., CHEUNG, M. C. & BROWN, B. G. 2011. Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. Journal of lipid research, 52, 566-571.

DeMichele, A., Yee, D., & Esserman, L. (2017). Mechanisms of resistance to neoadjuvant chemotherapy in breast cancer. *New England Journal of Medicine*, *377(*23), :2287-2289.

Dhillon, P. K. (2009). Breast cancer fact sheet. *Mortality*, *11*,(12.5): 10-8**.**

Dmitry, B., Juhaszova, M., & Sollot, S. (2014). Mitochondrial ROS-induced ROS release: an update and review. *Physiol Rev*, *94.*909-50.

Donepudi, M. S., Kondapalli, K., Amos, S. J., & Venkanteshan, P. (2014). Breast cancer statistics and markers. In *Journal of Cancer Research and Therapeutics* 10, (3):506–511. https://doi.org/10.4103/0973-1482.137927

Dreyfus, J., Brunet-de Carvalho, N., Duprez, D., Raulais, D., & Vigny, M. (1998). HB-GAM/pleiotrophin but not RIHB/midkine enhances chondrogenesis in micromass culture. *Experimental cell research*, *241*, (1), pp 171-180.

DO HAMMER, C. L. A. R. I. S. A., Fanning, A., & Crowe, J. (2008). Overview of breast cancer staging and surgical treatment options. *Cleveland Clinic journal of medicine*, *75*, (11).

Diaz-Santana, M. V., O'Brien, K. M., D'Aloisio, A. A., Regalado, G., Sandler, D. P., & Weinberg, C. R. (2020). Perinatal and postnatal exposures and risk of young-onset breast cancer. *Breast Cancer Research*, *22*,(1), :1-12.

References

Elmore, J. G., Armstrong, K., Lehman, C. D., & Fletcher, S. W. (2005). Screening for breast cancer. *Jama*, *293*(10), :1245-1256.

Elsherbiny, N. M., Sharma, I., Kira, D., Alhusban, S., Samra, Y. A., Jadeja, R., ... & Tawfik, A. (2020). Homocysteine induces inflammation in retina and brain. *Biomolecules*, 10,(3), :393.

Epstein, E., Van Holsbeke, C., Mascilini, F., Måsbäck, A., Kannisto, P., Ameye, L., ... & Testa, A. C. (2011). Gray-scale and color Doppler ultrasound characteristics of endometrial cancer in relation to stage, grade and tumor size. *Ultrasound in obstetrics & gynecology*, *38*,(5), :586-593.

Erten Şener, D., Gönenç, A., Akıncı, M., & Torun, M. (2007). Lipid peroxidation and total antioxidant status in patients with breast cancer. *Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease*, *25*(4), :377-382.

Elston CW, Ellis IO: Pathological prognostic factors in breast cancer I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991, 19: 403-410. 10.1111/j.1365-2559.1991.tb00229.x.

Ferlay, J., Colombet, M., I. Soerjomataram, Mathers, C., Parkin, D. M., M. Piñeros, A. Znaor, & F.Bray. (2018). *Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods* 20. https://doi.org/10.1002/ijc.3193768,(6) :394-424.

Ferroni, P., Palmirotta, R., Martini, F., Riondino, S., Savonarola, A., Spila, A., Ciatti, F., Sini, V., Mariotti, S., Del Monte, G., Roselli, M., & Guadagni, F. (2009). Determinants of homocysteine levels in colorectal and breast cancer patients. In *Anticancer Research* 29,(10): 4131–4138.

Figueira, P. V. G., Haddad, C. A. S., De Almeida Rizzi, S. K. L., Facina, G., & Nazario, A. C. P. (2018). Diagnosis of Axillary Web Syndrome in Patients after Breast Cancer Surgery. In *American Journal of Clinical Oncology: Cancer Clinical Trials* (Vol. 41, Issue 10, pp. 992–996). https://doi.org/10.1097/COC.0000000000000411

Friso, S., Lamon-Fava, S., Jang, H., Schaefer, E. J., Corrocher, R., & Choi, S. W. (2007). Oestrogen replacement therapy reduces total plasma homocysteine and enhances genomic DNA methylation in postmenopausal women. *British Journal of Nutrition*, *97*(4), :617-621.

References

Freigang S, Ampenberger F,Weiss A, Kanneganti TD, Iwakura Y, Hersberger M, et al. Fatty acid-induced mitochondrial uncoupling elicits inflammasome independent IL-1alpha and sterile vascular inflammation in atherosclerosis. Nat Immunol. (2013) 14:1045–53. doi: 10.1038/ni.2704

FRIEDEWALD, W. T., LEVY, R. I. & FREDRICKSON, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18, 499-502.

Gutierrez Blanco, C., Evans, E. B., & Porter, A. J. (2019). Breast cancer diagnosis in a specialised breast clinic: Are cancers detected by ultrasound alone less aggressive?. *Journal of Medical Imaging and Radiation Oncology*, *63*(1):40-46.

Gönenç, A., Özkan, Y., Torun, M., & Şmşek, B. (2001). Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. In *Journal of Clinical Pharmacy and Therapeutics* 26(2:)141–144). https://doi.org/10.1046/j.1365-2710.2001.00334.x

Greene, A. K., & Goss, J. A. (2018, February). Diagnosis and staging of lymphedema. In *Seminars in plastic surgery*32(01:) 012-016). Thieme Medical Publishers.

Gu, L., Ma, Y., Gu, M., Zhang, Y., Yan, S., Li, N., Wang, Y., Ding, X., Yin, J., Fan, N., & Peng, Y. (2015). Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. In *Peptides* 71:232–239. https://doi.org/10.1016/j.peptides.2015.07.018

Gulcin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of toxicology*, *94*(3): 651-715.

Gupta, R. K., Patel, A. K., Kumari, R., Chugh, S., Shrivastav, C., Mehra, S., & Sharma, A. N. (2012). Interactions between oxidative stress, lipid profile and antioxidants in breast cancer: A case control study. In *Asian Pacific Journal of Cancer Prevention* 13(12)6295–6298). https://doi.org/10.7314/APJCP.2012.13.12.6295

Gómez-Figueroa, E., Gutierrez-Lanz, E., Alvarado-Bolaños, A., Casallas-Vanegas, A., Garcia-Estrada, C., Zabala-Angeles, I., ... & Flores-Rivera, J. (2021). Cyclophosphamide treatment in active multiple sclerosis. *Neurological Sciences*, 42(9), : 3775-3780.

Goldstein JL, Brown MS. The low-density lipoprotein pathway and itts relation to

atherosclerosis. Annu Rev Biochem. 1977;46(1):897.

Hadida, W. M., Ali, D. A., ELnemr, A. A., & Nofal, H. E. (2019). The Role of Midkine in Breast Cancer. *The Egyptian Journal of Hospital Medicine*, *77* (3), :5089-5095.

Hagiwara, H., & Sunada, Y. (2004). Mechanism of taxane neurotoxicity. *Breast cancer*, 11(1), :82-85.

Hankey, G. J. (1999). *Homocysteine and vascular disease Graeme* 354:407–413.

Hasan, T., Arora, R., Bansal, A. K., Bhattacharya, R., Sharma, G. S., & Singh, L. R. (2019). Disturbed homocysteine metabolism is associated with cancer. *Experimental & molecular medicine*, *51*(2):1-13.

Hassanpour, M., Avci, Ç. B., Rahbarghazi, R., Rezabakhsh, A., Nourazarian, A., Nabat, E., ... & Khaksar, M. (2021). Resveratrol reduced the detrimental effects of malondialdehyde on human endothelial cells. *Journal of Cardiovascular and Thoracic Research*, *13*(2): 131.

Halsted, C. P., Benson, J. R., & Jatoi, I. (2014). A historical account of breast cancer surgery: beware of local recurrence but be not radical. *Future oncology*, *10*(9), :1649-1657.

He, Q., Yang, Z., Sun, Y., Qu, Z., Jia, X., Li, J., ... & Luo, Y. (2021). The Impact of Homocysteine on the Risk of Hormone-Related Cancers: A Mendelian Randomization Study. *Frontiers in nutrition*, *8*, 645371.

Hekim, M. G., Kelestemur, M. M., Bulmus, F. G., Bilgin, B., Bulut, F., Gokdere, E., ... & Ozcan, M. (2021). Asprosin, a novel glucogenic adipokine: a potential therapeutic implication in diabetes mellitus. *Archives of physiology and biochemistry*, : 1-7.

Henry, N. L., & Cannon-Albright, L. A. (2019). Breast cancer histologic subtypes show excess familial clustering. *Cancer*, *125*(18), :3131-3138.

Hidayat, K., Yang, C. M., & Shi, B. M. (2018). Body fatness at a young age, body fatness gain and risk of breast cancer: systematic review and meta-analysis of cohort studies. In *Obesity Reviews* 19(2:)254–268). https://doi.org/10.1111/obr.12627

Hoseini, H., Kerman Saravi, M., Shahraki, S., Sarani, A., & Sarabandi, A. (2021). Evaluation of Oxidative Stress Biomarkers in Patients with Breast cancer Compared with Healthy Subjects. *Journal of Chemical Health*

Risks, *11*(Special Issue: Bioactive Compounds: Their Role in the Prevention and Treatment of Diseases), :13-17.

Hoshmand, A. R. (2017). *Statistical methods for environmental & agricultural sciences*. CRC press.

Hu, T., Huang, J., & Fang, K. (2021). Overall Survival in Patients with Mucinous Carcinoma of Breast: A Population-Based Study. *International Journal of General Medicine*, *14:* pp 9991.

Hudis, A., Pusic, A. L., Elkin, E., & Bach, P. B. (2016). *Early Stage Breast Cancer : The Role of Breast Reconstruction* 135(6:)1518–1526).

Hardardóttir I, Grünfeld C, Feingold KR. Effects of endotoxin and cytokines on lipid Metabolism. Curr Opin Lipidol. 1994;5(3):207–15.

Ibusuki, M., Fujimori, H., Yamamoto, Y., Ota, K., Ueda, M., Shinriki, S., Taketomi, M., Sakuma, S., Shinohara, M., Iwase, H., & Ando, Y. (2009). Midkine in plasma as a novel breast cancer marker. In *Cancer Science* 100, (9:)1735–1739). https://doi.org/10.1111/j.1349-7006.2009.01233.x

In, I., Chio, C., & Tuveson, D. A. (2017). Feature Review ROS in Cancer : The Burning Question. In *Trends in Molecular Medicine:* :1–19). http://dx.doi.org/10.1016/j.molmed.2017.03.004

Judd, A. M., Matthews, M. K., Hughes, R., Veloz, M., Sexton, C. E., & Chaston, J. M. (2018). Bacterial methionine metabolism genes influence Drosophila melanogaster starvation resistance. *Applied and environmental microbiology*, *84*(17), e00 :662-18.

Jali, P. K., Donoghue, M., & Gadiwan, M. (2015). A rapid manual processing technique for resource-limited small laboratories. *Journal of Oral and Maxillofacial Pathology: JOMFP*, *19*(3),:306.

Jeon, B. J., & Chang, D. W. (2020). Breast cancer related lymphedema and surgical treatment. *Precision and Future Medicine*, *4*(2), :53-59**.**

Jones D. R. (2014). Measuring midkine: the utility of midkine as a biomarker in cancer and other diseases. *British journal of pharmacology*, 171(12), pp 2925–2939. https://doi.org/10.1111/bph.12601

Jurčić, P., Krušlin, B., Gatalica, Z., Sanati, S., & Vranić, S. (2016). Breast carcinoma with neuroendocrine features: a brief review. *Endo Oncology and Metab*, *2*:138-45.

Kadomatsu, K., & Muramatsu, T. (2004). Midkine and pleiotrophin in neural development and cancer. In *Cancer Letters* 204(2:)127–143). https://doi.org/10.1016/S0304-3835(03)00450-6

Kalli, S., Semine, A., Cohen, S., Naber, S. P., Makim, S. S., & Bahl, M. (2018). American joint committee on cancer's staging system for breast cancer: what the radiologist needs to know. *Radiographics*, *38*(7) 1921-1933.

Kalyanaraman, B., Cheng, G., Hardy, M., Ouari, O., Bennett, B., & Zielonka, J. (2018). Teaching the basics of reactive oxygen species and their relevance to cancer biology: Mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies. *Redox biology*, 15:347-362.

Kar, S. P., Andrulis, I. L., Brenner, H., Burgess, S., Chang-Claude, J., Considine, D., ... & Pharoah, P. D. (2019). The association between weight at birth and breast cancer risk revisited using Mendelian randomisation. *European journal of epidemiology*, *34*,(6):591-600.

Kilpatrick, E. S., & Lind, M. J. (2009). Appropriate requesting of serum tumour markers. *BMJ*, pp *339*.

Khasawneh J, Schulz MD, Walch A, Rozman J, Hrabe de Angelis M, Klingenspor M, et al. Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion. Proc Natl Acad Sci USA. (2009) 106:3354–9. doi: 10.1073/pnas.0802864106

Kim, J., & DeBerardinis, R. J. (2019). Mechanisms and implications of metabolic heterogeneity in cancer. *Cell metabolism*, *30*(3):434-446.

Kocaman, N., & Artaş, G. (2020). Can novel adipokines, asprosin and meteorin-like, be biomarkers for malignant mesothelioma? In *Biotechnic and Histochemistry* 95,(3:)171–175). https://doi.org/10.1080/10520295.2019.1656344

Koklesova, L., Mazurakova, A., Samec, M., Biringer, K., Samuel, S. M., Büsselberg, D., Kubatka, P., & Golubnitschaja, O. (2021). Homocysteine metabolism as the target for predictive medical approach, disease prevention, prognosis, and treatments tailored to the person. In *EPMA Journal* 12(4:). 477–505. https://doi.org/10.1007/s13167-021-00263-0

Kołodziejski, P. A., Pruszyńska-Oszmałek, E., Korek, E., Sassek, M., Szczepankiewicz, D., Kaczmarek, P., ... & Strowski, M. Z. (2018). Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin

resistance in women. *Physiological research*, *67*, (1), :45-56.

Kolodziejski, P. A., Pruszynska-Oszmalek, E., Micker, M., Skrzypski, M., Wojciechowicz, T., Szwarckopf, P., ... & Strowski, M. Z. (2018). Spexin: A novel regulator of adipogenesis and fat tissue metabolism. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1863*, Issue 10), pp 1228-1236.

Kresovich, J. K., Xu, Z., O'Brien, K. M., Weinberg, C. R., Sandler, D. P., & Taylor, J. A. (2019). Methylation-based biological age and breast cancer risk. *JNCI: Journal of the National Cancer Institute*, 111(10), :1051-1058.

Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. In *Nutrition Journal* 15(1). https://doi.org/10.1186/s12937-016-0186-5

Knapp ML, al-Sheibani S, Riches PG. Alterations of serum lipids in breast cancer: Effects of disease activity, treatment, and hormonal factors. Clin Chem 1991;37:2093-101.

Lin, J., Lee, I. M., Song, Y., Cook, N. R., Selhub, J., Manson, J. E., ... & Zhang, S. M. (2010). Plasma homocysteine and cysteine and risk of breast cancer in women. *Cancer research*, *70*(6), 2397-2405.

Lee, K. H., Kim, J. Y., Lee, M. H., Han, H. S., Lim, J. H., Park, K. U., ... & Im, S. A. (2016). A randomized, multicenter, phase II/III study to determine the optimal dose and to evaluate the efficacy and safety of pegteograstim (GCPGC) on chemotherapy-induced neutropenia compared to pegfilgrastim in breast cancer patients: KCSG PC10-09. *Supportive Care in Cancer*, *24*(4): 1709-1717.

Lee, R., Margaritis, M., M Channon, K., & Antoniades, C. (2012). Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. *Current medicinal chemistry*, *19*(16):2504-2520.

Lane DM, Boatman KK, McConathy WJ. Serum lipids and apolipoproteins in women with breast masses. Breast Cancer Res Treat 1995;34:161-9.

Leonard, A. N., Shill, A. L., Thackray, A. E., Stensel, D. J., & Bishop, N. C. (2021). Fasted plasma asprosin concentrations are associated with menstrual cycle phase, oral contraceptive use and training status in healthy women. In *European Journal of Applied Physiology* 121(3):793–801). https://doi.org/10.1007/s00421-020-04570-8

References

Li, F., Tian, P., Zhang, J., & Kou, C. (2015). The clinical and prognostic significance of midkine in breast cancer patients. *Tumor Biology*, *36*(12):pp 9789-9794.

Li, X., Liao, M., Shen, R., Zhang, L., Hu, H., Wu, J., ... & Zheng, H. (2018). Plasma asprosin levels are associated with glucose metabolism, lipid, and sex hormone profiles in females with metabolic-related diseases. *Mediators of inflammation*, *2018*.

Li S, Zhou T, Li C, Dai Z, Che D, Yao Y, et al. High metastaticgastric and breast cancer cells consume oleic acid in an AMPK dependent manner. PLoS ONE. (2014) 9:e97330. doi: 10.1371/journal.pone.0097330

Li, X., Yuan, J., Han, J., & Hu, W. (2020). Serum levels of homocysteine, vitamin B12 and folate in patients with multiple sclerosis: an updated meta-analysis. *International journal of medical sciences*, 17(6), pp 751.

Li, Y., Du, F., Zhu, W., & Xu, B. (2017). Neuroendocrine carcinoma of the breast: A review of 126 cases in China. In *Chinese Journal of Cancer* 36(1). https://doi.org/10.1186/s40880-017-0211-x

Liu, K., Zhang, W., Dai, Z., Wang, M., Tian, T., Liu, X., ... & Dai, Z. (2018). Association between body mass index and breast cancer risk: Evidence based on a dose–response meta-analysis. *Cancer management and research*, (vol *10*) pp 143.

Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemico-biological interactions*, 224:164-175.

Lauby-Secretan, B., Scoccianti, C., Loomis, D., Benbrahim-Tallaa, L., Bouvard, V., Bianchini, F., & Straif, K. (2015). Breast-cancer screening—viewpoint of the IARC Working Group. *New England journal of medicine*, *372*(24), pp 2353-2358.

Lassaletta, A., Zapotocky, M., Mistry, M., Ramaswamy, V., Honnorat, M., Krishnatry, R., ... & Tabori, U. (2017). Therapeutic and prognostic implications of BRAF V600E in pediatric low-grade gliomas. *Journal of Clinical Oncology*, *35* (25):2934.

Lehotský, J., Tothová, B., Kovalská, M., Dobrota, D., Beňová, A., Kalenská, D., & Kaplán, P. (2016). Role of Homocysteine in the Ischemic Stroke and Development of Ischemic Tolerance. *Frontiers in neuroscience*, (10) :538. https://doi.org/10.3389/fnins.2016.00538

References

Razavi, M., Sepidarkish, M., Maleki-Hajiagha, A., Vesali, S., Almasi-Hashiani, A., & Esmailzadeh, A. (2020). Preterm Birth and Breast Cancer Risk: A Systematic Review and Meta-Analysis.

Ma, A., Bai, J., He, M., & Wong, A. O. (2018). Spexin as a neuroendocrine signal with emerging functions. *General and comparative endocrinology*,(265) pp 90-96.

Makki, J. (2015). Diversity of breast carcinoma: histological subtypes and clinical relevance. *Clinical medicine insights: Pathology*, *8*, CPath-S31563.

Mamoor, S. (2021). *Differential expression of spexin hormone in cancers of the breast.* https://osf.io/xh2ng/

Mansoori, B. (2017). Mohammadi a, davudian S. *Shirjang S and Baradaran B: The different mechanisms of cancer drug resis-tance: a Brief review. adv Pharm Bull*, 7: pp 339-348.

Marrazzo, E., Frusone, F., Milana, F., Sagona, A., Gatzemeier, W., Barbieri, E., ... & Tinterri, C. (2020). Mucinous breast cancer: a narrative review of the literature and a retrospective tertiary single-centre analysis. *The Breast*, *49*:pp 87-92.

Masood, S. (2016). Breast cancer subtypes: morphologic and biologic characterization. *Women's Health*, 12, (1), : 103-119.

Masuda, N., Lee, S. J., Ohtani, S., Im, Y. H., Lee, E. S., Yokota, I., ... & Toi, M. (2017). Adjuvant capecitabine for breast cancer after preoperative chemotherapy. *New England Journal of Medicine*, *376*(22),:2147-2159.

Malek, A. E., Fife, C. E., Rasmussen, J. C., Karni, R. J., Morrow, J. R., Wanger, A., ... & Ostrosky-Zeichner, L. (2021, July). Lymphatic Dissemination and Axillary Web Syndrome in Primary Cutaneous Tuberculosis Secondary to Needlestick Injury. In *Open Forum Infectious Diseases* 8(7):ofab160). US: Oxford University Press.

McDonald, E. S., Clark, A. S., Tchou, J., Zhang, P., & Freedman, G. M. (2016). Clinical diagnosis and management of breast cancer. *Journal of Nuclear Medicine*, 57(1):pp 9S-16S.

Mitsuk, A. (2016). Breast cancer information for young women: a project for Terveysnetti.

Misra, A., & Dhurandhar, N. V. (2019). Current formula for calculating body

mass index is applicable to Asian populations. *Nutrition & diabetes*, *9*(1), 1-2.

Murphy, J. O., & Sacchini, V. S. (2016). Overall Survival, Disease-Free Survival, Local Recurrence, and Nipple–Areolar Recurrence in the Setting of Nipple-Sparing Mastectomy: A Meta-Analysis and Systematic Review. *Breast Diseases: A Year Book Quarterly*, *3*(27):201-203.

Mohamed, A., Krajewski, K., Cakar, B., & Ma, C. X. (2013). Targeted therapy for breast cancer. In *American Journal of Pathology* 183(4):1096–1112. https://doi.org/10.1016/j.ajpath.2013.07.005

Mohseni, H., Amani, R., Hosseini, S. A., Ekrami, A., Ahmadzadeh, A., & Latifi, S. M. (2019). Genetic variations in VDR could modulate the efficacy of vitamin D3 supplementation on inflammatory markers and total antioxidant capacity among breast cancer women: A randomized double blind controlled trial. In *Asian Pacific Journal of Cancer Prevention* 20(7):2065–2072. https://doi.org/10.31557/APJCP.2019.20.7.2065

Mozaffari, H., Daneshzad, E., Larijani, B., Surkan, P. J., & Azadbakht, L. (2020). Association of dietary total antioxidant capacity to anthropometry in healthy women: A cross-sectional study. *Nutrition*, *69*, 110577.

Mukendi, A. M., Van Den Berg, E., Pather, S., & Padayachee, R. S. (2018). Metachronous or synchronous male breast and prostate cancers a duality to lookout for. *F1000Research*, *7*.

Mejdahl, M. K., Wohlfahrt, J., Holm, M., Knoop, A. S., Tjønneland, A., Melbye, M., ... & Balslev, E. (2020). Synchronous bilateral breast cancer: a nationwide study on histopathology and etiology. *Breast Cancer Research and Treatment*, 182(1), pp 229-238.

Nascimento do, R. G., & Otoni, K. M. (2020). Histological and molecular classification of breast cancer: what do we know. *Mastology*, *30*, e20200024.

Navarro, María Ester Brandany, Y. V. (2006). *Brandan_RINCAN_2006 (1)*.

Nazha B, Moussaly E, Zaarour M, Weerasinghe C, Azab B. Hypoalbuminemia in colorectal cancer prognosis: nutritional marker or inflammatory surrogate? World J Gastrointest Surg. 2015;7(12):370-377.

Ndez-Garcı´a, L. A. lica M., Nava-Castro, K. E., Ochoa-Mercado, T. de L., Palacios-Arreola, M. I., Ruiz-Manzano, R. A., Segovia-Mendoza, M., Solleiro-Villavicencio, H., Zarez-Martı´nez, C. C., & Morales-Montor. (2019). *Breast Cancer Metastasis: Are Cytokines Important Players During Its Development*

and Progression? 39 https://doi.org/10.1089/jir.2018.0024

Nicolini, A., Carpi, A., & Rossi, G. (2006). Cytokines in breast cancer. In *Cytokine and Growth Factor Reviews* 17(5):325–337. https://doi.org/10.1016/j.cytogfr.2006.07.002

Nourazarian, A. R., Kangari, P., & Salmaninejad, A. (2014). Roles of oxidative stress in the development and progression of breast cancer. In *Asian Pacific Journal of Cancer Prevention* 15(12)4745–4751). https://doi.org/10.7314/APJCP.2014.15.12.4745

Narod, S. A., Iqbal, J., & Miller, A. B. (2015). Why have breast cancer mortality rates declined?. *Journal of Cancer Policy*, 5: 8-17.

OZCAN, S., YILDIRIM, N., YUR, M., OZDEDE, M. R., & OZCAN, M. (2021). *A novel adipose hormone asprosin as a potential breast cancer marker*. https://doi.org/10.21203/rs.3.rs-897211/v1 License:

US Preventive Services Task Force. **(**2016). Screening for breast cancer: US Preventive Services Task Force recommendation statement. *Ann Intern Med*, 164(4) 279-296.

Papa, G., Ferreira, C. F., Damasio, L., & Prigenzi, K. C. K. (2021). Metaplastic breast carcinoma: series of cases and literature review. *Mastology (Online)*, 1-8.

Perović, A., & Dolčić, M. (2019). Influence of hemolysis on clinical chemistry parameters determined with Beckman Coulter tests–detection of clinically significant interference. In *Scandinavian Journal of Clinical and Laboratory Investigation* 79(3):154–159. https://doi.org/10.1080/00365513.2019.1576099

Poli, G., Leonarduzzi, G., Biasi, F., & Chiarpotto, E. (2004). Oxidative stress and cell signalling. *Current medicinal chemistry*, 11(9):1163-1182.

Polidori, M. C., Savino, K., Alunni, G., Freddio, M., Senin, U., Sies, H., Stahl, W., & Mecocci, P. (2002). Plasma lipophilic antioxidants and malondialdehyde in congestive heart failure patients: Relationship to disease severity. In *Free Radical Biology and Medicine* 32(2):148–152. https://doi.org/10.1016/S0891-5849(01)00782-1

Pruner, I., Tomić, B., Dragojević, M., Gvozdenov, M., Kovač, M., Radojković, D., & Đorđević, V. (2019). Inherited thrombophilic risk factors in Serbian breast cancer patients. *Genetika*, 51 (2) : 463-472.

References

Pürnak, S., Külcü Çakmak, S., Çakir, B., Turhan, T., & Artüz, F. (2020). Serum midkine levels in patients with psoriasis. In *Turkiye Klinikleri Dermatoloji* 30, (1):1–5. https://doi.org/10.5336/dermato.2019-71481

Pathology Reporting of Breast Disease: A Joint Document Incorporating the Third Edition of the NHS Breast Screening Programme's Guidelines for Pathology Reporting in Breast Cancer Screening and the Second Edition of The Royal College of Pathologists' Minimum Dataset for Breast Cancer Histopathology. 2005, Sheffield; NHS Cancer Screening Programmes and The Royal College of Pathologists

Quora, H. A. A. R. A. (2021). Histopathological and Immunohistochemical Studies on The Breast Cancer Stem Cells Developed in The Microenvironment of Mouse Mammary Fat Pads.

Raffa, R. B., & Tallarida, R. J. (2010). Effects on the visual system might contribute to some of the cognitive deficits of cancer chemotherapy-induced "chemo-fog." In *Journal of Clinical Pharmacy and Therapeutics* 35(3): 249–255). https://doi.org/10.1111/j.1365-2710.2009.01086.x

Ramírez-Torres, A., Rodríguez-Ramos, R., Sabina, F. J., García-Reimbert, C., Penta, R., Merodio, J., ... & Preziosi, L. (2017). The role of malignant tissue on the thermal distribution of cancerous breast. *Journal of Theoretical Biology*, (426):152-161.

Ramírez-Parada, K., Garay-Acevedo, D., Mella-Abarca, W., Petric-Guajardo, M., Sánchez-Rojel, C., McNeely, M. L., Leao-Ribeiro, I., & Fernández-Verdejo, R. (2020). Axillary web syndrome among Chilean women with breast cancer: incidence and possible predisposing factors. In *Supportive Care in Cancer* 28(6): 2941–2947). https://doi.org/10.1007/s00520-019-05190-5

RB, B., NK, I., Olowoyeye OA, AA, A., & AT, O. (2010). *Knowledge , Attitude and Practice of breast self-examination among nursing students in the* 3(2) 2010).

Reczek, C. R., & Chandel, N. S. (2018). ROS promotes cancer cell survival through calcium signaling. *Cancer cell*, 33,(6), :949-951.

Razavi, M., Sepidarkish, M., Maleki-Hajiagha, A., Vesali, S., Almasi-Hashiani, A., & Esmailzadeh, A. (2020). Preterm Birth and Breast Cancer Risk: A Systematic Review and Meta-Analysis.

Rigby, K. M., & DeLeo, F. R. (2012). Neutrophils in innate host defense against

Staphylococcus aureus infections. In *Seminars in Immunopathology* 34(2):. 237–259. https://doi.org/10.1007/s00281-011-0295-3

Romere, C., Duerrschmid, C., Bournat, J., Constable, P., Jain, M., Xia, F., ... & Chopra, A. R. (2016). Asprosin, a fasting-induced glucogenic protein hormone. *Cell 165*(3):566-579.

Ross-Munro, E., Kwa, F., Kreiner, J., Khore, M., Miller, S. L., Tolcos, M., ... & Walker, D. W. (2020). Midkine: The who, what, where, and when of a promising neurotrophic therapy for perinatal brain injury. *Frontiers in neurology*, :1284.

Springfield, S., Odoms-Young, A., Tussing-Humphreys, L., Freels, S., & Stolley, M. (2019). Adherence to American Cancer Society and American Institute of Cancer Research dietary guidelines in overweight African American breast cancer survivors. *Journal of Cancer Survivorship*, 13(2) pp257-268.

Saad, M. E., Abdel–Megied, A. E., El–Mezayen, H., Farouk, O., Refaat, S., & Hamed, S. M. Evaluation of Oxidative Stress Involvement in Breast Cancer Carcinogenesis.

Saad, M. E., El-mezayen, H., Refaa, S., Tahmasebpour, N., Feizi, M. A. H., Ziamajidi, N., Pouladi, N., Montazeri, V., Farhadian, M., & Abbasalipourkabir, R. (2020). Association of omentin-1 with oxidative stress and clinical significances in patients with breast cancer. In *Advanced Pharmaceutical Bulletin* 10(1): 106–113. https://doi.org/10.15171/apb.2020.013

Sadeque, A. J. M., Wandel, C., He, H., Shah, S., & Wood, A. J. J. (2000). Increased drug delivery to the brain by P-glycoprotein inhibition. In *Clinical Pharmacology and Therapeutics* 68(3): 231–237. https://doi.org/10.1067/mcp.2000.109156

Sakurai, H., Bush, K. T., & Nigam, S. K. (2001). Identification of pleiotrophin as a mesenchymal factor involved in ureteric bud branching morphogenesis. In *Development* 128(17): 3283–3293). https://doi.org/10.1242/dev.128.17.3283

Samir, D., Naouel, A., & Safa, G. (2019). Assessment of Hematological Parameters, Enzymes Activities, and Oxidative Stress Markers in Salivary and Blood of Algerian Breast Cancer Patients Receiving Chemotherapy. *Journal of Biochemical Technology*, 10(4),:50.

Saorin, A., Di Gregorio, E., Miolo, G., Steffan, A., & Corona, G. (2020).

Emerging role of metabolomics in ovarian cancer diagnosis. In *Metabolites* 10, (10):1–15). https://doi.org/10.3390/metabo10100419

Sasanfar, B., Toorang, F., Maleki, F., Esmaillzadeh, A., & Zendehdel, K. (2021). Association between dietary total antioxidant capacity and breast cancer: a case–control study in a Middle Eastern country. *Public health nutrition*, 24, (5),:965-972.

Scharnagl, H., Matthias Nauck, H. W., & März, W. (2005). *The Friedewald Formula Underestimates LDL Cholesterol at low Concentrations*.

Schernhammer, E. S. (2002). In-utero exposures and breast cancer risk: Joint effect of estrogens and insulin-like growth factor? In *Cancer Causes and Control* 13(6): 505–508). https://doi.org/10.1023/A:1016348425833

Shah, F. D., Shukla, S. N., Shah, P. M., Patel, H. R., & Patel, P. S. (2008). Significance of alterations in plasma lipid profile levels in breast cancer. *Integrative cancer therapies*, (vol *7*, Issue1), pp 33-41.

Sibrian-Vazquez, M., Escobedo, J. O., Lim, S., Samoei, G. K., & Strongin, R. M. (2010). Homocystamides promote free-radical and oxidative damage to proteins. *Proceedings of the National Academy of sciences*, 107(2) :551-554.

Singh, K., Bhori, M., Kasu, Y. A., Bhat, G., & Marar, T. (2018). Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity–Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal*, *26*, (2),:177-190.

Sun, C. F., Haven, T. R., Wu, T. L., Tsao, K. C., & Wu, J. T. (2002). Serum total homocysteine increases with the rapid proliferation rate of tumor cells and decline upon cell death: a potential new tumor marker. *Clinica chimica acta*, *321*(1-2):55-62.

Smith, S. G., Sestak, I., Morris, M., Harvie, M., Howell, A., Forbes, J., & Cuzick, J. (2021). The impact of body mass index on breast cancer incidence among women at increased risk: an observational study from the International Breast Intervention Studies. *Breast cancer research and treatment*, *188*(1), :215-223.

Sorkun, H. Ç., Akbulut, M., Enli, Y., Tepeli, E., Özkan, S., & Erdem, E. (2016). Quantitative comparison of immunohistochemical and PCR analysis of midkine expression in breast cancer types and serum midkine level. *Turkish Journal of Medical Sciences*, 46(1):219-227.

References

Suami, H., & Scaglioni, M. F. (2018, February). Anatomy of the lymphatic system and the lymphosome concept with reference to lymphedema. In *Seminars in plastic surgery* 32(01): 005-011). Thieme Medical Publishers.

Stimpfel, M., & Virant-Klun, I. (2016). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. In *Journal of Cancer Stem Cell Research* 4 (3):1. https://doi.org/10.14343/jcscr.2016.4e1003

Stowe, D. F., Neufer, P. D., Sanz, A., Scialò, F., & Fernández-Ayala, D. J. (2017). Citation: Scialò F, Fernández-Ayala DJ and Sanz A (2017) Role of Mitochondrial Reverse Electron Transport in ROS Signaling: Potential Roles in Role of Mitochondrial Reverse Electron Transport in ROS Signaling: Potential Roles in Health and Disease. In *Front. Physiol* 8 (8).

Suami, H. (2017). Lymphosome concept: Anatomical study of the lymphatic system. *Journal of Surgical Oncology*, 115,(1), :13-17.

Suami, H., & Kato, S. (2018). Anatomy of the Lymphatic System and Its Structural Disorders in Lymphoedema. In *Lymphedema* :57–78). https://doi.org/10.1007/978-3-319-52423-8_5

Taniguchi, K., & Karin, M. (2018). NF-κB, inflammation, immunity and cancer: coming of age. *Nature Reviews Immunology*, 18, (5):309-324.

Tejasw, G. (2021). Insulin resistance and decreased spexin in Indian Patients with Type 2 Diabetes Mellitus. In *Bioinformation* 17,(9):790–797. https://doi.org/10.6026/97320630017790

Thouvenot, A., Bizet, Y., Baccar, L. S., & Lamuraglia, M. (2018). Primary breast cancer relapse as metastasis to the cervix uteri: A case report. *Molecular and Clinical Oncology*, 9(1), :96-97.

Toriyama, K., Muramatsu, H., Hoshino, T., Torii, S., & Muramatsu, T. (1997). Evaluation of heparin-binding growth factors in rescuing morphogenesis of heparitinase-treated mouse embryonic lung explants. *Differentiation*, 61 , (3), :161-167.

Tsuda, H. (2020). Histological classification of breast tumors in the General Rules for Clinical and Pathological Recording of Breast Cancer. *Breast Cancer*, *27*, (3),:309-321**.**

Tavassoli FA, Devilee P: World Health Organization classification of tumours. Pathology and Genetics Tumours of the Breast and Female Genital Organs.

2003, Lyon: IARC Press, 19-23.

Vranic, S., Schmitt, F., Sapino2, A., Costa, J. L., Reddy, S., Castro, M., & Gatalica5, Z. (2013). *Apocrine carcinoma of the breast: A comprehensive review* 3 https://doi.org/10201/61539

Vuong, D., Simpson, P. T., Green, B., Cummings, M. C., & Lakhani, S. R. (2014). Molecular classification of breast cancer. *Virchows Archiv*, 465(1) :1-14.

Vitols S, Björkholm M, Gahrton G, et al. Hypocholesterolaemia in malgnancy due to elevated low-density-lipoprotein-receptor activity in tumor cells: evidence from studies in patients with leukemia. Lancet. 1985;2(8465):1150–4

Veneracion G, Cabana JNS, Seymour M. Sabesin. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoprotein. J Lipid Res. 1989;30(1):39.

Wang, B., Cui, A., Tian, J., Zhang, Y., Jiang, Y., Xu, Y., & Liu, X. (2020). Characterization of a novel spexin gene (spx2) in the half-smooth tongue sole and regulation of its expression by nutritional status. *Aquaculture Reports*, *18* 100544.

Wei, T., Li, X., Yashir, N., Li, H., Sun, Y., Hua, L., ... & Guo, J. (2021). Effect of exogenous silicon and methyl jasmonate on the alleviation of cadmium-induced phytotoxicity in tomato plants. *Environmental Science and Pollution Research*, 28(37),:51854-51864.

Warnecke, P. M., & Bestor, T. H. (2000). Cytosine methylation and human cancer. *Current opinion in oncology*, *12*(1):68-73.

Xu, F., Xia, Y., Feng, Z., Lin, W., Xue, Q., Jiang, J., Yu, X., Peng, C., Luo, M., Yang, Y., Wei, Y., & Yu, L. (2019). Repositioning antipsychotic fluphenazine hydrochloride for treating triple negative breast cancer with brain metastases and lung metastases. In *American journal of cancer research* 9,(3):459–478).

Yamashita, E. K., Teixeira, B. M., Yoshihara, R. N., Kuniyoshi, R. K., Alves, B. C., Gehrke, F. S., ... & Fonseca, F. L. (2014). Systemic chemotherapy interferes in homocysteine metabolism in breast cancer patients. *Journal of clinical laboratory analysis*, *28*(2):157-162.

Yang, J., Li, H., Deng, H., & Wang, Z. (2018). Association of one-carbon metabolism-related vitamins (folate, B6, B12), homocysteine and methionine with the risk of lung cancer: systematic review and meta-analysis. *Frontiers in oncology*, *8*:493.

References

Yaméogo, N., Ouattara, A. K., Bazié, B. V., Ouédraogo, A. R., Djigma, F. W., & Simporé, J. (2022). Can Plasmodium falciparum Induce Homocysteinemia in Malaria Patients?. *Journal of Biosciences and Medicines*, *10*(5):117-128.

Zadeh, H. G., Haddadnia, J., Ahmadinejad, N., & Baghdadi, M. R. (2016). Assessing the potential of thermal imaging in recognition of breast cancer. In *Asian Pacific Journal of Cancer Prevention* 16(18):8619–8623). https://doi.org/10.7314/APJCP.2015.16.18.8619

Zhang, H., Qiu, L., & Peng, Y. (2018). The sonographic findings of micropapillary pattern in pure mucinous carcinoma of the breast. In *World Journal of Surgical Oncology* 16(1). https://doi.org/10.1186/s12957-018-1449-8

.**Zhang, W. W.,** Wu, S. G., Ling, Y. H., Sun, J. Y., Long, Z. Q., Hua, X., ... & Lin, H. X. (2018). Clinicopathologic characteristics and clinical outcomes of pure type and mixed type of tubular carcinoma of the breast: a single-institution cohort study. *Cancer Management and Research*, 10.pp 4509.

Zheng, T., Buka, S., Kelsey, K., Papandonatos, G., Oh, J., Sjodin, A., ... & Shi, K. (2021). The Hypothesis of Fetal Origin of Breast Cancer: An Update. *Women's Health Research*, *3*(2), Women-s.

الخلاصة

سرطان الثدي هو الورم الخبيث الأكثر شيوعًا عند النساء. وهو مسؤول عن أكثر من ثلث التشخيصات الجديدة عند النساء. يتم إجراء اختبارات إضافية عندما يتم التعرف على حالة منه أثناء الفحص الطبي أو عندما يظهر الشخص أعراضًا. يتم تشخيص سرطان الثدي عن طريق إجراء فحوصات تشريحية للأنسجة مرضية التي تم جمعها من عمق الورم. يتم استخدام الخزعات الدقيقة أو الكلّية عبر الجلد بشكل شائع لإزالة الورم من مصدره. يمكن دائمًا اقتراح علاج سرطان الثدي من خلال مجموعة من العلاجات ، بما في ذلك الجراحة والإشعاع والعلاج الهرموني والعلاج الكيميائي والعلاجات الموجهة

الاسبروسين :وهو سايتوكين مسكر حديث تم اكتشافه في عام 2016 عند الصيام يؤدي Aspدورًا معقدا في الجهاز العصبي المركزي ، تطور الخلايا الشحمية البيضاء والأنسجة الطرفية والأعضاء. يعتبر مرض السكري والسمنة واعتلال عضلة القلب والسرطان من الأمراض التي يؤثر فيها Asp على التطور والتقدم.

اسبكسين:- هو هرمون ببتيد يحتوي على 14 حمضًا أمينيًا تم تحديده في عام 2007 و هو مادة دهنية جديدة مرتبطة بحركات الجهاز الهضمي، والأنسولين وتوازن الجلوكوز، واستقلاب الدهون، وتوازن الطاقة.

ميدكين:- هو عامل نمو مرتبط بالهيبارين تم تشخيصه كجين مهم وظيفيًا أثناء التطور الجنيني في الفئران و الانسان. هو عامل نمو اندماجي يمكن أن يؤثر على الخلايا العصبية ، قلة العدلات، البلعمة، خلايا العضلات الملساء ، التليف ، الخلايا الطبيعية والخبيثة ، والخلايا السرطانية.

هوموسيستين :- وهو حمض أميني غير بروتيني يحتوي على سلفوهايدريل ، هو وسيط بيولوجي يتم انتاجه عن طريق إزالة الميثيونين من الجسم (Met) وهو ضروري من الناحية الفيسولوجية مثل تقدم دورة الخلية والحفاظ على التوازن الخلوي.

مالونودالديهايد :- هو احد نواتج اكسدة الدهون .وهو مركب عضوي له الصيغة CH2 (CHO) 2 ، وهو مادة كيميائية لتفاعل fenton. يستخدم MDA على نطاق واسع في تشخيص الإجهاد التأكسدي أثناء الأمراض الصحية الشديدة بما في ذلك السرطان لأنه مادة شديدة السمية للخلايا.

الغرض من هذه الدراسة هو التنبؤ بتطور السرطان من خلال التحقق من وجود مادة دهنية جديدة في مصل الدم للمريض مع الكشف المبكر عن السرطان لدى المرضى من أجل التنبؤ بتطور الورم. إجمالي 113 مشارك مقسمون إلى 68 مريضة مصابة بسرطان الثدي و 45 أمراة من الاصحاء. تضمنت دراسة الحالات من مرضى سرطان الثدي الذين تم تقسيمهم إلى أربع مجموعات: قبل العلاج ، وبعد الجرعة الأولى ، وبعد الجرعة الثانية ، وبعد الجرعة الثالثة. وأخذت العينات من مركز بابل لمعالجة الاورام في

مستشفى مرجان التعليمي بمدينة الحلة في الفترة ما بين أغسطس2021 ومارس 2022. وجميع المشاركين من الإناث (35≤ سنة). بعد ما يقرب من 12 ساعة من الصيام طوال الليل ، تم أخذ عينات الدم. لتقييم مستويات الدهون في المصل بطريقة القياس اللوني. تم تقييم تراكيز السعة الكلية لمضادات الأكسدة ((TAC ، malinodiahyde (MDA)،Asprosin ، ،Sepxin، midkine في الدم باستخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط (ELISA).

في هذه الدراسة ، تم التوصل الى أن زيادة بيروكسيد الدهون بشكل كبير ، المقدّرة بـ MDA ، تم إثباتها في مصل الدم لدى مرضى سرطان الثدي الذين لم يخضعوا لأي دواء كانت مستويات MDA (24.9193 ± 15.07722) نانومول / مل في مرضى سرطان الثدي و (14.4917± 7.91600) نانومول / مل من مستويات ال MDA في الاصحاء .

عند المقارنة بمستويات (MDA)في المرضى الذين لم يتلقوا أي علاج ، أظهرت الدراسة أن علاجات السرطان أدت إلى تحسن ملحوظ في مستويات (MDA) في بعض الأحيان أثناء العلاج ، علاوة على ذلك ، تم ملاحظة أن مرضى سرطان الثدي لديهم مستويات أقل بشكل ملحوظ من متوسط مضادات الاكسدة (TAC)مقارنة بمجموعة الاصحاء. كان هناك ارتباط إيجابي قوي بين نشاط العلاج الكيميائي ومستويات (MDA)لدى المرضى، بالإضافة إلى ارتباط مماثل بين مستويات (HCY)، ومستويات (T'AC)، في حين كانت هناك علاقة محددة بين (TAC) ومستويات (MDA) في مجموعة المرضى.

تم ملاحظة انخفاض مستويات Spx بشكل كبير في مرضى سرطان الثدي مقارنة بمجموعة الاصحاء، ولكن تميل مستويات Spx إلى الارتفاع في مرضى سرطان الثدي الذين يتلقون العلاج الكيميائي, في المقابل توصلت الدراسة الحالية أنه بينما وجد أنMK و Asp أكبر لدى مرضى سرطان الثدي مقارنة بالحالات الطبيعية العادية ، فقد ظهر أن Asp أقل في مرضى سرطان الثدي الذين تلقوا العلاج الكيميائي.

نظرًا لأن Spx انخفض في المرضى بينما زاد كل من منتصف العلاج وAsp في نفس المريض بسبب تغيير التمثيل الغذائي الجيد جدًا في الخلايا السرطانية، فقد تم افتراض أن السايتوكينات كانت علامات كيميائية حيوية تشير إلى تقدم سرطان الثدي في المرضى بالإضافة إلى ذلك، قد يساعد قياس السايتوكينات في مصل الدم في تقييم آثار العلاج الكيميائي لمرضى سرطان الثدي. تلعب مستويات MDA و Hcy و TAC، من بين أمور أخرى، دورًا حيويًا في انتشار السرطان قبل العلاج وبعده.

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دراسة مضادات الأكسدة، الهوموسيستين، السايتوكينات بدرجات مختلفة في مرضى سرطان الثدي

رسالة مقدمة الى مجلس كلية العلوم للبنات- جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في العلوم / الكيمياء

تقدمت بها الطالبة

ترتيل امجد حمزه

بكلوريوس علوم كيمياء /جامعة بابل / كلية العلوم

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