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FINDING THE CONCENTRATION OF THE LEVEL OF ABELIN AND SOME BIOCHEMICAL VARIABLES IN THE BLOOD SERUM OF WOMEN WITH BREAST CANCER IN BAGHDAD

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MARWAN AZEEZ HASAN

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By Marwan Azeez HASAN

August 2021

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

FINDING THE CONCENTRATION OF THE LEVEL OF ABELIN AND SOME BIOCHEMICAL VARIABLES IN THE BLOOD SERUM OF WOMEN WITH BREAST CANCER IN BAGHDAD

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Master of Science in Chemistry

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Abelin is instrumental in increasing the severity of the disease, as its levels increased with the further development of breast cancer. The aim of this study is evaluation measuring the concentration of the abelin and some biochemical variables in the blood serum of women with breast cancer in Baghdad. The disease was divided into four groups according to the severity of cancer, each group had clinical characteristics, analyzed the clinical and laboratory features of 120 cases in Baghdad. Our results showed that there is a clear increase in (glucose, insulin, cholesterol, triglyceride, LDL, VLDL, CA 15-3, and abelin), which is significant and statistically different except for zinc and HDL in the patient group when statistically compared with the control group. Abelin has a strong positive relationship with (glucose, insulin, cholesterol, triglyceride, LDL, VLDL and CA 15-3) and a negative relationship with (zinc). Our result indicated higher levels of CA15-3, abelin, and insulin with low zinc levels are associated with more severe disease development. The evolution of apelin will help study its regulative functions and physiological significance. We believe that this biological knowledge will start novel drug discoveries tomorrow.

2021, 49 page

Keywords: Breast cancer, Abelin, Biochemical markers

ÖZET

BAĞDAT'TA MEME KANSERLİ KADINLARIN KAN SERUMUNDA ABELIN DÜZEYİ VE BAZI BIYOKİMYASAL DEĞİŞKENLERİN KONSANTRASYONUNUN BULUNMASI

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Eylül 2021

Abelin, meme kanserinin daha da gelişmesiyle seviyeleri arttığından, hastalığın şiddetini artırmada etkilidir. Bu çalışmanın amacı, Bağdat'ta meme kanserli kadınların kan serumunda abelin ve bazı biyokimyasal değişkenlerin konsantrasyonunun ölçülerek değerlendirmektir. Hastalık, kanserin ciddiyetine göre dört gruba ayrıldı, her grup klinik özelliklere sahipti. Bağdat'ta 120 vakanın klinik ve laboratuvar özellikleri analiz edildi. Sonuçlarımız istatistiksel olarak karşılaştırıldığında kontrol grubu ile hasta grubunda çinko ve HDL dışında istatistiksel olarak anlamlı ve istatistiksel olarak farklı olan (glikoz, insülin, kolesterol, trigliserit, LDL, VLDL, CA 15-3 ve abelin) belirgin bir artış olduğunu göstermiştir. Abelinin (glikoz, insülin, kolesterol, trigliserit, LDL, VLDL ve CA 15-3) ile güçlü bir pozitif ilişkisi ve (çinko) ile negatif bir ilişkisi vardır. Sonuçlarımız, düşük çinko seviyelerine sahip daha yüksek CA15-3, Abelin ve insülin seviyelerinin daha şiddetli hastalık gelişimi ile ilişkili olduğunu gösterdi. Abelinin evrimi, düzenleyici işlevlerini ve fizyolojik önemini incelemeye yardımcı olacaktır. Bu biyolojik bilginin yarın yeni ilaç keşiflerine başlayacağına inanıyoruz.

2021, 49 Sayfa

Anahtar Kelimeler: Meme kanseri, Abelin, Biyokimyasal belirteçler

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Marwan Azeez HASAN
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LIST OF SYMBOLS

%	Percent
±	Plus minus
°C	Degrees Celsium
µg	Migrogram
µL	Microliter
g	gram
h	hour
kg	Kilogram
mg	Miligram
mL	Mililitter
rpm	Part per milliıon
Zn	Zinc

LIST OF ABBREVIATIONS

AIB1	Amplified in breast cancer 1
BRCA2	Breast cancer type 2
CAMP	Cyclic adenosine monophosphate
cm	centimeter
CSCs	Certified strength and conditioning specialists
DNA	Deoxyribonucleic acid
E2	Estrogen
EGFR	Epidermal growth factor receptor
GF	Growth factor
GLU	Glucose test
HDL	High density lipoprotein
IL-6	Interleukin-6
LDL	Low density lipoproteins
LPL	Lipoprotein lipase
MCF-7	Michigan cancer foundation-7
NSCLC	Non small cell lung cancer
PAI-1	Plasminogen activator inhibitor-1
TNT	Troponin tests
USA	United states of america
VLDL	Very low density lipoprotein
WHO	World health organization

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1. INTRODUCTION

Breast cancer is the most common type of cancer in women worldwide. This type of cancer is one of the cancer types with the highest incidence of cervical cancer (cervix) worldwide, after lung cancer. According to statistics, one out of every eight women is diagnosed with breast cancer at a certain time in their life. Cancer cells usually form in the lobules of the breasts, where milk is produced, or in the milk drainage ducts of the breast. Breast cancer most commonly occurs in postmenopausal women but can occur at any age. Although breast cancer can also occur in men, it is extremely rare. It is seen in one man for every 100 women. When symptoms of breast cancer are suspected, action should be taken quickly. When cancer is diagnosed at an early stage (first stage), the success rate of breast cancer treatment is over 90%. You may find suspicious firmness when checking your breast, your physician may feel a firmness on a clinical breast exam, or an abnormal area of breast tissue may show up on a screening test such as a mammogram or breast MRI (Gueye *et al.* 2016). Adipose tissue in the breast secretes many growth factors that cancer cells use for their survival. Obesity is a well-established risk factor for the development of breast cancer, exerting its effect via several different biological (Grupińska *et al.* 2021).

As an endocrine organ, adipose tissue secretes molecules called adipocytokines that act in an endocrine, paracrine, and autocrine manner and may promote breast cancer's malignant progression (Chu *et al.* 2019). Apelin and retinol-binding protein 4 (RBP4) are adipocytokines that may serve a role in carcinogenesis (Luo *et al.* 2013). Evaluation of their role may be useful in predicting survival times and cancer recurrence (Uribesalgo *et al*. 2019). Apelin was remote as an endogenous ligand from bovine belly epithelial cells in 1998 (Tatemoto *et al.* 1998). Further research has validated that Apelin is likewise expressed in coronary heart muscles, brain, kidneys, liver, lungs, and spleen, in addition to in mammary glands, placenta, and gastric mucosa (Kleinz 2005, Davenport 2005). The small peptide is worried in numerous essential physiological processes, which include angiogenesis, fluid homeostasis, and glucose metabolism (Mughal 2018, O'Rourke 2018).

Growing proof has counseled that APLN induces the maturation of tumor blood capillaries and activates tumor vascularization (Wang *et al.* 2019). Moreover, abelin additionally indicates lymphangiogenic capability in terms of tumor increase and lymph node metastasis (Berta *et al.* 2014). Upregulated expression of apelin has been determined in numerous forms of most cancers, consisting of breast most cancers, wherein its degrees had been proven to be correlated with shorter survival instances and a better occurrence of most cancers recurrence (He *et al.* 2015).

Apelin goes to the adipokines family, which are built from adipose tissue which is not only a place to stock fat, but consider as endocrine gland secrete several active substances such as TNF, IL-6, Leptin, and others, which has many features such as they act as inflammatory agents, regulate blood pressure, glucose Homeostasis, fat metabolism, formation new blood vessels from being vessels (Trayhurn 2005).

2. LITERATURE REVIEW

The mammary gland (Latin: glandular mammaria) is an organ in mammals that feeds the young with the milk it produces. Mammals get their name from this organ. In humans, the mammary gland is located in the breasts located in the chest. A woman's breast consists mainly of adipose tissue and connective tissue that includes the mammary glands, each connected to a milk duct that connects the mammary gland to the nipple, a circle located in the middle of the breast that is darker in color than the rest of the breast and contains multiple openings from the milky ducts (Edmondson 2010). The breast contains several lobes, and each lobe contains smaller lobules at the end of which dozens of follicles are capable of producing milk.

The breast tissue consists of milk glands (lobules), milk ducts (ducts) that carry the produced milk to the nipple, and the supporting tissues, fat, nerves, blood, and lymph vessels between them. Lobules come together and gather in structures called lobes. Each breast has 15 - 20 lobes. In shape, each lobe resembles a bunch of grapes. Lobes, lobules, and milk sacs are connected by thin milk ducts (Sharma 2011).

Breasts are one of the organs that undergo the most changes in the female body throughout life. About 1 - 2 years before the first menstruation, the breasts begin to grow with the effect of the female hormones estrogen and progesterone. The size of the breast is not only related to genetic predisposition but also affected by the body fat ratio, more detials are illustrated in Figure 2.1. Until the menopause period, the breasts undergo monthly changes, which are in parallel with the menstrual periods and are affected by the levels of female hormones in the blood. Every month, as if preparing for pregnancy, the mammary glands become active and the breast tissue swells, and the mammary glands become inactive once the hormone values return to normal. For this reason, swelling and tenderness may occur in the breasts before and during the menstrual period (Sharma *et al.* 2011).

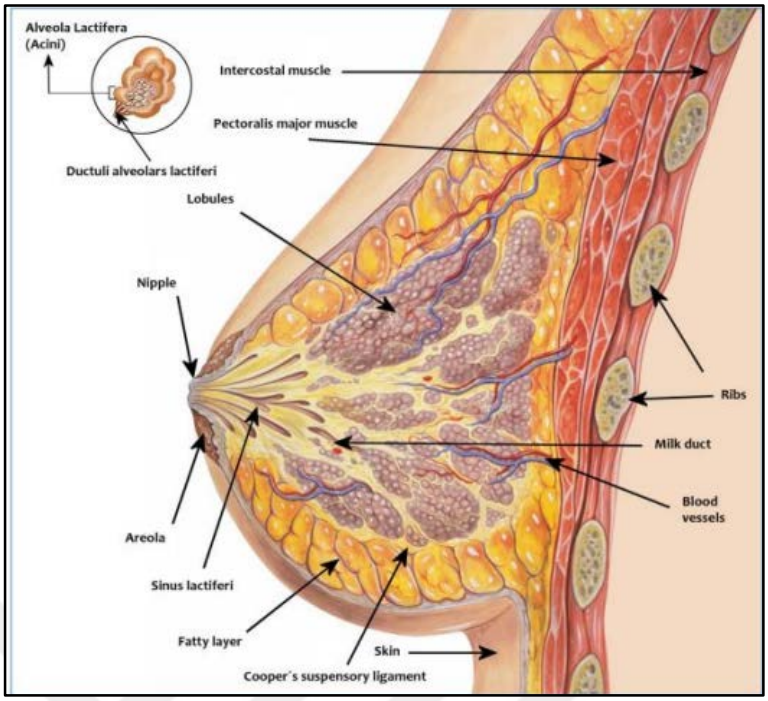

Figure 2.1 Anatomy of a healthy breast

2.1 Breast Cancer Disease

It can be list the symptoms of breast cancer as follows (Vicini *et al.* 1997).

- Usually painless, hard structured, movement on the breast.
- In the breast; they are usually painless, hard, movable, or non-displaceable, palpable swellings that can grow over time.
- A visible change in breast size or shape.
- Point recessions such as redness, bruises, wounds, vasodilation, inward depression, widespread small swellings, orange peel appearance on the breast skin.
- Change in color and shape of the nipple and its surroundings, enlargement of the nipple, flattening, inward collapse, change of direction, crusting, cracks, and wounds.
- Bloody or bloodless discharge from the nipple.
- Painful or painless swellings can be seen in the armpit and can be noticed by the hand.

2.1.1 Breast cancer stages

Breast cancer is staged from stage 0 (zero) to stage 4 (four), it is illustrated in Figure 2.2. Stage 0 (zero) does not define breast cancer. Abnormal cells in the lobule in the breast are defined as lobular carcinoma in situ and may cause the risk of developing cancer in both breasts. Another lesion is ductal carcinoma in situ. Ductal carcinoma in situ is also abnormal cells inside the ducts. These cells are not very common outside the canal. If both lesions are not treated, they can turn into cancer later in life (Mariotto *et al.* 2017).

- Stage 1; The tumor is less than 2 cm in size and the tumor has not spread elsewhere.
- Stage 2; Tumor size 2 and 5 cm. that between. However, in some subgroups, tumor cells can spread to the armpit glands, although the amount is not high.
- Stage 3; If the tumor is larger than 5cm, or a tumor smaller than 5 cm, with involvement of many packages in the axillary glands, involvement of the chest muscle wall, involvement of the nipple or spread to the lymph nodes in the neck, stage- It is defined as 3.
- Stage 4 is when breast cancer has spread to other organs.

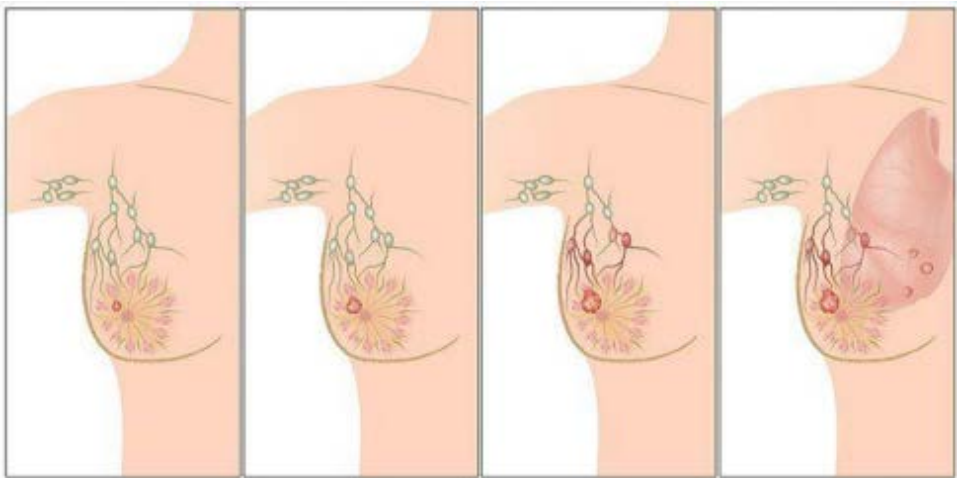

Figure 2.2 The stages of breast cancer

2.1.2 Types of breast cancer

Breast cancer types are determined as a result of the pathology examination performed on the tissue taken by biopsy. Although there are many types of breast cancer, it is generally evaluated under two headings:

- Ductal carcinoma that forms in the cells that line the breast ducts.
- Lobular carcinoma of the mammary glands.

Ductal and lobular carcinomas are divided into non-invasive/in situ tumors that do not spread among themselves, and invasive tumors that can spread.

Ductal carcinoma in situ: It is a type of cancer that cannot be detected by manual examination and is manifested by its irregular structure and calcifications on mammography. The patient with this disease may also have nipple discharge.

Lobular carcinoma in situ: It is an important symptom that increases the risk of developing breast cancer in both breasts 8 - 10 times. Patients in this situation are kept under regular control and close follow-up, and at the same time, protective drugs are given to the patient.

In some patients, procedures such as removing both breasts and emptying the breast tissue for protective purposes can be performed. Surgical procedures such as prostheses and breast reconstruction, which are applied to prevent the patient from experiencing any cosmetic problems, also contribute positively to the social lives of the patients. Breast cancer, which could only be classified into 2 groups until 10years ago, is now gathered in 4 different subgroups. In addition, individual and individual tumor-specific treatment methods have been developed with different treatment strategies. Breast cancer, which could only be classified into 2 groups until 10 years ago, is now gathered in 4 different subgroups. In addition, individual and individual tumor-specific treatment methods have been developed with different treatment strategies (Mariotto *et al.* 2017).

2.2 Breast Carcinomas

2.2.1 Etiology and pathogenesis

Breast cancer shows biological and clinical heterogeneous features. The uncontrolled cell proliferation that occurs in breast cancer often exhibits changes such as signs of genomic instability and the disappearance of certain epithelial features. Therefore, it is of great importance to determine the molecular mechanisms that cause cancer development and the characteristics of each patient's tumor and to apply the most appropriate treatment method. Studies on experimental animals and in vitro mammary tumors revealed that three major effects may play an important role in the development of breast cancer (Welsc 1985).

2.2.1.1 Genetic changes

BRCA1 and BRCA2 genes located on chromosomes 17 and 13, p53 tumor suppressor gene defect in Li-Fraumeni syndrome, loss of a locus at 10q in Cowden syndrome, and ataxia-telangiectasia (AT) gene defect are responsible for the majority of autosomal inherited familial cases. These genes are involved in tumor suppression or DNA repair. As in other cancers, mutations in breast cancer cause an increase in oncogene expression and loss in the functions of tumor suppressor genes. One of the most characteristics of these genes is the epidermal growth factor receptor (Yao *et al.* 2015).

2.2.1.2 Hormonal effects

Known hormonal risk factors for breast cancer development are thought to be related to the cumulative exposure of the breast to estrogen and possibly progestins. Normal breast epithelium has estrogen and progesterone receptors. Estrogen and Progesterone receptors are located in the luminal region of ductal and lobular cells and have been applied for more than 25 years to evaluate the ability to respond to hormonal therapy. Although 60 - 70% of breast tumors are ER (+), only half to two-thirds of them respond

to hormonal therapy. On the other hand, some of the ER(-) patients benefit from hormone therapy. The reason for this is not known, and it is not yet known whether the receptors in normal and malignant cells have the same structural and functional characteristics. PR expression, which plays an important role in response to endocrine therapy, is regulated by the interaction of the estrogen hormone and its receptor. It is unclear whether the PR in normal human breast epithelium is ER-dependent and whether these two receptors coexist in luminal cells. 70% of PR(+) cancers respond to hormonal therapy while 25 - 30% of PR(-) cancers benefit from hormonal treatment.

Growth promoters (transforming GF, epidermal GF, platelet-derived GF, fibroblast GF) and growth factor inhibitors are secreted by breast cancer cells and play a role in the autocrine mechanism of tumor progression. The formation of these growth factors is estrogen-dependent, suggesting that the interactions between circulating hormones, hormone receptors secreted by cancer cells, and autocrine growth factors formed by tumor cells are involved in breast cancer progression (Welsch 1985, Yao *et al.* 2015).

2.2.1.3 Environmental factors

The fact that the incidence of breast cancer is 4 - 7 times higher in the USA and western countries, and the increase in the rate of cancer in those who immigrated to these countries after a few generations, supports the idea that life habits and nutritional characteristics (rich in fat, alcohol intake, etc.) play a role in the etiology. It is mentioned that the risk of breast cancer increases up to 20 - 30% in 10 - 15 years due to DNA damage in those exposed to radiation. Although research on viruses has been carried on since 1936, it has not become definitive (Nikolic-Vukosavljevic *et al.* 2002).

2.3 Epidermal Growth Factor Receptor

The epidermal growth factor receptor (EGFR) is overexpressed in many epithelial tumors. Agents targeting EGFR are successful drugs that have taken their place in the treatment of various cancers, especially colorectal cancer, head and neck cancer, lung

cancer, and breast cancer. Although EGFR inhibitors are generally well-tolerated, they may cause serious deterioration in the quality of life due to cutaneous side effects, changes in optimal doses of antitumoral drugs, and thus a decrease in antitumor efficacy. For this reason, dermatologists should be aware of these side effects and know the appropriate dermatological treatment approaches to ensure adequate drug dosage for antitumoral efficacy and to prevent deterioration of the patient's quality of life. In this article, the cutaneous side effects caused by EGFR inhibitors used in the treatment of malignancies and the treatment approaches to the lesions will be discussed (Kim *et al.* 2017).

2.4 Adipose Tissue

As in all other vertebrates, humans have adipose tissue (adipose tissue). Adipose tissue is a special connective tissue in which fat cells-adipocytes are denser in number. In adipose tissue, adipocytes store fat in the form of triglycerides and are found in adipose tissue alone or small or large clusters in the connective tissue. Between the fat cells, there is a partly tight partly loose connective tissue that varies according to the anatomical regions of the body. This connective tissue consists of fibroblast cells, fibrous ligaments, blood, and lymphatic vessels, and nerves.

We know that there are fibroblasts in the adipose tissue in the embryonic period and they turn into lip blasts and adipocytes, which are fat cells. Lipocytes that are mature and have begun to store fat are round, annular, and appear to have pushed the fat cell nuclei to the cell margin. Fibroblasts transform into preadipocytes. Even in cases where calories are reduced in the body, adipocytes in the adipose tissue can turn back to fibroblasts.

Each adipocyte has a capillary circulation system around it. These capillaries show high sensitivity to adrenaline and vasoconstriction-constriction occurs with adrenaline. This explains the purpose of using adrenaline in solutions used for regional anesthesia during liposuction. Visceral fat constitutes up to 10% of total body fat, and this rate can

increase up to 20% with aging. Adipose tissue and adipose cells are closely associated with blood vessels and have a well-developed capillary network. Adipose tissue capillaries are more permeable and rich in lipoprotein lipase (LPL) than skeletal muscle capillaries. Adipose tissue cells are continuous with each other, with capillary endothelium and vascular smooth muscle cells. is in communication. The number of fat cells continues to increase until puberty. From adolescence, mitosis does not occur in the fat cell, the cells do not increase in number, only the cell size changes. Therefore, prepubertal obesity is hyperplastic (in the form of an increase in cell number and size), post-puberty hypertrophic (in the form of growth only in cell diameter and volume). Triglyceride synthesis (lipogenesis) and storage from glucose and fatty acids in adipose and liver cells is stimulated by insulin. Insulin increases fat cell membrane LPL activity and fatty acid entry into the cell. The destruction of triglycerides (lipolysis) in the fat cell occurs when adrenaline and noradrenaline activate the hormone-sensitive lipase enzyme, and fatty acids are released into the circulation. Adipose tissue is a tissue that shows constant volume variability in terms of cell number and size throughout life, depending on energy need and consumption. Fat cells are controlled by very complex systems for these functions in the process of energy storage and secretion. The fat cell is not passive, on the contrary, it is an extracellular fluid that shows continuous volume variability depending on daily energy intake. It is a cell that secretes cytokines and hormones. It communicates with other cells in endocrine, paracrine, and autocrine ways with these secretory products. It responds to hormones and cytokines by secreting fatty acids through membrane receptors or by taking fatty acids into the cell and secreting cytokines. The fat cell has adapted to energy storage and secretion, fat lipid droplets are stored as triglycerides, and these droplets make up about 90% of the cell, and other cell organelles make up the rest. Adipose tissue can be divided into brown fat and white fat. Brown fat cells differ from white fat cells in that they contain a large number of mitochondria, very few in adults, and take part in thermoregulation.

2.5 Adipokines

We can group adipokines in different ways. In the classification we will make; adipokines associated with insulin sensitivity: Leptin, adiponectin. Adipokines

associated with insulin resistance: Resistin, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), visfatin, apelin. Adipocyte proteins and lipid metabolism: Adipsin, acylation stimulating protein. Adipokines and homeostasis: Plasminogen activator inhibitor-1 (PAI-1), adipocyte renin-angiotensin system. Other Adipocyte Proteins: Metallothionine, fasting-induced adipose factor. We can examine it in six different groups (Fiorio *et al.* 2008).

2.5.1 Leptin

The size and location of the fat cells in the body affect the secretion of leptin. However, with a decrease in body weight, its level in the blood decreases. The leptin production of subcutaneous adipose tissue is higher than that of visceral adipose tissue. Women have higher leptin production than men. Leptin is secreted rhythmically from adipose tissue cells. In addition, while overeating, insulin and glucose levels, glucocorticoids, and proinflammatory cytokines increase leptin production; fasting, cold, β-adrenergic agonists, and testosterone decrease leptin production. Leptin regulates many important biological functions such as energy balance, neuroendocrine function, hematopoiesis, angiogenesis, wound healing, immune and inflammatory response, sexual development, reproduction, regulation of gastrointestinal functions, sympathetic nervous system activity, and bone metabolism. Along with the mentioned effects, the main physiological role of leptin is to stimulate the central nervous system and brain in terms of available energy stores and to reduce food intake. Leptin deficiency or insensitivity leads to increased appetite, overeating, and weight gain, and ultimately obesity (Sultana *et al.* 2017).

2.5.2 Adiponectin

Adiponectin is one of the most studied adipocytokines in recent years. It is a hormone that regulates energy balance, glucose, and lipid metabolism and is secreted with the effect of insulin. Adiponectin level in the blood; is negatively correlated with body mass index, plasma triglyceride level, fasting insulin concentration, leptin level, and amount

of visceral adipose tissue, while it is positively correlated with plasma HDL and glucose levels. It has been shown that adiponectin performs its physiological functions related to an energy balance through muscle and liver tissue by increasing insulin sensitivity (Liu *et al*. 2017, Sultana *et al.* 2017).

2.5.3 Resistin

It is a cysteine-rich protein consisting of amino acids. Resistin is so named because of its insulin resistance. Resistin is a hormone associated with obesity and metabolic syndrome. It is the most important factor that causes an increase in serum resistin levels in obesity, especially in the body center. Its level is higher in women than in men. Studies have found a positive correlation between serum resistin levels and leptin levels. TNF-α has been shown to have a strong negative effect on the release of resistin. Resistin is suggested to be protective against atherosclerotic vascular damage (Liu *et al.* 2017).

2.5.4 Apeline

Apelin is an adipokine that demonstrations through the apelin receptor (APJ), an angiotensin II sort 1 receptor with whom it is normal co-communicated, and intercedes a few impacts, like decrease of pulse, apoptosis, and angiogenesis; advancement of cell expansion; and guideline of glucose homeostasis (Kleinz 2005, Uribesalgo *et al.* 2019). Apelin is essentially actuated in different tumors also, has a job in cell multiplication (particularly on CSCs), tumor advancement and metastasis by invigorating angiogenesis), and medication opposition. Apelin invigorates MCF-7 BC cell expansion through the portion subordinate enlistment of cyclin D1 and enhanced in bosom disease 1 (AIB1) and attack potential by upregulating MMP1 articulation, an instrument likewise portrayed in A549 lung adenocarcinoma cells. Apelin is upregulated in NSCLC analyzed to solid lung tissue and is related to tumor development and movement. In BC and cellular breakdowns in the lungs, the consolidated restraint of apelin and angiogenesis (i.e., sunitinib) effectively decreases tumor development and

angiogenesis rates. Different examinations have shown that apelin is a vital controller of separation, expansion, and endurance of mesenchymal immature microorganisms (MSCs), which thus control the number of inhabitants in CSCs all through the emitted cytokines and chemokines (Tatemoto *et al.* 1998).

Role of apelin in breast cancers: Breast cancer is common among women and is one of the leading causes of cancer-related deaths. It has been reported by the World Health Organization that breast cancer is the most common type of cancer seen in women. The prevalence of the disease is because it results in death and death, research on the treatment of this disease is still up-to-date and increasing.

It has been reported that some hormones, especially estrogen (E2), play a role in the emergence and development of breast cancers and that breast cancer will not occur without the effect of hormones. Therefore, breast cancer is seen 100 times more frequently in women than in men (Santen *et al.* 2007). It has been suggested that obesity is a risk factor for breast cancer and that dietary fat intake plays an important role in the development of cancer. In 1998, Tatemato et al. discovered Apelin, which is a new member of the adipose tissue family and exerts its effects by binding to APJ receptors. Apelin, first isolated from bovine gastric tissue, is a preproapelin with a sequence of 77 amino acids. It is derived from its precursor and there are different forms of apelin in different tissues (such as apelin-10, 13, 16, and 36). In vitro studies have shown that apelin-13 is the most biologically active form among all apelin derivatives.

Habata et al. determined that there was a significant increase in apelin expression in rat mammary tissue during pregnancy and lactation. Studies have shown that apelin is abundant not only in cattle and rat milk samples but also in human milk. It has been demonstrated that abelin mRNA is expressed at high levels in Hs 578T human breast cancer cell cultures. In addition, it has been shown that apelin mRNA expression continues in malignant ductal and lobular tumor cells (Habata *et al*. 1999).

2.6 Methods of Breast Cancer Treatment and the Importance of Adinopectin

2.6.1 Surgical methods

In breast cancer surgery, the primary aim is to remove the tumor so that it does not remain behind and to remove the lymph nodes completely in cases that spread to the armpit.

2.6.2 Mastectomy

Simple mastectomy: This procedure is also called total mastectomy. The entire breast, including the nipples, is removed, but not the axillary lymph nodes or the muscle tissues under the breast. It is a method that is not preferred much nowadays.

Skin-sparing mastectomy: In some female patients, the breast can be reconstructed during surgery. This procedure is called a skin-sparing mastectomy. Most of the skin above the breast (including around the nipple (areola) and nipple) is left untouched.

Radical mastectomy: In this large-scale operation, the entire breast, armpit glands, and pectoral (chest wall) muscles under the breast are removed. Radical mastectomy is a method that has been used quite frequently in the past.

2.6.3 Breast-conserving surgery

When breast cancer is detected early, more of the affected part of the breast is removed. However, the portion to be removed depends on the size and location of the tumor and other factors. In a lumpectomy, only the mass in the breast and surrounding tissues are removed. Radiotherapy is a treatment method applied after lumpectomy. If the patient will also be given adjuvant chemotherapy, radiotherapy is usually delayed until the chemotherapy treatment is complete. In quadrantectomy, one-quarter of the breast is

removed. Radiotherapy is usually given after surgery. Again in this method, if chemotherapy is to be given, radiotherapy is delayed.

2.6.4 Lymph node surgery

To determine the spread of breast cancer to the axillary lymph nodes, one or more lymph nodes are taken and examined under a microscope. This examination is important for staging cancer, determining the treatment modality and its results. If cancer cells are found in the lymph nodes, there is a high chance that cancer has spread through the bloodstream to other parts of the body.

2.6.5 Radiotherapy treatment

With radiotherapy in breast cancer, it is aimed to destroy cancer cells that may remain with the beam given to the armpit and breast area after surgery. In the treatment of breast cancer, radiotherapy is applied to protect the remaining tissue of the breast, especially in patients with a high risk of regeneration and in patients undergoing breast-conserving surgery.

2.6.6 Drug treatments

2.6.6.1 Chemotherapy

Chemotherapy in breast cancer is mostly applied after surgery. Although there is no cancerous cell left after the surgery, chemotherapy treatment can continue for a while as a preventive measure.

2.6.6.2 Hormonotherapy

Hormone therapy aims to reduce the hormone amount of the cancer patient in cases of breast cancer sensitive to female hormones. Some cancer cells that are sensitive to the hormone estrogen grow and multiply faster. This method of treatment prevents the development of cancer by eliminating the effect of estrogen.

2.6.6.3 Smart therapies

Breast cancer is a disease that requires individual and tumor-specific treatment with different treatment strategies. In the past, there were no options other than classical chemotherapy drugs and hormone treatments, but today, the use of newer and more effective chemotherapy drugs, targeted smart drugs that can be taken intravenously and orally, and new hormonal therapy drugs together bring successful results (Mukama *et al*. 2020). Adiponectin is a cytokine protein that contains 244 amino acids, weighs 30 kDa, and is secreted only from adipose tissue. It was first identified in the 1990s as a protein called 3T3-L1 secreted from the mouse adipocyte cell line. On the other hand, human adiponectin was identified 1 year later and named APM1 (AdiPose Most abundant gene transcript = gene transcript intensely secreted by adipose tissue). Although it is also called GBP28 (gelatin binding protein 28 genes), APM1, adipic, and ACRP30 (adipocyte complement related protein 30) in the literature, it is most commonly referred to as adiponectin. Adiponectin is encoded in the Gene transcript-1 (APM1) gene region. Important with collagen VIII, X, and Clq degree of structural similarity. Adiponectin, which is 2–25 µg/mL in plasma, binds to collagen I, III, V, and does not bind to II and IV in plasma after it is secreted. 18 amino acids followed by a short hypervariable region of 22 repeats at the N-terminus that is not homologous to any other known sequence long signal peptide. At the C end, there is a spherical region with 22 repeats. At the C-terminus, there is a spherical region similar to the complement cascade molecule C1q. There are a surprising similarity between type VII and X collagens, the C1q part of complement, precerebellin, and hibernation regulation proteins 20, 25, and 27. The C-terminal globular region also shares similarities with the TNF-alpha trimeric cytokine family (Yamauchi *et al.* 2002). Adiponectin, a new member of the soluble

collagen family, has been reported to be a negative regulator of macrophage function and myelomonocytic progenitor cell proliferation. Antiapoptotic bcl-2 gene expression was decreased in adiponectin-treated cells and adiponectin had a regulatory effect. It has been shown to provide apoptotic mechanisms. It is thought that adiponectin inhibits endothelial NF-κβ (Nuclear transcriptional factor-kappa beta) signal transduction by blocking adenylate cyclase or protein kinase A via a cAMP-dependent pathway and participates in the inflammatory response about atherogenesis. All these studies indicate that adiponectin is an important regulatory protein in hematopoiesis and inflammatory response (Ouchi *et al.* 2000, Yokota *et al*. 2002).

2.7 Breast Reconstruction

Breast reconstruction after removal of the breast due to a tumor or another disease is one of the most successful operations of plastic surgery. Thanks to new technologies in medicine, surgeons can now create a breast that closely resembles a natural breast. Today, these operations can be performed simultaneously with breast removal surgery (mastectomy). Thus, when the patient comes out of the surgery, he has a new breast and gets rid of the psychological distress that a period without breasts can cause. However, it should be kept in mind that; Breast reconstruction after a mastectomy is not a simple operation. There are many options available to be decided by you and your doctor. Below you are given basic information about the surgery; such as when the surgery was performed, how it was performed, and what results would be obtained. However, it is not possible to answer all questions. Therefore, speaking one-on-one with your surgeon will provide you with better information. Breast reconstruction can be performed in almost any woman who has lost her breast due to cancer. However, as with any surgery, some problems may arise after this surgery. Bleeding, fluid collection, or anesthesia problems, which are the general problems of surgery, can also be seen after this surgery, but are rare. In smokers, wound healing may be delayed, impaired, or more scars may appear. Sometimes these problems may require secondary surgery. If a prosthesis is to be used, there is rarely a risk of developing an infection within two weeks. In some such cases, it may be necessary to remove the prosthesis and put it back in months later. The most common problem, capsular contracture, occurs when the scar tissue around the

prosthesis compresses the prosthesis. Thus, the feeling of breast hardness occurs. There are some treatment methods for capsule contracture; sometimes it may require removal and relief of scar tissue or replacement of the prosthesis. Reconstruction does not affect cancer recurrence and does not interfere with radiotherapy/ chemotherapy. Your surgeon may recommend that you continue with periodic mammograms of your normal breast and your reconstructed breast.

3. MATERIALS AND METHODS

This chapter explains the type of study used and the number of participants, as well as statistical methods, materials, and equipment needed to analyze samples.

3.1 Materials

Materials and equipments that used in the present study are illustrated in Appendices.

3.2 Methods

3.2.1 Study design

The study was a case control study

3.2.2 Study site

The study was conducted at the Hospitals of the Iraqi Ministry of Health in Baghdad.

3.2.3 Target population

Breast cancer-infected adults aged 26 years, and above females were included in the study.

3.2.4 Study population

All persons who met the inclusion criteria (indicated below) were enrolled in the study. A study control group of females who were non-breast cancer was constituted. Study participants consisted of the following four groups:

- Stage 1; The tumor is less than 2 cm in size and the tumor has not spread elsewhere.
- Stage 2; Tumor size 2 and 5 cm. that between. However, in some subgroups, tumor cells can spread to the armpit glands, although the amount is not high.
- Stage 3; If the tumor is larger than 5 cm, or a tumor smaller than 5 cm, with involvement of many packages in the axillary glands, involvement of the chest muscle wall, involvement of the nipple or spread to the lymph nodes in the neck, It is defined as 3.
- Stage 4 is when breast cancer has spread to other organs.

3.2.5 Specimen preparation and storage

In the laboratory, each specimen serial number was recorded on a compilation summary sheet. The blood specimens in the sodium citrate tubes were centrifuged at 3000 rounds per minute (3000 rpm) to separate the plasma from the blood cellular component. Clear tubes were centrifuged at 1500 rpm for 15 minutes to isolate serum from the blood cellular parts. Serum and plasma were gathered using pasture pipettes and shifted to 2 mL cryovials with sealable screw caps, saved in a freezer at -80^{o}C until the specimens were needed for analysis.

3.2.6 Quality control

To warrant that the results are good and accurate, the quality and efficiency of the devices and equipment used during the analysis of the samples were checked, and by the quality control guidelines, the analyst before each test calibrated the analytical instruments.

3.2.7 Preparing laboratory tests

Assay procedure of cholesterol and triglyceride

1- Blank: Mix 1000 µL of R1 with 10 µL of water.

2- Sample: Mix 1000 µL of R1 with 10 µL of the sample.

3- Standard: Mix 1000 µL of R1 with 10 µL of standard.

4- Mix incubate for 5 min at $37C^0$ temperature.

5- Read absorption of sample and standard against blank reagent then add.

Assay procedure of glucose

1- Blank: Mix 1000 µL of R1 with 10 µL of water.

2- Sample: Mix 1000 µL of R1 with 10 µL of the sample.

3- Standard: Mix 1000 µL of R1 with10 µL of standard.

4- Mix incubate for 10 min at 37 ℃ temperature.

5- Read absorption of sample and standard against blank reagent then add.

Assay procedure of HDL, LDL, and VLDL

1- Take a tube and put in it 400 microliters of HDL from the Spanish company Liner.

2- Put with the tube above 200 microliters of the form (serum).

3- Mix well and leave at room temperature for 10 minutes.

4- Then it is placed in the centrifuge for 10 minutes at a high speed.

5- Here, attention will come to a defect, a clear one and us.

6- We take out 50 microliters of the liquid and put it in another clean tube that contains 1 mL of cholesterol (meaning cut cholesterol for the same company).

7- Mix well and leave at room temperature for 10 minutes.

8- Double on the device is 500 nm.

9- Calculating T/S x S concentration, we get mg/ dL.

10- To convert it to mmol/ L, we multiply by 0.0259.

Assay procedure of human apelin

1- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2- Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3- Add 50 µL standard to standard well. Note: Do not add antibodies to a standard well because the standard solution contains biotinylated antibodies.

4- Add 40 µL sample-to-sample wells and then add 10 µL anti-APLN/AP antibody to sample wells, and then add 50 µL streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5- Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 mL wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

6- Add 50 µL substrate solution A to each well and then add 50 µL substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7- Add 50 µL Stop Solution to each well, the blue color will change into yellow immediately.

8- Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

Assay procedure of zinc

1- Blank: Mix 800 µL of R1 with 550 µL of water.

2- Sample: Mix 800 µL of R1 with 50 µL of the sample.

3- Standard: Mix 800 µL of R1 with 50 µL of standard.

4- Mix incubate for 1 min at room temperature.

5- Read absorption of sample and standard against blank reagent then add.

Assay procedure of insulin

1- The aia-pack IRI is intended for in vitro diagnostic use only.

2- Test cups from different lots should not be mixed within a tray.

3- Do not use it beyond the expiration date.

4- The AIA-PACK IRI has been designed so that the high-dose hook effect is not a problem for the vast majority of samples.

5- The materials provided by Tosoh contain solution azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

6- Human sera is not used in the preparation of this product, however, since human specimens will be used for samples, standard laboratory safety procedures should be used in handling all specimens and controls.

4. RESULTS AND DISCUSSION

Cancer is a disease that affects a large number of women, where abnormal cells divide and become complex and uncontrollable cells (Khajoueiab *et al.* 2011). Cancer also causes many complications in those affected by it. The channels that carry milk to the infant are affected by the severity of cancer. Breast cancer is characterized by its prevalence in most countries of the world and is considered the most dangerous type of cancer in women because it is the main cause of death in more than 100 countries *(*Gelsomino *et al.* 2019). It is important to diagnose the disease early and provide support and advice to people who suffered from breast cancer to increase recovery and reduce the severity of infection with this disease. The vital indications of severe patients predicted the risk of infection. The description of predictive variables may support Physicians in the evidence-based medication of breast cancer. In this research, the disease was divided into four groups according to the severity of cancer, each group had clinical characteristics, analyzed the clinical and laboratory features of 120 cases in Baghdad. The statistics program (SPSS) version 22 was used.

4.1 The Anthropometric and Biochemical Characteristics of the Study Groups

120 samples were collected from the hospitals of the Iraqi Ministry of Health in Baghdad, these samples were divided into two groups, the first group is women suffering from breast cancer in all stages, numbering 80 samples, and the second group is healthy women, numbering 40 samples, groups are illustrated in Table 4.1 and Figure 4.1. The average age was 48.5 ± 10.3 with the control group but with breast cancer group was 48.2 ± 13.8, The p-value was equal to 0.9 which does not indicate a statistical difference, while the p-value of (glucose, insulin, zinc, cholesterol, triglyceride, LDL, VLDL, CA 15-3, and abelin) was equal to (<0.001) respectively, which is significant and statistically different except for HDLwas (p= 0.84). Obesity is nearly related to a metabolic shortage in adipocytes that points to several chronic diseases. Obesity too affects breast cancer in women by excretion of some adipokines, which are also named “released hormones”, particularly estrogen, adiponectin, leptin, and insulin, also big level fatty acid, cholesterol, glycerol, triglyceride, serum accumulation effect, breast

tumor secretion (Chu *et al.* 2019). The level of CA15-3 is significantly elevated in women with breast cancer due to the increased secretion of carbohydrate antigen (CA15-3) in the blood from the cancer cell, which is excellent as a tumor marker to control the tumor pathway. Our study is consistent with a Norwegian study conducted at Radium Hospital (Norum *et al.* 2001). Of 221 women with breast cancer who had elevated tumor marker CA125, level. Our study also agrees with Canadian research published in 2010 (Yerushalmi *et al.* 2012), That documented elevated levels of CA 15-3.

Table 4.1 The anthropometric and biochemical characteristics of the study groups

Variable	**Control (N= 40)**	**Breast cancer (N=80)**	**P Value**
Age	48.5 ± 10.3	48.2 ± 13.8	0.9
Glucose mg/ dL	98.4 ± 11.7	133.2 ± 34.1	< 0.001
Insulin mu/ L	7.3 ± 2.6	10.3 ±4.1	< 0.001
Zinc mcg/ mL	85.3 ± 9.8	55.7 ± 13.7	< 0.001
Cholesterol mg/ dL	182.9 ± 27.1	207.4 ± 35.8	< 0.001
Triglyceride mg/ dL	125.9 ± 21.7	155.1 ± 41.9	< 0.001
HDL mg/dL	42.6 ± 6.5	42.3 ± 6.4	0.84
LDL mg/ dL	113.3 ± 21.1	134.6 ± 24.3	< 0.001
VLDL mg/ dL	25.3 ± 4.3	31.0 ± 8.3	< 0.001
Ca 15-3 u/ mL	8.6 ± 3.9	108 ± 119.5	< 0.001
Apelin h. Ng/ L	424.5 ± 150.5	665.9 ± 161.3	< 0.001

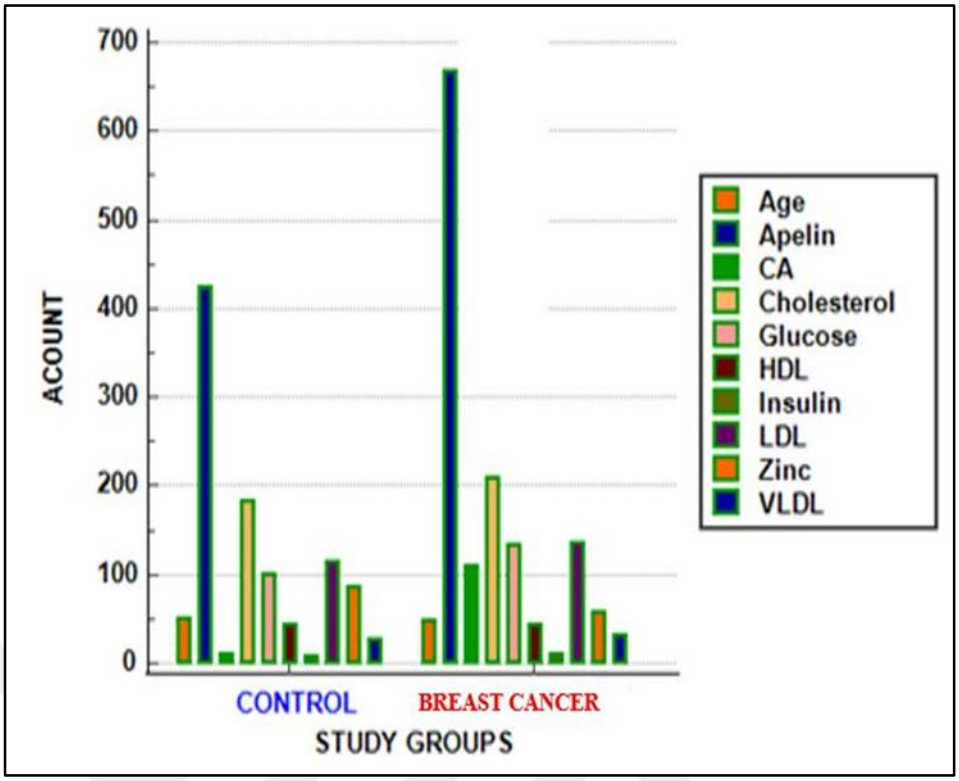

Figure 4.1 The anthropometric and biochemical characteristics of the breast cancer stages

By analyzing (Anova one way), the breast cancer group was divided into four groups according to the severity of the disease; the first stage was 33 patients, the second stage 19 diseases, the third stage 18 patients, and the fourth stage 10 patients. According to the results that appeared in Table 4.2 and Figure 4.2. All biochemical, markers were statistically significant (age p=0.002, glucose P = 0.001, insulin P=0.001, zinc P=0.01, cholesterol P=0.02, triglyceride P=0.001, HDL P=0.02, LDL P=0.003, VLDL P=0.001, CA 15-3 P=0.002, and Apelin P=0.004).

In our study, the effect of apelin was instrumental in increasing the severity of the disease, as its levels increased with the further development of breast cancer, and the highest level of apelin was in the fourth stage. Our results were in agreement with a study presented by the researcher Luo in 2013, which explains that Apelin and retinol-binding protein 4 (RBP4) are adipocytokines that may serve a function in carcinogenesis (Luo *et al.* 2013). Our result indicated higher levels of CA15-3, Apelin, and Insulin with low zinc levels are associated with more severe disease development.

The evolution of apelin will help study its regulative functions and physiological significance. We believe that this biological knowledge will start novel drug discoveries tomorrow.

Table 4.2 The anthropometric and biochemical characteristics of the BC stages

Variables	**N = 10 Stage (4)**	**N= 18 Stage (3)**	**N = 19 Stage (2)**	**N = 33 Stage (1)**	**P. Value**
Age	64.2 ± 7.5	59.1 ± 10.7	52.0 ± 10.8	43.8+8.9	0.002
Glucose mg/ dL	167.0 ± 41.7	157.6 ± 33.1	135.2 ± 14.8	127.2 ± 24.5	0.001
Insulin mL	13.0 ± 4.5	12.8 ± 2.5	11.4 ± 2.1	9.4 ± 3.3	0.001
Zinc mg/ mL	43.7 ± 12.9	55.6 ± 12.4	51.2 ± 8.9	57.6 ± 15.1	0.01
Cholesterol mg/ dL	238.1 ± 14.9	241.7 ± 25.1	224.6 ± 24.5	193.4 ± 23.7	0.002
Triglyceride mg/ dL	183.4 ± 39.3	188.1 ± 26.1	167.0 ± 26.5	143.4 ± 34.4	0.001
HDL mg/dL	49.2 ± 4.4	47.1 ± 3.5	45.8 ± 5.7	38.9 ± 3.1	0.02
LDL mg/dL	152.6 ± 9.1	156.2 ± 20.1	145.2 ± 19.2	128.5 ± 19.3	0.003
VLDL mg/dL	36.8 ± 7.6	37.2 ± 5.2	33.6 ± 5.4	28.6 ± 6.8	0.001
CA 15-3 µg/ mL	273.8 ± 208	140.3 ± 59.2	96.4 ± 35.1	65.2 ± 37.7	0.002
Apelin H g/ L	870.2 ± 76.8	789.1 ± 77.9	639.3 ± 67.9	592.4 ± 90.5	0.004

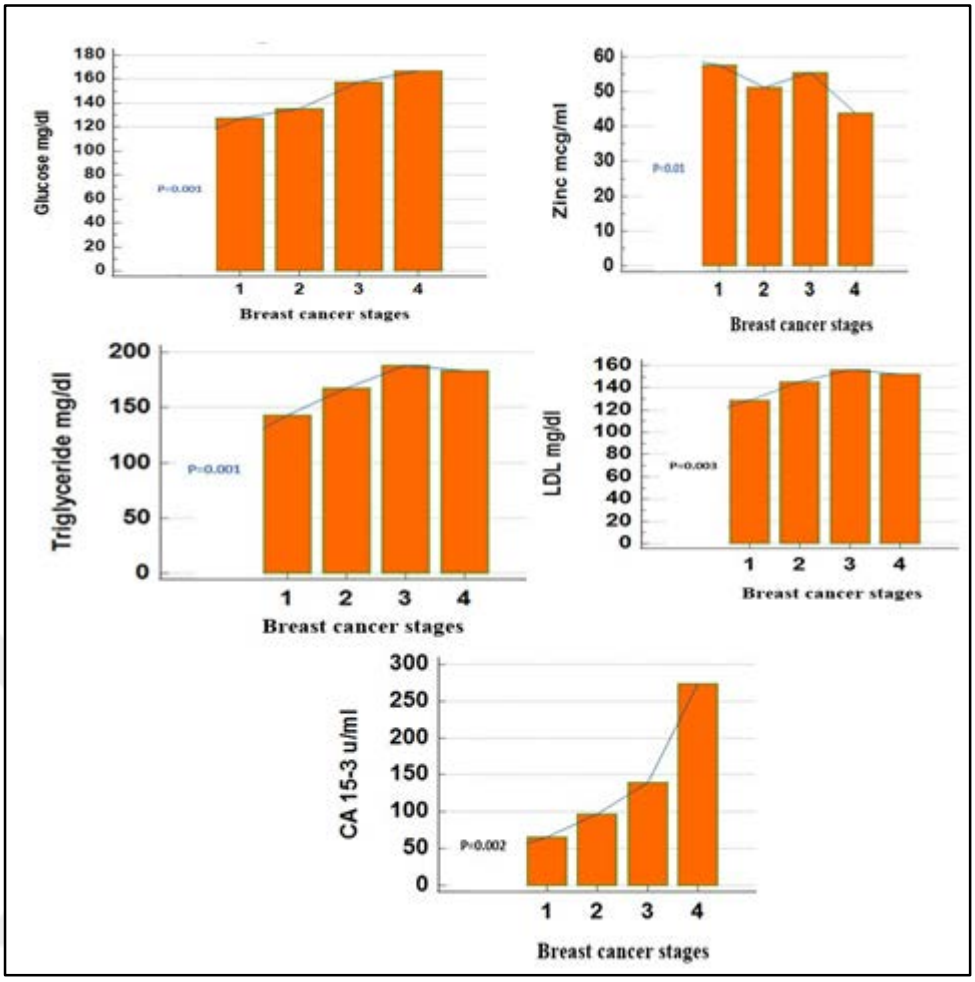

Figure 4.2 The anthropometric and biochemical characteristics of the breast cancer stages

4.2 Comparison of Blood Groups between Control and Chemotherapy Groups

By analyzing (Chi square) the percentage of blood groups, each according to the percentages of the study groups. In the healthy group, the percentage of each of (A-, A+, B-, B+, AB-, AB+, O-, and O+) were (2.5%, 27.5%, 2.5%, 22.5%, 2.5%, 2.5%, 2.5%, and 37.5%) Respectively, while breast group the percentage of each of (A-, A+, B-, B+, AB-, AB+, O-, and O+) were (2.5%, 27%, 1.25%, 28.75%, 2.5%, 3.75%, 1.25%, and 32.5%) Consecutive as illustrated in Table 4.3 and Figure 4.3. Our study showed that there is no statistical difference for blood groups when comparing patients and healthy people, and the percentage A+, B+, and AB+ was higher than the other group, this is consistent with the study that was published by (Costantini *et al.* 1990). The study

(Massimo Costanlini and colleagues) had a different interpretation from our study, which supports that The function of blood groups as danger factors for breast cancer and their predictive value after radical surgery have been retrospectively estimated in 315 breast cancer patients (Costantini *et al.* 1990).

Table 4.3 Comparison of blood groups between control and chemotherapy groups

Groups Study	A-	A+	B-	B+	AB-	AB+	O-	O+	Total	P value
Control	1 2.5%	11 27.5%	1 2.5%	9 22.5%	1 2.5%	1 2.5%	1 2.5%	15 37.5%	40 33.3%	0.06
Breast Cancer	2 2.5%	22 27%	1 1.25%	23 28.75%	2 2.5%	3 3.75%	1 1.25%	26 32.5%	80 66.7%	
	3 2.5%	34 28.3%	1 0.8%	33 27.5%	2 1.7%	5 4.2%	1 0.8%	41 34.2%	120 100%	

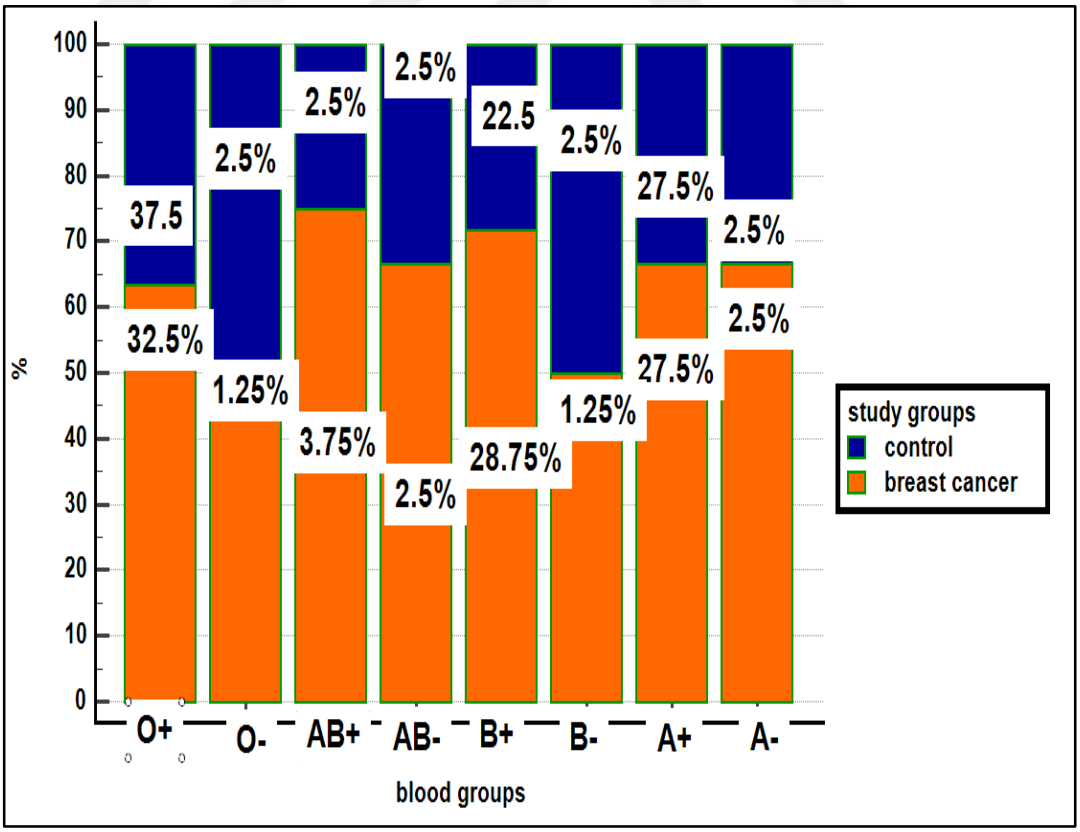

Figure 4.3 Comparison of blood groups between control and chemotherapy groups

4.3 Comparison of Age between Breast Cancer Stages

For the breast cancer group (80 cases), the results of the studied group presented in Table 4.4 and Figure 4.4, both of them showed the distribution of cases by age, and the average age of women with breast cancer was 48.2 years so that the patient was suffering greatly between 50-59 years (P≤ 0.001). In addition, our data explained that older people get greater levels of illness severity. Postmenopausal women with early breast cancer, and to a minor extent also pre-menopausal patients, had raised serum levels of C-peptide than control women (Bruning *et al.* 1992).

Table 4.4 Comparison of age between breast cancer stages

Variable		N	%	Mean	SD	P-Value
Age, years	26-29	10	12.5%	27.8000	1.0328	< 0.001
	30-39	16	20%	34.6250	3.3040	
	40-49	14	17.5%	44.2857	3.4514	
	50-59	20	25%	54.7000	2.3418	
	60-69	14	17.5%	63.5714	2.7656	
	70-72	6	7.5%	71.0000	0.8944	

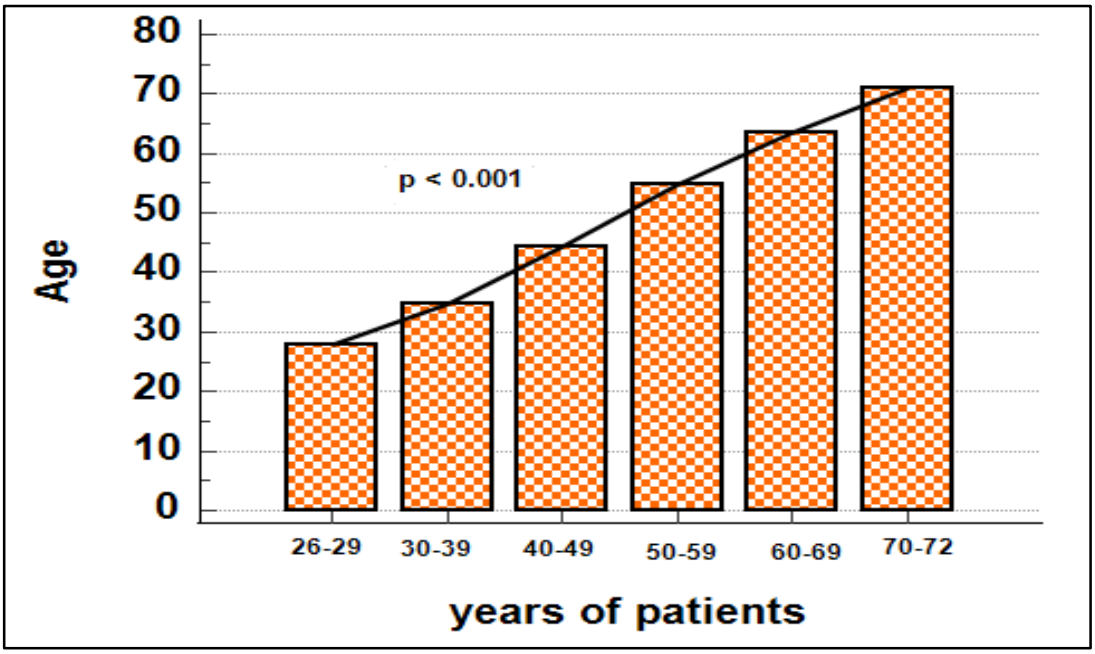

Figure 4.4 Comparison of age between chemotherapy groups

4.4 Identification of Risk with Multivariable Logistic Regression Analysis for the Relation between Parameters and Patients

Multivariate logistic regression models adjusted for several diseases which related to risk factors at admission, including (glucose P=0.001, insulin P=0.002, zinc P=0.002, cholesterol P=0.0005, triglyceride P=0.003, HDL P=0.8, LDL P=0.0001, VLDL P=0.0005, CA 15-3 P=0.9, and abelin P<0.0001), it is illustrated Table 4.5. The present study explained that the HDL level was not significant (p>0.05) in breast cancer patients these results were agreement by (Ray 2001). Who explained that there was no significant effect of HDL on breast cancer patients? This result came in agreement with Ballard-Barbash (1994) who submitted that although very small or no correlation has also been notified Hyperinsulinemia. Experimentation has explained that insulin is a majority factor for breast cancer cells (Foekens *et al.* 1989). C-peptide, a marker of hyperinsulinemia, is a great predictor of breast cancer hazard (Bruning *et al.* 1992).

Table 4.5 Identification of risk with multivariable logistic regression analysis for the relation between parameters and patients

Regression Coefficient	Wald	P. Vale	Odds Ratio	95% Confidence Limits	Variable
0.06	20.5	0.001	1.06	0.74-0.85	Glucose mg/ dL
0.2	14.5	0.002	1.2	0.63-0.79	Insulin mg/ L
0.3	14.1	0.002	0.6	0.90-0.96	Zinc mg/ mL
0.02	12.1	0.0005	1.02	0.61-0.71	Cholesterol mg/ dL
0.02	13.3	0.0003	1.02	0.61-0.71	Triglyceride mg/ dL
- 0.006	0.04	0.8	0.99	0.41-0.60	HDL mg/ dL
0.03	16.1	0.0001	1.0	0.63-0.80	LDL mg/ dL
0.1	12.3	0.0005	1.11	0.59-0.77	VLDL mg/ dL
2.8	0.08	0.9	17.4	0.97-1.01	CA 15-3 µg/ mL
0.009	28.3	< 0.0001	1.01	0.63-0.80	Apelin H. ng/ L

4.5 Correlation Coefficient (r) of the Comparisons between (Apelin H. Ng/L) with Every Measured Parameter

According to Table 4.6 and Figure 4.5, there was a large correlation coefficient between the variables (Glucose, Insulin, Cholesterol, Triglyceride, HDL, LDL, VLDL, and CA

15-3) and (Apelin H. ng/ L); while there was no clear relationship with ZINC. Abelin has a strong positive relationship with (Glucose, Insulin, Cholesterol, Triglyceride, LDL, VLDL, and CA 15-3) and a negative relationship with (zinc). Apelin is involved in cardiovascular function, metabolic syndrome, and homeostasis disorder and this is consistent with our study (Wang *et al.* 2019).

Table 4.6 Correlation coefficient (r) of the comparisons between (Apelin H. ng/ L) with every measured parameter

Variable	**Total Patient (N =80)**	
	r value	p-Value
Glucose mg/ dL	0.63	<0.0001
Insulin mg/ L	0.62	<0.0001
Zinc mg/ mL	-0.07	0.48
Cholesterol mg/ dL	0.53	<0.0001
Triglyceride mg/ dL	0.71	<0.0001
HDL mg/ dL	0.67	<0.0001
LDL mg/ dL	0.78	<0.0001
VLDL mg/ dL	0.61	<0.0001
CA 15-3 µg/ mL	0.77	<0.0001

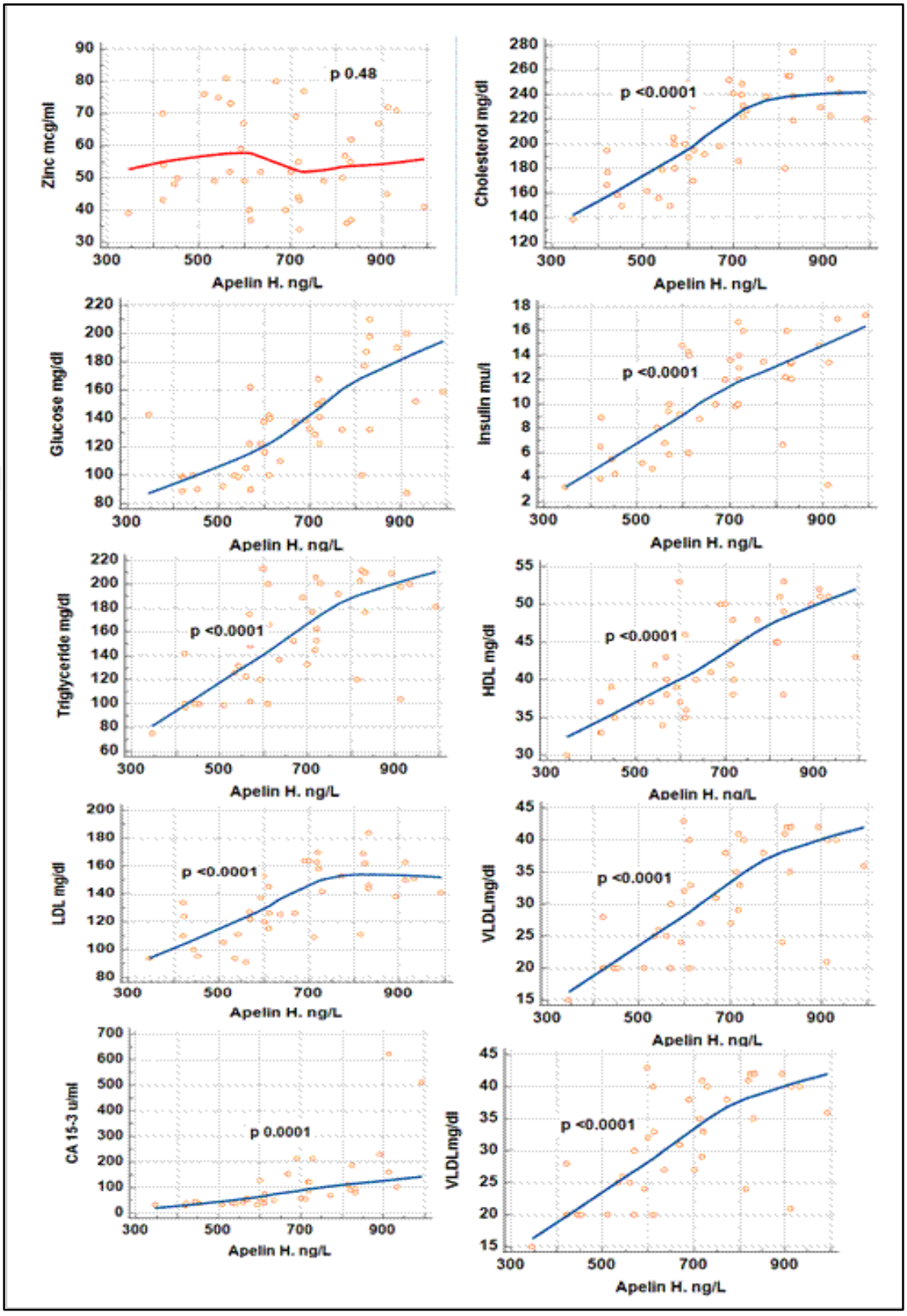

Figure 4.5 Correlation coefficient (r) of the comparisons between (Abelin H. ng/L) with total patients groups

4.6 Correlation Coefficient (R) of the Comparisons between Biochemical Markers with Total Patients

Table 4.7 shows the correlations among the variables of breast cancer patients and disease severity, using the Spearman rank correlation coefficient. The following variable showed a significant positive correlation to the disease severity ($p < 0.01$): Glucose, Insulin, Cholesterol, Triglyceride, LDL, VLDL, and CA 15-3, ($p<0.05$): HDL. However, zinc had a negative correlation with all biochemical markers. Apelin and CA 15-3 levels had a particularly strong correlation, while HDL, Glucose, and Insulin levels had a particularly medium correlation. There was also a low negative correlation between zinc and each HDL, Cholesterol, LDL, VLDL, and Triglyceride. Still, a high negative correlation with Abelin and CA 15-3 levels. We now describe the outcomes of serum insulin (C-peptide) analyses, which prove data from the report on their relation with obesity and central-body fat concentration in special. A new research is that C-peptide levels and an clear insulin insensitivity are significantly associated to breast-cancer danger, individualistic of adiposity or fat distribution (Bruning *et al.* 1992).

Table 4.7 Correlation coefficient (r) of the comparisons between biochemical markers with total patients

Cholesterol		0.84	0.84	0.95	0.71	0.65	0.59	0.73	0.402	-0.29
Triglyceride	0.84		0.99	0.78	0.78	0.80	0.59	0.61	0.30	-0.24
VLDL	0.84	0.99		0.71	0.78	0.80	0.58	0.61	0.29	-0.22
LDL	0.95	0.71	0.71		0.67	0.55	0.56	0.59	0.39	-0.35
Glucose	0.71	0.78	0.78	0.67		0.73	0.68	0.39	0.35	-0.37
Insulin	0.65	0.80	0.80	0.55	0.73		0.57	0.36	0.32	-0.28
Apelin	0.59	0.59	0.58	0.56	0.66	0.58		0.41	0.64	-0.48
HDL	0.73	0.61	0.61	0.59	0.39	0.36	0.47		0.3	-0.01
CA	0.40	0.30	0.29	0.39	0.3	0.32	0.61	0.30		-0.42
Zinc	-0.2	-0.24	-0.22	-0.3	-0.37	-0.27	-0.4	-.01	-0.42	
	Choles.	Trig.	VLDL	LDL	Glu.	Ins.	Apelin	HDL	CA15-3	Zinc

Spearman rank correlation coefficient

5. CONCLUSION

This study shows an increasing number of women in Iraq who suffer from a high level of apelin, especially women who have obesity associated with diabetes mellitus. The evolution of apelin will help study its regulative functions and physiological significance. We believe that this biological knowledge will start novel drug discoveries tomorrow.

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APPENDICES

APPENDIX 1. System URIT-660 URIT elisa analyzer fully automatic elisa

APPENDIX 2. BioSystems - BTS 350 semi-automatic analyzer

APPENDIX 3. TD-5M Portable medical centrifuge

APPENDIX 4. Medical test tubes

APPENDIX 5. Lt contains a substance that helps blood

APPENDIX 6. Centrifuge

APPENDIX 7. Thermo scientific

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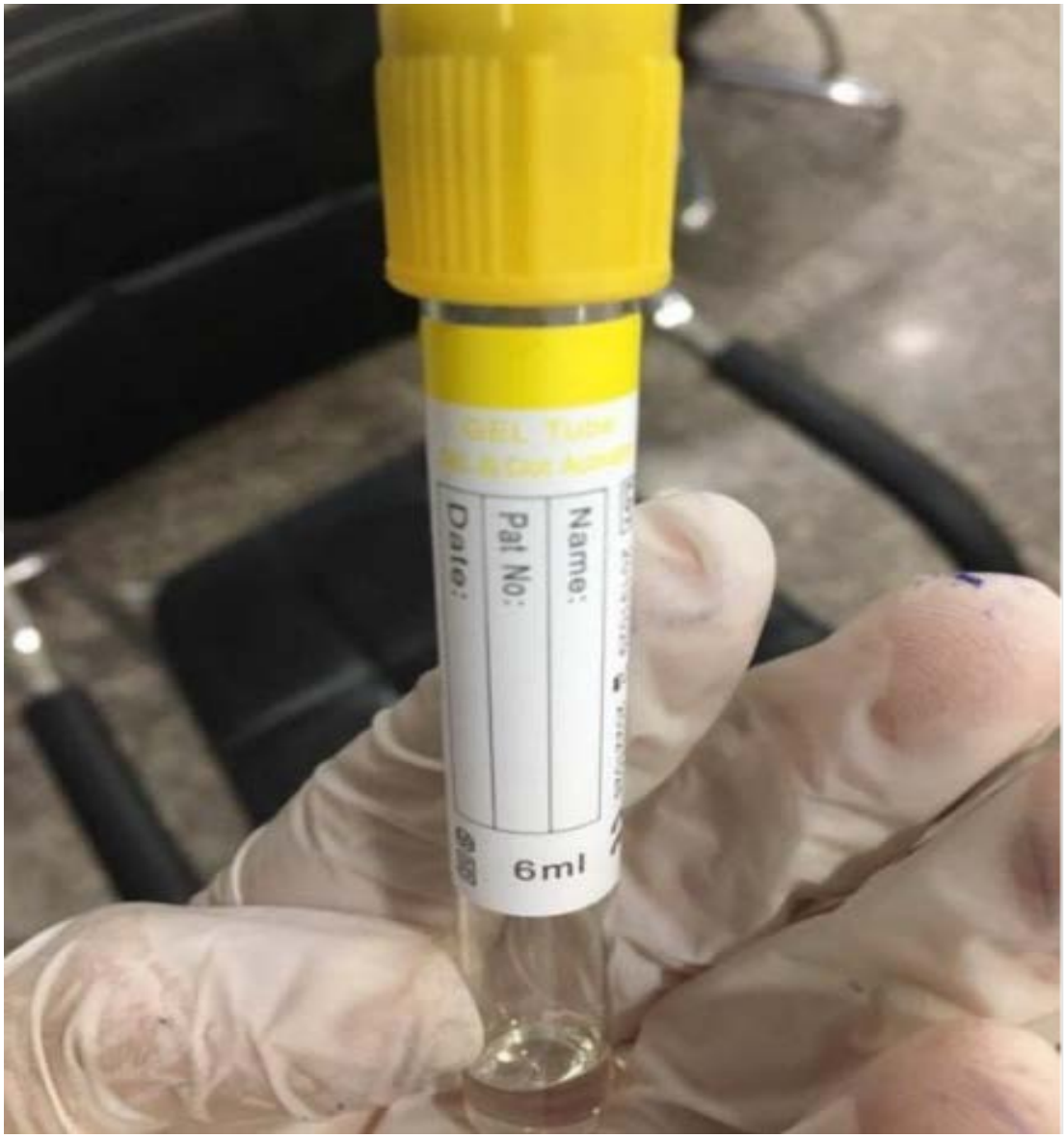

APPENDIX 6. Centrifuge

APPENDIX 7. Thermo scientific

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