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EVALUATION OF THE LEVELS OF CDK-5, INTERLUKIN-2 AND ANOTHER BIOCHEMICAL PARAMETER FOR PATIENTS WITH BREAST CANCER IN BAGHDAD CITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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IN
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ESRAA QADDOORI ABTTAN ABTTAN

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EVALUATION THE LEVELS OF CDK-5, INTERLUKIN-2 AND ANOTHER BIOCHEMICAL PARAMETER FOR PATIENTS WITH BREAST CANCER IN BAGHDAD CITY

By Esraa Qaddoori Abttan ABTTAN

March 2022

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

Advisor : Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor : Dr. Mahana Oied HUSSEIN

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Beytullah EREN
Environmental Engineering
Sakarya University

Member : Prof. Dr. Volkan EYUPOGLU
Chemistry
Çankırı Karatekin University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

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Esraa Qaddoori Abttan ABTTAN

ABSTRACT

EVALUATION THE LEVELS OF CDK-5, INTERLUKIN-2 AND ANOTHER BIOCHEMICAL PARAMETER FOR PATIENTS WITH BREAST CANCER IN BAGHDAD CITY

Esraa Qaddoori Abttan ABTTAN
Master of Science in Chemistry
Advisor: Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor: Dr. Mahana Oied HUSSEIN
March 2022

Women with breast cancer who took part in this study were classified into pre and post-menopausal. Postmenopausal women comprised two-thirds of the healthy, recently diagnosed, and treated groups. Non-obese, overweight, and obese women were categorized according to their body mass index (BMI). A significant correlation a p-value of less than 0.05, patients have lower leukocyte numbers, lymphocytes counts, monocyte counts, and basophil counts than the control group. Cdk5 protein levels in recently diagnosed women with breast cancer rise disproportionately to treatment (63 percent of 40 treated patients have a low rate of CDK-5 (17.2 ± 4.90). Cdk5 protein levels increased by 50% in the tumor specimens of 30 patients. Tumor samples from 50 newly diagnosed patients with breast cancer were tested for IL-2 protein levels for the first time, and the results showed that 80 percent of the 40 treated patients had a low level of IL-2 900 ± 191.90 compared to 90 percent of those who had previously been affected by the disease. As a result of this study, a statistical link was found between the newly diagnosed (NMB) and normal breasts' total estradiol concentrations (1111.73 ± 170.5, 494.9 ± 129, 614.72 ± 180.08, respectively).

2022, 37 pages

Keywords: Cancer, Breast cancer, Cyclin-dependent kinase 5 (CDK-5), Interleukin-2 (IL-2), Leucocyte breast cancer

ÖZET

BAĞDAT ŞEHRINDE MEME KANSERLI HASTALARDA CDK-5, IL-2 VE DIĞER BIYOKIMYASAL PARAMETRELERIN DEĞERLENDIRILMESI

Esraa Qaddoorı Abttan ABTTAN
Kimya, Yüksek Lisans
Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU
Eş Danışman: Dr. Mahana Oied HUSSEİN
Mart 2022

Bu çalışmaya katılan tam meme kanserli kadınlar menopoz öncesi ve sonrası olarak sınıflandırıldı. Menopoz sonrası kadınlar, sağlıklı, yakın zamanda teşhis edilmiş ve tedavi görmüş grupların üçte ikisini oluşturuyordu. Tedavi ve kontrol grupları arasında anlamlı olmayan farklar bulundu, radyasyon tedavisi uygulanan grupta obez kadınlarda tedavi edilen hasta grubunun %36'sı tedavi edildi ve bu durum yeni tanı grubunda anlamlı bir fark yaratıyor (radyoterapisiz %50). P değeri 0,05'ten düşük olan hastalarda lökosit sayıları, lenfosit sayıları, monosit sayıları ve bazofil sayıları kontrol grubuna göre daha düşüktür. Yakın zamanda meme kanseri teşhisi konmuş kadınlarda Cdk5 protein seviyeleri tedaviye orantısız bir şekilde yükselir (tedavi edilen 40 hastanın yüzde 63'ünde düşük bir CDK-5 oranı 17,2 ± 4,90). 30 hastanın tümör örneklerinde Cdk5 protein seviyeleri %50 arttı. Meme kanserli yeni teşhis edilmiş 50 hastadan alınan tümör numuneleri ilk kez IL-2 protein seviyeleri için test edildi ve sonuçlar, tedavi edilen 40 hastanın yüzde 80'inin 90'a kıyasla düşük IL-2 900 ± 191,90 seviyesine sahip olduğunu gösterdi. Bu çalışmanın sonucunda, yeni tanı konmuş (NMB) ve normal memelerin toplam östradiol konsantrasyonları arasında istatistiksel bir bağlantı bulundu (sırasıyla 111,73 ± 170,5, 494,9 ± 129, 614,72 ± 180,08).

2022, 37 sayfa

Anahtar Kelimeler: Kanser, Meme kanseri, Sikline bağımlı kinaz 5 (CDK-5), İnterlökin-2 (IL-2), Lökosit meme kanseri

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Esraa Qaddoori Abttan ABTTAN
Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus minus
°C	Degrees Celcium
µg	Migrogram
µL	Microliter
g	Gram
h	Hour
kg	Kilogram
mg	Miligram
mL	Mililitter

LIST OF ABBREVIATIONS

A	Absorbance
ADMA	Asymmetric dimethylarginine
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
BMI	Body mass index
CBC	Complete blood counts
CDC	Centers for disease control
EIZA	Enzyme ımmunoassay
ERK	Extracellular signal-regulated kinase
HIF-1	Hypoxia factor-1
NOS	Nitric oxide species
OD	Optical density
TAMs	Tumor-associated macrophages
WBCAs	White blood cells

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1. INTRODUCTION

Among the most frequent and malignant tumors in women, breat malignancy is amongst the most prevalent and deadly. In the tumour microenvironment, a complex tissue composed of a variety of stromal cell types, neoplastic cells are surrounded by a variety of stromal cell types (Quandt *et al.* 2020).

Oncogenic cancer cells must have a paracrine relationship with macrophages to invade and spread in vivo and intravenously, according to research on immune cells such as Tumor-Associated Macrophages (TAMs). Breast cancer-associated macrophage CDK5 involvement was previously unknown (Plava *et al.* 2019).

An essential regulation of signal transduction, including neuronal development, migration, and degeneration, has been revealed in CDK5, which seems to be a proline-directed serine/threonine kinase.. When CDK5 is overexpressed, it is connected to poor prognosis in a number of cancers, as well as to tumor growth and migration, as well as invasion (Liang *et al.* 2013). Thus, CDK5 can be employed as a therapeutic cancer target. A probable CDK5-mediated carcinogenesis mechanism is discussed in this paper and potential therapeutic approaches. These findings should serve as a solid platform for further investigation into CDK5's potential therapeutic role in cancer treatment (Do and Lee 2020).

Several studies have found a novel role for CDK5 in the genesis and progression of cancer. Gene amplification of CDK5 and its activators and a polymorphism in the CDK5 protein promoter region were discovered in cancer cells. Many malignancies, including head and neck squamous cell carcinoma, breast and hepatocellular carcinoma, colorectal, pulmonary, thyroid, pancreatic, and prostate cancer, have been associated with CDK5 dysregulation and over (Pozo and Bibb 2016).

1.1 Objectives of Study

The main aim of the current study is to learn more about how CDK-5 contributes to the spread of breast cancer and how it interacts with other immunological and metabolic factors.

1.2 Specific

1- Determine the levels of CDK-5, IL-2 in patients at the dissimilar phase of breast cancer.
2- Find a correlation of CDK-5, and IL-2 with some demographaic factor like obesity and ageing also with some clinical pathological cases.

2. LITERATURE REVIEW

2.1 Cancer

Uncontrolled cell proliferation is a hallmark of cancer. It takes time for a cancer cell to evolve from a simple to a complex form. Ten unique "cancer hallmarks" have been devised to help remember the qualities common to most malignancies (Figure 2.1) Cancer is characterized by genetic predisposition and mutation, tumour-induced inflammation, proliferative signals, and resistance to increased control. The growing cancer cell must also avoid programmed cell death, promote angiogenesis, invasion, and metastasis, conserve energy, and avoid immune destruction in (Hanahan and Weinberg 2011). The accumulation of genetic and epigenetic aberrations that give cancer an edge over nearby cells makes it a hereditary disease. Most malignant illnesses have improved due to tumour suppressor genes and proto-oncogenes. Most cancers are driven by proto-oncogenes, unlike tumour suppressor genes that prevent malignant cell formation (becoming oncogenes). A tumour suppressor gene can be inactivated, or an oncogene can be activated or amplified. These changes are called "drivers" because they directly contribute to cancer growth, whereas most modifications seen in cancer cells are called "passengers" since they do not increase cancer growth (Hanahan and Weinberg 2011).

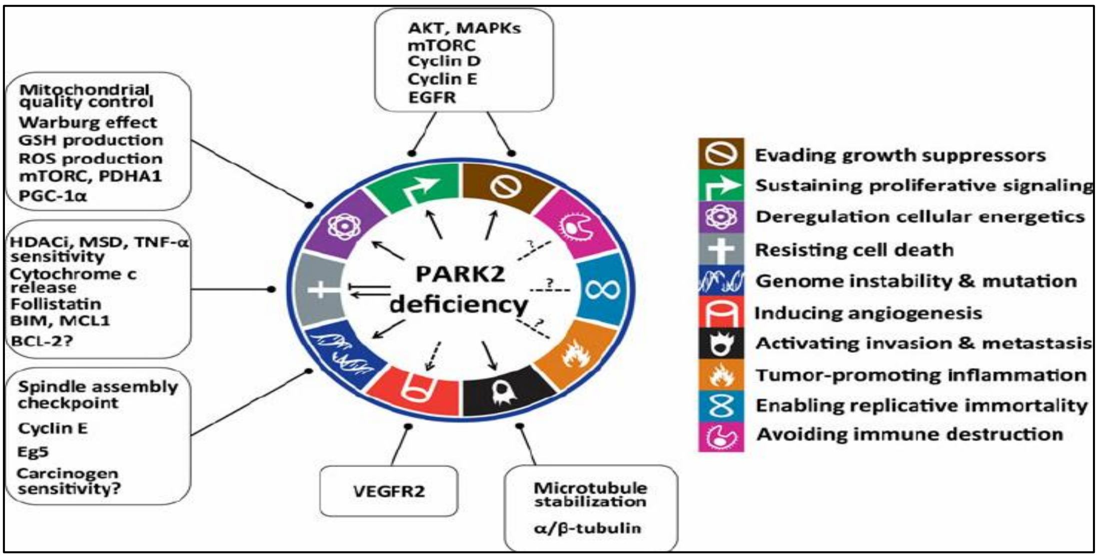


Figure 2.1 The traits are acquired gradually through genetic and/or epigenetic changes in the DNA (Hanahan and Weinberg 2011)

2.1.1 Breast cancer

Breast cancer malignant will continue to be a substantial threat to public health. This type of cancer accounts for more than 23% of all cancers in women, making it the most common. According to the American Cancer Society, 411,000 people died of breast cancer in 2002, with 1.15 million new cases. As a result, the exact cause of breast cancer remains a mystery. The development of breast cancer has been linked to various variables, but it is now known that a combination of genetic and environmental factors is responsible. Recent years have seen significant progress in identifying the risk factor that characterizes a person's susceptibility to breast cancer and the genetic factors that influence this susceptibility. Although more people are becoming aware of the disease, the disease's complex genetic and environmental efficacy is still a work in progress. Increasing our understanding of the genetic pathways that promote tumour growth could improve breast cancer prevention, detection, and treatment (Igene 2008).

2.1.2 Breast incidence

In 2008, it was detected in 1.38 million new cases, breast cancer represents 23% of all new gynaecological cancer cases globally. Recent studies have identified the most common cancers in underdeveloped countries, the death rate is no longer more (about 6-19, corresponding to 100,000. In 2013, an estimated 232,340 women in the United States developed breast cancer, and 39,620 died (Kuchenbaecker *et al.* 2017).

East Africa has a prevalence of 19.3 per 100,000 women, while Western Europe is 89.7. Breast cancer rates vary between these two sites (less than 40 per 100,000). Iraq's rate of return was 31.1/100,000 in 2008, as is Kuwait, Saudi Arabia (22.4), Jordan (47.0) and Syria (19.0). Breast cancer is the most common malignancy among Iraqi women. According to the Iraqi Cancer Registry, one-third of Iraqi girls with cancer will have breast cancer (Yeole and Kurkure 2003).

2.1.3 Risk factor

Many factors have a role in breast cancer. The disease progresses due to a complex combination of inherited and environmental factors. Breast cancer is not always curable, regardless of the cause. Breast cancer, like most malignancies, increases with age. Postmenopausal women report this happening in roughly 80% of cases. Breast cancer in women in their twenties or early thirties is quite rare. After menopause, the threat grows. Breast cancer is associated with advanced age. Breast cancer impacts women in their 60s at a rate that is 100 times greater than that of women in their 20s. Approximately about one in eight women would've been diagnosed with the disease assuming they survived to the age of 95. In a lifetime, 90% of women die before 95, lowering the risk of heart attacks and strokes (Harbeck *et al.* 2019).

The effects of circulating estradiol generated by testosterone precursors in fatty tissue in postmenopausal women with a body fat percentage of 27 to 29 kg/m^2 are regarded to be activating and proliferating of cancer, other factors, most likely include irritation, may have a role in the development of breast cancer in menopausal overweight women, according to research (Makuwa 2015). The weight problems infection aromatase axis improved in postmenopausal breast tissue, explaining the relationship between obesity and HR-high-quality breast cancer as illustrated in Figure 2.2 (Taylor *et al.* 2012).

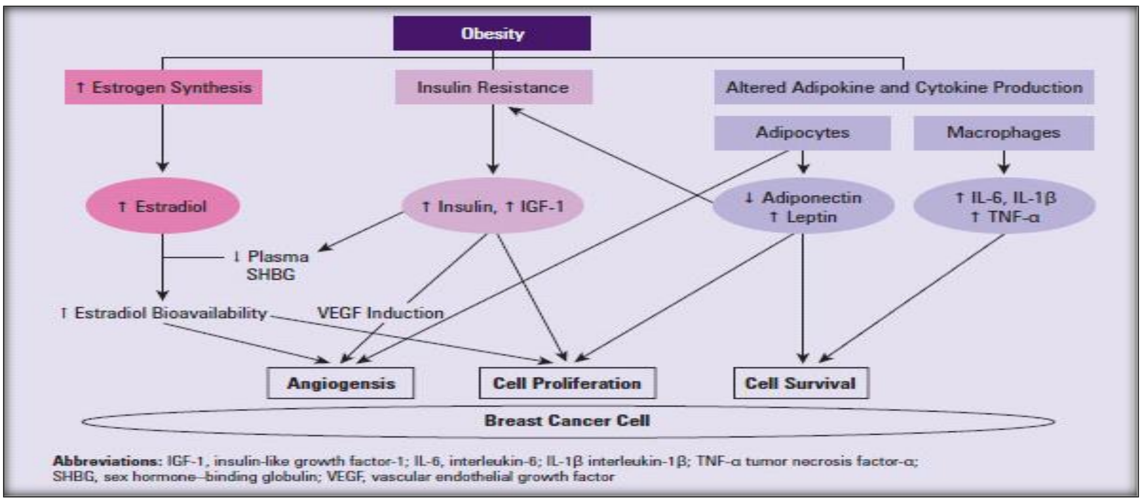


Figure 2.2 White adipose tissue has hormonal and metabolic activities linked to breast cancer (Taylor *et al.* 2012)

Insulin resistance, which makes it difficult for the body to process glucose and raises insulin levels, is associated with obesity. Most cancers need to have angiogenesis and programmed cell death, both of which are induced by insulin. In addition, activated macrophages in white adipose tissue release several seasoned inflammatory mediators associated with tumour growth, such as TNF- and IL-1, when they become obese. Many pro-inflammatory mediators play a role in obesity-related mastitis. These pro-inflammatory mediators also play a role in the astrologically poorer prognosis of obese women with breast malignancies (Li *et al.* 2017).

Recent research suggests that breastfeeding about a year after childbirth reduces the incidence of breast cancer. The longer length reduces baseline risk. A meta-analysis of 47 studies from 30 countries found that nursing reduces the risk of breast cancer by 4.3% annually. Breastfeeding suppresses the menstrual cycle, which may alter menstrual variability (Daly *et al.* 2021). Exposure to estrogen is an intrinsic and critical risk factor for breast cancer. Some research has indicated a 2.5-fold increase in the chances of estrogen publicity (Lewis-Wambi *et al.* 2011). Breast cancer risk has been determined in young women exposed to high amounts of ionizing radiation in the chest. She notes that the maximum age for public exposure is between 10 and 14 years. There doesn't seem to be any better odds after 45 (Land *et al.* 1980).

Recent research links endogenous hormone levels to an increased risk of breast cancer. Apart from estrone, estradiol, and androstenedione, testosterone, dehydroepiandrosterone, and prolactin levels were all considered significant in this study. Exposure to endogenous sex hormones is influenced by a woman's age, number of pregnancies, and approach to menopause. Exposure to estrogen and higher estrogen levels in women during the beginning of their menstrual cycle may lessen the risk of breast cancer (Hankinson *et al.* 1988).

2.1.4 Family history

Availability of family history information is vital in predicting the possibility of clinical genetic conditions and unrelated genetic issues. Other early diseases, such as breast and colon cancer, run in some families. Only thromboembolism, coronary artery disease, type 2 diabetes, and depression have a family history. The American general practitioner's family history initiative began in 2004. This research aims to promote awareness among medical professionals and patients about the costs of collecting and analyzing a circuit of kin history as a screening tool (Scheuner *et al.* 2008).

The human genome project has improved our understanding of how genetic diversity affects health and fitness. Decades of research have focused on establishing links between genotype and phenotype and predicting which individuals contribute to inherited scientific conditions. Preventive approaches and more excellent monitoring can improve outcomes if the value of using an individual's family history is recognized. Women (and men) who have a first-degree relative with breast cancer have a higher risk. The risk of breast cancer increases if two first-degree relatives have it. Girls with grade 1 breast cancer are 1.8 times more likely to develop it. Those with three or more relatives outperformed women without relatives about four times (Kaput *et al.* 2005).

2.2 CDK-5 and Breast Cancer

Neurite outgrowth, migration, and neuronal degeneration are all controlled by CDK5, a proline-directed serine/threonine kinase. In several malignancies, CDK5 has been linked to a poor prognosis as well as tumor proliferation, migration, and invasion. As a consequence, CDK5 is a promising therapeutic target for the treatment of cancer. This research focuses on CDK5, its role in cancer development, a putative CDK5-mediated carcinogen pathway, and prospective treatment avenues. Research on CDK5 as a cancer immunotherapy target (Dhariwala and Rajadhyaksha 2008).

2.2.1 Biology of CDK5

In 1992 Hellmich first identified it as a kinase-like neuronal division 2 control because of its substantial sequence similarity to cdc2. Two hundred ninety-two amino acids and about 5,000 nucleotides make up a protein. Activators and substrates of CDK5 can be found close in mammalian tissues and culture cells. CDK5 does not require T-loop phosphorylation, as with other cyclin-dependent kinases In order for CDK5 to function properly, Ser159 phosphorylation on the T-loop and p35 interaction appear to be necessary (Tang and Wang 1996).

2.2.2 CDK5 and cancer

The characteristics of tumour development have been recognized so far, cancer is characterized by a wide range of symptoms, a potential anti-cancer target is CDK5, which affects these indicators, these informations are illustrated in Figure 2.3. Based on these findings, we speculate a possible role for CDK5 in the carcinogenesis pathway (Wang 2021).

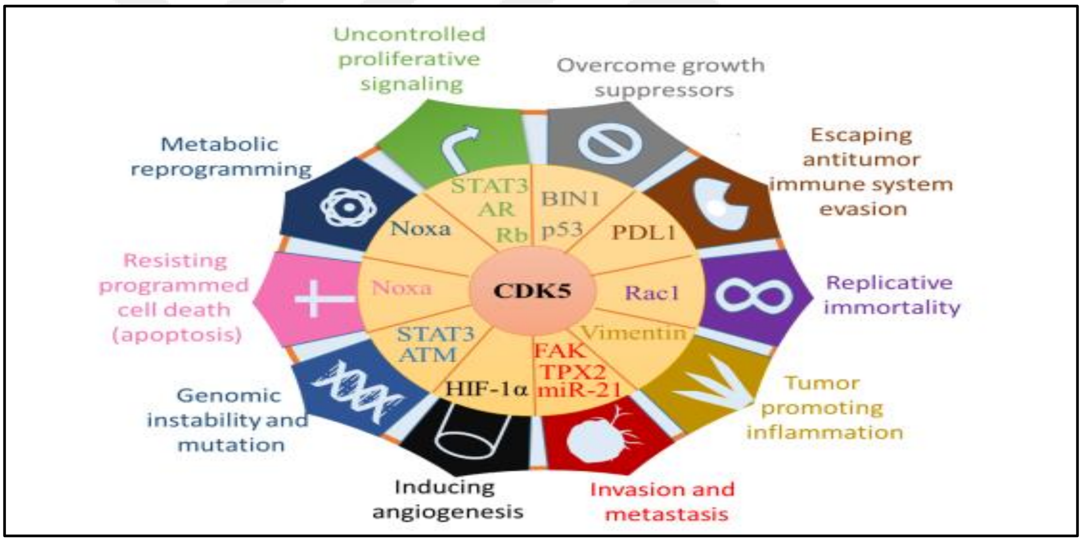


Figure 2.3 shows the effect of CDK5 on hallmarks of cancer (Wang 2021)

Uncontrolled proliferative signals include the escape of growth inhibitors and evasion of the immune system. Impact of CDK5 on cancer markers: angiogenesis, genomic instability and mutation, resistance to apoptosis, and metabolic reprogramming are all examples of dysregulated proliferative signals. With its target protein, CDK5 promotes tumour growth and metastasis. Noxa, androgen receptor, retinoblastoma, bridging integrator 1, programmed cell death ligand 1, focal adhesion kinase, protein targeting of Xklp2 (TPX2), hypoxia factor-1 (HIF-1), hypoxia factor-1 (HIF- 1) (Wang 2021).

Cyclin stimulates enzyme activation. Cyclin-dependent kinase. CDK5 is unique from other CDKs in the CDK family in that it is not activated by cyclins or the T-loop. It depends on the non-cyclin proteins p35 and p39 or their shorter homologs, p25 and p29, for kinase activity. Cyclin I and cyclin I-like activators were recently discovered, CDK5 is expressed in all human tissues, but neural tissues have the highest expression and activity levels. Axon guidance, synaptic transmission, and neuronal migration are just a few of its roles. In other words, CDK5 is essential for neuronal survival, thus, CDK5 dysregulation is associated with various neurodegenerative disorders, including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease and Parkinson's disease as illustrated in Figure 2.4 (Malumbres 2014, Parkin 2011).

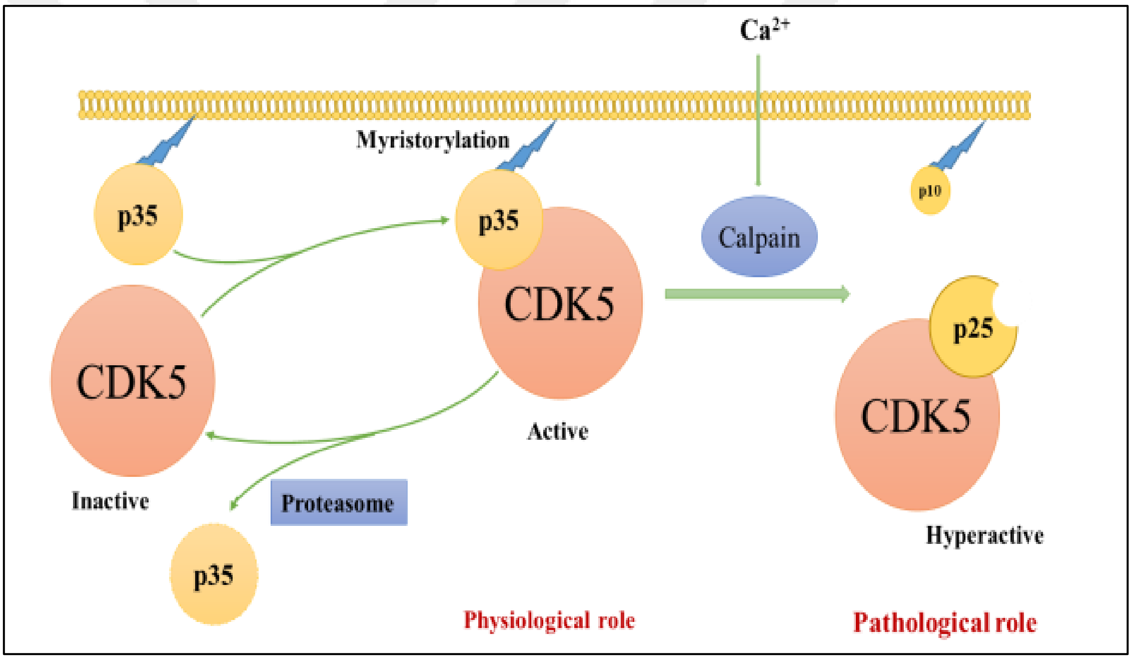


Figure 2.4 CDK5 is an inactive promoter that is activated by the p35 activator CDK5 and translocates to the membrane when p35 myristoylated the N-terminal region Malumbres 2014)

Several studies have revealed that CDK5 plays a vital role in the development of cancer When it comes to medullary thyroid cancer, CDK5 increases cell proliferation by interfering with the Rb/E2F and STAT3 pathways. Breast cancer tumour formation is influenced by STAT3 phosphorylation. CDK5 also phosphorylates and directly stabilizes the androgen receptor. CDK5 can target Notch-dependent endothelial cell proliferation in the angiogenic pathway. In hepatocellular carcinomas, CDK5 stabilizes hypoxia factor-1

(HIF-1). CDK5 inhibition slows tumour angiogenesis because HIF-1 is a vital transcription factor. It is also essential for cells to migrate and invade. Lung cancer migration is regulated by the human CDK/p35 core helix-loop-helix-loop-transcription factor. HASH1 is an achaete-scute homolog discovered (Pozo *et al.* 2016).

2.3 IL2 and Breast Cancer

Interleukin 2 (IL-2) enhances T and B cell proliferation and differentiation while increasing the activity of natural killer cells. It may also affect the growth of non-lymphocytes. There are three IL-2 receptor complex sub-genres F. Intracellular expression of IL-2R subunits determines the affinity of the IL-2R: intermediate affinity receptors are generated by the combination of strands, while high-affinity receptors require the subunit (Itoh *et al.* 1994). IL-2 is found in endothelial cells, gut epithelial and nerve cells. Activation of cancers such as gastric, renal, and malignant spinal cell carcinomas, melanomas, neuroblastomas, and prostate cancers elevated IL-2 and IL-2R, a expression in this result (A Baudino 2015).

IL-2 promotes cell growth in healthy tissues, but it also promotes tumour growth in cancers such as stomach, kidney, and head and neck squamous cell carcinomas, it is found in mitotic cells more than any other stage. Head and neck squamous cell carcinomas respond favourably to inhibition of anti-allergic IL-2 or IL-2R in preclinical trials (Song *et al.* 2008). Injection of systemic IL-2 can activate tumour necrosis factors and antiangiogenic molecules such as IP-10, which have been shown to diminish metastatic kidney cancer in animals. On the other hand, exogenous IL-2 was found to inhibit cell growth (Herrera-Aco *et al.* 2019). Increased expression of interleukin-2 (IL-2) and its receptor (IL-2R) in breast cancer cells has been linked to tumour progression in various organs Lilly claims that the proliferation index increased with cell proliferation in benign breast tissue. There was a higher expression of IL-2 during mitosis in head and neck squamous cell carcinomas than in other mitotic stages such as G0, G1 and S (Herrera-Aco *et al.* 2019).

3. MATERIAL AND METHOD

3.1 Materials

3.1.1 Instruments

Some of important interuments and kits which used in vitro during the present studyare illustrated in Table 3.1.

Table 3.1 List of instrument and kits

No	Instrument.	Country.	Company.
1	Biopette Variable.Volume 2-20, 20-100, 100-1000 ulv	China	
2	UV transmission	Germany	Vilberlourmat
3	Incubation	China	Jrad
4	Spectrophotometer	Jaban	apple
5	Centrifuge	Japan	H-19F Kokusan,
6	Water bath	Germany	Arneold ,
7	Autoclave	Germany	Human
8	Tips (blue, yellow)	Maliza	-,
9	Lab. Water bath	China	mindery
10	Complete blood picture(CBC)	china	Genex
11	EIA	Germany	Human
12	EDTA Tubes	China	-
Kits			
1	ABO blood group	USA	BIOTEC
2	ELIZA Estradiol Kit	Germany	Human
3	ELIZA Testosterone Kit	Germany	Human
4	ELIZA DHEA Kit	Germany	Demeditec Diagnostics GmbH
5	ELIZA CDK-5	Germany	Human
6	ELIZA IL-2	Germany	Human

3.1.2 Subjects

Breast cancer patients who visited the Medical City oncology hospital between December 2021 and February 2022 will be included in this study, and healthy women as a control group and those currently being treated for the disease. Patients who underwent surgery for breast cancer ectomy the samples needed for this study. Detailed physical examinations were performed on all patients. Aspiration of the cysts, cytology, histology (biopsy) and mammography were used to conclude. In addition to interviewing patients in private, researchers also collect data by asking them. Premenopausal and postmenopausal patients A subgroup were taken according to age, Table 3.2 provides a breakdown of the different groups.

Table 3.2 Classification of study groups according to their age range

Groups	Sub-Group	No.	Age Range (year)
Beast cancer with newly diagnosis No.50	Pre-menopausal	21	<45
	Post-menopausal	29	45-75
Breast cancer with trated No. 50	Pre-menopausal	18	<45
	Post-menopausal	32	45-75

3.1.3 Diagram of project

The groups of the current study are illustrated in Figure 3.1

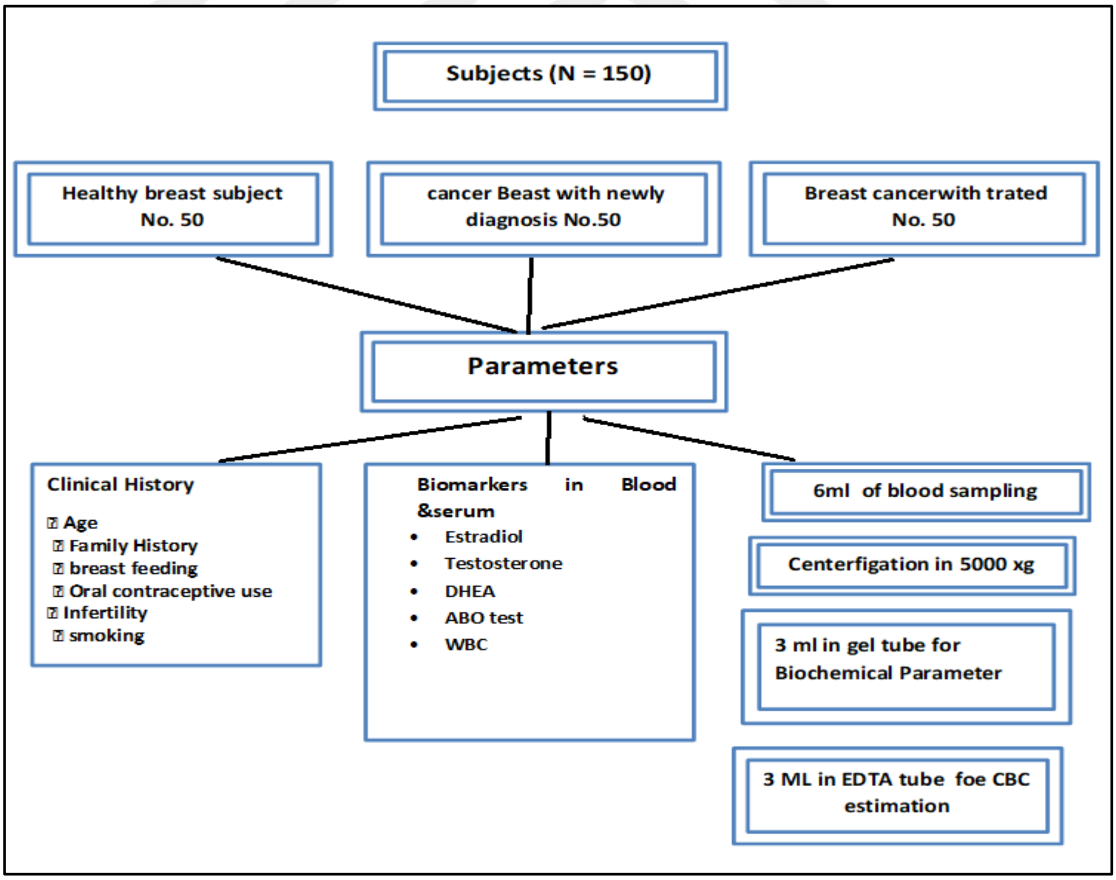


Figure 3.1 Studied groups

3.2 Methods

3.2.1 Blood sampling

Two divisions of 3 and 2mL of patients and healthy controls were obtained via venipuncture, and blood samples were stored at four °C until further analysis. We coagulated the first sample using a gel tube and 15 min at room temperature. Serum was obtained by centrifugation for 10 min at 3000 rpm and stored in the freezer (-20 °C) until needed. The second aliquot is dissolved in EDTA and kept at -20 °C until blood tests are required to avoid coagulation.

3.2.2 White blood cells count

A multi-parameter cell counter can be used to measure, derive, and/or calculate WBC types (Hematology Analyzer, Frankford, Germany, 2013). System status monitoring, barcode recognition, and computer-enhanced data processing are all features of the automated sampling system at Medical City Oncology Hospital.

3.2.3 Determination of labratoray biomarker by ELIZA

All biomarkers in serum samples have been carried out once in all subjects of the three major groups (control, newly diagnosis of breast cancer, and treatment), these biomarkers are illustrated in Table 3.3.

Table 3.3 The type of technique)ELIZA) used to measure biomarker in this study

No	Apparatus	Type
1	Estradiol	Competitive
2	Testosterone	Competitive EIA
3	DHEA	Competitive binding
4	CDK-5	Sandwach Eliza
5	IL-2	Sandwach Eliza

3.2.4 Stradiol. (E2)

Principle

People who work in health care can use the human estradiol ELISA. ELISA is based on the assumption that estradiol and a hormone enzyme group compete for a limited number of antibodies (rabbit) on a plate. Since the concentration of estradiol is inversely proportional to the amount of conjugated hormone-enzyme conjugate in the sample, after the specimen is incubated and the hormone-enzyme conjugate in the well, the unbound conjugate is removed by washing. Step 2 introduces the substrate solution, which results in a blue dye that fades to yellow upon termination of the reaction. The intensity of the forms is inversely related to the amount of estradiol in the substance.

3.2.5 DHEA

PRINCIPLE

Solid-phase enzyme-linked immunosorbent test (ELISA) accordance with the principle of competitive binding is used in the DEMEDITEC DHEA ELISA Kit. Anti-DHEA antibodies are used to cover microtiter wells. DHEA peroxidase and horseradish competition to interact with both the antibody-coated with such an unspecified amount of DHEA present in the sample, which is coated with an antibody. The unattached conjugate is removed after incubation by rinsing with distilled water. As DHEA concentration decreases, peroxy-date conjugate binding increases. Peroxy-date conjugate binding is inversely related to DHEA concentration in the sample.

3.2.6 CDK-5

This assay has high sensitivity and excellent specificity for detection of Human CDK5. Manufactured in an ISO 9001:2015 Certified Laboratory.

Principle

The ELISA kit uses sandwich-ELISA technology. They were pre-covered on the kit-included micro-ELISA plate with the Human CDK5 antibody. The wells of the ELISA micro-plate are coated with a specific antibody that binds to the samples or standards. Next, a Biotinylated, Avidin-Horseradish Peroxidase (HRP) anti-human CDK5 detection antibody is added to each microplate. They are rinsed and cleaned of any unnecessary components. The substrate solution is injected into each of the wells. The biotinylated antibody detecting human CDK5 and the Avidin-HRP conjugate will only stain wells containing these components blue: it turns yellow when sulfuric acid is introduced into the enzyme-substrate reaction. In order to determine optical density (OD), a spectrophotometric method using a laser with a wavelength of 450 nm is used. The OD value is affected by the concentration of human CDK5. The optical density (OD) of the samples can be used to calculate the amount of human CDK5 in the models.

3.2.7 IL2

IL-2 is measured in human blood, plasma, cultured cell supernatants, amniotic fluid, synovial fluid, urine, and other body fluids were collected using human interleukin-2 (HuIL-2) ELISA. Both original and recombinant HuIL-2 will be recognized by screening.

Principle

An ELISA (enzyme-linked immunosorbent assay) sandwich human solid-phase IL-2 is used to determine how much a target is bound between two antibodies. Target-specific antibodies were already pre-coated into the wells of the provided microplate. Those samples or controls attached to the fixed antibody (capture) are then added to the wells. The second antigen (reagent) antibody recognizes an epitope distinct from the first (capture) using a sandwich approach. The enzyme-conjugated antibody binds the resulting sandwich. The substrate solution is then added to the microplate after the enzyme target and antibody combination is incubated and washed to eliminate any unbound molecules. The amount of prey in the initial sample affects the intensity of this signal.

3.3 Statistical Analysis

It was determined that the data had been normalized, comparable, and distributed normally. The likelihood was also assessed using IBM SPSS version 25.0 and a student independent T-test with median standard deviation. When the probability was less than 0.05, it was considered statistically significant.

4. RESULTS AND DISCUSSION

4.1 A Pathological Examination in a Medical Facility

1. There were two parts to the research: Comparing associated risk factors for breast cancer onset was done in the first section.
2. Women in the "control" group (NB=50).
3. The study comprised 80 women who had just been diagnosed with breast cancer BCN (No. 50). Breast cancer patients who received BCT radiation treatment.
4. Blood hormone and hematological analysis in these groups constitutes this part.

4.1.1 Age and its relation with studied groups

In this study, the age range for women with intact breasts was between (30-75). It is important to note that this is just a control group. The results showed that the test subjects were appropriate for the age group of patients (p-value <0, 01). Table 4.1 and Figure 4.1 illustrate the the average and stander diveation of age into. Pre- and postmenopausal women were divided into two groups for this study. There appears to be a significant positive association (P 0.002) between the age of the three base groups, and the data showed that (2/3) of the healthy subject, newly diagnosed, and treated groups were postmenopausal (62 percent, 65 persent, and 60 percent, respectively).

Table 4.1 Avarege of age groups in present study

		Frequencey	Mean ± SD	P value
Healthy breast subject No. 50	Premenopausal	19 (38%)	38.68 ± 6.30 (Range 30-45)	0.002
	Postmenopausal	31 (62 %)	56.5 ± 7.05 (Range 46-70)	
	Total	50	46.06 ± 10.25	
Breast cancer with newly diagnosis No.50	Premenopausal	18 (35%)	36.62 ± 5.82 (Range 30-45)	0.0021
	Postmenopausal	32 (65%)	61.75± 6.55 (Range 46-75)	
	Total	50	54.38 ±11. 8	
Breast cancer with Treated No. 50	Premenopausal	20 (40%)	40.38 ± 5.48 (Range 30-45)	0.001
	Postmenopausal	30 (60%)	57.16 ± 10.03 (Range 46-75)	
	Total	50	50.11 ± 12.35	
$X^2 = 33.118$, df = 5, P value ≤ 0.01				

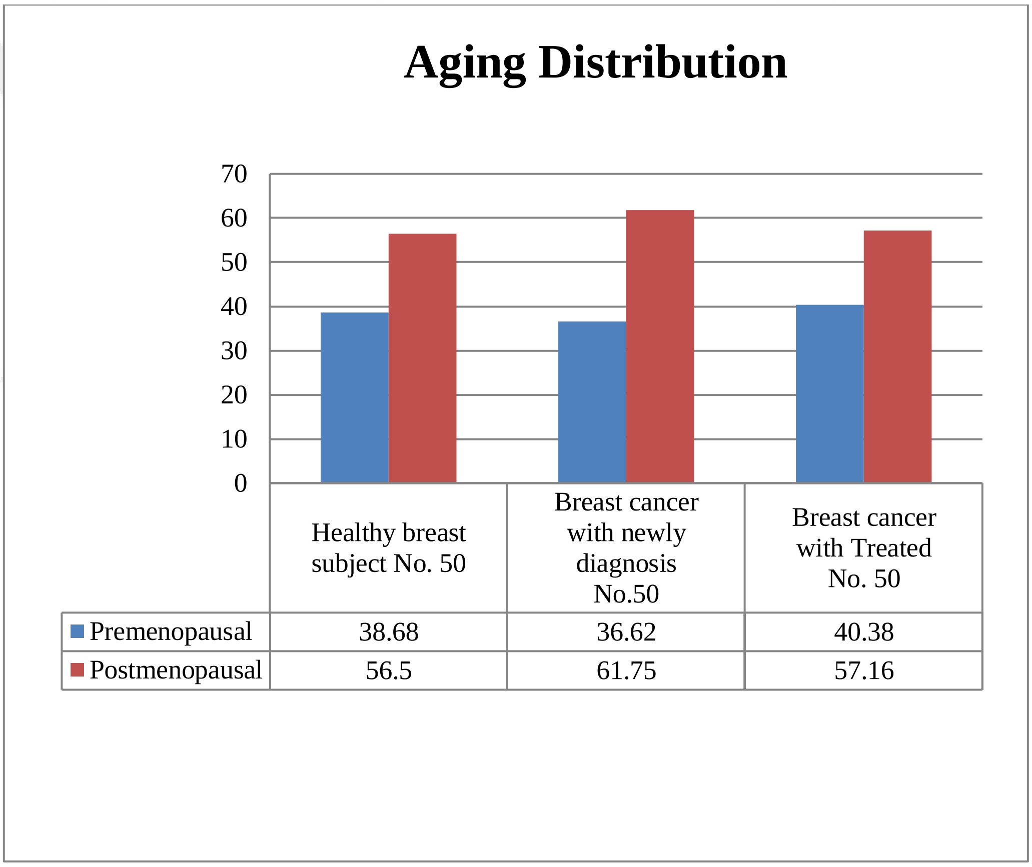


Figure 4.1 Age distrubition in studied groups

Perimenopause and menopause are signs of ovarian onset and menopause, respectively. The ovaries produce steroid hormones around the menstrual period and reduce production during menopause. Most women reach menopause between the ages of 45 and 54. Early menopause may increase the risk of breast cancer.

Atypical postmenstrual menstruation occurs in about 35% of women with premenopausal breast cancer versus 38% of normal women (Table 4.1). In contrast to premenopausal women (38%), most Iraqi women diagnosed with breast cancer (65%) were postmenopausal (61.75 ± 6.55) (Table 4.1). A woman in her 60s is more than 100 times more likely than a woman in her 20s to have breast cancer, according to a global survey Breast cancer risk lowers with each year after menopause and rises with every year younger prior to menopause, according to the Collaborative Group on Hormonal Factors in Collaborative Breast Cancer (2012), which also concluded that there is a relationship between age and the risk of breast cancer. Many conceptions and deliveries expose breast tissue or cells to this investigation in a reservist capacity (Cortazar *et al.* 2012).

4.1.2 BMI and its relation with studied groups

Both being overweight and obese are risk factors for breast cancer, the most preventable type of cancer. Women's obesity was classified into three categories based on body mass index (BMI): non-obese, overweight (BMI 25-29.9 kg/m^2), and obese (BMI 30 kg/m^2). Worldwide, breast cancer kills more people than any other form of cancer-related death. P-value >0.01 showed no statistically significant difference in BMI between patient subgroups. Comparison of the mean and standard deviation of BMI for women with normal breasts (NB = 50), newly diagnosed breast cancer patients (BCN = 50), and breast cancer patients undergoing radiation (BCT = 50) compared to the control group. The results are shown in Table 4.2 and Figure 4.2. Obesity was found in 51% of newly diagnosed patients, a significant increase over the previous year (P > 0.05). Radiation therapies were used to treated group in obese women are 36% of treated patients group, and this making a significant difference in the newly diagnostic group (50% without radiotherapy).

Table 4.2 BMI (Kg/m²) for control, newly diagnosis and treated groups

BMI			
	Normal	Overweghit	Obese
Healthy subject Mean ± SD	22.96±0.83(63%)	26.63±1.4(28%)	30.6 ± 1.07(9%)
newly diagnosis No.50	23.9 ±1.55(17 %)	27.33±1.7 (33%)	35.0 ± 2.11(50%)
Treated group Mean ± SD	24.6 ± 1.43 (42%)	28.0 ± 1.9 (22%)	34.7 ±1.5 (36%)
Chi square = 16.3, P value 0.04			

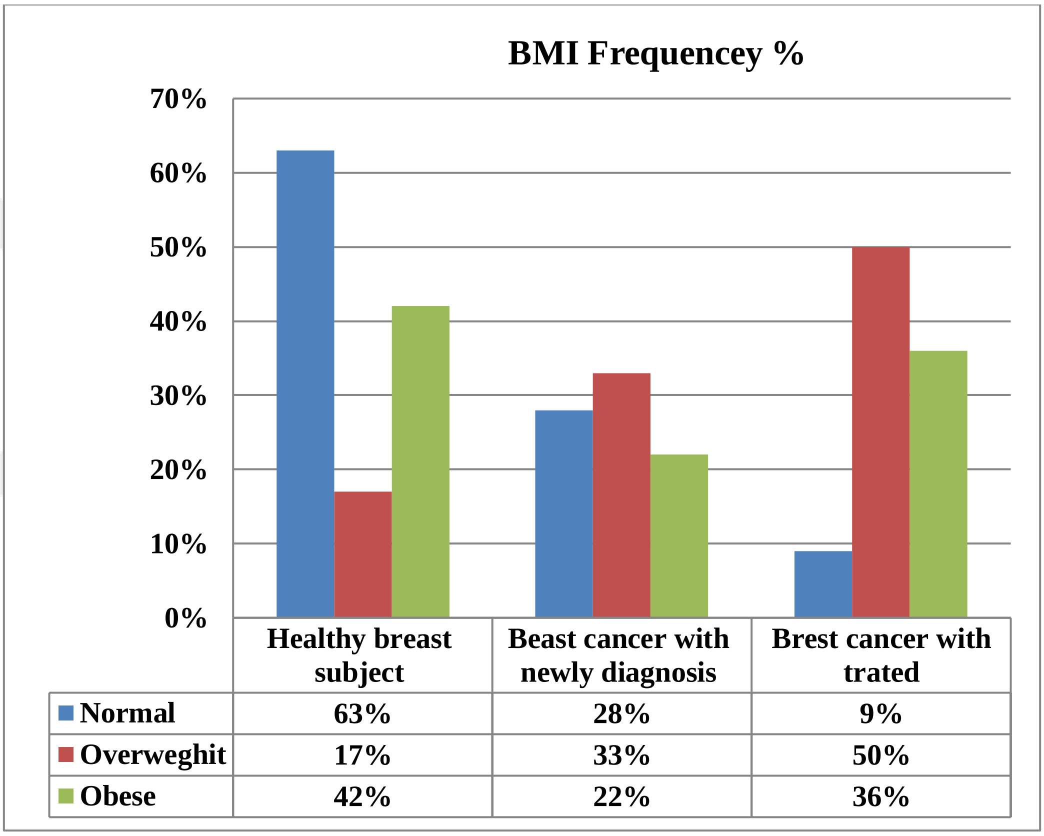


Figure 4.2 BMI (Kg/m²) frequencey in studied groups

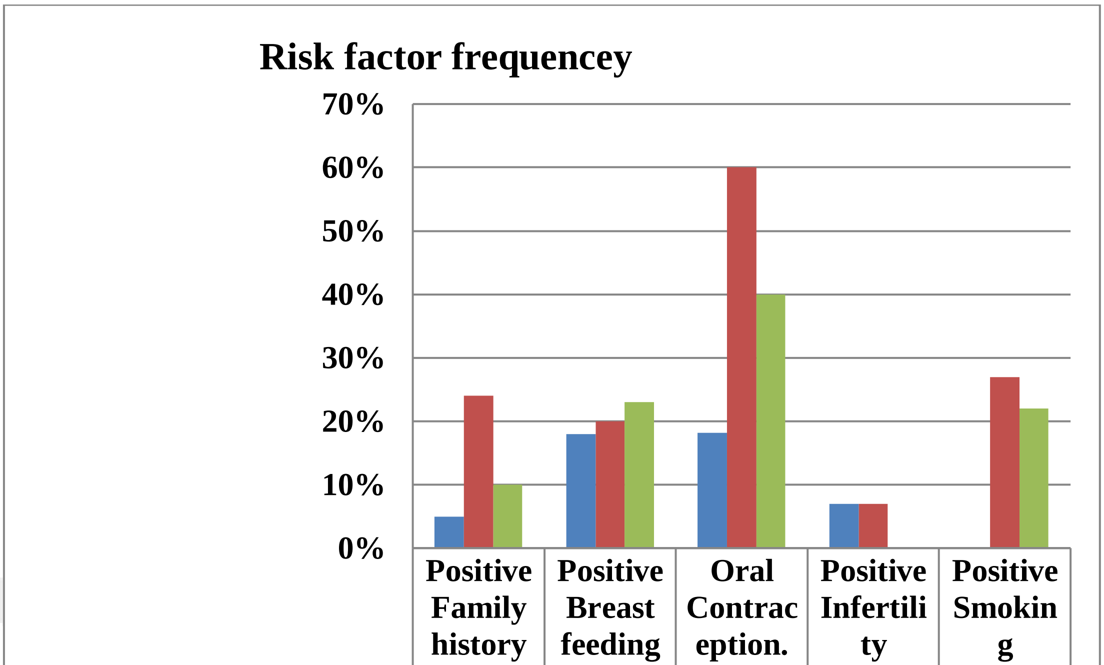
Adipose tissue in obese women contains estrogen, which increases the risk of breast cancer. Because women have more fatty tissue (obese), higher estrogen levels may encourage the production of breast cancer (Lorincz and Sukumar 2006). Increased body fat is connected with the metabolic syndrome, insulin resistance, and estrogen production, all of which raise the risk of breast cancer. Obesity and metabolic syndrome are associated with insulin and insulin-like growth factor-1 (IGF-1). Recent research focuses on the functions of autokinetic, paracrine, and endocrine adipocytes. Adipocytes are dynamic endocrine cells that emit hormone-like substances, cytokines, polypeptides, and other hormone-like molecules (Lahmann *et al.* 2004).

4.1.3 Risk Factor in present cancer

Other risk variables were noted as a result of the questions that were asked of the participants in this study There were substantial disparities among the major groups as shown in Table 4.3, with 12 patients (24 percent) having a positive family history of breast cancer and 38 cases (76 percent) having negative family history. Two other groups (P value < 0.05) were significantly different from normal breast and treated women (5% and 10%, respectively).

Table 4.3 Frequencey statistical examination of normal, malignant, and treated patients' risk factors

	Positive Family history	**Positive Breast feeding**	**Oral Contraception.**	**Positive Infertility**	**Positive Smoking**	**p-value**
Healthy subject	(5%)	(18.0%)	(18.2%)	(7%)	-	0.22
Newly diagnosis	(24 %)	(20 %)	(60%)	(7%)	(27%)	0.001
Treated group	(10 %)	23%	40 %	-	(22%)	0.31
p-value	0.032	0.11	P<0.001	0.44	0.76	



	Positive Family history	Positive Breast feeding	Oral Contraception.	Positive Infertility	Positive Smoking
Healthy breast subject	5%	18.00%	18.20%	7%	0%
Beast cancer with newly diagnosis	24%	20%	60%	7%	27%
Brest cancer with trated	10%	23%	40%	0%	22%

Figure 4.3 Statistical analysis of normal and malignant breast disease risk variables SPSS 10

Women with newly diagnosed breast cancer had similar rates of breastfeeding, oral contraceptives, infertility, and smoking compared to women with normal breast cancer (18%, 18%, 75%, 60 %). (p-value 0.005). Oral contraceptive use is intrinsically associated with natural breast cancer and newly diagnosed breast cancer, as shown in Figure 4.3. Inherited. However, not all patients have a family history of breast cancer (5-10%) (about three-quarters have a negative family history). Women with a first- or second-degree breast cancer history are much more certain to be have risk factors, according to many studies. If the mother or sister has it, the risk goes up by 1.5 to 3 times. The risk of breast cancer increased six-fold if both the mother and sister had breast cancer. Increased risk if this relative had both breast cancers or was diagnosed at a young age (Loman *et al.* 2011).

Breastfeeding was observed in 15 (24%) of the 80 breast cancer cases examined. This finding matched a recent Turkish study that indicated a non-significant relationship between breastfeeding and a lower risk of breast cancer. According to a 2016 study, normal breastfeeding have a vital role in decline of chance of breast cancer (Norsa adah *et al.* 2005) reported a strong association between breastfeeding habits and a lower incidence of breast cancer. For example, 60 percent of women newly diagnosed with breast cancer have used oral contraceptives at some point in their lives, compared to 18 percent of users in natural breasts and 40 percent of users in breast cancer treatment groups (Table 4.3).

Contraceptives may be lead to promote the chance of breast cancer by 7% compared to non-users, but not in postmenopausal women, these women were more likely to develop malignant breast cancer if they started using hormonal contraceptives before the age of 20 (Parkin 2011).

According to a prior study, the link between cancer and tobacco is inconclusive. Re-analysis of more than 80% of global epidemiological data by the collaborative group on hormonal factors in breast cancer found that smoking is no longer connected with breast cancer. Breast cancer risk was shown to be higher in Japanese women who had smoked. Neither the altered endogenous estrogen repute nor the distribution of positive genes related with metabolic enzymes could account for the differences between Japanese and non-Japanese women (Parkin 2011).

Infertility was found in 5 of the 50 breast cancer cases studied. There is no evidence of a connection between breast cancer and infertility in this study because it focused on postmenopausal infertility. Many studies had established a strong correlation between the two, but our data showed the opposite. Chemotherapy and/or radiotherapy are the most common methods of treating breast cancer. The reproductive system and consequently the ability harmed by certain medication, chemotherapy is the application of chemical agents to the destruction of malignant cells. Chemotherapy eliminates cancerous cells by targeting fast dividing cells. Healthy cells that divide fast, such as those involved in egg production, are similarly harmed by these medications. There are a number of factors that affect fertility, including the type of chemotherapy agent or medications used and the length of treatment. Chemotherapy has been known to briefly decrease fertility in certain women. However, it has the potential to be long-lasting (Giacalone *et al.* 2003).

4.2 ABO Blood Groups

When blood types were distributed according to the ABO system, there were no significant differences in the incidence of blood groups A, B, AB, and O among women of the three base groups (Table 4.4). Only 63.4 percent of newly diagnosed breast cancer patients belong to group A, but 43 percent of normal breast cancer patients and 22 percent of those undergoing treatment belong to group B. From Rh -) a strong relationship according to Rh.

Table 4.4 Distribution of blood groups according to ABO

	Blood group system			
	A	B	O	AB
Control (50)	(43%)	(18%)	(31%)	(6.6%)
Newly diagnosis (N=50)	% 50	(14%)	17%)	(6%)
Treated group (N= 50)	22%	(15%)	(27%)	(3%)

A study shown relationship of "Rhesus +ve" and "Rhesus -ve" blood groups and breast cancer. "Rhesus +ve" blood type is associated with high incidence of breast cancer (74.31%) and "Rhesus -ve" blood group has least association with breast cancer (26.68%) (Figure 4.4).

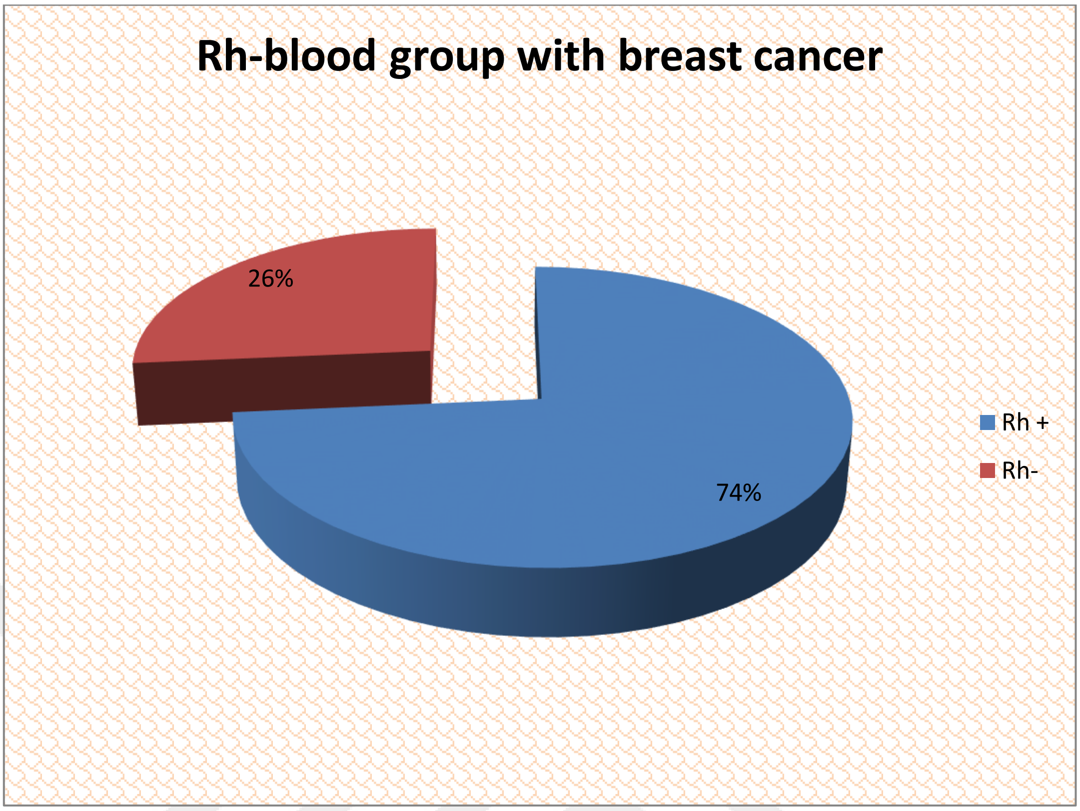


Figure 4.4 Rh-blood groups and incidence with breast cancer

Those with blood type A are more likely to develop breast cancer, while those with blood type AB are less likely to develop the disease. Breast cancer is more common in women with the 'Rhesus + ve' blood type.

Our progect demonstrated and agreement with Yuksel et al. Who found the involvement of ABO blood group in breast cancer risk. Among women with blood type A, cancer was detected in 44% of cases, 32% of cases, 16% of cases, 8% of cases, and 88% of women with blood type Rh +. Other researchers have investigated the relationship between blood type and breast cancer risk by conducting a study on the topic. 53.1 percent of females with blood group A, 21.8 percent of females with blood group O, 17.5 percent of females with blood group B, 7.5 percent of females with breast cancer. , according to the study. The study authors showed that blood type A is associated with an increased risk of breast cancer. A study by Akhtar et al. In India, I looked at the relationship between 'ABO' blood type and several types of cancer. ABO blood types and ages of each patient were recorded. Blood type A was more prevalent in breast cancer patients (42.4 percent).

4.3 WBC and present cancer

Results of hematological parameters include white blood cells count obtained from (50) breast cancer patients and (50)control. We find leukocytes count, lymphocytes count, monocytes count, and basophiles count are lower in patients than the control, with a significant difference (p value ≤ 0.05). While the eosinophils and neutrophils count count is significantly higher in patients than the control (p value ≤ 0.05), Table 4.5.

Table 4.5 Compaction of some blood parameters in patients with breast cancer with treatment and control group

Blood parameter	Group	No	Mean x 10^3	SD	P-value
WBC	Breast cancer	50	6.834	3.33	0.16
	Treatment	50	4.20	2.66	0.09
	Control	50	8.90	4.62	
Lymphocytes	Breast cancer	50	0.61	1.31	0.02
	Treatment	50	0.44	1.12	0.02
	Control	50	0.3	0.19	
Neutrophils	Breast cancer	50	2.19	0.861	0.001
	Treatment	50	1.85	0.69	0.001
	Control	50	1.97	0.74	
Monocytes	Breast cancer	50	0.081	0.052	0.012
	Treatment	50	0.099	0.04	0.02
	Control	50	0.09	0.55	
Eosinophils	Breast cancer	50	6.39	2.99	0.005
	Treatment	50	6.9	3.12	0.005
	Control	50	3.74	2.038	
Basophils	Breast cancer	50	0.286	0.27	0.03
	Treatment	50	0.280	0.273	0.03
	Control	50	0.33	0.91	

These screening tests are very accurate when done correctly, but none of them can detect all breast cancers. To increase the likelihood of detecting breast cancer early, when it is most curable, doctors are constantly looking for new signs associated with cancer. The immune system relies on white blood cells to fight infections and diseases. The number of white blood cells in your body increases when you are sick and your body tries to fight whatever it attacks. White blood cell counts in the body have long been studied to see if they are associated with an increased risk of certain diseases, such as breast cancer (Facione *et al.* 2000).

New research shows that chronic, low-grade inflammation has a significant impact on cancer development. There is a relationship between menopause, obesity and the development of breast cancer; Although this correlation depends on the status of menopause. Postmenopausal women who are obese are more likely to develop breast cancer. However, premenopausal women are at an increased risk of developing breast cancer due to obesity. According to our data, WBC counts were not associated with breast cancer burden in obese premenopausal women (Facione *et al.* 2000).

White blood cells (WBCs), which are characterized by their high levels of reactive oxygen species (ROS) and nitric oxide species (NOS), produce these chemical reactants (ROS) and NOS (NOS), if the antioxidant defence system fails to neutralize ROS and NOS insufficiently, they can damage cellular proteins, lipids, and DNA, leading to genomic instability, altering single nucleotide polymorphisms (SNPs), and regulating the PI3K-Akt pathway of carcinogenesis. Results have been inconsistent in large-scale studies investigating the relationship between WBC counts and breast cancer risk without stratification by menopause or obesity. Leukocyte count was found to be a predictor of breast cancer in a prospective analysis, but only postmenopausal women were included. According to Akinbami and his team in 2013, although menopausal status was not included in their investigations, WBC numbers were higher in breast cancer patients than in the control group. According to neutrophil counts were associated with breast cancer risk, especially stage IV breast cancer (Akinbami *et al.* 2013).

4.4 Results of CDK-5 and IL-2 in Studied Group

The distribution of Cdk5 protein levels in 90 breast cancer patient’s tumor specimens was shown in Table 4.6 (for the quantified results and supplementary level of Cdk5 protein levels in the 30 patients' tumor specimens with Cdk5 overexpression increasing in newly diagnosis whereas a decline with treatment (63 % from 40 treated patient have a low level of CDK-5 (17.2 ± 4.90).

Table 4.6 Distribution of CDK-5 in studied groups

Preast Cancer	CDK-5 LEVELS (ng/mL)					
	Newly	Low	Moderate	High	TOTAL	P-Value
	Breast cancer	15 (31%)	10 (19%)	25 (50%)	50 (100%)	0.03
	M ±SD	28.1±7.90	46±10.5	70.4± 20.8	68.0±12.1	
	Treatment	25 (63%)	8 (20%)	7 (17%)	40 (100%)	0.048
	M ±SD	17.2±4.90	20±5.3	56.1± 19.8	68.0±12.1	
Normal	Control	50 (100%)	-	-	50 (5.13%)	-
	M ±SD	1.66 ± 0.188			1.66 0.188	
	Range	0.3- 20 ng/mL				

The distribution of IL2 protein levels in 50 newly breast cancer patients' tumor sample was shown in Table 4.7 for the quantified results level of IL-2 protein levels in the 30 patients' tumor collect with IL-2 overexpressioly increaseing in newly diagnosis wherase a decline with treaement (80% from 40 treated patient have a low level of IL-2 (900 ± 191.9).

Table 4.7 Distrbution of IL-2 in studied group

Preast Cancer	IL-2 LEVELS (ng/mL)					
	Newly	Low	Moderate	High	Total	P-Value
	Breast cancer	9 (18%)	11 (22%)	30 (60%)	50 (100%)	0.03
	M ± SD	28.1 ±7.90	46±10.5	70.4± 20.8	68.0 ±12.1	
	Treatment	32 (80%)	8 (20%)	-	40 (100%)	0.001
	M ± SD	900 ±191.90	1380±195		68.0 ±12.1	
Normal	Control	50 (100%)	-	-	50 (100%)	-
	M ± SD	600 ± 122.3			600 ±122.3	
	Range	18.8-1,200 pg/mL				

Cancers, especially human malignancies, have been found to have higher levels of CDK5 Mechanisms that include angiogenesis and cell proliferation as well as the immune system are involved. Because CDK5 stimulates pRb phosphorylation and thus cell cycle progression, as previously reported. Apoptosis, DNA repair and cell cycle progression are regulated by CDK5. Researchers are investigating whether elevated levels of CDK5 protein targets could serve as markers of cancer. In breast cancer, for example, elevated CRMP2 phosphorylation could be a diagnostic (Shupp *et al.* 2017).

Additional variants, such as anti-apoptotic gene products of the Bcl-2 family, may contribute to tumorigenesis by stimulating cell growth and/or suppressing apoptosis, along with increased IL-2 production in breast cancer according to Lelle, the index increased as the number of cells in the benign breast tissue doubled, the work of (Konigsberg *et al.* 1999).

4.5 Results of Hormonal in Studied Group

Three major groups were studied: normal breast cancer, recently diagnosed breast cancer and breast cancer requiring therapy. Estradiol (E2), testosterone (T) and DHEA were found in the blood of all women in these three groups.

Blood was found to contain all of the biomarkers. A substantial statistical association was found between recently diagnosed (NMB) and normal breast (NB) estradiol concentrations and TBC with NB and the NMB, but not between TBC with NB.

This study found a significant statistical correlation between newly diagnosed malignancies (NMB) and normal breasts (NB) in terms of testosterone concentrations while finding no correlation may be shown in normal breasts (NB) in terms of testosterone concentrations during treatment.

Table 4.8 Estimation of some hormonal in studied groups

Biomarker	Healthy subject	Newly cancer	Treated	P-Value
E2 M ± SE	620.62±190.18	1122.43± 160.2	524.9 ± 117.3	P<0.001
Testo M ± SE	2.5 ± 1.05	8.34 ±1.32	3.8 ± 2.17	P<0.001
Dhea M ± SE	5.8 ± 1.22	6.11 ± 2.01	7. 61 ± 1.72	0.095

These data show no significant change in estradiol levels ($p > 0.05$) when comparing control groups with treatment (Table 4.8). Previous studies in Malaysia and Egypt support this conclusion. The serum E2 levels of premenopausal breast cancer patients increased, but this was not statistically significant. According to previous research, the mean E2 concentration in premenopausal women increased but was not statistically significant compared to the control group. An environment with a high estrogen content affects the biological characteristics of breast cancer and thus its clinical course. Since estrogen enhances proliferation while decreasing apoptosis, it may aid the proliferation of cells with genetic defects. Postmenopausal women with higher amounts of estrogen in their blood have an increased risk of developing breast cancer. Women with raised estradiol levels did not have an increased risk of developing breast cancer as a result of this study's findings, which corroborate this hypothesis (Dorgan *et al.* 2010).

Newly diagnosed (NMB) and normal breast (NB) had a significant relationship, but TBC and NB had no ties. There has been a long history of testosterone (T). Although it is the most abundant and biologically active hormone in women, the importance lies in the "male" hormone. In the ovaries, adrenal glands and cells, testosterone is produced in huge quantities (more than 50%) from steroid precursors. Androgen receptors (AR) can be found in practically every tissue and organ in the body, including the breast. Testosterone and its active metabolite, trans-testosterone, directly affect AR. Estradiol (E2) is synthesized from testosterone by an aromatizing process, which indirectly affects the estrogen receptor (ER). Testosterone levels must be adequate for optimal mental and physical health, immune function, glycemic regulation, and reduced inflammation. Women with low levels of testosterone have received little attention. In their twenties, both men and women had high testosterone levels and maternal hormones, which declined with age. Symptoms of androgen insufficiency can begin in middle-aged women, clinicians are just beginning to understand the advantages of testosterone supplementation in this group, and subcutaneous testosterone injections have been used successfully for more than 70 years in both sexes. A significant source of E2 in postmenopausal women is the androgen released from the implants by aromatization (Theut Riis *et al.* 2016).

Present study shown no correlation or any change in DHEAas compared in studied group, therfore our finding dissagrement with Labrie and his friends, they studied that refere to C19 adreno stimulants appear to play an essential role in modulating breast cancer rates in humans. A 100-fold higher amount of dehydroepiandrosterone sulfate (DHEAS) was detected in breast secretions collected through nipple suction compared to plasma, indicating that DHEA levels are elevated in normal breast tissue and in benign and malignant carcinomas. The elevated levels of DHEAS also confirmed the ability of breast tissue to synthesize C19 adrenal steroids and DHEA detected in breast sac fluid compared to plasma layers. There is doubt as to where the C19-derived adrenal steroids originate. Plasma-based attention and a priori-indicator shifting are possible hypotheses. DHEA, androstenedione, and rose-five-ene-three and 17-diol have been uptaken by breast cancer tissues from plasma. The sulfatase pathway is used by malignant breast tissue to convert DHEAS to DHEA. It is not known precisely how much and what type of C19 adrenal steroids are produced in the breast from plasma (Labrie *et al.* 2000).

No one knows why extensive prospective studies provide such a mixed set of results, but even though premenopausal women in these studies had high estrogen levels, comprehensive future studies have consistently identified a link between elevated estrogen concentrations. Dehydroepiandrosterone and breast cancer indicate that these hormones may not act as anti-estrogens in premenopausal women (Way *et al.* 2004).

5. CONCLUSIONS AND RECOMMENDATIONS

According to the findings of this research, it is possible to draw the following conclusions:

4.1 Conclusions

1- Estradiol levels have been linked to an increased risk of breast cancer.

2- Increasing age, particularly above the age of 55, was associated with an increased risk of breast cancer.

3- IL-2 and CDK-5 chain expression appear to be associated with the formation of breast tumors, and this expression also appears to be linked to the tumor's aggressiveness, according to recent findings.

4- There was a statistically significant link between estradiol concentrations in newly diagnosed malignancies and normal breasts (NMB) while no such correlation was seen between TBC and NMB in patients with newly diagnosed malignancies and those who were treated.

4.2 Recommendations

1- A correlation between malignant growth and hormonal status has been found between free testosterone, prolactin, and insulin like growth factor (ILGF) levels in breast cancer patients.

2- Molecular techniques for malignant growth analysis (constant PCR, Microarray and tissue culture), for example.

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CURRICULUM VITAE

Personal Information

Name and Surname : Esraa Qaddoori Abttan ABTTAN

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Bio Chemistry	2022-Present
Undergraduate	Al-Anbar University College of Science Department of Chemistry	2005-2009