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The Impact of Leptin and Ferritin on the Severity of Breast Cancer in Obese Postmenopausal Women

A Thesis

**Submitted to the Council of College of Medicine, University of Babylon
in Partial Fulfillment of the Requirements for the Degree of
Master in Science/ Medical Physiology**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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صَدَقَ اللَّهُ الْعَلِيِّ الْعَظِيمِ

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Dedication

To my family.....

Sarah

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Summary

Leptin and ferritin are important parameters that have an association with more severe cases of breast cancer in postmenopausal women especially obese ones. Obese women with breast cancer tend to have poor prognostic features at the time of presentation compared to non-obese and were associated with advanced stage and grade of cancer at diagnosis. This work had aimed to examine the role of elevated levels of leptin and ferritin in postmenopausal women with breast cancer and its relationship with increased body mass index and to correlate their levels on the severity of the disease.

This cross-sectional study was conducted on 240 postmenopausal women diagnosed with breast cancer who were then divided into two groups according to the scores of their body mass indexes.

The study extended from the beginning of September 2022 to the end of March 2023. Participants in this study were collected from Babylon Oncology Treatment Center in Merjan Medical City in Al-Hillah City, Babylon Governorate.

Ferritin values were determined by the use of the ElectroChemiLuminescence method with Roche Cobas E411 auto-analyzer system, while leptin levels were measured by Enzyme-Linked Immunosorbent Assay technique using human leptin ELISA kit.

Statistically significant differences were present between the study groups regarding the levels of leptin and ferritin (for leptin, p value=0.001 and for ferritin, p value=0.0001).

Random blood sugar and hemoglobin A1C values also showed significant differences between the patients groups (for random blood sugar, p value=0.011 and for hemoglobin A1C, p value=0.007).

| Summary -----

Age, stage, grade and immunohistochemistry of breast cancer all of them showed statistically significant differences between the studied groups. For age distribution, p value=0.008. P values=0.033 for cancer's stage and 0.0001 for cancer's grade; while for immunohistochemistry, p value=0.002.

Strongly positive correlations were found between random blood sugar and hemoglobin A1C ($r=0.615$, $p=0.0001$); between random blood sugar and leptin ($r=0.141$, $p=0.029$) and between hemoglobin A1C and leptin ($r=0.168$, $p=0.009$).

Other measured parameters (subtype of breast cancer, history of hypertension and diabetes, history of medication usage, hemoglobin, packed cell volume, mean cell volume and mean cell hemoglobin concentration) all showed no significant differences between the study groups.

To conclude, increased body mass index is a major risk factor studied for breast cancer in postmenopausal women and measuring the levels of leptin and ferritin can predict the severity of this cancer in those patients.

Table of Contents

No.	Subject	Page
	Summary	I
	Table of contents	III
	List of tables	VII
	List of figures	VIII
	List of abbreviations	IX
Chapter One: Introduction		1-2
1.1.	Introduction	1
1.2.	Aim of the study	2
Chapter Two: Review of Literature		3-25
2.1.	Physiology of the breast	3
2.2.	Breast cancer	3
2.2.1.	Types	4
2.2.2.	Incidence	5
2.2.3.	Mortality	5
2.2.4.	Risk factors	6
2.2.4.1.	Non-modifiable factors	6
2.2.4.1.1.	Sex	6
2.2.4.1.2	Age	6
2.2.4.1.3.	Family history	7
2.2.4.1.4.	Genetic mutations	7
2.2.4.1.5.	Race/ ethnicity	8
2.2.4.1.6.	Reproductive factors	8
2.2.4.1.7.	History of breast cancer and benign breast diseases	9
2.2.4.1.8.	Blood group	9
2.2.4.2.	Modifiable factors	9
2.2.4.2.1.	Hormonal factors	9

2.2.4.2.1.1.	Contraceptive methods	9
2.2.4.2.1.2.	Ovulation-stimulating drugs	10
2.2.4.2.1.3.	Hormone replacement therapy	10
2.2.4.2.2.	Obesity and overweight	10
2.2.4.2.3.	Physical activity	10
2.2.4.2.4.	Insufficient vitamin supplementation	10
2.2.5.	Classification of breast cancer	11
2.2.5.1.	Histological classification	11
2.2.5.2.	Luminal breast cancer	11
2.2.5.3.	Human epidermal growth factor receptor 2-enriched breast cancer	12
2.2.5.4.	Triple-negative breast cancer	12
2.2.6.	Prognostic biomarkers	13
2.2.6.1.	Estrogen receptor	13
2.2.6.2.	Progesterone receptor	14
2.2.6.3.	Human epidermal growth factor receptor 2	14
2.2.7.	Tumor staging (TNM classification)	14
2.3.	Obesity	16
2.3.1.	Main mechanisms involved in obesity-associated breast cancer	17
2.3.1.1.	Chronic inflammation	17
2.3.1.2.	Sex hormone deregulation	17
2.3.1.3.	Insulin	18
2.3.1.4.	Adipokines	18
2.3.1.4.1.	Leptin and breast cancer	19
2.3.1.4.2.	Leptin receptors	21
2.3.1.4.3.	Leptin and estrogen	21
2.3.1.4.4.	Leptin and therapeutic targets in breast cancer	22

2.4.	Diabetes and breast cancer	22
2.4.1.	Diabetes and breast cancer: The molecular link	24
2.4.2.	Insulin and insulin-like growth factor	25
Chapter Three: Materials and Methods		26-32
3.1.	Materials	26
3.1.1.	Patients	26
3.1.2.	Sample size calculation	26
3.1.3.	Inclusion and exclusion criteria	27
3.1.3.1.	Inclusion criteria	27
3.1.3.2.	Exclusion criteria	27
3.1.4.	Ethical approval and consent	27
3.1.5.	Confirmation of the diagnosis	27
3.1.6.	Laboratory apparatuses, instruments and disposable materials	28
3.1.7.	Kits	29
3.2.	Methods	29
3.2.1.	History	29
3.2.2.	Blood collection and serum preparation	30
3.2.3.	Hematological and biochemical studies	31
3.2.3.1.	Hematological studies	31
3.2.3.2.	Biochemical studies	31
3.2.3.2.1.	Random blood sugar	31
3.2.3.2.2.	Hemoglobin A1C	31
3.2.3.2.3.	Serum ferritin	31
3.2.3.2.4.	Serum leptin	32
3.3.	Statistical analysis	32
Chapter Four: Results		33-46
4.1.	Age distribution	34
4.2.	Staging and grading of breast cancer	34

4.3.	Subtype and immunohistochemistry of breast cancer	37
4.4.	History of diabetes mellitus and hypertension	39
4.5.	History of medication usage	40
4.6.	Hematological parameters	41
4.7.	Random blood sugar and hemoglobin A1C	42
4.8.	Leptin and ferritin	43
4.9.	Correlation analyses	44
4.9.1.	Correlation between RBS and HbA1C	44
4.9.2.	Correlation between RBS and leptin	44
4.9.3.	Correlation between HbA1C and leptin	45
Chapter Five: Discussion		47-62
5.1.	Age distribution	48
5.2.	Staging and grading of breast cancer	49
5.3.	Subtype and immunohistochemistry of breast cancer	51
5.4.	History of diabetes mellitus and hypertension	52
5.5.	History of medication usage	53
5.6.	Hematological parameters	54
5.7.	Random blood sugar and hemoglobin A1C	55
5.8.	Leptin and ferritin	57
5.9.	Correlation analyses	59
5.9.1.	Correlation between RBS and HbA1C	59
5.9.2.	Correlation between RBS and leptin	60
5.9.3.	Correlation between HbA1C and leptin	61
Chapter Six: Conclusions and Recommendations		63-63
6.1.	Conclusions	63
6.2.	Recommendations	63
Chapter Seven: References		64-86

List of Tables

Table No.	Title	Page
3-1-A	Scientific laboratory apparatuses	28
3-1-B	Technical instruments and disposable materials	28
3-2	Biochemical kits used in this study with their companies and countries of origin	29
3-3	Enzyme-linked immunosorbent assay (ELISA) kits used in this study with their companies and countries of origin	29
4-1	Age distribution for the study groups	34
4-2	Distribution of breast cancer's stage among the study groups	35
4-3	Distribution of breast cancer's grade among the study groups	36
4-4	Distribution of breast cancer's subtype among the study groups	37
4-5	Distribution of breast cancer's immunohistochemistry among the study groups	38
4-6	Distribution of clinical history among the study groups	40
4-7	Distribution of medication usage among the study groups	41
4-8	Values of hematological parameters for the study groups	42
4-9	Values of random blood sugar and hemoglobin A1C for the study groups	43
4-10	Values of leptin and ferritin for the study groups	43

List of Figures

Figure No.	Title	Page
2-1	The association of multiple cancer types with BRCA1 and BRCA2 mutation.	8
2-2	Classification of molecular subtypes of breast cancer	13
2-3	American Joint Committee on Cancer's Staging System for Breast Cancer, Eighth Edition.	15
3-1	File sheet questionnaire	30
4-1	Distribution of the study's patients according to body mass index (BMI). Group 1 ($BMI \geq 30 \text{ Kg/m}^2$); Group 2 ($BMI < 30 \text{ Kg/m}^2$).	33
4-2	Frequency of cancer's stage among the two groups of breast cancer patients	35
4-3	Frequency of cancer's grade among the two groups of breast cancer patients	36
4-4	Frequency of cancer's subtype among the two groups of breast cancer patients	38
4-5	Frequency of cancer's immunohistochemistry among the two groups of breast cancer patients	39
4-6	Correlation between random blood sugar (RBS) and hemoglobin A1C (HbA1C) for the patients of the study	44
4-7	Correlation between random blood sugar (RBS) and leptin for the patients of the study	45
4-8	Correlation between hemoglobin A1C (HbA1C) and leptin for the patients of the study	46

List of Abbreviations

Abbreviation	Key
ACS	American Cancer Society
ADA	American Diabetes Association
AJCC	American Joint Committee on Cancer
BMI	Body mass index
BC	Breast cancer
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CYP450	Cytochrome p450
DM	Diabetes mellitus
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Estrogen receptor
Hb	Hemoglobin
HbA1C	Hemoglobin A1C
HER2	Human epidermal growth factor receptor 2
HRT	Hormone replacement therapy
HT	Hormonal therapy
IDC	Invasive ductal cancer
IGF-1	Insulin-like growth factor 1
IR	Insulin resistance
ILC	Invasive lobular cancer
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
OCP	Oral contraceptives
PCV	Packed cell volume

PR	Progesterone receptor
RBS	Random blood sugar
T2DM	Type 2 diabetes mellitus
TNBC	Triple-negative breast cancer
TNM	Tumor, lymph node, and metastasis
WHO	World Health Organization

Chapter One

Introduction

Chapter One

Introduction

1.1. Introduction:

Breast cancer is currently one of the most prevalent diagnosed cancers and the 5th cause of cancer-related deaths with an estimated number of 2.3 million new cases worldwide according to the GLOBOCAN 2020 data (Sung *et al.*, 2021).

According to the Iraqi Cancer Registry report of 2021, breast cancer is the most prevalent malignant tumor, with an incidence rate of about 22.2% (GLOBOCAN, 2021).

Additionally, breast cancer is regarded as the second-leading cause of death from malignancy in the entire Iraqi population, and the first leading cause of death from malignancy in Iraqi women (Alrawi, 2022).

The World Health Organization (WHO) defines overweight and obesity as abnormal or excessive fat accumulation that presents a risk to health. Body mass index (BMI) is a simple metric used to indicate overall body fatness was calculated using patient's height and weight (BMI = weight in kg/height in meter²). BMI was classified according to the WHO into underweight (BMI <18.5); normal weight (BMI 18.5-24.9); overweight (BMI 25.0-29.9) and obese (≥ 30) (WHO, 2015).

Several mechanisms have been proposed for the increased risk of breast cancer development, including common risk factors (such as obesity), the specific metabolic abnormalities of diabetes (i.e., hyperglycemia, hyperinsulinemia, and insulin resistance), and the use of insulin, particularly insulin analogs (Overbeek *et al.*, 2019).

The local and systemic alterations induced by obesity may influence breast cancer through direct effects on neoplastic epithelial cells as well as indirect effects on tumor microenvironment. All these obesity-related factors can impact tumor initiation, metabolic reprogramming, angiogenesis, progression, and/or response to therapy (Kang *et al.*, 2017).

Leptin, an adipokines that's produced by adipose tissue. Leptin has several biological effects in addition to its neuroendocrine function, including the development of breast tumors. The ability of this adipokine to influence various aspects of breast cancer biology in an endocrine, paracrine, and autocrine manner has been demonstrated clearly. It is primarily produced by distant and local adipocytes, but it is also produced by epithelial tumor cells as well as by other cells within the tumor-stroma (i.e., cancer associated fibroblasts) (Andò *et al.*, 2014; Giordano *et al.*, 2016).

Ferritin, which is the iron storage form within cells and is also found in the circulation. Ferritin levels in the body and tissues rise in breast cancer and are linked to disease development (George *et al.*, 2021).

Systemic ferritin is a marker of inflammation or the body's iron reserves. Elevated ferritin levels in breast cancer patients' blood and tumor tissues were associated with a poor prognosis and an advanced histological grade (Alkhateeb *et al.*, 2013).

1.2. Aim of the study:

This study aims to investigate whether elevated levels of leptin and ferritin are correlated to a more severe form of breast cancer in those women.

Chapter Two
Review of Literature

Chapter Two

Review of Literature

2.1. Physiology of the breast:

The mammary gland is a highly evolved and specialized organ present in pairs, one on each side of the anterior chest wall. The organ's primary function is to secrete milk. Though it is present in both sexes, it is well developed in females and rudimentary in males. It is also a vital accessory organ of the female reproductive system. The mammary gland is situated in the pectoral region in the superficial fascia. However, a part of it called the axillary tail pierces the deep fascia and lies in the axilla. The parenchyma of the glandular tissue of the mammary gland is made up of branching ducts and terminal secretory lobules. There are 15 to 20 lobes, and a lactiferous duct drains each of them. These ducts enlarge to form the lactiferous sinus before they open separately into the nipple. The milk is collected in the lactiferous sinuses and let out on the suckling of the baby. The lactiferous ducts are arranged radially in the nipple. Hence the surgeon needs to take radial incisions to avoid cutting through multiple lactiferous ducts (NIH, 2022).

2.2. Breast cancer:

Cancer is one of the leading causes of death globally (Momenimovahed and Salehiniya, 2017). Malignant diseases are thought to have caused 8 million fatalities in 2008, and by 2030, that number is expected to rise to 11 million (Benson and Jatoui, 2012).

An important global health issue is breast cancer, which is the most often diagnosed cancer worldwide with an expected 2.26 million cases in 2020 and the main cause of cancer death among women. Over half of

breast cancer diagnosis and two-third of breast cancer-related fatalities occur in the less developed countries in 2020, despite previously being thought of as a disease of mostly developed countries (Sung *et al.*, 2021).

2.2.1. Types:

Various factors such as etiology, clinical presentation, molecular characteristics, and response to treatment, divide the breast cancer into different groups (Li *et al.*, 2017).

Breast cancers are classified based on their molecular signature determined by the absence or presence of receptors, which determines the type of therapeutic intervention (Rivenbark *et al.*, 2013).

The most generally used categorization of invasive breast cancer is the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), which is used to give treatment methods and predict clinical outcomes (Momenimovahed and Salehiniya, 2019).

The presence/ overexpression of either one or a combination of the hormonal receptors; ER, PR and HER2 render these types of breast cancers susceptible to hormonal/ HER2 targeted therapy (Mohamed *et al.*, 2013). The receptor status thus has a major implication on therapy and prognosis among breast cancer patients.

The prevalence of various hormone receptor-positive breast cancers varies by different regions. Milne *et al.* (2017) stated that there is a link between BRCA1 mutation carriers and the development of triple negative breast cancer (TNBC).

The heterogeneity in breast cancer with wide variations in gene expression determines the course of the disease, response to and the clinical outcome of treatment modalities and patient survival (Rivenbark *et al.*, 2013).

2.2.2. Incidence:

Every American woman's lifetime risk of developing breast cancer is 12.4%, or one in eight (Desantis *et al.*, 2017).

In the last 25 years, the incidence of breast cancer has been increased more than 30% in western countries. Although this is due in part to changes in reproductive patterns and improved detection tools, it may also be due to the rising incidence of obesity and physical inactivity in the population (Matthews and Thompson, 2016).

The incidence rate of breast cancer is higher in developed countries, and it's varies greatly with race and ethnicity (DeSantis *et al.*, 2014). It's also varies among different parts of the world (Ghoncheh *et al.*, 2016).

In the United States in 2017, around 252,710 new cases of invasive breast cancer and 6,341 cases of breast cancer in situ were diagnosed (Desantis *et al.*, 2017).

2.2.3. Mortality:

Breast cancer was the second cause of death in developed countries after lung cancer, with 197,000 deaths accounting for 15.4% of all deaths (Ferley *et al.*, 2015).

By 2020, breast cancer death rates are expected to rise in various regions of Europe (Clèries *et al.*, 2018). Although the frequency of breast cancer is higher in developed countries, higher fatality rates are observed in less developed regions (Ghoncheh *et al.*, 2016).

In 2017, 89% of breast cancer fatalities in the United States happened in women aged 50 and older (Desantis *et al.*, 2017). Breast cancer death rates in high-income countries have decreased significantly as a result of improved treatment and diagnostic procedures and promotion of breast cancer care (Carioli *et al.*, 2018).

2.2.4. Risk factors:

There are many risk factors for breast cancer, which include both modifiable and non-modifiable factors.

2.2.4.1. Non-modifiable factors:

2.2.4.1.1. Sex:

Female sex is one of the key variables linked with an increased risk of breast cancer, owing to greater hormonal stimulation. Women have breast cells that are extremely sensitive to hormones (estrogen and progesterone in particular) as well as to any changes in their balance, in contrast to males who exhibit negligible estrogen levels (HEBCCG, 2013).

Breast cancer in males is an uncommon illness that tends to be more advanced at the time of diagnosis than in women, about less than 1% of all breast cancers occur in men. However; older age, BRCA1/BRCA2 mutations, elevated estrogen levels, Klinefelter syndrome, a family history of breast cancer, and radiation exposure are significant risk factors for breast cancer in men (Giordano, 2018).

2.2.4.1.2. Age:

Currently, almost 80% of breast cancer patients are over the age of 50 years, while more than 40% are over the age of 65 years (Siegel *et al.*, 2014; McGuire *et al.*, 2015).

Age is the most important recognized risk factor for breast cancer (Thakur *et al.*, 2017). Breast cancer incidence increases dramatically with age, peaks during menopause, and then progressively declines or remains constant (Kim *et al.*, 2015).

A link was discovered between a certain molecular subtype of cancer and a patient's age: aggressive resistant triple-negative breast

cancer is most usually identified in groups under 40 years old, whereas luminal breast cancer is most typically diagnosed in patients over 70 years (McGuire *et al.*, 2015).

2.2.4.1.3. Family history:

One of the major risk factors for breast cancer that has been noted in many research is a family history of the disease (Bhadoria *et al.*, 2013; Thakur *et al.*, 2017).

Regardless of age, the incidence of breast cancer is much increased in all individuals with a family history. This relationship is fueled by epigenetic modifications as well as environmental variables that function as potential triggers (Wu *et al.*, 2018).

A higher risk of breast cancer may also result from a family history of ovarian cancer, especially with BRCA1 or BRCA2 mutations (Çelik *et al.*, 2015).

2.2.4.1.4. Genetic mutations:

A number of genetic mutations have been linked to an increased risk of breast cancer. BRCA1 and BRCA2 (located on chromosome 17 and chromosome 13 respectively), are two key genes with strong penetrance (Shiovitz and Korde, 2015). Mutations in the BRCA1 and BRCA2 genes inherited through the autosomal dominant method and are responsible for approximately 40% of hereditary breast cancer cases (Cobain *et al.*, 2016).

According to a study's findings, between 55% and 65% of BRCA1 mutation carriers and 40% of BRCA2 mutation carriers would acquire breast cancer by the age of 70 (Godet and Gilkes, 2017).

Cancer Type	General Population Risk	Mutation Risk	
		BRCA1	BRCA2
Breast	12%	50%-80%	40%-70%
Second primary breast	3.5% within 5 years Up to 11%	27% within 5 yrs	12% within 5 yrs 40%-50% at 20 yrs
Ovarian	1%-2%	24%-40%	11%-18%
Male breast	0.1%	1%-2%	5%-10%
Prostate	15% (N. European origin) 18% (African Americans)	<30%	<39%
Pancreatic	0.50%	1%-3%	2%-7%

Figure (2-1) demonstrate the association of multiple cancer types with BRCA1 and BRCA2 mutation.

2.2.4.1.5. Race/ ethnicity:

There are still significant racial and ethnic disparities among breast cancer patients, but the mechanisms associated with this phenomenon are not clear yet. In general, white non-Hispanic women continue to have the greatest prevalence of breast cancer (Hill *et al.*, 2019; Yedjou *et al.*, 2019). In contrast, the death rate from breast cancer is substantially greater in black women, who also have the lowest survival rates (ACS, 2014).

2.2.4.1.6. Reproductive factors:

The link between reproductive factors and breast cancer is due to the action of ovarian hormones, which begin during puberty and persist throughout the monthly cycle. These hormones are also affected by the

number of pregnancies and ultimately decrease in menopause (Thakur *et al.*, 2017).

2.2.4.1.7. History of breast cancer and benign breast diseases:

Previous history of breast cancer is associated with an increased risk of recurrence within the breasts (Schacht *et al.*, 2014). Besides, a history of any other non-malignant diseases such as atypical hyperplasia, carcinoma in situ, or many other proliferative or non-proliferative lesions, also increases the risk of breast cancer significantly (Dyrstad *et al.*, 2015).

2.2.4.1.8. Blood group:

The results of a review study showed that women with blood group A and Rhesus positive have a higher risk of developing breast cancer, while women with blood group AB and Rhesus negative have a lower risk of developing breast cancer (Meo *et al.*, 2017).

2.2.4.2. Modifiable factors:

2.2.4.2.1. Hormonal factors:

2.2.4.2.1.1. Contraceptive methods:

Several research have looked into the role of contraceptive pills in the occurrence of breast cancer (Beaber *et al.*, 2014). The use of oral contraceptive pill is linked to a higher chance of getting breast cancer. (Bhadoria *et al.*, 2013).

Williams *et al.* (2018) showed a positive relationship between the current use of contraceptives and lobular breast cancer. The risk of developing breast cancer decreases 5-10 years after the use of hormonal contraception is discontinued (Zolfaroli *et al.*, 2018).

2.2.4.2.1.2. Ovulation-stimulating drugs:

Using ovulation-stimulating drugs for more than 6 months raises the chance of getting breast cancer (Taheripanah *et al.*, 2018).

2.2.4.2.1.3. Hormone replacement therapy:

Numerous studies show that females who utilize hormone replacement therapy (HRT) for more than 5 or 7 years are at an elevated risk of developing breast cancer (Narod, 2011; Vinogradova *et al.*, 2020).

2.2.4.2.2. Obesity and overweight:

Obese postmenopausal women (BMI 30 kg/m²) had a worse disease-free survival and overall survival than non-obese women at the time of breast cancer diagnosis (Scholz *et al.*, 2015).

Increased body fat may exacerbate the inflammatory state and change the levels of circulating hormones, allowing pro-carcinogenic processes to occur (James *et al.*, 2015).

Body mass index-related increased breast cancer risk is also correlated with concurrent family history of breast cancer (Hopper *et al.*, 2018).

2.2.4.2.3. Physical activity:

Regular physical exercise is thought to be a preventive factor against the occurrence of breast cancer, even if the mechanism is not yet understood (Kyu *et al.*, 2016).

2.2.4.2.4. Insufficient vitamin supplementation:

High levels of 25-hydroxy vitamin D are linked to a decreased risk of breast cancer in both pre and postmenopausal women (Atoum and Alzoughool, 2017; Estébanez *et al.*, 2018). Also an increased expression

of vitamin D receptors has been linked to decreased breast cancer death rates (Huss *et al.*, 2019).

2.2.5. Classification of breast cancer:

2.2.5.1. Histological classification:

About 25% of invasive breast tumors have different developmental patterns and cytological features, hence they are classified as separate subtypes (e.g., invasive lobular carcinoma, tubular, mucinous A, mucinous B, neuroendocrine) (Erber and Hartmann, 2020).

2.2.5.2. Luminal breast cancer:

Luminal breast cancers are ER-positive tumors that account for over 70% of all breast cancer cases in Western countries (Howlader *et al.*, 2014).

Luminal-like tumors are divided into Luminal A and B subtypes which has different clinical outcomes. The presence of ER and/ or PR and the lack of HER2 characterize Luminal A subtype. In this subtype, ER transcription factors activate genes that are expressed in luminal epithelium lining the mammary ducts (Prat *et al.*, 2013). They are clinically low-grade, slow growing, and have the best prognosis.

Luminal B subtype are ER positive and may be PR negative and/or HER2 positive. In contrast to luminal A, Luminal B tumors are higher grade and has worse prognosis. (Ades *et al.*, 2014; Raj-Kumar *et al.*, 2019).

2.2.5.3. Human epidermal growth factor receptor 2-enriched breast cancer:

The HER2-enriched group accounts for 10-15% of breast cancers. It is characterized by strong HER2 expression in the absence of ER and PR (Roberts *et al.*, 2013; Raj-Kumar *et al.*, 2019)

Prior to the development of HER2-targeted medicines, HER2-enriched tumors grew faster than luminal tumors and had the worst prognosis of any subtype (Plasilova *et al.*, 2016).

2.2.5.4. Triple-negative breast cancer:

Triple-negative breast cancer (TNBC) is a diverse group of breast cancers that are ER-negative, PR-negative, and HER2-negative. They account for about 20% of all breast cancers. Triple-negative breast cancer is more frequent in women under the age of 40 and in African-American women (Plasilova *et al.*, 2016).

Triple-negative breast cancer account for the vast majority (about 80%) of breast cancers caused by BRCA1 germline mutations, whereas 11-16% of all TNBC have BRCA1 or BRCA2 germline mutations. It is an aggressive disease and it's often associated with a poor prognosis (Newman *et al.*, 2015).

Triple-negative breast cancers account for 10-30% of all breast cancers are associated with younger age and higher grade at diagnosis, and do not benefit from hormonal or HER2 targeted therapies since they lack ER, PR and HER2 receptors (Hon *et al.*, 2016; Bianchini *et al.*, 2016).

Molecular Subtypes	Luminal A	Luminal B		HER2+	TN
		(HER2-)	(HER2+)		
Biomarkers	ER+ PR+ HER2- Ki67low	ER+ PR- HER2- Ki67high	ER+ PR-/+ HER2+ Ki67low/high	ER- PR- HER2+ Ki67high	ER- PR- HER2- Ki67high
Frequency of Cases (%)	40–50	20–30		15–20	10–20
Histological Grade	Well Differentiated (Grade I)	Moderately Differentiated (Grade II)		Little Differentiated (Grade III)	Little Differentiated (Grade III)
Prognosis	Good	Intermediate		Poor	Poor
Response to Therapies	Endocrine	Endocrine Chemotherapy	Endocrine Chemotherapy Target Therapy	Target Therapy Chemotherapy	Chemotherapy PARP Inhibitors

ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.

Figure (2-2) classification of molecular subtypes of breast cancer.

2.2.6. Prognostic biomarkers:

2.2.6.1. Estrogen receptor:

Estrogen receptor (ER) expression is an essential diagnostic factor since it is found in about 70-75% of invasive breast cancer (Colomer *et al.*, 2018; Li *et al.*, 2020). The assessment of ER expression in both primary invasive tumors and recurrent lesions is required in current practice. This approach is necessary to identify the patients who will get benefit from the use of endocrine treatment, particularly selective estrogen receptor modulators, pure estrogen receptor down-regulators, or third-generation aromatase inhibitors (Duffy *et al.*, 2017).

Patients with high ER expression often have much better clinical outcomes, therefore even while the diagnosis of altered ER expression is extremely important for choosing the right medication, ER expression may also serve as a prognostic factor (Nasrazadani *et al.*, 2018).

A link was discovered between ER expression and a family history of breast cancer, which expands the value of ER expression as a diagnostic biomarker of breast cancer, particularly in situations of familial risk (Tse *et al.*, 2015).

2.2.6.2. Progesterone receptor:

Progesterone receptor is overexpressed (>50%) in ER-positive breast cancer patients and rarely in ER-negative breast cancer patients. Progesterone receptor expression is regulated by ER therefore, physiological values of PR inform about the functional ER pathway (Obr and Edwards, 2012).

However, both ER and PR are widely expressed in breast cancer cells and are used as diagnostic and prognostic indicators (especially ER-positive subtypes) (Wu *et al.*, 2020).

Over expression of PR is significantly correlated with overall survival, time to recurrence, and time to treatment failure or advancement, whereas lower PR levels are often linked with a more aggressive course of the illness and worse prognosis and recurrence rates (Patani *et al.*, 2013).

2.2.6.3. Human epidermal growth factor receptor 2:

About 15-25% of breast tumors express HER2, and the presence or absence of this protein is significant in the selection of appropriate therapy for breast cancer patients, HER2 overexpression is one of the first processes in breast cancer development (Kohler *et al.*, 2015).

Furthermore, HER2 raises the detection rate of metastatic or recurring breast cancer from 50% to over 80% (Kontani *et al.*, 2013).

2.2.7. Tumor staging (TNM classification):

In 1959, the American Joint Committee for Cancer Staging and End-Results Reporting, now the American Joint Committee on Cancer (AJCC), standardized the tumor, node, and metastasis (TNM) cancer staging system. The first edition of the AJCC Staging Manual, published in 1977, allowed clinicians to standardize treatment and evaluate

treatment results between different institutions. Since then, the manual has been periodically updated to reflect clinical and technological advancements in the field (Amin *et al.*, 2017; Plichta *et al.*, 2018).

Until the implementation of the 8th edition of the AJCC Staging Manual in 2018, a purely anatomic staging method, which uses primary tumor (T) size, nodal (N) involvement, and metastasis (M) based on clinical and pathological evaluations, was employed. Advancements in tumor biology and prognostic biological markers [estrogen receptor (ER) and progesterone receptor (PR), HER2/neu, and Ki-67] have allowed clinicians to understand why similarly staged patients had significantly different outcomes. The most recent update to the staging system integrates anatomic staging with prognostic staging, which uses tumor grade, hormone receptors and oncogene expression, and multigene testing (Hortobagyi and Connolly, 2016).

Overall Stage	T category	N category	M category
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage IIB	T2	N1	M0
	T3	N0	M0
Stage IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage IIIB	T4	Any N	M0
Stage IIIC	Any T	N3	M0
Stage IV	Any T	Any N	M1

Figure (2-3) American Joint Committee on Cancer’s Staging System for Breast Cancer, Eighth Edition.

2.3. Obesity:

According to WHO (2019), epidemiological research conducted in 2016 revealed that around 1.9 billion of people were overweight, with over 650 million of these being obese. Further, 40% of females aged 18 years and above were overweight and out of these adults females 15% were obese according to WHO during 2016.

Obesity is linked to an increased risk of several chronic illnesses, including diabetes, heart disease, and hypertension, as well as an increased chance of cancer. Abdominal obesity contributes to the development of an environment that favors cancer growth (Chang and Eibl, 2019).

The association between obesity and cancer, especially breast, endometrial, ovarian, thyroid, and prostate cancers, was reported (Gallagher and LeRoith, 2015).

Obesity has a link to breast cancer due to higher rates of androgenic precursor conversion to estrogen in adipose tissue via peripheral aromatization. High levels of insulin and insulin-like compounds in response to obesity, on the other hand, can promote cancer cell growth (Chen *et al.*, 2016).

Obese breast cancer patients are more likely to have bigger tumors, be at a more advanced stage at the time of their initial clinical examination, have a higher likelihood of metastasis, and develop resistance to hormonal therapy (Santa-Maria *et al.*, 2015; Gerard and Brown, 2018).

Clinical data demonstrates that obese breast cancer patients who receive chemotherapy or aromatase inhibitors have a higher risk of recurrence and a worse response to treatment in contrast to thin individuals (Karatas *et al.*, 2017).

2.3.1. Main mechanisms involved in obesity-associated breast cancer:

2.3.1.1. Chronic inflammation:

Adipocyte hypertrophy and cell death caused on by excessive adiposity result in persistent, subclinical inflammation of adipose tissue (Iyengar *et al.*, 2015).

Adipose chronic inflammation has been shown to increase cancer cell survival and proliferation, promote angiogenesis, metastatic spread, modify antitumor immune responses, and interfere with hormonal or chemotherapeutic therapies that affect the prognosis of cancer. A pro-inflammatory microenvironment is created at the tumor site as a result of the existence of crown-like structures, which are histological focal inflammatory regions, and the close proximity of adipose stromal cells. Therefore, infiltrating M1 macrophages, cytokines, and pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), TNF- α , IL-6, monocyte chemoattractant protein-1 (MCP-1) and interleukin (IL)-1 β are elevated (Iyengar *et al.*, 2016).

2.3.1.2. Sex hormone deregulation:

Increased adiposity promotes the formation and progression of breast cancer in postmenopausal women by increasing estrogen production, resulting in increased estrogen exposure to breast tissue, estrogen exposure is the primary cause of breast carcinogenesis (Gerard and Brown, 2018).

Adipose tissue becomes the primary source of estrogens beyond menopause due to aromatase, an enzyme produced by stromal cells, which converts androstenedione to estrone. In obese women, enhanced estrogen synthesis and relatively high plasma levels are the results of increased aromatase activity. Therefore the risk of breast cancer and its poor prognosis increase in this group of population (Rose *et al.*, 2015).

Factors created during inflammation, as well as the paracrine loop formed between macrophages and adipose stromal cells, may stimulate the aromatase enzyme activity, resulting in ER-positive breast cancer epithelial cell development via locally produced estrogens. Therefore, a link between obesity, inflammation, and hormone receptor-positive cancer appears to be quite significant (Crespi *et al.*, 2016).

2.3.1.3. Insulin:

Insulin is another obesity-related factor that stimulates the activity of adipose aromatase while directly promoting the proliferation and invasion of breast cancer cells as part of the adipose metabolic changes (Howe *et al.*, 2013).

Deregulated insulin signaling in obesity has been extensively documented for decades. Body mass index is directly related to circulating insulin and insulin-like growth factor-1 (IGF-1). An increased risk of post-menopausal breast cancer as well as an increased risk of cancer recurrence and death are also linked to high levels of insulin in obese women as a result of insulin resistance (Rose *et al.*, 2015).

Elevated insulin levels have been linked to an increased risk of breast cancer in postmenopausal women even with normal BMI (Gunter *et al.*, 2015).

2.3.1.4. Adipokines:

Over the past few years, there has been a considerable increase in knowledge regarding adipose tissue. Even while adipose tissue has traditionally been thought of as an inactive tissue used to store lipids, it is now recognized as a functional organ with both metabolic and endocrine activities that secretes a wide range of substances into the circulation to

connect with other organs and tissues (Poulos *et al.*, 2010; Gerard and Brown, 2018).

Those substances, collectively known as adipokines, are required for a variety of physiological reactions, including glucose and lipid metabolism, homeostasis, angiogenesis, inflammation, and satiety (Khan and Joseph, 2014; Gerard and Brown, 2018)

Overweight or obesity is caused by the unregulated expression of adipokines in adipose tissue and hormonal dysregulation, which together increase the risk of breast cancer (Lafontan, 2014; Gerard and Brown, 2018).

One of the main characteristics of fat metabolism and the physiopathology of obesity-related disorders is altered adipokine and cytokine signaling. Obese people's adipocytes release endocrine, inflammatory, and angiogenic substances that have an impact on nearby breast cancer cells. Leptin and adiponectin are the most important adipokines linked to the development of breast cancer caused by obesity (Khan *et al.*, 2013).

Adiponectin has a negative regulatory role in the development of breast cancer linked to obesity. Therefore, low levels of adiponectin, which are indicative of obesity, are linked to higher proliferative activity, which raises the risk of development of cancer. Furthermore, decreased blood adiponectin levels are linked to increased tumor size and a worse prognosis of breast cancer (Nagaraju *et al.*, 2016).

2.3.1.4.1. Leptin and breast cancer:

Hyperleptinemia is one of the pathophysiological causes of breast cancer in obesity. Leptin is classified as both a hormone and an adipokine, and it is primarily generated and released into circulation by adipose tissue. The placenta also synthesizes and secretes it, as do the

mucosa of the gastric fundus, skeletal muscle, brain, bone marrow, lymphoid tissues, immunological cells, ovaries, endometrium during embryo implantation, and both normal and cancerous breast tissue (Ray, 2012).

The primary role of leptin is to maintain energy homeostasis by engaging in the anorexigenic pathway via a central feedback mechanism at the hypothalamic level. In this way, it regulates adipose tissue development via intermediate hormonal pathways that regulate food intake. In addition to its primary role, it is known that leptin influences fetal development, reproduction, breastfeeding, bone formation, hematopoiesis, immunological response, angiogenesis, and the proliferation of several cell types, including breast tissue cells (Barone *et al.*, 2016).

Leptin has another regulatory role in the interaction between energy metabolism and the immune system, which is partially responsible for the inflammatory conditions associated with obesity (Pérez-Pérez *et al.*, 2017).

According to reports, leptin levels may raise or decrease the risk of breast cancer depending on menopausal state. Postmenopausal women with greater plasma leptin levels had a higher chance of getting breast cancer, whereas premenopausal women with lower leptin levels had a higher risk of developing breast cancer. Furthermore, a rise in leptin levels enhances breast tumor survival and development (Harris *et al.*, 2011; Khan *et al.*, 2013).

According to reports, leptin stimulates intracellular signaling pathways and aids breast cancer cells in proliferation, migration, angiogenesis, invasion, and metastasize (Guo *et al.*, 2012).

2.3.1.4.2. Leptin receptors:

The mechanism of leptin action starts with the activation of its transmembrane receptor, called Ob-R or LEPR, which has a helical structure and is related to class I cytokine receptors. LEPR is present in tissues such as pancreas, placenta, adrenal glands, stomach, hematopoietic cells, liver, heart, lung as well as breast cells (Sánchez-Jiménez *et al.*, 2019).

Leptin can influence breast cancer cells not only by endocrine and/or paracrine actions, but also through autocrine pathways. High levels of leptin and overexpression of its receptors in obese women can lead to an increase in signaling, key in the development of breast cancer (Ray, 2012).

2.3.1.4.3. Leptin and estrogen:

A significant amount of data has indicated that estrogen and its receptor play a critical role in the genesis and progression of breast cancer. Estrogens are thought to contribute significantly to breast cancer by promoting oxidative metabolism and cellular proliferation through a variety of cytochrome P450 (CYP) enzymes, including CYP1A1 and CYP1B1. The primary estrogen in breast tissues is 17 β -oestradiol (E2), which binds to the estrogen receptor α (ER α) and feeds the phase I enzyme cytochrome P450 CYP1B1 (Gérard and Brown, 2018).

Leptin increases the tumorigenicity of estrogen-positive breast cancer cells particularly (Strong *et al.*, 2015). Furthermore, leptin from adipose stromal/stem cells promotes a distinct pattern of gene expression in ER α -positive compared ER α -negative breast cancer cells (Binai *et al.*, 2013).

2.3.1.4.4. Leptin and therapeutic targets in breast cancer:

In certain breast cancer follow-up studies, leptin network interactions have been linked to prognosis as well as pharmacological responses. Patients with breast cancer, for example, who have high levels of leptin but negative expression of the estrogen hormone receptor have a greater survival rate. As a result, people with a positive leptin/negative hormone receptor status have a better response to chemotherapy and a better prognosis compared to those with a positive leptin/positive hormone receptor profile have a poor response (Simone *et al.*, 2016).

Furthermore, it has been proven that leptin interferes with the activity of tamoxifen in ER-positive breast cancer cells when induced by betaestradiol (Chen *et al.*, 2013).

2.4. Diabetes and breast cancer:

Type 2 diabetes mellitus is one of the most common chronic diseases in the world, affecting people at any age, including children, women, youth, and adults (Hu, 2011).

Diabetes affected roughly 9.3% of the US population in 2012 (CDC, 2014). Diabetes is classified into two types: type I and type II, with type II accounting for 95% of all clinical diagnosis (CDC, 2014).

Breast cancer and diabetes mellitus are chronic illnesses that are becoming more prevalent worldwide (Heleen *et al.*, 2017). Women with type 2 diabetes mellitus (T2DM) are more likely to develop breast cancer, the most frequent malignant tumor in females (Torre *et al.*, 2015).

Several mechanisms have been proposed for the increased risk of breast cancer in women with T2DM, including common risk factors (such as obesity), specific metabolic derangements of diabetes (i.e., hyperglycemia, hyperinsulinemia, and insulin resistance), and the use of insulin, specifically insulin analogs (Jetty *et al.*, 2019).

In comparison to women without diabetes, women with diabetes have a 40% higher risk of death from breast cancer; the comorbidity and consequences from diabetes may also play a role in the high all-cause of mortality rate. However, diabetes also raises the chance of mortality specifically related to breast cancer (Lorraine *et al.*, 2015).

Another factor contributing to the poorer breast cancer survival rates may be the more aggressive or less responding tumor subtypes that emerge in women with diabetes. It has been demonstrated that the development of estrogen receptor (ER)-negative breast cancer subtypes is correlated with hormone-related breast cancer and diabetes risk factors, such as obesity (Heleen *et al.*, 2017). There is evidence that insulin may increase estrogen production, promoting the development of ER-positive breast cancer (Rose and Vona, 2012).

Compared to those without diabetes, women with diabetes are notably more likely to present with advanced-stage breast cancer (Lorraine *et al.*, 2015). Furthermore, women with T2DM are more likely to be diagnosed with a more aggressive type of breast cancer than women without T2DM (Jetty *et al.*, 2019).

The incidence of advanced-stage breast cancer was highest in younger women and those who had diabetes for a longer period of time; these findings suggest that diabetes may predispose women to more rapidly advancing breast cancer, resulting in more advanced-stage illness upon diagnosis (Lorraine *et al.*, 2015).

According to a new comparative risk assessment study, the global incidence of cancer linked to diabetes and obesity will continue to rise unless the prevalence of these disorders decreases (Pearson-Stuttard *et al.*, 2018).

Also according to evidence, 16% of breast cancer subjects have diabetes, implying a 10-20% increased risk of developing breast cancer in diabetic women (Vona-Davis and Rose, 2012).

Moreover, diabetes/ impaired glucose tolerance worsens breast cancer prognosis (Schrauder *et al.*, 2011; Goodwin *et al.*, 2012) and has a positive association to breast cancer related mortality in a 10-year follow-up study (De Bruijn *et al.*, 2013).

2.4.1. Diabetes and breast cancer: The molecular link:

Clinical studies dating back to 1932 revealed a link between diabetes and cancer. Diabetes and breast cancer have complex relationships, according to preclinical and clinical research (Renehan *et al.*, 2010; Tudzarova and Osmanm 2015).

Pre-diabetes is defined by insulin resistance and glucose intolerance, as well as higher-than-normal blood glucose levels that are not high enough to be classified as diabetes. During diabetes progression, insulin resistance-related metabolic re-programming causes three major metabolic changes in circulation: (1) dyslipidemia/ hyperlipidemia (high levels of triglycerides, fatty acids, and cholesterol), (2) hyperinsulinemia, and (3) hyperglycemia, resulting in a low-grade chronic inflammatory condition (Sun and Kashyap, 2011; Basha *et al.*, 2012).

Insulin and hyperinsulinemia are indicators of obesity, and hyperinsulinemia is linked to higher IGF-1 levels (Bowers *et al.*, 2015). IGF plays a multifaceted role in cancer, with research indicating a slight elevated risk of some malignancies due to enhanced IGF system activity.

In overweight/obese females, there is a bidirectional association between dysregulated/imbanced glucose/insulin metabolism and breast cancer (Luque *et al.*, 2017). Hyperinsulinemia is associated with

increased IGF-1 production, resulting in cancer-promoting effects of obesity (Bowers *et al.*, 2015).

2.4.2. Insulin and insulin-like growth factor:

In breast cancer models, *in vitro* and *in vivo* studies revealed that the IGF-I signaling cascade promotes proliferation, migration, angiogenesis, and survival (Weroha and Haluska, 2012; Christopoulos *et al.*, 2018).

According to Duggan *et al.* (2011), elevated insulin and IGF-1 levels were linked to higher mortality. Therefore, lowering IGF-1 levels and focusing on IGF-1 receptors in various breast cancer subtypes may be helpful for treating the disease in that subgroup (Christopoulos *et al.*, 2018).

Obesity, diabetes, and metabolic syndrome all have a significant impact on breast cancer risk (Rose *et al.*, 2015; Simone *et al.*, 2016), however the pathophysiological mechanisms and molecular and cellular variables underlying these associations are still poorly understood (Klil-Drori *et al.*, 2017). Among the suggested players, IR stands out as a strong candidate because it plays a significant role in the metabolic syndrome, diabetes, other metabolic illnesses, as well as various cancer types, including breast cancer.

Chapter Three
Materials and Methods

Chapter Three

Materials and Methods

3.1. Materials:

3.1.1. Patients:

This cross-sectional study was conducted on a group of postmenopausal women (woman hasn't experienced a period for over a year) diagnosed with breast cancer in Babylon Oncology Treatment Center in Merjan Medical City in Al-Hillah City, Babylon Governorate; under the supervision of the Department of Medical Physiology in Babylon Medical College.

The study extended from the beginning of September 2022 till the end of March 2023. The cases were selected according to the inclusion and exclusion criteria listed below in addition to the decision of their attendant physician.

3.1.2. Sample size calculation:

The study's sample size was calculated according to the following formula:

$$N = Z^2 P (1-P) / d^2$$

Where:

N: Sample size

Z: Statistic corresponding to level of confidence which equals to 1.96 as the level of confidence is 95%.

P: Expected prevalence of breast cancer among obese postmenopausal women which equals to about 20% (Giaquinto *et al.*, 2022).

d: Precision which equals to 5% as the prevalence of disease is between 10-90%.

Accordingly, the calculated sample size will be about 245 patients.

3.1.3. Inclusion and exclusion criteria:

3.1.3.1. Inclusion criteria:

Post-menopausal woman presented with histopathologically-confirmed breast cancer, with or without type II diabetes mellitus and with any body weight.

3.1.3.2. Exclusion criteria:

1. Premenopausal women.
2. Women with type I diabetes mellitus.
3. Women with other type of malignancy.

3.1.4. Ethical approval and consent:

All patients involved in this work were informed and the agreement was obtained verbally from each one before the collection of samples. This study was approved by the Committee on Publication Ethics at the College of Medicine, University of Babylon, Iraq under the reference number 3-1 in 30/6/2022.

3.1.5. Confirmation of the diagnosis:

The diagnosis of breast cancer and its pathologic subtypes was determined based on the results of breast tissue biopsy samples (Goldhirsch *et al.*, 2011); and the diagnosis of diabetes was made according to ADA, (2014); based on the patient's age, body weight overweight/obesity, fasting

blood sugar of 126 mg/dL or more, 2-hour postprandial blood sugar of 200 mg/dL or more, random blood sugar of 200 mg/dL or more, glycosylated hemoglobin of 6.5% or more, normal or elevated serum insulin and C-peptide levels, and absence of specific antibodies against islet β -cells.

3.1.6. Laboratory apparatuses, instruments and disposable materials:

The laboratory apparatuses, instruments and disposable materials used in this study are mentioned in tables (3-1 A and B).

Table (3-1-A) Scientific laboratory apparatuses.

No.	Item	Company	Country
1	CBC auto-analyzer	Orphée	Switzerland
2	Respons 920	Dia Sys	Germany
3	Centrifuge	Hittech	Germany
4	Cobas C111	Roche	Japan
5	Cobas C411	Roche	Japan
6	Refrigerator	Concord	Lebanon
7	Deep freeze	GFL	Germany
8	Microplate reader	Biotek	USA

Table (3-1-B) Technical instruments and disposable materials.

No.	Item	Company	Country
1	Glass EDTA tubes	Afco	Jordan
2	Glass gel tubes 10 ml	Afco	Jordan
3	Disposable syringes 5 ml	Universal	China
4	Eppendrof tubes 1.5 ml	Universal	China

3.1.7. Kits:

The kits used in this study are mentioned in tables (3-2) and (3-3).

Table (3-2) Biochemical kits used in this study with their companies and countries of origin.

No.	Item	Company	Country
1	Elecsys ferritin kit	Roche	Germany
2	A1C-3 kit	Roche	Germany

Table (3-3) Enzyme-linked immunosorbent assay (ELISA) kit used in this study with its company and country of origin.

No.	Item	Company	Country
1	Human leptin ELISA kit	BT lab	China

3.2. Methods:

3.2.1. History:

Basic information was recorded as follows: Name, age, height and weight to record the body mass index as follows: $BMI = \text{Weight (kg)} / \text{Height (m}^2\text{)}$. Marital status, stage and grade of breast cancer at the time of diagnosis, histologic and luminal subtypes of breast cancer, history of recurrence of breast cancer, family history of breast cancer and other cancers, history of diabetes and hypertension, history of medication usage such as contraceptive pills and hormonal therapy and physical activity. All

these parameters was assessed and recorded in a special file sheet questionnaire (Figure 3-1).

Patient's name :
Age:
Hight: **weight:**
BMI:
Marital status:
Stage and grade of breast cancer at time of diagnosis: **T**
Stage **grade**
N
M
Histologic and luminal subtypes of breast cancer: **HPR**
ER **LUMINAL**
PR
HER2
History of recurrence of breast caner:
Family history of breast cancer or other cancers:
History of diabetes or hypertension:
History of medication (cop or hormonal therapy):
Physical activity:
Blood test
Hb ***PCV*** ***MCV***
MCHC
RBS
HbA1C ***Leptin*** ***Ferritin***

Figure (3-1) File sheet questionnaire.

3.2.2. Blood collection and serum preparation:

Five milliliters of fresh blood were drawn at 8:30 AM from participants, two milliliters for hematological and the rest for biochemical analyses. Two types of labeled tubes were used; the first contain EDTA as an anti-coagulant to prevent clotting of blood to be used for hematological studies. The second type of tubes were without anti-coagulant as gel tubes, for preparing sera by putting blood in the tubes and allowing it to clot for 15-30 minutes, then separating it by centrifugation for 10 minutes at 3000 round

per minute to be used in the subsequent biochemical tests (Bishop *et al.*, 2000). Each sample was labeled and given a serial number together with the participant's name. The separated sera were divided into two parts; the first one is used for the measurement of serum ferritin and the second one was placed in a labeled Eppendorf tube and were kept frozen at -20 C° for subsequent measurement of serum leptin.

3.2.3. Hematological and biochemical studies:

3.2.3.1. Hematological studies:

Hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) were all done by the use of an automated auto-analyzer (Orphée, Switzerland).

3.2.3.2. Biochemical studies:

3.2.3.2.1. Random blood sugar:

Random blood sugar was measured by DiaSys respons® 920 through an enzymatic UV test using hexokinase (Bakker and Mücke, 2007).

3.2.3.2.2. Hemoglobin A1C:

Hemoglobin A1C was measured by the use of A1C-3 kit (Roche, Germany) and was done according to the Cobas C111 HbA1c kit insert (Zander *et al.*, 1984; Wolf *et al.*, 1984 and Little *et al.*, 1992).

3.2.3.2.3. Serum ferritin:

Serum ferritin concentrations were measured using Elecsys ferritin kit (Roche, Germany) by the ElectroChemiLuminescence method with Roche Cobas E411 autoanalyser system (Roche-Hitachi Diagnostics, Japan) and

was done according to Elecsys ferritin kit (Roche, Germany) instructions (Blackmore *et al.*, 2008).

3.2.3.2.4. Serum leptin:

Serum leptin concentrations were measured using human leptin elisa kit (Bioassay technology laboratory, China), and was done according to elisa microplate reader (Biotek, Usa) instructions (Kalmarzi *et al.*, 2017).

3.3. Statistical analysis:

Continuous variables were reported as means \pm standard deviations (SD), while the other categorical variables were addressed as percentages (no. (%)). The comparisons between the study groups were done using independent-samples t-test for the continuous variables and chi-square test for the categorical ones. In addition; correlation analyses were done between all the continuous variables to report the strength of the relationship between them.

All analyses were done by the use of a statistical package of social sciences (SPSS) version 18.0 software (SPSS Inc., Chicago, IL, USA). *P* value of less than 0.05 was regarded as statistically significant (Daniel, 2009).

Chapter Four
Results

Chapter Four Results

This study was done on postmenopausal women having breast cancer. It included a total of 240 patients. They were divided into two groups according to the scores of their body mass indexes. The first group of patients (n=138) included those with BMI \geq 30 Kg/m², while the second group (n=102) included those with BMI<30 Kg/m² (figure 4-1).

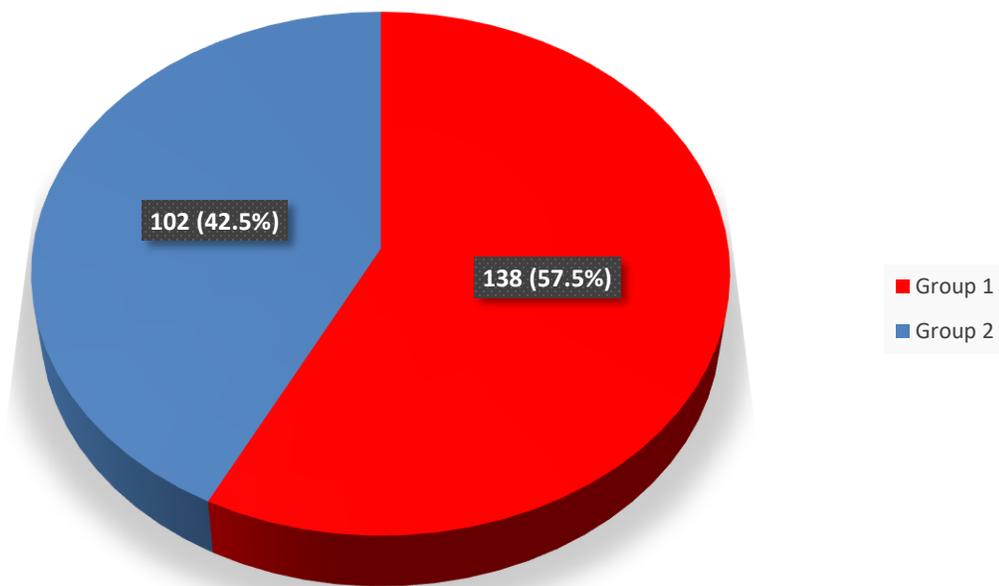


Figure (4-1) Distribution of the study's patients according to body mass index (BMI). Group 1 (BMI \geq 30 Kg/m²); Group 2 (BMI<30 Kg/m²).

4.1. Age distribution:

Table (4-1) demonstrates the age distribution for the two groups of patients in this study. The ages range between 48-85 years. A statistically significant difference was present between the two groups of patients regarding age (p value=0.008).

Table (4-1) Age distribution for the study groups.

Age	Group 1 (BMI \geq 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	<i>p</i> value
Age (years) Mean \pm SD	57.26 \pm 6.823	59.68 \pm 8.251	0.008

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- SD: Standard deviation.

4.2. Staging and grading of breast cancer:

Tables (4-2), (4-3) and figures (4-2), (4-3) demonstrate the distribution of breast cancer’s stage and grade among the two groups of the study, showing that statistically significant differences were present between the study’s groups regarding them (p values=0.033 for cancer’s stage and 0.0001 for cancer’s grade).

Table (4-2) Distribution of breast cancer’s stage among the study groups.

Groups Stage	Group 1 (BMI ≥ 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	p value
Stage I No. (%)	14 (10.1)	10 (9.8)	24 (10.0)	0.033
Stage II No. (%)	64 (46.4)	30 (29.4)	94 (39.2)	
Stage III No. (%)	36 (26.1)	32 (31.4)	68 (28.3)	
Stage IV No. (%)	24 (17.4)	30 (29.4)	54 (22.5)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- No.: Number.
- %: Percent.

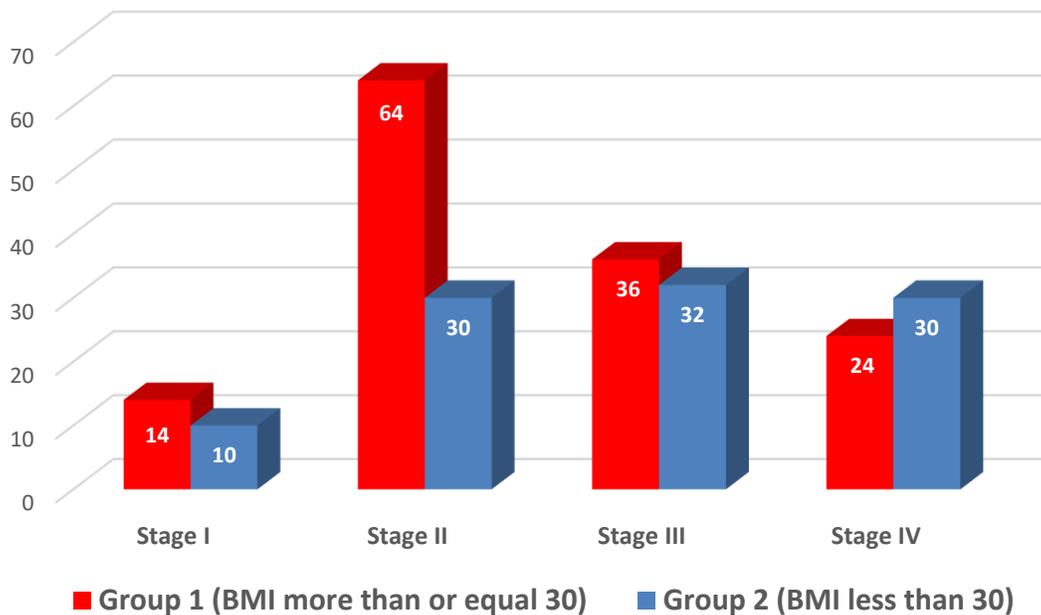


Figure (4-2) Frequency of cancer’s stage among the two groups of breast cancer patients.

Table (4-3) Distribution of breast cancer’s grade among the study groups.

Groups Grade	Group 1 (BMI ≥ 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	p value
Grade I No. (%)	10 (7.2)	30 (29.4)	40 (16.7)	0.0001
Grade II No. (%)	88 (63.8)	54 (52.9)	142 (59.2)	
Grade III No. (%)	40 (29.0)	18 (17.6)	58 (24.2)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- No.: Number.
- %: Percent.

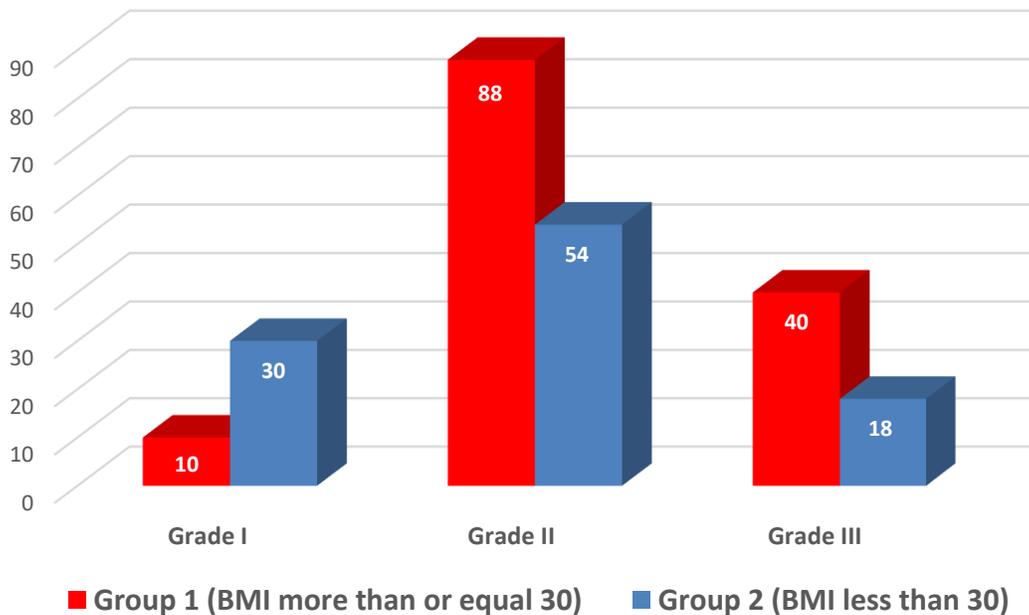


Figure (4-3) Frequency of cancer’s grade among the two groups of breast cancer patients.

4.3. Subtype and immunohistochemistry of breast cancer:

Tables (4-4), (4-5) and figures (4-4), (4-5) point out the distribution of breast cancer’s subtype and immunohistochemistry among the two groups of the study. Statistically significant difference was present between the study’s groups regarding immunohistochemistry only (*p* values=0.436 for cancer’s subtype and 0.002 for cancer’s immunohistochemistry).

Table (4-4) Distribution of breast cancer’s subtype among the study groups.

Groups	Group 1 (BMI ≥ 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	<i>p</i> value
Invasive ductal No. (%)	126 (91.3)	90 (88.2)	216 (90.0)	0.436
Invasive lobular No. (%)	12 (8.7)	12 (11.8)	24 (10.0)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- No.: Number.
- %: Percent.

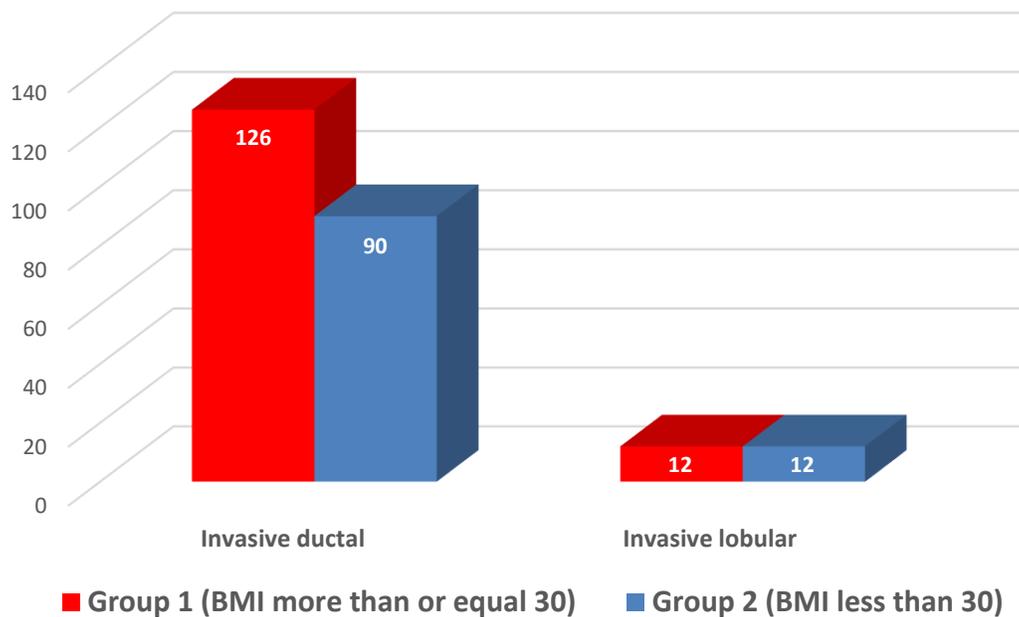


Figure (4-4) Frequency of cancer’s subtype among the two groups of breast cancer patients.

Table (4-5) Distribution of breast cancer’s immunohistochemistry among the study groups.

Immunohistochemistry	Group 1 (BMI ≥ 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	p value
Luminal A No. (%)	76 (55.1)	36 (35.3)	112 (46.7)	0.002
Luminal B No. (%)	34 (24.6)	34 (33.3)	68 (28.3)	
Triple negative No. (%)	22 (15.9)	16 (15.7)	38 (15.8)	
HER2 No. (%)	6 (4.3)	16 (15.7)	22 (9.2)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- HER2: Human epidermal growth factor receptor 2.
- No.: Number.
- %: Percent.

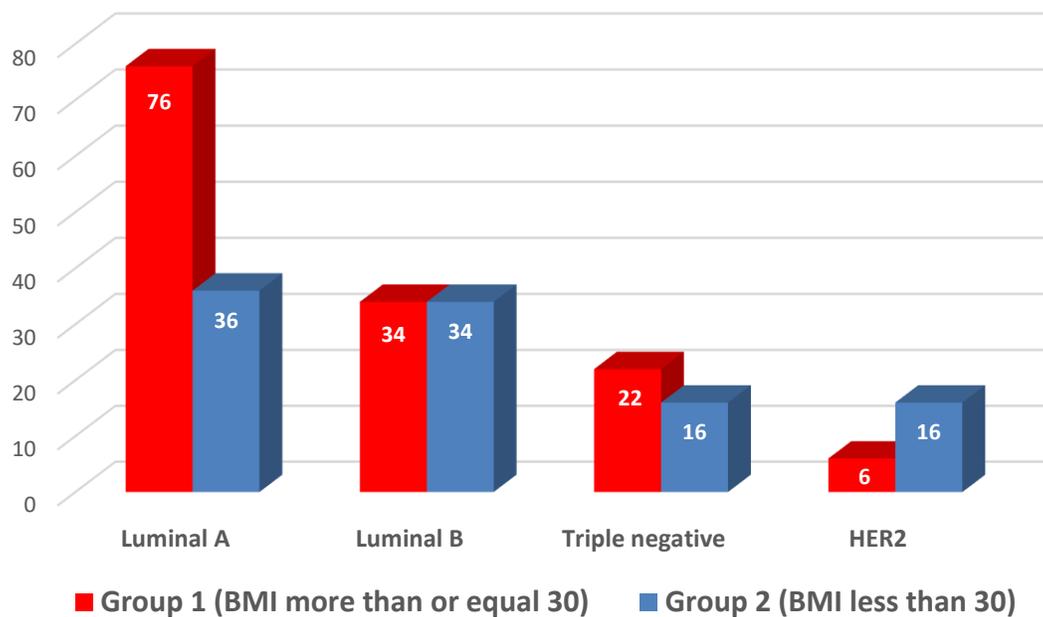


Figure (4-5) Frequency of cancer's immunohistochemistry among the two groups of breast cancer patients.

4.4. History of diabetes mellitus and hypertension:

The distributions of clinical history parameters (diabetes mellitus and hypertension) among the patients of the study are shown in table (4-6). No statistically significant difference was present between the two groups of the study (p value=0.073).

Table (4-6) Distribution of clinical history among the study groups.

Groups	Group 1 (BMI \geq 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	p value
Clinical history				
Diabetes mellitus No. (%)	20 (14.5)	10 (9.8)	30 (12.5)	0.073
Hypertension No. (%)	46 (33.3)	24 (23.5)	70 (29.2)	
Both of them No. (%)	22 (15.9)	14 (13.7)	36 (15.0)	
Negative No. (%)	50 (36.2)	54 (52.9)	104 (43.3)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- No.: Number.
- %: Percent.

4.5. History of medication usage:

The distributions of medication usage (oral contraceptives and hormonal therapy) among the patients of the study are shown in table (4-7). No statistically significant difference was present between the two groups of the study (*p* value=0.895).

Table (4-7) Distribution of medication usage among the study groups.

Medication usage	Group 1 (BMI ≥ 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	Total No. (%)	p value
Oral contraceptives No. (%)	28 (20.3)	18 (17.6)	46 (19.2)	0.895
Hormonal therapy No. (%)	24 (17.4)	16 (15.7)	40 (17.6)	
Both of them No. (%)	4 (2.9)	4 (3.9)	8 (3.3)	
Negative No. (%)	82 (59.4)	64 (62.7)	146 (60.8)	
Total No. (%)	138 (100)	102 (100)	240 (100)	

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- No.: Number.
- %: Percent.

4.6. Hematological parameters:

Values of hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) are presented in table (4-8). No statistically significant difference was found between the two groups of patients and controls regarding those parameters (*p* values=0.172 for Hb; 0.215 for PCV; 0.086 for MCV and 0.327 for MCHC).

Table (4-8) Values of hematological parameters for the study groups.

Groups Parameters	Group 1 (BMI \geq 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	p value
Hb (g/dl) Mean \pm SD	11.861 \pm 1.390	11.610 \pm 1.422	0.172
PCV (%) Mean \pm SD	36.419 \pm 3.998	35.767 \pm 4.044	0.215
MCV (fl) Mean \pm SD	85.813 \pm 5.379	87.137 \pm 6.514	0.086
MCHC (%) Mean \pm SD	32.536 \pm 1.304	32.353 \pm 1.585	0.327

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- Hb: Hemoglobin, g/dl: Gram/deciliter.
- PCV: Packed cell volume, %: Percent.
- MCV: Mean cell volume, fl: Femtoliter.
- MCHC: Mean cell hemoglobin concentration.
- SD: Standard deviation.

4.7. Random blood sugar and hemoglobin A1C:

Table (4-9) demonstrates the value of random blood sugar (RBS) and hemoglobin A1C (HbA1C), both of them exhibit statistically significant differences between the two groups of the study and were higher in the first group of patients compared to the second one (p values=0.011 for RBS and 0.007 for HbA1C).

Table (4-9) Values of random blood sugar and hemoglobin A1C for the study groups.

Groups Parameters	Group 1 (BMI \geq 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	p value
RBS (mg/dl) Mean \pm SD	162.07 \pm 68.599	141.66 \pm 49.099	0.011
HbA1C (%) Mean \pm SD	6.123 \pm 1.384	5.677 \pm 1.088	0.007

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- RBS: Random blood sugar, mg/dl: Milligram/deciliter.
- HbA1C: Hemoglobin A1C, %: Percent.
- SD: Standard deviation.

4.8. Leptin and ferritin:

Values of leptin and ferritin are shown in table (4-10), which reveals that they were higher in the first group of patients in comparison to the second group and a statistically significant differences were present between the two groups regarding those parameters (p values=0.001 for leptin and 0.0001 for ferritin).

Table (4-10) Values of leptin and ferritin for the study groups.

Groups Parameters	Group 1 (BMI \geq 30 Kg/m ²) (n=138)	Group 2 (BMI < 30 Kg/m ²) (n=102)	p value
Leptin (ng/ml) Mean \pm SD	1.386 \pm 0.658	1.125 \pm 0.475	0.001
Ferritin (ng/ml) Mean \pm SD	346.004 \pm 451.232	177.892 \pm 155.019	0.0001

- BMI: Body mass index.
- Kg: Kilogram.
- m²: Square meter.
- ng/ml: Nanogram/milliliter. - SD: Standard deviation.

4.9. Correlation analyses:

4.9.1. Correlation between RBS and HbA1C:

Figure (4-6) shows the correlation between RBS and HbA1C among the patients of the study, and it is clear that a significant positive correlation was present between them ($r=0.615$, $p=0.0001$).

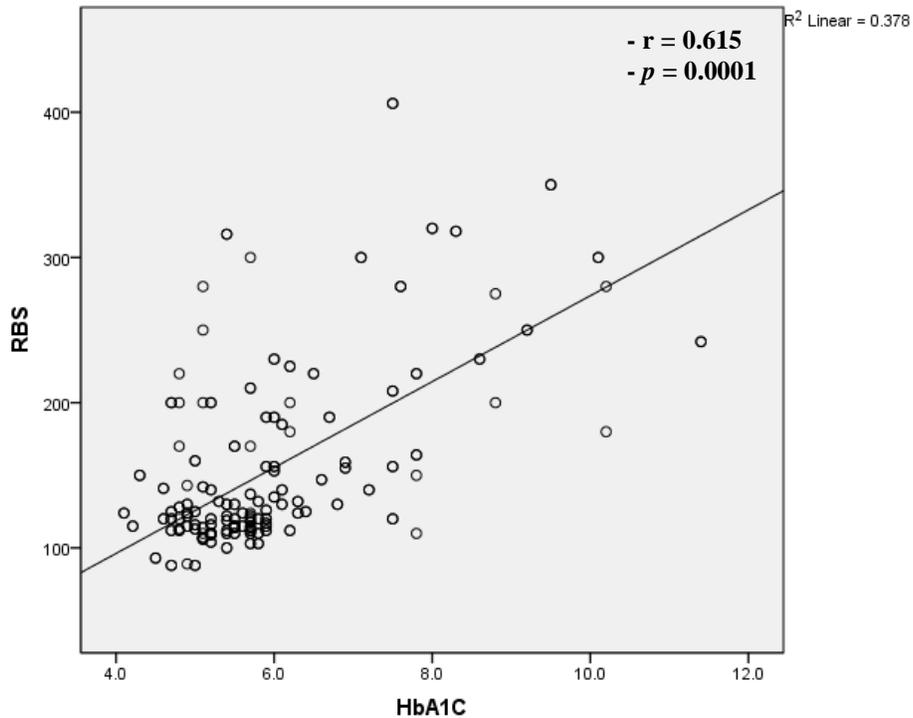


Figure (4-6) Correlation between random blood sugar (RBS) and hemoglobin A1C (HbA1C) for the patients of the study.

4.9.2. Correlation between RBS and leptin:

Figure (4-7) shows the correlation between RBS and leptin among the patients of the study, and it is clear that a significant positive correlation was present between them ($r=0.141$, $p=0.029$).

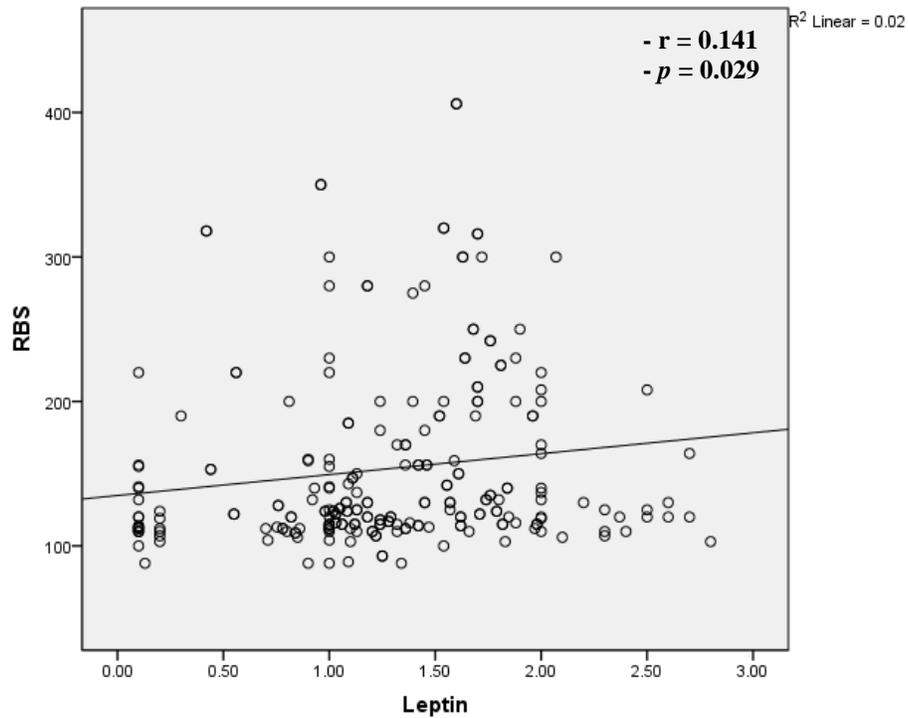


Figure (4-7) Correlation between random blood sugar (RBS) and leptin for the patients of the study.

4.9.3. Correlation between HbA1C and leptin:

Figure (4-8) shows the correlation between HbA1C and leptin among the patients of the study, and it is clear that a significant positive correlation was present between them ($r=0.168$, $p=0.009$).

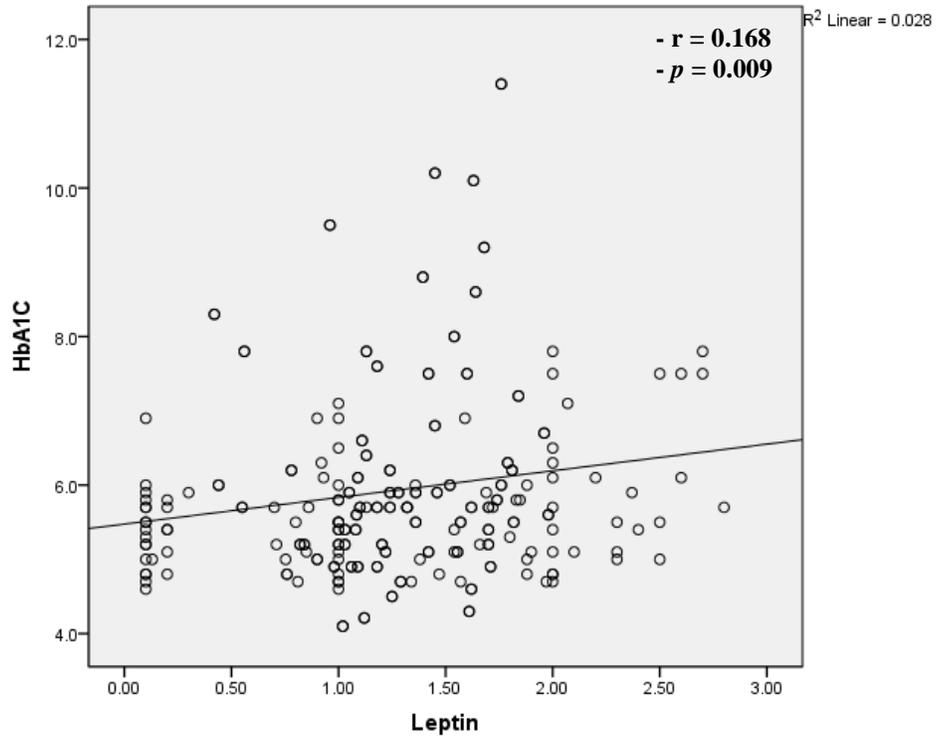


Figure (4-8) Correlation between hemoglobin A1C (HbA1C) and leptin for the patients of the study.

Chapter Five

Discussion

Chapter Five

Discussion

Although the tight epidemiological connection between cancer development and obesity is well documented, the molecular mechanisms underlying the obesity-breast cancer link are still under investigation due to the complexity of obesity condition and the different oncogenic alterations that may sustain all breast cancer subtypes. Obesity is characterized by an expanded, metabolically active and reprogrammed fat tissue that induces local inflammation and altered levels of cytokines/adipokines. These local alterations contribute to and cooperate with systemic physiological changes regarding the levels of insulin, IGF-1, steroid hormones, adipokines, and inflammation-related molecules. Furthermore, hypertrophy and hyperplasia of white adipocytes reduces their vascularization, resulting in a decrease of the oxygen availability. This hypoxia state induces an increased oxidative stress, insulin resistance, ischemia, adipocyte necrosis and release of inflammatory as well as angiogenic proteins (Laudisio *et al.*, 2018).

The local and systemic alterations induced by obesity may influence breast cancer through direct effects on neoplastic epithelial cells as well as indirect effects on tumor microenvironment. All these obesity-related factors can impact tumor initiation, metabolic reprogramming, angiogenesis, progression, and/or response to therapy (Kang *et al.*, 2017).

5.1. Age distribution:

As represented in table (4-1), age distribution showed a significant difference between the two groups of patients in this study, reflecting that increasing age plays a significant role and acts as a potential factor in the development of breast cancer.

Aging is one of the most important risk factors of breast cancer, because the incidence of breast cancer is highly related to the increasing age. In 2016, approximately 99.3% and 71.2% of all breast cancer-associated deaths in America were reported in women over the age of 40 and 60, respectively (Siegel *et al.*, 2017).

Al-Hashimi (2021) stated that in Iraq, according to the cancer registry office; almost one-third of the registered cancer amongst female is a breast cancer and a significant increase in the incidence has been noticed among 50-59 year age group.

Another similarity to the study's results was found with the outcomes of Wang and his co-workers (2019), who showed that females above 50 years old with greater BMI are at a greater risk of cancer compared to those with low BMI.

On the other hand, Kyu-Won Jung and lee (2018) showed that breast cancer incidence rates decrease with age after menopause in Japan, while the opposite trend is observed in the UK. South Korean women show a trend very similar to that of the Japanese population. Meanwhile, obesity rates in adult females are quite low in both Japan and South Korea. This low rate of obesity might contribute to the low breast cancer incidence in Japan.

5.2. Staging and grading of breast cancer:

The staging and grading of breast cancer were demonstrated in tables (4-2), (4-3) and figures (4-2), (4-3), showing that statistically significant differences were present between the two groups of the study. The results showed that most of patients were diagnosed at stage II and stage III breast cancer because breast lump at this stage is significantly observed by the patient's herself leading to seek medical advice also may be related to the lack of screening program. The results also showed that the grade of breast cancer was significantly associated with increased BMI, in which most obese patients were at advanced grade at presentation this related to increase incidence of breast cancer in obese women. Some discrepancy in the results may be attributed to environmental and socioeconomic factors of the society in addition to slightly atypical environment of sample collection.

The American Joint Committee on Cancer (AJCC) Cancer Staging Manual, Eighth Edition (2018) defined early breast cancer as stage I-II, and the stage III as locally advanced. The staging is the most important component on the prognosis than the other considerations. The higher the stage at diagnosis, the poorer the prognosis (Alawadi *et al.* 2019).

This manual includes 2 staging systems: anatomic stage and prognostic stage. Although TNM staging remains especially important for local-regional treatments such as surgery and radiation, endocrine therapy and other targeted therapies are increasingly recommended on the basis of tumor phenotype. Thus, the new prognostic stage now incorporates the historical anatomic TNM factors, and also tumor grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status, and tumor multigene panel testing (Plichta *et al.*, 2020).

A close finding to the study's result was stated by Ayoub *et al.* (2019) who revealed an association between obesity and more aggressive breast cancer phenotypes, Advanced presentation of obese breast cancer patients could be related to low rates of breast cancer screening among obese women and the possibility that obese patients have more biologically aggressive tumors as indicated by higher expression of cellular proliferation markers.

Vernaci and his colleagues (2019) also revealed that a higher BMI was correlated with advanced stage and nodal involvement at diagnosis.

Another similarity was shown by Sun *et al.* (2018) who observed that greater BMI is associated with more aggressive biological features of tumor including a higher percentage of lymph node metastasis and greater size.

Regarding the grade of breast cancer, the results were significantly associated with the high BMI group. This finding is in agreement with multiple earlier studies, suggesting an association between obesity and more aggressive breast cancer phenotypes. Obesity was associated with advanced stage and grade of breast cancer at diagnosis. Obese breast cancer patients tend to have poorer prognostic features at disease presentation compared to non-obese. The impact of obesity on clinic-pathologic characteristics and prognostic features was largely confined to postmenopausal cases (Ayoub *et al.*, 2019).

A similar finding was found by Rojas *et al.* (2019) who presented that obese women are affected by advanced breast cancer with histologic grade 2 or 3.

5.3. Subtype and immunohistochemistry of breast cancer:

Tables (4-4), (4-5) and figures (4-4), (4-5) pointed out the distribution of breast cancer's subtype and immunohistochemistry among the two groups of the study. Statistically significant difference was present between the study's groups regarding immunohistochemistry only. The results showed that the luminal A is the most commonly diagnosed breast cancer followed by luminal B, TNBC and HER2 type, although the luminal type is the commonest, the TNBC increased in incidence because there was a significant number of patient diagnosed at younger age most properly due to hereditary cause in Iraqi patients and due to lack of gene assay. While breast cancer subtypes, the results show that there is no difference between the two groups because breast cancer subtype not affected by BMI.

The mechanisms underlying the increased risks of obese women in developing postmenopausal receptor positive breast cancer are multi-factorial and mainly linked to hormonal pathways (Agurs-Collins *et al.*, 2019).

Most of the invasive breast cancers (about 80%) are infiltrating (or invasive) ductal cancers (IDCs). These tumors start in the duct of the breast, break through the wall of the duct and invade the surrounding fat tissue, from where they can spread through the lymphatic system or bloodstream. The other main type of invasive breast cancers (about 10-15%) is the infiltrating (or invasive) lobular cancer (ILC). These cancers begin in the lobules of the breast and act then similarly to the IDCs (Alsaimary *et al.*, 2020).

Similar finding to the study's results shown by Ahmed *et al.* (2020) who stated a considerable association between obese postmenopausal breast cancer patients and HR+/HER- subtype.

Alawadi and his colleagues (2019) also presented that the IDC recorded in (81.2%) of women as most common histopathology; Regarding the molecular status of breast cancer, the HR+/HER- was the prominent one in this study (50.2%), followed by weak HR+/HER- (29.9%), triple-negative/basal-like (9.6%), and HER2-enriched (7.9%).

On contrary, Ayoub *et al.* (2019) showed a lack of association between obesity and breast cancer molecular subtypes, regardless of menopausal status.

5.4. History of diabetes mellitus and hypertension:

The distributions of clinical history parameters (diabetes mellitus and hypertension) among the patients of the study are shown in table (4-6). No statistically significant difference was present between the two groups of the study. These finding may all be attributed to the fact that both of them are risk factor for breast cancer development and this risk is almost even in both groups of patients.

Dong *et al.* (2021) show that DM and its related hyperinsulinemia and insulin resistance are risk factors for breast cancer. However, the correlation between DM and breast cancer subtypes has inconsistent conclusions among studies and requires further exploration and research.

A similar finding to the study's result shown by Verma and Hussain (2017) who suggested that around 90% of diabetes is related to excessive body weight and BMI.

Regarding the relation between breast cancer and hypertension, Han and his colleague (2017) reported a 7% higher risk for total breast risk in hypertensive individuals especially for postmenopausal women.

Also Rausch *et al.* (2017) stated that the suggestive mechanisms to explain the association between hypertension and breast cancer involve blocking of apoptosis, adipose tissue related hypoxia and chronic inflammation promoting reactive oxygen species formation.

5.5. History of medication usage:

The distributions of medication usage (oral contraceptives (OCP) and hormonal therapy (HT)) among the patients of the study are shown in table (4-7). No statistically significant difference was present between the two groups of the study. The results of the study showed that about 40% of patients used OCP and/or HT in their premenopausal lives, suggesting a relationship between medication usage and development of breast cancer regardless of BMI.

The use of exogenous estrogen and progesterone/progestin medications spans the reproductive and post reproductive lives of millions of women providing control over pregnancy timing, management of premenopausal and postmenopausal symptoms, and prevention of disease, including endometrial and ovarian cancer. These same hormones can also increase the risk of breast cancer in some settings and their use in breast cancer survivors may increase the risk of breast cancer recurrence. Given both the benefits and risks of these exogenous hormones (Pearlman, 2022).

A similar result shown by Morch *et al.* (2017) who explained in his study that the relative risk of breast cancer was 20% higher among women who currently or recently used contemporary hormonal contraceptives than among women who had never used them. There was a suggestion that risk may persist more than 5 years after discontinuation of HC among women who had used HC for at least 5 years. The increased risk would not have

been significant with adjustment for multiple comparisons involving different categories of duration and time since last use. It's therefore appears that the excessive risk will diminish with time and will go back to baseline: this will happen after 5 years for shorter use of HC and after 10 years for women using HC for a longer time.

On the other side, Grandi *et al.* (2017) reviewed data from 2527 women in a retrospective cohort study and concluded that the use of HC was not associated with an increased risk of breast cancer regardless of the duration of use.

5.6. Hematological parameters:

Values of Hb, PCV, MCV and MCHC are presented in table (4-8). The results showed that no statistically significant difference was found between the two groups of patients regarding those parameters which could be explained based on that the two groups of patients complained from mild anemia regardless of their BMI.

Breast cancer cause a condition of oxidative stress. This disorder results in an increase in free radicals, an imbalance between the production and elimination of active species in the body, and a decrease in the strength of antioxidant defense system. Oxidative stress may affect the functions of blood cells, resulting in acute and chronic infections, anemia, and hypercoagulability. The complete blood count (CBC) reflects the cellular immune response in a cancer patient and any changes in hematological parameters influence cancer progression (Danesh *et al.*, 2022).

A similarity to the results of the study was showed by Muthanna *et al.* (2020) who hypothesized in their study that most of the patients with breast cancer suffered from mild anemia.

Also Muthanna and his colleagues (2021) stated that, anemia is a frequent complication in critically ill and cancer patients. It has been linked to a reduction in quality of life as well as a poor prognosis.

Moreover, Muthanna and his co-workers (2022) indicated that obesity increases the risk of anemia in cancer patients. About 57.5% of obese breast cancer patients develop anemia, whereas only 16.9% of non-obese patients do. Logistic regression analysis confirmed these findings, revealing that obese patients were 12.4 times more likely to develop anemia than non-obese breast cancer patients.

5.7. Random blood sugar and hemoglobin A1C:

Table (4-9) demonstrates the value of RBS and HbA1C, both of them exhibit statistically significant differences between the two groups of the study and were higher in the first group of patients compared to the second one. Since in individuals who are obese, higher amounts of fatty acids, hormones, and pro-inflammatory cytokines that could participate in the development of insulin resistance are released by adipose tissue which can result in the development of T2DM.

Insulin resistance is the key factor in the pathogenesis of type 2 diabetes and the most typical and serious phenomenon. It is defined as decreased sensitivity to insulin-mediated glucose disposal and inhibition of hepatic glucose production and presents as dysfunction of insulin transduction in glucose uptake and utilization in body skeletal muscles, adipocytes and hepatocytes, which leads to hyperglycemia, hyperinsulinemia and various disorders (Dong *et al.*, 2021).

Also Pan *et al.* (2020) stated that among postmenopausal women, higher insulin resistance is associated with significantly higher breast cancer incidence and a significantly higher risk of death after breast cancer. These findings appear to identify insulin resistance as a potential breast cancer risk factor.

Momenimovahed and Salehiniya (2019) showed in their study that diabetes is associated with the risk of breast cancer progress among postmenopausal women and those with higher BMI. Also showed that women with diabetes (especially those with type II diabetes) are at 20% increased risk of developing breast cancer, and these results could show some agreement to the results of the study.

Another similarity was showed by Peila and Rohan (2020) who observed that an increased risk of breast cancer among postmenopausal women with relatively high levels of HbA1c among those with BMI ≥ 30 kg/m².

On another hand, Mellergård *et al.* (2020) found no relationship between obesity and glycemc control status as measured by HbA1C.

5.8. Leptin and ferritin:

Values of leptin and ferritin are shown in table (4-10), which reveals that they were higher in the first group of patients in comparison to the second group. This could be attributed to the fact that obesity induce a state of tissue inflammation and adipocyte hypertrophy resulting in the release of pro-inflammatory factors like leptin and ferritin leading to increase their levels in obese women.

Serum leptin levels significantly increased independently in breast cancer cases for BMI > 25 kg/m². Although the mechanism remains unclear, the elevated levels of leptin were consistent with the amount of body fat in overweight/obese groups of patients, which could positively correlate with hyperleptinemia, insulin-sensitizing, and synthesis and activity of IGF-I and II. The IGF-I, II and leptin could have substantial effect on tumorigenesis through various intracellular pathways, which may synergize with other growth factors to enhance their mitogenic effects in obese hyperinsulinemic patients (Atoum, 2022).

Nathan and Gustian (2022) stated that leptin enhance aromatase activity and increases estrogen synthesis in breast cancer patients who are obese and have estrogen receptor positive (ER+) cells. Estrogen activates a variety of processes in breast cancer, including cell division, angiogenesis, and proliferation. According to the findings of their study, obese females having ER+ breast cancer are at a high risk to produce leptin, a hormone that promotes breast cancer growth.

A close observation to the findings of the study was presented by Abd *et al.* (2022) who stated that participants with breast cancer and obesity showed significantly higher serum leptin levels compared to non-obese

breast cancer. This result may be explained by the high quantity of leptin expressed at the gene level in adipose tissue in people with obesity.

Also Koprivčić and his colleague (2022) revealed in their study that postmenopausal obese women had significantly highest level of leptin in comparison to other postmenopausal, as well as premenopausal women.

Another similarity to the study's results was showed by Pan (2018) who observed that leptin levels are higher in patients with breast cancer compared to patients who are healthy, particularly in women who are overweight or obese.

Regarding serum ferritin, the increased levels of ferritin in breast cancer have been observed, which could be due to excess iron stores, or the underlying inflammation, or both. Tissue inflammation in obese patients can disturb iron homeostasis by inhibiting its absorption, and by its retention in the reticuloendothelial system leading to increase in its storage.

In the pathogenesis of breast cancer, inflammation has an important impact. Pro-inflammatory state leads to the generation of a tumor-related inflammatory microenvironment. Studies have reported the usefulness of inflammatory biomarkers for predicting the prognosis and outcome of breast cancer. The storage form of iron inside the cells is ferritin and is also found in the circulation. The origin of serum ferritin in physiological and pathological conditions could be hepatocytes, macrophages, and cancer cells. The serum ferritin is elevated in inflammation and cancers, this is related to its enhanced synthesis in macrophages. The macrophages that infiltrate breast tumors can release ferritin, which is implicated in tumorigenesis by triggering inflammation. The tissue and systemic levels of ferritin increase in breast cancer and are associated with disease progression (George *et al.*, 2021).

Some consistency was observed by Raya *et al.* (2022) who said that ferritin stimulates the growth of cancer cells through an iron-independent mechanism. Increased infiltration of ferritin-rich macrophages into breast tumors may have a direct effect on tumorigenesis through ferritin production and secretion.

Sukhbaatar and Weichhart (2018) also displayed in their study that in inflammation, the iron recycling from macrophages is inhibited by increased hepcidin levels. This increases serum ferritin levels, systemic iron deficiency and anemia.

While Suárez-Ortegón *et al.* (2019) said that high circulating ferritin levels to be related to metabolically unhealthy obesity.

5.9. Correlation analyses:

5.9.1. Correlation between RBS and HbA1C:

Figure (4-6) shows the correlation between RBS and HbA1C among the patients of the study, and it is clear that a significant positive correlation was present between them. In general, higher random blood sugar levels tend to be associated with higher HbA1C levels, but there can be individual variations depending on factors such as insulin resistance, medication use, and dietary habits, because HbA1C test measures the amount of hemoglobin with attached glucose and reflects the average blood glucose levels over the past 3 months. The A1C test result is reported as a percentage, so the higher the percentage, the higher the levels of blood glucose.

It is necessary to have a clear understanding of the relationship between RBS and HbA1C for both T2DM patients and their health care providers which may be used in adjusting management interventions based

on regular RBS monitoring with the expectation of achieving glycemic control rather than relying solely on HbA1C (Romakin *et al.*, 2021).

Zaidi and his co-workers (2019) revealed that the measurement of HbA1C has become an integral tool for the diagnosis and treatment of diabetes mellitus since its widespread introduction into clinical practice nearly two decades ago. It also serves as a surrogate marker for glycemic control and is a key indicator of the risk of micro-vascular and macrovascular complications and diabetes mortality.

A similar finding to the outcome of the study was conducted by Kazmi *et al.* (2013) on 106 randomly selected T2DM patients and by Rasmussen *et al.* (2014) on 78 T2DM patients where they found a significant linear positive correlation between RBS and HbA1C level.

Also Nasir and his colleagues (2013) who stated that blood levels of HbA1C have a significant correlation with levels of blood sugar and can give a clear idea about glycemic control in the past three months and hence can be used as a preferred method to assess glycemic control in diabetics.

5.9.2. Correlation between RBS and leptin:

As was shown in figure (4-7) there was a significant positive correlation between RBS and leptin among the patients group of the study. This is may be attributed to the disturbances of adipocytokine secretion induced by obesity that may contribute to insulin resistance and its consequences.

Although the relationships between obesity and T2DM have not yet been fully clarified, adipocytokines may play an important role in this interaction. Adipocytokines are secreted by adipose tissue and serve important roles in energy balance and homeostasis. Leptin closely related to

T2DM, as it influence both insulin sensitivity and inflammation. It is commonly believed that leptin are pro-inflammatory cytokines. Although this adipocytokines have been known for many years, their roles in the pathophysiology of T2DM remain controversial (Lee *et al.*, 2019).

Liu and his colleagues (2020) also stated that significant higher serum concentrations of leptin was present in patients with newly diagnosed T2DM who were obese than in patients with newly diagnosed T2DM who had normal BMI.

Opalenyk and Patskun (2020) also detected a possible role of leptin and leptin resistance in the pathogenesis of type 2 diabetes mellitus and indicate that the level of leptin can be used as a predictor of the risk of developing type 2 diabetes mellitus in obese patients.

Also Moonishaa *et al.* (2017) stated in their study that hyperleptinemia reflecting leptin resistance plays an important role in the development of insulin resistance in T2DM patients, making leptin a possible biomarker for assessing insulin resistance levels in T2DM patients, especially in the obese.

5.9.3. Correlation between HbA1C and leptin:

Figure (4-8) shows the correlation between HbA1C and leptin among the patients of the study, which show a significant positive correlation was present between them. Leptin is a protein hormone that regulates the balance of food intake and energy expenditure through communication with the central nervous system. Leptin levels decrease during fasting or starvation and increase after eating. Leptin deficiency or resistance can result in severe obesity followed by insulin resistance, conversely, obesity can also cause leptin resistance. Leptin resistance occurs because leptin secretion continues

to increase in obese individuals so that it reaches saturation levels at its receptors.

Close opinion was shown by Akinjinmi *et al.* (2023) who indicated that obese individuals with type 2 diabetes exhibited elevated HbA1C and leptin levels.

Another similarity was shown by Jatoi *et al.* (2022) who stated in their research that there was a positive correlation between obesity and HbA1C levels in type 2 diabetes mellitus.

Also Sisodia and Chouhan (2019) showed a significant relationship between BMI and HbA1C values.

On another hand, Limijadi and his colleagues (2023) observed that leptin was not associated with HbA1C values.

Vinitha *et al.* (2015) also stated that no significant correlation between leptin levels and HbA1C values.

Chapter Six
Conclusions and
Recommendations

Chapter Six

Conclusions and Recommendations

6.1. Conclusions:

From the present work we can conclude the following:

1. Obese postmenopausal women are at high risk of developing more severe form of breast cancer, evidenced by advanced cancer metrics at the time of presentation.
2. Higher circulating levels of leptin were found to be involved in breast cancer development and progression in postmenopausal women.
3. Ferritin is found to be elevated in postmenopausal women having breast cancer; in whom this elevation was associated with additional severity and progression of the disease.

6.2. Recommendations:

1. Leptin and ferritin are recommended to be used for the prediction of breast cancer's severity in obese postmenopausal women. They could also serve as guidelines to identify high risk patients and to plan appropriate management approaches.
2. Taking attention for patients with major risk factors who are susceptible to develop more advanced form of the disease and giving particular care if more than one risk factor is present.
3. Further studies with larger cohorts and including other parameters not reached in this study, are recommended.

Chapter Seven

References

Chapter Seven

References

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الخلاصة

يعتبر اللبتين والفريتين من العوامل المهمة التي لها علاقة بالحالات الأكثر خطورة من سرطان الثدي لدى النساء بعد سن اليأس وخاصة البدينات منهن. تميل النساء البدينات المصابات بسرطان الثدي إلى أن يكون لديهن سمات تنبؤية ضعيفة في وقت تشخيص مقارنةً بغير البدينات ويكُنَّ مرتبطات كذلك بمرحلة ودرجة متقدمة للسرطان عند التشخيص. يهدف هذا العمل إلى فحص دور المستويات المرتفعة من اللبتين والفريتين في النساء بعد سن اليأس المصابات بسرطان الثدي وعلاقته بزيادة مؤشر كتلة الجسم.

أجريت هذه الدراسة المقطعية على ٢٤٠ امرأة بعد سن اليأس تم تشخيصهن بسرطان الثدي وتم تقسيمهن بعد ذلك إلى مجموعتين وفقاً لنتائج مؤشرات كتلة الجسم.

امتدت الدراسة من بداية أيلول ٢٠٢٢ إلى نهاية آذار ٢٠٢٣. تم جمع المشاركين في هذه الدراسة من مركز علاج أورام بابل في مدينة مرجان الطبية في مدينة الحلة بمحافظة بابل.

تم تحديد قيم الفريتين باستخدام طريقة ElectroChemiLuminescence مع نظام المحلل التلقائي Roche Cobas E411، بينما تم قياس مستويات اللبتين باستخدام تقنية مقايسة الممتز المناعي المرتبط بالإنزيم باستخدام عدة ELISA لقياس اللبتين لدى البشر.

كانت هناك فروق ذات دلالة إحصائية بين مجموعات الدراسة فيما يتعلق بمستويات اللبتين والفريتين (اللبتين، قيمة $p = 0.001$ وللـفريتين، قيمة $p = 0.0001$).

أظهرت قيم سكر الدم العشوائية وهيموجلوبين A1C أيضاً فروقاً معنوية بين مجموعات المرضى (بالنسبة لسكر الدم العشوائي، قيمة $p = 0.011$ وللهيموجلوبين A1C، قيمة $p = 0.007$).

أظهر كل من العمر والمرحلة والدرجة والكيمياء النسيجية المناعية لسرطان الثدي فروق ذات دلالة إحصائية بين مجموعات الدراسة. للتوزيع العمري، قيمة $p = 0.008$. قيم $P = 0.033$ لمرحلة السرطان و 0.0001 لدرجة السرطان؛ بينما بالنسبة للكيمياء النسيجية المناعية، قيمة $p = 0.002$.

تم العثور على ارتباطات إيجابية قوية بين سكر الدم العشوائي والهيموجلوبين A1C ($r = 0.615$)؛ بين سكر الدم العشوائي واللبتين ($r = 0.141$)، وبين الهيموجلوبين A1C واللبتين ($r = 0.168$)، $p = 0.009$.

لم تظهر المؤشرات الأخرى المقاسة (النوع الفرعي لسرطان الثدي، تاريخ ارتفاع ضغط الدم والسكري، تاريخ استخدام الأدوية، الهيموغلوبين، حجم الخلايا المكسدة، متوسط حجم الخلية ومتوسط تركيز الهيموغلوبين في الخلية) أي فروق ذات دلالة إحصائية بين مجموعات الدراسة. ختاماً، زيادة مؤشر كتلة الجسم هو خطر كبير للإصابة بسرطان الثدي لدى النساء بعد سن اليأس ومن الممكن أن يتنبأ قياس مستويات اللبتين والفريتتين بخطورة هذا السرطان لدى هؤلاء المرضى.



وزارة التعليم العالي والبحث العلمي

جامعة بابل

كلية الطب

فرع الفسلجة الطبية

تأثير اللبتين والفريتين على شدة سرطان الثدي لدى النساء البدينات بعد سن اليأس

رسالة

مقدمة إلى مجلس كلية الطب/ جامعة بابل
كجزء من متطلبات نيل درجة الماجستير في العلوم/ الفسلجة الطبية

من قبل

ساره عبد الحسن حمزة جريذي

بكالوريوس طب وجراحة عامة، جامعة بابل (٢٠١٧)

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