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Role of Genetic Polymorphisms of CYP2C19 on Tamoxifen Response in a Sample of Iraqi Breast Cancer Women

A Thesis

Submitted to the Council of College of Pharmacy /University of Kerbala as Partial Fulfillment of the Requirement for the Master Degree in Pharmacology and Toxicology Science

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

((وَفَوْقَ كُلّ ِذِي عِلْمٍ عَلِيمٌ))

صَدَقَ اللة الْعَلِيُ الْعَظِيمُ

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DEDICATION

TO……

Iraqi martyrs

My dear parents

My Lovely husband

My angels' daughters

(Yakoot and Zumored)

My lovely sisters and brother

Zahraa 2023

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List of Abbreviations

Abbreviations	Meaning
4-AAp	4 aminoantipyrin
4-OH TAM	4-hydroxy tamoxifen
ABCB1	ATP-binding cassette transporter family B member 1
AIs	Aromatase Inhibitor
ARMS-PCR	Amplification Refractory Mutation System Polymerase Chain Reaction
BC	Breast cancer
BCS	Breast-Conserving Surgery
BMI	Body mass index
Bp	base pair
BRCA1	Breast Cancer gene1
BRCA2	Breast Cancer gene 2
CA15.3	Cancer Antigen 15.3
CA27.29	Cancer Antigen 27.29
CE	Cholesterol esterase
CEA	Carcinoembryonic Antigen
CHOD	Cholesterol oxidase enzyme
CLIA	Chemiluminescent Immunoassy
CL	Chemiluminescent
CYP450	Cytochrome P450
CYP1B1	Cytochrome P450 Family 1 Subfamily B Member1
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member6
CYP3A4	Cytochrome P450 Family 3 Subfamily A Member4

CYP3A5	Cytochrome P450 Family 3 Subfamily A Member5
CYP2C19	Cytochrome P450 Family 2subfamily C Member19
DCIs	Ductal carcinoma in situ
DNA	Deoxyribonucleic acid
DVT	Deep venous thromposis
E1	Estrone
E2	Estradiol
EDTA	Ethylene diaminete traacetic acid
EMR	Eastern mediterian region
ELFA	Enzyme linked fluorescent assay
ER	Esterogen receptor
FDA	Food and drug administration
G2	Gap 1 phase
GnRH agonist	Gonadotropin releasing hormone agonist
HDL	High density lipoprotein
HER-2	Human Epidermal Growth Factor Receptor 2
HR	Hormonal receptor
IRC	Iraqi Cancer Registery
IDC	Invasive ductal carcinoma
I-F	Inter forward
IgG	Immunoglobulin G
ILC	Invasive lobular carcinoma
I-R	Inter reward
Kg	Kilogram
LDL	Low density lipoprotein
MUC-1	Mucin -1
MRI	Magnetic Resonance Imaging

NDM TAM	N –desmethyl tamoxifen
OATP1B1	Organic anion transporting polypeptides family member 1B1
O-F	Outer forward
O-R	Outer reward
PCR	Polymerase chain reaction
Pg /ml	Picogram per milliliter
Pmol/μL	Picomole per microliter
POD	Peroxidase enzyme
PR	Progesteron receptor
RBC	Red blood cell
RLUS	Relative light units
Rpm	Rotation per minute
SERDs	Selective Estrogen Receptor Degrader
SERMs	Selective Estrogen Receptor Modulators
SNPs	Single nucleotide polymorphisms
SPR	Solid phase receptacle
TAM	Tamoxifen
TBE	Tris-borate-EDTA
TC	Total cholesterol
TG	Trigliceride
TNM	Tumor (T),Nodes (N),Metastasis (M)
US	United State
UV	Ultraviolet
v/cm	Volt per centimeter
μL	Microliter

Abstract

Background: Breast cancer is considered a global problem of health that affects the breast. Tamoxifen which is non-steroidal selective estrogen receptor modulator. It is considered standard adjuvant therapy and widely used endocrine therapy for a patient with steroid hormone receptor-positive breast cancer. CYP2C19 plays an essential role in forming a proportion of tamoxifen metabolites including converting 4-OH-tamoxifen to endoxifen. Pharmacogenomics studies the impact of the individual's genome on response to medication and describes how genes influence the metabolism of drugs.

Aims of the study: The aim of present study is to identify the single nucleotide polymorphism of (G >A, rs4986893) and (C >T, rs11188072) in a sample of Iraqi breast cancer women and to investigate the effect of CYP2C19 genetic polymorphisms on tamoxifen therapy response.

Patients and methods: This is across-sectional observational study carried out at Imam AL-Hussein Medical City/Oncology center in kerbala and laboratories of College of Pharmacy / University of Kerbela . The study was conducted on total 100 females Iraqi women aged 45-65 years with (Esterogen and /or Progesteron- receptor positive breast cancer, taken tamoxifen tablet 20 mg daily dose at least 3 months were included without any diseases.

Results: The obtained results from this study have detected multiple genotypes of CYP2C19*3(G > A, rs4986893) alleles that include wild type (GG), homozygous mutant type (AA) and heterozygous mutant type (GA)

genotype, while for CYP2C19*17 (C > T, rs11188072) wild type (CC), mutant type (TT) and heterozygous mutant type (CT) also detected in Iraqi breast cancer patients participated in this study. The serum levels of estradiol (E2), tumor marker (CA15.3), vitamin D3, calcium and lipid profile showed non-significant association ($p>0.05$) with the studied SNPs of CYP2C19.

Conclusion: The current study concluded that genetic variation in phase I metabolizing enzyme CYP2C19 not contribute to variability in response to tamoxifen therapy in a sample of breast cancer Iraqi women.

Chapter one

Introduction

1. Introduction

1.1 Breast cancer

Breast cancer is atumor disease; its pathogenesis mainly related to estrogen.It is the most common neoplastic disease in women worldwide and is the main cause of cancer death in women (1).

Breast cancer most commonly develops in cells lining the milk ducts and the lobules that supply the ducts with milk(2).

1.1.1 Epidemiology

Numerous studies have found that Iraqi women are more likely than their Western counterparts to develop breast cancer at a younger age, in advanced stages and with aggressive behavior. The latest annual report of the Iraqi Cancer Registry (ICR) for the year 2019 indicated that breast cancer has increased in the last two decades and has become the first among the top ten malignancies affecting society (19.7%) , followed by bronchi and lungs, while cancer-related deaths are due to bronchi and lungs (16%), breast (11.3%), and leukemia (8.6%) (3).

Breast cancer is a serious disease that spreads and causes significant physical illness. Data collected in worldwide estimated that breast cancer had a higher incidence of 287,850 new cases (representing 31% of all cancers) compared to lung cancer's 118,830 cases (13%), despite the fact that lung cancer's mortality rate is 21% (of all cancer deaths) , breast cancer deaths which is 15% (4) .Breast cancer incidence trends vary by country, due to ethnicity, cultural and geographical difference (5).

Breast cancer rates are high in developing Middle Eastern countries such as Egypt, Lebanon, Syria, Jordan, Iran, Iraq and Saudi Arabia. Although the incidence of breast cancer in the Middle East is lower than in Western countries, ranging from 22 to 71/100,000 women. Women aged 40-49 years

accounted for nearly a third of all breast cancer diagnoses in the Eastern Mediterranean Region (EMR).The incidence of breast cancer is highest in Asia Premenopausal women in their 40s, while in Western countries it peaks among postmenopausal women in their 60s (6).Whereas, western countries have higher incidence rates of breast cancer with stable and declining mortality rates (7).

1.1.2 Pathogenesis

Normal breast development is controlled by specific genes and signaling pathways that control cell division, death, differentiation, and motility.Genetic dysregulation of multiple signaling pathways leads to the formation of primary tumors and metastases. The metastatic cascade is a multi-step process that includes invasion of local tumor cells, entry into blood vessels, exit of malignant cells from circulation, and finally colonization in distant organs (8). Loss of control of apoptosis causes cancer cells to survive longer, gives more time for mutation accumulation, stimulates angiogenesis, increases tumor invasion, and dampens cell proliferation and differentiation (9).

There are two types of breast tumors: benign and malignant. The most common type of benign form (fibroadenoma) is caused by abnormal growth of cells in the ducts of the breast, causing a bump that is unusual and does not appear to spread to other parts of the body, which is usually removed by surgery. Whereas in malignancies (Invasive ductal carcinoma, and Invasive lobular carcinoma) when left untreated, cancer cells can metastasize beyond the breast to the lymph nodes, bone, brain, lung, and liver (10).

The development of breast cancer is caused by mutations in BRCA1 or BRCA2, which are tumor suppressor genes. Cells that lack BRCA1 function are unable to arrest in the G2 phase of the cell cycle after DNA damage. BRCA2 deficiency is responsible for the error of chromosome segregation ;thus after several divisions, chromosomal abnormalities develop (11).

Estrogen is essential for the normal growth and growth of breast tissue. It is also considered a significant risk factor for breast cancer when exposed to a high level of estrogen(12).Estrogen is a phenolic steroid hormone produced by ovaries and secreted into the blood and also produced peripherally in cells expressing the aromatase enzyme, has lipophilic nature that allows it to pass through cell membranes; estradiol (E2), oestrone(E1) and oestriol(E3) are the primary physiological estrogen form(13).

Estrogen signals through its two receptors (ERα and ERβ), but only ERα is essential for the development of the breast and activates proliferative signalling in common and breast cancer. In contrast ERβ generally antagonizes ERα in the breast(14).Two mechanisms involved in the carcinogenesis of estrogen:

A- Receptor dependent: estrogen receptor (ER) mediated stimulation of breast cell proliferation through stimulating transcription of genes involved in the proliferation of cells with a concomitant enhanced rate of mutations(during mitosis for each cell cycle, a new DNA synthesized and there is the point of mutation if not repaired as the process can lead to the development of breast cancer)(15)

B- Receptor independent: because estrogen contains aromatic A-ring, it undergoes oxidative metabolism by CYP1B1 which is present in breast tissue resulting in the formation of unstable genotoxic metabolite 3,4-quinones that form adducts with amino acid bases of DNA (adenine and guanine) can result in a point of mutation(16).

Overexpression of the human epidermal growth factor receptor-2 (HER2) predisposes to poor differentiation, high-grade tumors, increased rates of cell proliferation and lymph node involvement, and relative resistance to certain types of chemotherapy and endocrine therapy. This led to a poor prognosis

due to increased invasion and metastasis as well as increased vascular activity (17).

1.1.3 Clinical presentation

The main presentation of breast cancer is typically a lump that is different from the rest of the breast tissue, while other presentations include: one breast becomes bigger or smaller and changes in the position or shape of the nipples, skin dimpling, nipple discharge, pain in part of the breast or swelling beneath the axilla(18).

Breast cancer can present as a metastatic disease. Common sites of metastasis include bone, liver, lung and brain. Symptoms depend on the site of metastasis and may include weight loss, fever, bone pain, jaundice or neurological symptoms(19).

1.1.4 Risk factors

Risk factors are things that will increase the probability of developing BC condition in women (20) , which include:

1.1.4.1 Gender

Unlike men, who present insignificant estrogen levels, women have breast cells which are very sensitive to hormones (estrogen and progesterone in particular) and any change in their balance (21).

Changes within the physiological levels of endogenous sex hormones result in a higher risk of breast cancer in the case of premenopausal and postmenopausal women (22) (23). Less than 1% of all breast cancers occur in men ; breast cancer in men is a rare disease that at the time of diagnosis tends to be a more advanced condition than in women (24).

1.1.4.2 Age

Besides sex, ageing is one of the important risk factors for breast cancer as the incidence of the disease largely increases with age. About 80% of patients with breast cancer are individuals aged >50 years, while at the same time, more than 40% are more than 65 years old female (25)(26).

1.1.4.3 Family history

About 13–19% of patients diagnosed with breast cancer are those of a first-degree relative affected by the same condition. The risk of breast cancer significantly increases with the number of first-degree relatives affected. The risk is higher when the affected relatives are at younger age dignosis (27)(28).The incidence rate of breast cancer is significantly higher in all patients with a family history despite their age(29).Many genetic mutations were reported to be highly associated with increased risk of breast cancer. There are two significant genes characterized by BRCA1 (located on chromosome 17) and BRCA2 (located on chromosome 13) they are primarily linked to elevated risk of breast carcinogenesis (30).

1.1.4.4 Breastfeeding

Breastfeeding mother for two years will reduce breast cancer risk , so breastfeeding has a protective effect. A possible explanation for this effect is that prolonged breastfeeding will cause ovulatory suppression reduce the exposure of breast cells to the reproductive hormone by reducing women total number of lifetime menstrual cycles (31).

1.1.4.5 Lifestyle factors

Modifiable risk factors including the excessive use of alcohol, obesity and physical inactivity account for 21% of all breast cancer deaths worldwide (32).

1.1.4.5.1 Obesity

Obesity might be a reason for greater mortality rates and a higher probability of breast cancer(33).Poorer clinical outcomes are primarily obtained in females with BMI 25 kg (34).

Postmenopausal women tend to be poorer clinical outcomes due to excessive fat volume(35).This association is mostly intensified in obese postmenopausal females who tend to develop estrogen-receptor-positive breast cancer (36).

1.1.4.5.2 Physical activity

Regular physical activity is a protective factor from breast carcinogenesis(37).Because physical activity will reduce body fat, decrease the peripheral conversion of androgens to estrogens through the enzyme aromatase and increase sex hormone–binding globulin so that reduce the total amount of free circulating estrogen(38).

1.1.4.5.3 Smoking

Cigarette smoking and exposure to smoke can increase the hazard for BC and worsen survival outcomes; a longer smoking history is an additional risk factor found in females with a family history of breast cancer(39)(40).

1.1.4.5.4 Alcohol consumption

Excessive alcohol consumption was linked to the risk of breast cancer. The explanation for this association ; increased levels of circulating estrogens induced by alcohol intake through suppression of hepatic estrogen metabolism and by enhancing the conversion of androgens to estrogen . Thus, hormonal imbalance affects the risk of carcinogenesis within the female(41).Alcohol intake results in excessive fat gain with higher BMI levels that will increase the condition of risk(42).

1.1.5. Types of breast cancer

Depending on histological appearance, breast cancer is initially divided according to its originated lesion from either the ductal epithelium or lobular epithelium(43).

1.1.5.1 .Non‑invasive breast cancer

It is a type of cancer that has not extended away from the lobular ducts where it is situated(44). Atypical cells have not extended to tissues outer

the lobules or ducts; they can progress and grow into invasive breast cancer. Non-invasive breast cancer, includes: Ductal carcinoma in situ is the most general non-invasive breast cancer limited to the breast duct(45).

Lobular carcinoma in situ is identified as non-invasive breast cancer that has developed into breast lobules(46).

1.1.5.2 . Invasive breast cancer

It exists when abnormal cells within the lobules or milk ducts split out into proximity to breast tissue(47). Cancer cells can pass through the breast to different body parts through an immune system or systemic circulation(48).

1.1.5.2.1. Invasive lobular carcinoma

Infiltrating lobular carcinoma is also recognized as invasive lobular carcinoma. It originated in the breast's milk glands (lobules) but frequently extended to other body areas(49).

1.1.5.2.2. Invasive ductal carcinoma

Infiltrating ductal carcinoma is also recognized as invasive ductal carcinoma. It originated in the breast milk ducts and extended to the duct wall, invading the breast fatty tissues and probably other body parts. It is considerd as a most prevalent type of invasive breast cancer account for about up to 75% (50).

1.1.5.3. Inflammatory breast cancer

Inflammatory breast cancer is the form of swollen breasts (red and warm) with dimples and broad ridges because cancer cells block lymph vessels or channels in the skin over the breast. Inflammatory breast cancer is uncommon and extremely fast-growing(51).

1.1.6. Prognostic factors

Prognostic factors can be defined as those that measure at the time of diagnsois, which are related to disease-free or overall survival and can frequently be used to expect the nature of the tumor (52).

1.1.6.1. Hormonal receptors

Estrogen is one of the successful tumor markers and important diagnostic biomarkers of female breast cancer, as approximately 70–75% of invasive breast carcinomas that are characterized by significantly enhanced ER expression(53)(54).ER is a member of the family of nuclear steroid receptors(55). ER expression is also a predictive factor—patients with high ER expression usually present significantly better clinical outcomes(56).

A relationship was observed between ER expression and the family history of breast cancer, which facilitates the utility of ER expression as a diagnostic biomarker of breast cancer, especially in cases of familial risk(57)(58).

The progesterone receptor is a member of the family of nuclear hormone receptors that bind distinct to progesterone(59). ER and PR are abundantly expressed in breast cancer cells and both are considered diagnostic and prognostic biomarkers of breast cancer (especially ER-positive ones)(60).

1.1.6.2. Human Epidermal Growth Factor Receptor 2 (HER2)

It is a member of tyrosine kinase receptor type and is involved in several regulatory pathways in breast proliferation, survival, cell motility and invasion. It is an essential prognostic factor for outcomes in both node-negative and node-positive and a predictive factor in proper choice management with breast cancer patients(61).

HER2 amplification leads to further over-activation of the pro-oncogenic signalling pathways leading to uncontrolled growth of cancer cells, which corresponds with poorer clinical outcomes in the case of HER2-positive cancers(62).

1.1.6.3. Tumor nodes and metastasis classification of breast cancer

Tumor node metastasis classification describes the size of the tumor (T): T0 (no evidence of tumor), T1 (the invasive part of the tumor has size ≤ 20 mm), T2 (the invasive part of the tumor is 20–50 mm), T3 (the invasive part >50mm) and T4 (the tumor has grown into the chest wall and skin with signs of inflammation). Lymph node status (N): N0 (no cancer cells are found in the lymph nodes); N1(cancer has spread to three nodes); N2 (cancer has spread to four to nine nodes); and N3 (cancer has spread to ≥10 nods) and distant metastasis presence (M): M0 (cancer has not metastasized); and M1 (there is an evidence of metastasis to another part of the body)(63)(64).

1.1.6.4. Stages of breast cancer

Breast cancer staging is essential in local and systemic treatment determination and provides prognostic information (65). There are four stages of breast cancer depending on the TNM system that includes: stage 0, which indicates apre-cancerous, either ductal carcinoma in situ or lobular carcinoma

in situ. Stages 1-3 are within the breast or original lymph node and stage 4 is considered metastatic cancer with a poor prognosis (66).

1.1.6.5. Histological grade

The histological grade is a measure of the differentiation of breast cancer. Grading can describe the breast cancer cell as a low grade which means well differentiated; intermediate grade which means moderately differentiated and high grade which means poorly differentiated(67).

1.1.6.6 Tumor marker

A tumor marker is a biomarker found in blood, urine or body tissues that can be elevated by the presence of one or more types of cancer. It is produced either by the tumor itself or the host in response to a tumor(68). The tumor marker recommended for use in practice includes CA 15.3, CA27.29, and Carcinoembryonic antigen (CEA)(69)

1.1.6.6.1 Cancer antigen 15.3

Cancer antigen (CA) 15.3 is a carbohydrate-containing protein antigen called mucin (MUC). CA 15.3 belongs to the MUC1 family. The MUC1 gene is found in several tissues and is overexpressed in BC allowing the use of gene product CA 15.3 as tumor marker for breast cancer. CA 15.3 concentrations in the blood can be used for screening breast cancer and other malignancies including pancreatic, lung, ovarian, colon and liver (70)(71).

1.1.6.6.2 Cancer antigen 27.29

Cancer antigen 27.29 is a carbohydrate-containing protein antigen that serves as a tumor marker for breast cancer. CA27.29 can also be found in patients with other malignancies or benign breast, liver and kidney disorders and in patients with ovarian cysts. It is also called breast carcinoma-associated antigen(72).

1.1.6.6.3 Carcinoembryonic antigen

Carcinoembryonic antigen (CEA), which belongs to a family of related cell surface glycoproteins is the most widely used tumor marker for colorectal, gastrointestinal, lung and breast cancer. Continuous rising level of CEA in breast cancer may be explained either that cancer is not responding to treatment or recurrence after treatment(73).

1.1.7 Detection and Diagnosis

1.1.7.1 Mammography

Mammography is considered the standard gold test for the early detection of breast cancer in women; it helps to find lumps in dense tissue and gives two-dimensional images that can be easily stored and transmitted to radiologist for opinion(74). Many factors affect mammographic accuracies such as breast tissue density. A woman with higher breast density is related to decreased mammography sensitivity(75).

1.1.7.2 Magnetic resonance imaging

MRI has been considered an appropriate screening method for breast cancer detection for many years. It is a technology based on magnetic fields. The sensitivity of MRI is more than mammographic while the specificity of mammographic is higher(76).

1.1.7.3 Ultrasound

Ultrasound permits physicians to display breast tumors not measured by long-established mammography(77).Ultrasound breast imaging shows the size and position of tumor whether filled with fluid or solid and needs to be biopsied to rule out cancer. This examination is characterized by quickly becoming a routine procedure for diagnosing lumps in young women(78).

1.1.8 Management

Breast cancer management aims to preserve the quality of life with prolonged life expectancy. Patients with early breast cancer choose therapy dependent on tumor size, the feasibility of surgery and clinical phenotype (79).

1.1.8.1 Surgery

Surgery is the most local management strategy for a female whose breast cancer has not extended to different areas of the body and is the choice for the complex stage of the condition. There are two major types of surgical procedures breast-conserving surgery (BCS) and mastectomy(80).

BCS also called (partial/segmental mastectomy or Lumpectomy) is wide local excision, removal of the cancerous tissue with simultaneous preservation of intact breast tissue. The mastectomy involves the removal of part of the breast and the axillary lymph node without the removal of muscle so, this procedure is more famous(81).

1.1.8.2 Radiation therapy

Radiation therapy is an adjuvant treatment for most BC after lumpectomy or mastectomy to reduce the chance of recurrence. Radiation therapy involves using high-energy X-rays or gamma rays that target a tumor or tumor site. It effectively kills cancer cells that may remain after surgery or recur when the tumor is removed(82).

1.1.8.3 Chemotherapy

Chemotherapy is a systemic treatment of BC and chooses the most appropriate one according to the characteristics of the breast tumor can be provided intravenously or orally like(paclitaxel,cyclophosphamide and 5-fluorouracil) (83). Chemotherapy is considered effective, it has several side

effects like: hair loss, nausea/vomiting, diarrhea, mouth sores, fatigue , increased susceptibility to infections and bone marrow suppression(84).

1.1.8.4 Biological Therapy

Biological therapy (targeted therapy) can be provided at early stage of breast cancer therapy–before surgery as neoadjuvant therapy or adjuvant therapy. Biological therapy is quite common in HER2-positive breast cancer patients major drugs including (trastuzumab and pertuzumab)(85).

1.1.8.5 Hormonal (Endocrine) Therapy

Endocrinal therapy aims to lower estrogen levels or prevent breast cancer cells from being stimulated by estrogen. Estrogen and progesterone are essential in regulating breast tissue growth and differentiation both steroid hormones are mainly generated in the ovaries and exert their cellular consequences by binding to and activating specific nuclear receptors (ERs and PRs)(86). About 60% of premenopausal women and 75% of postmenopausal females are estimated to have breast cancer hormone (estrogen and/or progesterone) receptor-positive tumors(87).

1.1.8.5.1 Selective estrogen receptor degraders

Fulvestrant is a SERDs agent, a highly insoluble compound with poor oral bioavailability and a short intravenous half-life therefore must be given intramuscularly(88).

1.1.8.5.2 Aromatase inhibitors

Aromatase inhibitors (AIs) are antiestrogenic compounds that aim to lower estrogen levels which includes (letrozole, anastrazole and exemestane). In postmenopausal women, estrogen is no longer released by the ovary. It is mainly synthesized from non-glandular sources by an enzyme called aromatase that can be found in many tissues such as fat, liver, muscle and in breast cancer cells(89). So,aromatase inhibiter will reduce both endogenous

estrogen levels and breast cancer incidence also reduce the endometrial cancer risk(90).

1.1.8.5.3 Gonadotropin-releasing hormone agonist

Suppression of estrogen in premenopausal females is achieved by radiation to the ovaries or surgical oophorectomy and by using GnRHa such as goserelin (Zoladex) that is used monthly as a depot injection which acts by decreasing the gonadotropins and sex hormones production by the gonads. So they lower endogenous estrogen production(91)(92).

1.1.8.5.4 Selective estrogen receptor modulators

Selective estrogen receptor modulators (SERMs) are a diverse group of non-steroidal compounds that function as ligands for ER. SERMs can selectively act as agonists or antagonists depend on target tissue (93).

The chemical structure of SERMs can be the basis for their classification: triphenylethylene (tamoxifen), benzothiophenes (raloxifene), phenylindoles (bazedoxifene,pipindoxifene)and tetrahydronaphthalenes (lasetofoxifene)(94).

1.2. Tamoxifen

Tamoxifen sold under the brand name (Nolvadex) is a non-steroidal selective estrogen receptor modulator.TAM is considered standard adjuvant therapy and the most widely used endocrine therapy for a patient with steroid hormone receptor-positive breast cancer(95). Morever 5 years of adjuvant endocrine treatment has been more effective than 1–2 years of treatment . The recurrence rate of patients receiving tamoxifen (TAM) increases from 15% at 5 years to 33% at 15 years and cancer mortality increases from 8.3% at 5 years to 26% at 15 years (96).

It was discovered in 1967 and approved by the Food and Drug Administration in 1977 for treating patients with advanced breast cancer and later for adjuvant treatment of primary breast cancer(97).

1.2.1 Mechanism of action of tamoxifen

Estradiol binds to the ER in a target cell, which initiates a chain of events. The estrogen-ER complex homologs and binds to separate DNA sequences in regulatory regions of estrogen-sensitive genes known as estrogen response elements (ERE). The two transcriptional activating functions of the estrogen-ER complex, AF1 and AF2 interact with other proteins (transcriptional activators) to stimulate RNA polymerase II (RNA Polymerase) activity thereby regulating gene activity(98).

Tamoxifen inhibits estrogen binding to the ER in a competitive manner. Tamoxifen-ER complex also homodimerizes and binds to an estrogen-responsive element in estrogen-sensitive genes. Moreover, only AFI remains active where the inactivation of AF2 reduces the transcriptional activity of the estrogen-responsive gene and activator binding. As a result, tamoxifen inhibits the G1 phase of the cell cycle and limits cell growth(99).

1.2.2 Pharmacokinetics of tamoxifen

The usual dose of tamoxifen is (20 mg) daily.Tamoxifen is easily absorbed following oral administration,with peak plasma concentration 4-7 hours and steady state level attained in 3-4 weeks of treatment. After a single oral dosage of 20 mg tamoxifen,concentration in breast tissue and lymph nodes were high exceed serum ratio(100).

Tamoxifen undergoes extensive hepatic metabolism with about 65% of the dose excreted from the body by the fecal route and mainly excreted as polar conjugates with the unconjugated metabolites and the unchanged drug accounting for less than 30% of total fecal excretion(101).

1.2.3 Pharmacodynamics of tamoxifen

The antitumor effects of tamoxifen are thought to be due to its antiestrogenic affecting that is mediated by competitive inhibition of estrogen binding to estrogen receptors(102). Tamoxifen is a prodrug with relatively little affinity for its target protein, estrogen receptor (ER). It is mainly metabolized in the liver by the cytochrome P450 isoforms into active metabolites and other metabolites which have 30-100 times more affinity than tamoxifen with ER(103)(104).

These active metabolites compete with estrogen for binding to the ER in breast tissue. These active metabolites act as an ER antagonist leading to the inhibition of transcription of estrogen-responsive genes(105).

1.2.4 Medical uses

1.2.4.1 Breast cancer

Tamoxifen is mainly used to treat early and advanced estrogen receptor-positive breast cancer in pre-and postmenopausal females(106).

1.2.4.2 Infertility

Tamoxifen treats infertility in women with ovulatory disorders(107). It also improves fertility in males by disinhibiting the hypothalamic-pituitary-adrenal axis and there by increasing the secretion of luteinizing hormone and follicle-stimulating hormone and testicular testosterone production(108).

1.2.4.3 Gynecomastia

Tamoxifen is used to treat gynecomastia, it is taken as a preventative measure in small doses or used at the onset of any symptoms such as nipple soreness or sensitivity(109) . Tamoxifen has become the most frequently used endocrine in breast cancer of men(110).

1.2.5 Drug interaction

Many patients are on medications that may interfere with the effectiveness of tamoxifen such as Antidepressants (paroxetine and duloxetine) and certain antimicrobials such as (terbinafine and quinidine) that reduce the effectiveness of tamoxifen by inhibiting the conversion of tamoxifen to its active metabolites by inhibition of the cytochrome P450 2D6 (111)(112)(113).Other antimicrobials such as; moxifloxacin and ciprofloxacin may impact cardiac function(114).

1.2.6 Adverse effect

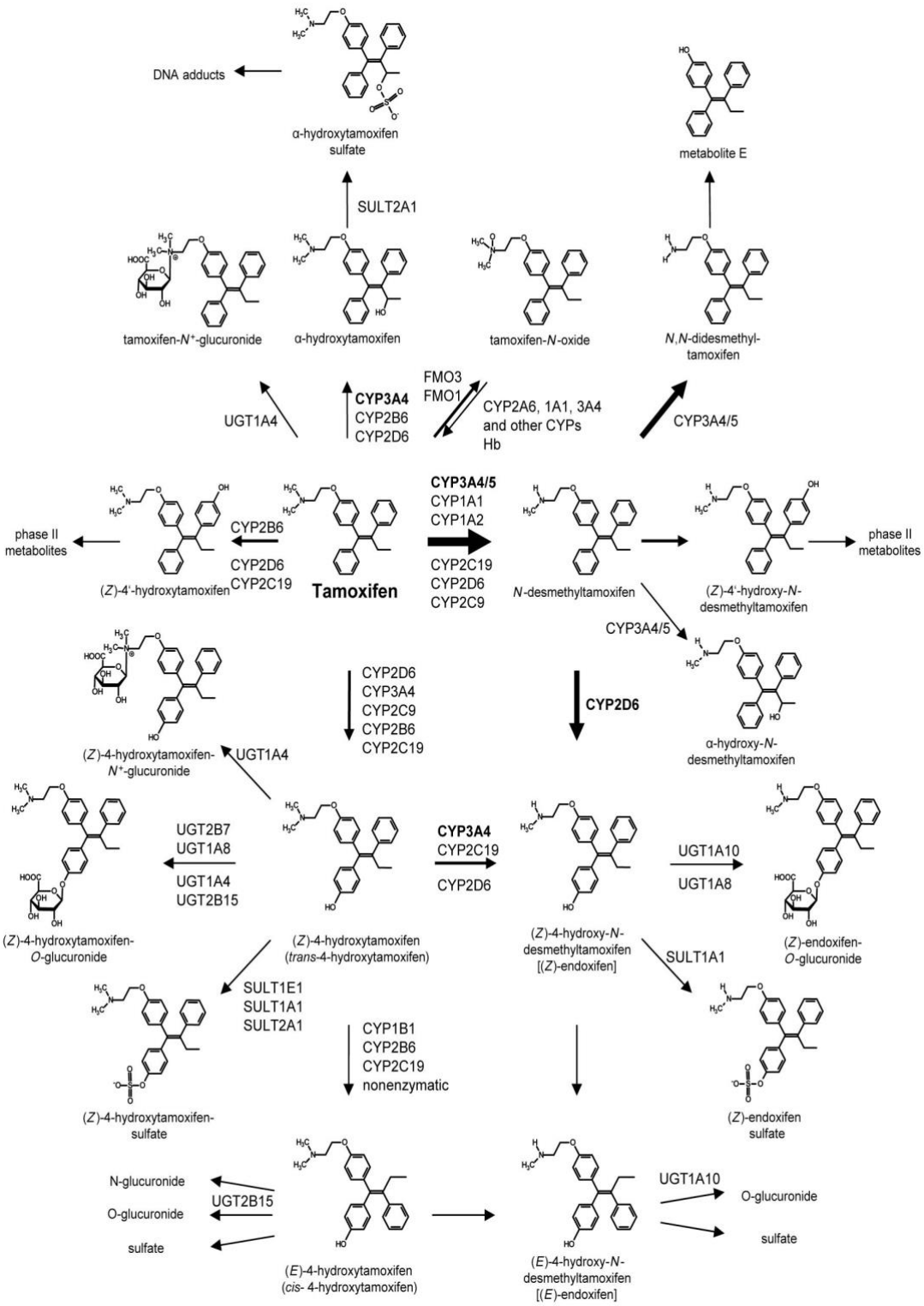
The adverse effect of tamoxifen includes menopausal symptoms (hot flashes, atrophic vaginitis, irregular menses), ocular toxicity, thrombocytopenia or leukopenia and gynecologic complications (endometrial cancer, endometrial hyperplasia and polyps)(115).

1.2.7 Metabolism of tamoxifen

Tamoxifen is a prodrug extensively metabolized by phase 1 and 2 metabolic pathways in the liver that requires metabolic activation(116). The primary route of tamoxifen metabolic activation occurs through the demethylation of tamoxifen to N-desmethyl-tamoxifen (NDtam), which is catalyzed mainly by (CYP3A4, CYP3A5) with minor contributed (CYP2D6, CYP1A2, CYP2C9, and CYP2C19) followed by 4-hydroxylation of ND tam to the active metabolite 4-hydroxy-N-desmethyl-tamoxifen (endoxifen) which is generated manily by the action of CYP2D6. Second active metabolite, 4-hydroxy-tamoxifen(4OH tam) is formed by 4- hydroxylation of tamoxifen . Endoxifen and 4OH tam have ~100 higher affinity to the estrogen receptor than the parent drug tamoxifen(117).

Previous pharmacological studies indicate that these two active metabolites may be responsible for tamoxifen's anti-estrogenic effect and

efficacy(102)(118). A significant source of endoxifen variability is attributed to the activity of CYP2D6, the rate-limiting enzyme in the conversion of NDtam to endoxifen(119).



Figure(1.1):Metabolic pathways of tamoxifen metabolite(120).

1.2.8 The pharmacogenomics of tamoxifen

Pharmacogenomics studies the impact of the individual's genome on response to medication and describes how genes influence the metabolism of drugs(121)(122). Pharmacogenomics uses genetics to optimize drug therapies, maximize drug efficacy and minimize adverse drug reactions(123). Pharmacogenomics is a DNA-based test that detects genetic variations associated with the risk of adverse response or drug response(124).

After standard dosing, there is significant interindividual variability in the steady-state levels of the two strongest tamoxifen metabolites, 4-hydroxy-tamoxifen and endoxifen. The majority of these differences can be attributed to CYP2D6 genetic polymorphisms. There are currently more than 100 variants of CYP2D6 alleles with different metabolic activities(125). The predicted phenotype allows metabolites to be classified into four groups: weak, intermediate, extensive and supra metabolite. Patients carrying CYP2D6 alleles with elevated enzymatic activity had elevated serum levels of 4OHTAM and endoxifen. Low endoxifen and N-desmethyl-tamoxifen concentrations will be expected to be associated with CYP3A4*22, CYP3A5*3, and CYP3A5*6 polymorphisms indicating reduced or no metabolic activity(125).

Tamoxifen metabolism is altered by CYP2C19*2 and CYP2C19*17 variant alleles that do not have or increase metabolic activity(126). The enzymes responsible for the elimination and inactivation of tamoxifen and its metabolites by conjugating with sulfate or glucuronide may be subject to significant genetic variation. Patients with breast cancer treated with adjuvant tamoxifen who had SULTIA1*2/*2 and either UGT2B15*1/*2 or UGT2B15*2/*2 had a significantly lower 5-year survival rate(127).

Previous studies found that a gene polymorphism of OATP1B1 (the efflux transporter) resulted in decreased uptake of tamoxifen and endoxifen into

cells, which may represent a potential therapeutic strategy for the treatment of breast cancer(128).

Many effectiveness breast cancer treatments including tamoxifen, are also affected by genetic variants of ABCB1 (the efflux vector) which will be critical in the future for development of personalized anti-cancer therapies(129).

1.3. Genetic polymorphism of CYP2C19

SNPs are mutations that usually occur at 1% or higher frequency. SNPs can occur every 100-300 base pairs and account for about 90% of all differences in human DNA. These genotypes affect an individual's ability to metabolize certain drugs(130).

CYP2C19 gene (cytochrome P450, family 2, subfamily C, polypeptide 19).CYP2C19 enzyme is encoded by the CYP2C19 gene, located on chromosome 10q24 along with the other CYP2C genes in the order of CYP2C18-CYP2C19-CYP2C9-CYP2C8 from centromere to telomere(131).

CYP2C19 gene is highly polymorphic,CYP2C19 enzyme is a protein of 490 amino acids that binds to substrates that are generally neutral or weakly essential molecules. The drugs metabolized by the CYP2C19 enzyme are classified as CYP2C19 substrates; it is an essential member of the CYP450 superfamily, which plays a substantial role in the metabolism of approximately 10% of commonly prescribed drugs such as proton pump inhibitors, antipsychotics, antidepressants, and clopidogrel. CYP2C19 plays an essential role in tamoxifen metabolites including converting 4-OH-TAM to endoxifen(132).

CYP2C19*3(G>A, rs4986893) gene has a mutation 636G>A in exon 4, creating a premature stop codon and producing a truncated inactive enzyme, the *3 allele occurs in 5–10% of Asians (133)(134).

CYP2C19*17(C > T, rs11188072) gene has two linked mutations, -806C >T and -3402C >T, with high linkage in the 5'-regulatory region leading to increasing expression and activity of the enzyme. The *17 alleles are found in about 4% of Asians and 18–24% of Caucasians and Asians(135)(136).

1.4 Aims of the study:

- To detect genetic polymorphism in phase I metabolizing enzyme CYP2C19 of (G > A, rs4986893) and(C > T, rs11188072) in Iraqi breast cancer women taking tamoxifen therapy.
- To identify genetic distribution of CYP2C19 (rs4986893and rs11188072) genotype between Iraqi breast cancer women .
- To determine the effect of genretic polymorphism of metabolizing enzyme (CYP2C19) on tamoxifen response in breast cancer women by measuring serum level of estradiol (E2) and tumor marker (cancer antigen CA15.3), in addition to lipid profile, vitamin D3 and calcium levels.

Chapter two

Patients, Materials and Methods

2. Patients ,Materials and methods

2.1 Patients

This study was across-sectional observational study carried out at Imam AL-Hussein Medical City/Oncology center in kerbala and laboratories of College of Pharmacy / University of Kerbela during the period from September 2021 till February 2022. The protocol of the study was approved by the Scientific and Ethical Committee of Pharmacy College / Kerbala University and informed signed consent form was given by each subject after explaining the nature and purpose of study. The study was conducted on total 100 females Iraqi women aged 45-65 years with (ER and /or PR) positive breast cancer who were taken tamoxifen tablet (20 mg per day) orally at least 3 months were included without any diseases. All precautions have been taken in clinical settings to prevent infection of COVID-19**.**

2.1.1 Inclusion criteria

Female patients with breast cancer were receiving 20 mg /day tamoxifen orally for at least 3 months after using tamoxifen therapy with no other diseases.

2.1.2 Exclusion criteria

1. Patients were excluded if they had started tamoxifen therapy simultaneously with either adjuvant chemotherapy or adjuvant radiation therapy (or both).

2. Women who were taking inducer drugs (phenytoin ,rifampin and prednisolone) or inhibitors drugs (cimetidine,ciprofloxacin and erythromycin) that affect the activity of CYP2C19 enzyme were excluded.

3. Patients with previous history of hepatic disorder or liver surgery, deep vein thrombosis , pregnant and breast feeding woman were excluded.

2.2. Materials

2.2.1. Instruments

The instruments that used in this study with their manufacture and origin are listed in table (2-1).

Table (2-1): Instruments used in this study with their manufacture and origin.

Instruments	Manufacture / Origin
Autoclave	LabTek/ Korea
Digital camera	Canon / England
Electrophoresis apparatus	Consort / Belgium
High speed centrifuge	Mikro 200R Hettich / Germany
Hood	LabTech / Korea
Hot plate stirrer	LabTech DAIHAN / Korea
Micropipettes	Eppendorf / Germany
Refrigerator/ freezer (-20 C)	Concord / Lebanon
Sensitive balance	AND/ Taiwan
Thermos cycler (PCR apparatus)	Cleaver / USA
UV trans illuminator	UV-VIS /JAPAN
Vortex mixer	Human Twist / Germany
Water bath	LabTech / Korea

2.2.2. Chemicals and kits

Specific chemicals and kits that used in this study are listed in table (2-2).

Table (2-2): Kits and chemicals used in this study with their manufacture origin.

Kits and Chemicals	Manufacture / Origin
Absolute ethanol	SDI/Iraqi
Agarose	BDH / England
CA15.3 Kit	Biomerieux / France
Calcium kit	Mindray/China
DNA ladder(100 bp)	Bioneer / Korea
DNA extraction kit	Favorgen Bio Tech /China
Estradiol Kit	Mindary / China
Ethidium bromide	Sigma / USA
Lipid profile kit	Mindray/China
Nuclease free water	Promega / USA
PCR master mix	Promega / USA
Primers	Macrogen / Korea
Tamoxifen tablet 20mg	AstraZeneca/UK
TBE (Trise Borate EDTA) buffer solution	Bioneer / Korea
Vitamin D kit	Snibe /Germany

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2.3. Methods

2.3.1.Clinical data collection

During the time of blood sample collection ; the data were obtained from the medical records of consenting patients and from the patients themselves and these include: age, weight, height, education , workplace, marital status, breast feeding, family history of breast cancer and number, date of breast cancer diagnosis, site (left ,right, bilateral), type of breast cancer, stage and grading, immunhistochemically status (ER,PR,HER2), surgery, chemotherapy, radiation, presence of osteoporosis, liver disease or any other diseases ,time on tamoxifen therapy and duration and other drugs used.

2.3.2. Sample collection and analysis

After an overnight fast, blood samples were taken from females who had signed informed consent. About 6 ml of venous blood were withdrawn from all females contributed in this study. Two ml of blood was placed in EDTA-tube for molecular analysis and three ml was placed in gel tube. Serum was obtained after centrifugation of blood at 3000 rpm for 10 minutes to separate serum.The serum was used for measuring estradiol, tumor marker (CA15.3), lipid profile, calcium and vitamin D3 levels.

2.3.3. Genetic analysis

2.3.3.1. DNA extraction

Genomic DNA was extracted from blood sample as stated by the protocol Favorgen for blood genomic DNA extraction kit. The following method was used for DNA isolation from blood:

1. About 200 µl of blood was transferred to a 1.5ml microcentrifuge tube .
2. We added 30µl of proteinase K to the sample and briefly mix then incubated for 15 minutes at 60ºC.
3. About 200µl of FABG buffer was add to the sample and mix by vortex and incubated in a 70ºC water bath for 15 minutes to lyse the sample.
4. We added 200 µl of 100% ethanol to the sample and vortexed for 10 seconds.
5. FABG column was placed to the collection tube.
6. The sample mixture was transferred carefully to FABG column and centrifuged it at speed 14,000 rpm for 1 min.
7. The mixture in the collection tube was discarded and the FABG column was placed to a new collection tube.
8. About 400 µl of W1 buffer was added to the FABG column and then centrifuged at speed 14,000 rpm for 1 min.
9. The flow through was discarded and then the FABG column was placed back again to the collection tube.
10. About 600 µl of wash buffer was added to the FABG column and then centrifuged it at speed 14,000 rpm for 1 min.
11. The flow through was discarded and then the FABG column was placed back again to the collection tube.
12. The dry FABG column was placed to a new eppindrof tube.

13.Preheated Elution buffer(100 µl) was added to the membrane center of FABG column and then centrifuged it at speed 14,000 rpm for 1 min to elute the DNA.

14.The DNA was collected and long term stored at -20 °C (deep freezing).

2.3.3.2. Polymerase chain reaction

Polymerase chain reaction is the process that involves DNA regions replication through using an enzyme DNA polymerase which permits amplification of desired DNA fragments from one molecules to several million copie . Many requirements need to proceed PCR reaction such as two primers which are complementary to the target DNA one primer bind to each side of DNA and between them the desired DNA sequences will be amplified. There are three main steps for any PCR that are cycled about 25-45 times, which include:

A. Denaturation: this step occurs at 94-95°C and requires decoding of double strand DNA into single strands.

B. Annealing: this step occurs at 55-65°C, the reaction begins by stiffen the pair of short oligonucleotide sequences to the ends of the strands of DNA template.

C. Extension: this step occur at 72-74°C, it needs extension of the primers to form new strand complementary to the template table and this happens in the presence of DNA Taq polymerase.

In addition to DNA templet and primers there are other components which are essential for techniques include DNA polymerase (Taq polymerase), deoxy nucleotide tri-phosphates and buffer solution((137).

There are several polymerase chain reactions (PCR) techniques differ in the principle. This study use Amplification refractory mutation system (ARMS-PCR) technique use: refers to mutation detection method based on

specific PCR primers and also called amplification of specific alleles, in which specific set of primer; two forward primers (forward wild and forward mutant type) and two reveres primers which are complementary to the DNA template which include the region to be amplified(138).

2.3.3.3 Primers design

Polymerase chain reaction was performed using a specific primer pairs designed for CYP2C19*3(G>A) (rs4986893) alleles and CYP2C19*17 (C> T)(rs11188072)alleles using primer blast software and depending on NCBI database. The primer sequences are shown in tables (2.3) and (2.4) respectively.

Table (2.3): Primers sequences of CYP2C19*3(G>A)(rs4986893) genetic polymorphism.

Primer sequence (5 ’- 3 ’)	Primers	Product size
CTCCATTATTTTCCAGAAACGTTTCAT	O-F	258 bp
TGCCATCTTTTCCAGATATTCACCC	O-R	258 bp
AGGATTGTAAGCACCCCCGGA	I-F allele A	180 bp
AAAAAACTTGGCCTTACCTGGCTCC	I-R allele G	124 bp

Table(2.4): Primers sequences of CYP2C19*17(C>T)(rs11188072)genetic polymorphism.

Primer sequence (5 ’-3 ’)	Primers	Product size
GGCACAATCCATGAAATAAAGAAT	O-F	408 bp
AATAGTTCTCCTTGCTGCATATCC	O-R	408 bp
AACGGGTCTGAACAGACCCT	I-F allele T	200 bp
TTTGGTATCTGTATGTCTTCTTGGTG	I-R allele C	256 bp

Lyophilized primers were dissolved with a specific volume of nuclease free water to obtain primary concentration of (100 Pmol/µL) (as stock solution). Table (2.5) and (2.6) represent the volume of nuclease free water to each primer to obtain 100 Pmol/µL.

Table (2.5):The volume of nuclease free water added to each primer of CYP2C19*3(G> A,rs4986893) to obtain 100 Pmol/µL.

Primers of CYP2C19*3(rs4986893)	Volume of nuclease water added (µL)
O-F	250
O-R	250
I-F allele A	250
I-R allele G	250

Table (2.6): The volume of nuclease free water added to each 'primer of CYP2C19*17 (C> T) (rs11188072) to obtain 100 Pmol/µL.

Primers of CYP2C19*17 (rs11188072)	**Volume of nuclease water added(µL)**
O-F	250
O-R	250
I-F allele T	250
I-R allele C	250

For working solution, 10µL of stock solution was diluted with 90 µl of nuclease free water to get (10 pmol/ µL) as final concentration (working solution) the primer kept in -20 °C until farther use.

2.3.3.4. Polymerase chain reaction optimization conditions

Optimization of polymerase chain reaction was attained after several trails.

2.3.3.4.1Optimization of CYP2C19*3(G>A,rs4986893) genetic polymorphism conditions.

CYP2C19*3(G>A rs4986893) optimization of PCR conditions was prepared by using:

- Different volumes of primers (1µL, o.5 µL)
- Different annealing temperatures (63.3°C,65.8°C)
- Different volumes of DNA sample (3 µL,5 µL)

The best results of this SNP were obtained in the following conditions:

A. 1 µl of outer forward primer

B. 1 µl of outer reverse primer

C. 1 µl of inner reverse primer

D. 1 µl of inner forward primer

E. 3 µl DNA sample

F. 12 µl Master mix

G. 6 µl nuclease free water

2.3.3.4.2 Optimization of CYP2C19*17 (C> T) (rs11188072) genetic polymorphism conditions.

CYP2C19*17 (C> T) (rs11188072) optimization of PCR conditions was prepared by using:

- Different volumes of primers (1 µL.1.2 µL)
- Different volumes of DNA sample (3 µL, 5 µL)
- Different annealing temperatures (55.7°C, 60°C)

The best result of this SNP was obtained in the following conditions:

A. 1.2 µl of outer reverse primer

B. 1.2 µl of outer forward primer

C. 1.2 µl of inner forward primer

D. 1.2 µl of inner reverse primer

E. 3 µl DNA sample

F. 12 µl Master mix

G. 5.2 µl nuclease free water

A total volume of reaction was (25 µL) which centrifuged at 2000 rpm for 10 seconds in a micro centrifuge for mixing the sample tubes and then placed in thermocycler.

2.3.3.5. Running and working solution of PCR

2.3.3.5.1. ARMS-PCR running and working solution

For CYP2C19*3(G > A) (rs4986893) ARMS-PCR working solution was prepared as the following :

• 1µl of 10 poml/µL from each outer forward and outer revers primer, 1µL of 10 pmol/ µL from each inner forward and inner reverse primer, and 3µL of extracted DNA in PCR premix tube

• The volume was completed to 25 µL with nuclease free water.

• The thermal program For CYP2C19*3(G > A) (rs4986893) demonstrated in table (2.7).

Table (2.7): PCR thermocycler program for for CYP2C19*3(G > A) (rs4986893) polymorphism.

Steps	Temperature (°C)	Minute:second	Cycles
Initial denaturation	95°	05:00	1
Denaturation	95°	00:30	35
Annealing	65°	00:35	
Extension	72°	00:55	
Final extension	72°	05:00	1

For of CYP2C19*17 (C> T) (rs11188072) ARMS-PCR working solution was prepared as the following :

- 1.2 µl of 10 poml/µL from each outer forward and outer revers primer, 1.2µL of 10 pmol/ µL from each inner forward and inner reverse primer, and 3µL of extracted DNA in PCR premix tube
- The volume was completed to 25 µL with nuclease free water.
- The thermal program for CYP2C19*17 (C > T) (rs11188072) demonstrated in table (2.8)

Table (2.8): PCR thermocycler program for CYP2C19*17 (C > T) (rs11188072) genetic polymorphism.

Steps	Temperature (°C)	Minute: second	Cycle
Initial denaturation	95°	05:00	1
Denaturation	95	00:30	35
Annealing	57°	00:35	
Extension	72°	00:55	
Final extention	72°	05:00	1

2.3.3.6. Agarose Gel Electrophoresis

1. Agarose gel 1.5% was prepared by dissolving 0.5 gm of agarose powder was put in beaker, and then 5mL of 10 X TBE buffer (tris- borate-EDTA) and 45 mL of distilled water was added.

2. The mixture was warmed on hot plate, and left for few seconds when the mixture began to boil.

3. The solution was left to cool and 3µL of ethidium bromide was added.

4. The comb was fixed on one end of the tray to make holes where the samples were loading.

5. After the agarose solution had poured to tray, it has been left to congeal at 25℃.

6. The comb was removed lightly away from the tray.

7. The try was stabled into the device chamber, and the chamber was filled with 1X TBE buffer.

8. One of the wells of agarose gel was loaded with 5 µl of DNA ladder

9. While the others were loaded with 5 µl of each PCR products.

10. The voltage of the electrophoresis apparatus was adjusted at 45 volts to ensure an electrical field adjusted with (5) v/cm for 10 cm distance between cathode and anode.

11. At the end of the run, ultraviolet treans-illuminator was used for detection of the bands.

12. The gel was photographed using digital camera (139)(140).

2.4 Biochemical Assay

2.4.1 Estimation of serum Estradiol

The quantitative determination of concentration of estradiol level in human serum by chemiluminescent immune assay (CLIA), which is a competitive binding immunoenzymatic assay. Chemiluminescent (CL) series E2 assay is a competitive binding immunoenzymatic assay to determine the level of estradiol. In the first step, sample paramagnetic micoparticle coated with goat anti-rabbit IgG, sample treatment solution, and polyclonal anti-estradiol antibody (rabbit) were added into a reactive vessel. After incubation, estradiol in the sample bound to anti-estradiol antibody. In the second step, estradiol alkaline phosphatase conjugate was added to the reaction vessel. Estradiol in the sample competes with estradiol alkaline phosphatase conjugate for binding sites on the antiestradiol antibody. The resulting antigen: antibody complexes were bound to goat-rabbit IgG on the micro plate, which was magnetically captured while other unbound substances were removed by washing. In the third step, the substrate solution was added to their action vessel. It was catalyzed by estradiol-alkaline phosphatase conjugate in the immune complex retained on the micro particle. The resulting chemiluminescent reaction was measured as relative light units (RLUs) by a photo multiplier built into the system. The amount of estradiol present in the sample was inversely proportional during the reaction, the estradiol concentration could be determined via a calibration curve (141). The expected values of estradiol level are Postmenopausal female < 25-84 pg/ml, Follicular phase 20-138 pg/ml, ovulation phase 100-400pg/ml, luteal phase 31-317 pg/ml.

2.4.2. Estimation of serum tumor marker CA 15-3

The quantitative measurement of CA 15.3 levels in human serum using the Enzyme Linked Fluorescent Assay (ELFA). The assay principle was combined a 2-step enzyme immunoassay sandwich method with a final fluorescent detection (ELAF). The solid phase receptacle (SPR) was served as the solid phase as well as the pipetting device for the assay.All of the assay steps were performed automatically by the instrument. The reaction medium was cycled in and out of the SPR several times.The sample was cycled in and out of the SPR several times.This operation enables the 155D8 fixed onto interior wall of the SPR to capture the reactive antigenic determinants present in the sample. Unbound component was eliminated during the washing steps.Then alkaline phosphatase-labeled DF3 antibody was incubated in the SPR where it was bonded with the DF3antibody reactive antigenic determinants.Unbound conjugate was eliminated during the washing steps.During the final detection step, the substrate (4-Methyl umbelliferly phosphate) was cycled in and out of the SPR. The conjugate enzyme was catalyzed the hydrolysis of this substrate into a fluorescent product (4-Methyi-umbelliferone) the fluorescence of which is measured at 450nm. The intensity of fluorescence is proportional to the concentration of CA 15.3 present in the sample.At the end of assay, the results are automatically calculated by the instrument in relation to calibration curve stored in memory and then printed out (142)(143).

2.4.3.Estimation of serum lipid profile

A. Estimation of serum total cholesterol

The enzymatic colorimetric method was used for the determination of total cholesterol (TC) in serum on BS240Pro in vitro. By the effect of the cholesterol esterase enzyme (CE), cholesterol esters are cleaved to free

cholesterol and fatty acids. Then by cholesterol oxidase enzyme (CHOD) which oxidizes cholesterol to cholest -4-en-3-one and hydrogen peroxide. The hydrogen peroxide affects oxidative coupling of phenol and 4-aminoantipyrin (4-AAP) to form red quinone –imine dye by the effect of peroxidase enzyme (POD).

Cholesterol esters+ $H2O \xrightarrow{CE} cholesterol + RCOOH$

$$cholesterol + O2 \xrightarrow{CHOD} cholest-4-en-3-one + H_2O_2$$

$$2H_2O_2 + 4-AAP + phenol \xrightarrow{POD} quinone-imine\ dye + 4H_2o$$

The cholesterol concentration is directly proportional to the color intensity of the dye formed and measured by the increase in absorbance at 512 nm(144).

B. Estimation of serum triglyceride

In vitro quantitative enzymatic colorimetric method was used for determination of triglyceride in serum on cobas integra systems(144).

$$triglyceridec + 3\ H_2o \xrightarrow{triglyceridec} glycerol + 3\ RCOOH$$

$$glycerol + ATP \xrightarrow{Glycero\ Kinase} glycerol - 3\ Phosphate + ADP$$

$$glycerol - 3 - Phosphate + O_2 \xrightarrow{glycerol-3\ Phosphate\ Oxidase} dihydroxyacetone\ phosphate + ADP$$

$$H_2O_2 + 4-aminophenazone + 4-chlorophenol \xrightarrow{*peroxidase} 4-(p-benzoquinone-monoimino)-phenazone + 2H_2O + HCL$$

C.Estimation of serum high-density lipoprotein

In vitro quantitative enzymatic colorimetric method was used for the determination of HDL in serum on cobas integra systems. In the presence of magnesium ions and dextran sulfate, water-soluble complexes with LDL, VLDL, and chylomicrons are formed which are resistant to PEG-modified enzymes. The cholesterol concentration of HDL-cholesterol was determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approximately 40 %). Cholesterol esters are broken down quantitatively into free cholesterol and fatty acid by cholesterol esterase. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide(145).

$$HDL - cholesterol + H_2o \xrightarrow{PEG-Cholesterol\ esterase} HDL - cholesterol + RCOOH$$

$$HDL\_cholesterol + O2 \xrightarrow{PEG_{cholesteroloxidase}} \Delta 4\text{-}cholestenone + H2O2$$

$$2H2O2 + 4\_aminoantipyrine + HSDAa) + H + H2O \xrightarrow{peroxidase}$$ Purple blue pigment (a) Sodium N- (2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline)$+5H2O$

The color intensity of the blue quinoneimine dye formed is directly proportional to the HDL-cholesterol concentration. It was determined by measuring the increase in absorbance at 583 nm.

D.Estimation of serum low-density lipoprotein (LDL)

Cholesterol esters and free cholesterol in low-density lipoprotein (LDL) are determined using a cholesterol enzymatic method that involves the use of cholesterol esterase and cholesterol oxidase in the presence of surfactants that selectively solubilize the only LDL. Surfactants and a sugar compound inhibit

the enzyme reactions to lipoproteins other than LDL.Cholesterol levels in HDL, VLDL, and chylomicron is not recognized.

$LDL_{cholesterolester} + H2O \xrightarrow{detergent}$Cholesterol + free fatty acid (selective micellary solubilization)

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$LDL_{cholesterol} + O2 \xrightarrow{cholesterol\ oxidase} \Delta 4\text{-}cholestenone + H2O2$$

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ4-cholestenone and hydrogen peroxide.

$$2H2O2 + 4_{aminoantipyrine} + EMSEa) + H2O + H \xrightarrow{peroxidase} red\ purple\ pigment(a)$$

$$N\text{-}ethyl\text{-}N\text{-}(3\text{-}methylphenyl)\text{-}N\text{-}succinylethylenediamin$$

The hydrogen peroxide produced reacts with 4 aminoantipyrine and EME to form a red-purple dye in the presence of peroxidase. This dye'scolor intensity is proportional to cholesterol concentration and is measuredusing a photometer(146).

2.4.4.Estimation of serum vitamin D3

Competitive chemiluminescence immunoassay: use a purified 25-OH vitamin D antigen to label ABEI and use 25-OH vitamin D monoclonal antibody to label FITC. Sample Calibrator or Control with Displacing reagent, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Then add ABEI Label, incubation and washing for the 2nd time, sample antigen and ABEI labeled antigen compete to combine with FITC labeled monoclonal antibody, forming antibody-antigen complexes. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of 25-OH vitamin D present in controls or samples(147).

2.4.5 Estimation of serum calcium

$$Calcium + Arsenazo\ III \xrightarrow{PH=7} ablue\ colored\ complex$$

By using B - hydroxyquinoline - 5 - sulfonic acid to eliminate the interference of magnesium, calcium ions combine with Arsenazo III to produce a blue colored complex at a neutral solution. The absorbency increase is directly proportional to the concentration of calcium(148).

2.4.6 Determination of body mass index

Body Mass Index (BMI) is a value obtained from the weight and height of an individual. The BMI was described as the body weight divided by the square of the body height and is globally expressed in units of kg/m2, resulting from the mass in kilograms and height in meters(149).

BMI=Weight (Kg) / {Height (m)} 2

Normal weight falls between BMI values of 18.5-24.9, overweight between 25-30 and obese above 30.

2.5. Statistical analysis

The data of participants in this study were converted into a computerized database, and analysed by using the statistical package for social sciences (SPSS) version 28, IBM, US. Scale variables presented in mean, standard deviation (SD), while descriptive statistics for (categorical) variables represented as frequency (number of participants) and proportion (percentage). Student's test for two independent samples was used to compare means between groups. One way ANOVA was used to compare more than two means. Chi square was used to measure the association between categorical variables. Fisher's exact test was used as an alternative when the chi square was not appropriate.

Chapter Three

Results

3.1. Demographic characteristic of patients

A total of 100 women participated in this study, were divided into subgroups based on Age, BMI and duration of disease and tamoxifen treatment. The clinical demographic characteristics and laboratory parameters of patients group were summarized in table (3.1) , (3.2) and (3.3) .

The descriptive table was also shown an adjustment of other characteristics and risk factors which were collected , these factors included: BMI, marital status, family history, lymph node involvement, number of patients who have previous surgery or chemotherapy, duration of disease and dignosis, location of cancer and results of histochemical tests.

(3.1) :Descriptive of demographic characteristics of variables studied patients

Variables	Values (mean ± SD)
Age (Years)	51.08 ± 4.85
BMI (Kg/m^2)	28.30 ± 5.57
Duration of tamoxifen (Years)	3.41 ± 2.36
Duation of disease (Years)	4.18 ± 2.5

Table (3.2):Descriptive demographic characteristics of studied patients.

Variables		Frequency (%)	
Age group	44 - 49 Years	45%	
	50 - 55 Years	38%	
	56 - 65 Years	17%	
BMI group	Obese	61%	
	Non Obese	39%	
Marital status	Married	93%	
	Single	7%	
Family history	Yes	44%	
	NO	56%	
Breast cancer site	Left breast	56%	
	Right breast	44%	
Lymph node involvement	Yes	64%	
	NO	36%	
Surgery	Yes	94%	
	NO	6%	
Chemotherapy	Yes	91%	
	NO	9%	
Radiotherpy	Yes	79%	
	No	21%	
Histochemical test	Human epidermal growth factor receptor2 (HER-2)	Negative test	33 %
		Positive test	67 %

	Positive (ER)/ (PR)	98%
	Positive (ER) / Negative for (PR)	2%
Side effect	Hot flashes	13%
	Joint pain	76%
	Both(hot flashes & joint pain)	9%
	Endometrial hyperplasia	2%
Recurrence	Yes	7%
	No	93%

Table (3.3): Description of laboratory biomarkers of the study patients (n=100)

Biomarkers	Mean±SD	Reference range
S. Estradiol	33.14±11.25	10-66 pg/ml
S.CA15-3	17.99±6.29	Up to 35 ng/ml
S. Vitamin D	11.81±5.69	30-100 ng/ml
S. Calcium	8.87 ±2.58	8.1-10.4 mg/dl
S.Cholesterol	176.8 ± 31.6	50-200 mg/dL
S.TG	166.53 ± 61.99	35-150 mg/dL
S.HDL	46.03 ± 11.35	35-65 mg/dL
S.LDL	94.98 ± 28.82	0-140 mg/dL

3.2. Genetic analysis

3.2.1. Results of genotyping of CYP2C19*17 (rs 11188072)

The results of genotype rs 11188072 was clear band with a molecular size 200 and 256 bps (Figure 3-1) .The size of amplicon was determined by comparing with DNA ladder 100 - 1500 bp.

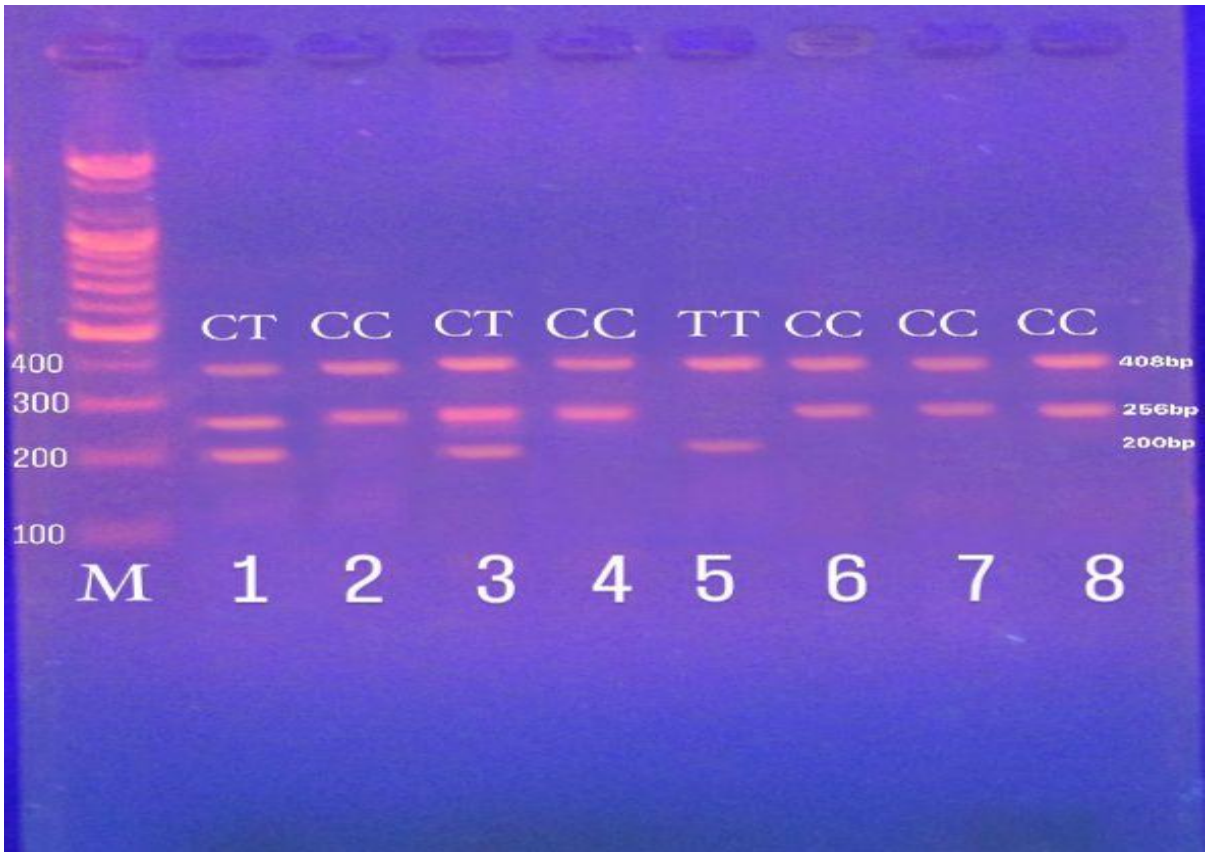


Figure (3.1):PCR (C >T,rs 11188072) showing:line M represented the DNA ladder (100 - 1500 bp),Where lines 2,4,6,7,8 represented of CC (Wild) genotype, line 5 represented of TT (homozygous mutant) and lines 1,3 represented of CT (heterozygous mutant) .

Genotyping of CYP2C19*17 (C > T rs 11188072) alleles were classified into:

1. The major (Wild) genotype groups (CC) which had frequency71 and percentage 71% ,was the the most common genotype among the studied BC patients

2. The homozygous genotype groups (TT) which had frequency 12 and percentage 12% ,was the the least common genotype among the studied BC patients

3. The heterozygous (CT) had frequency 17 and percentage 17%.

Table (3.4): Description genotype of CYP2C19*17(C> T,rs 11188072) genotypes in breast cancer patients.

Genotype	**Group**	**Frequency**	**Percentage**
rs 11188072	CC (wild)	71	71%
	TT (homo)	12	12%
	CT (hetero)	17	17%
Data Presented by numbers and percentage			

The result of comparison between observed and anticipated value for CYP2C19*17 (C > T, rs 11188072) tested population were shown in figure (3.2) and table (3.5). The distribution and percentage of individuals having rs 11188072 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: CC (71, 63.2); TT (12, 4.2); CT (17, 32.6) (goodness-of-fit $\chi 2$ for rs 11188072; 22.891, $P < 0.001$} and therefore it was statistically significant.

Table (3.5): Hardy–Weinberg equilibrium for rs 11188072 genotype in BC samples.

Genotypes			Alleles		Hardy–Weinberg equilibrium X^2 test
			T	C	
Genotype N= 100	Frequency	%	0.795	0.205	22.891 P < 0.001 [S]
CC (Wild Type)	71	71			
CT(heterozygoustype)	17	17			
TT(homozygous type)	12	12			

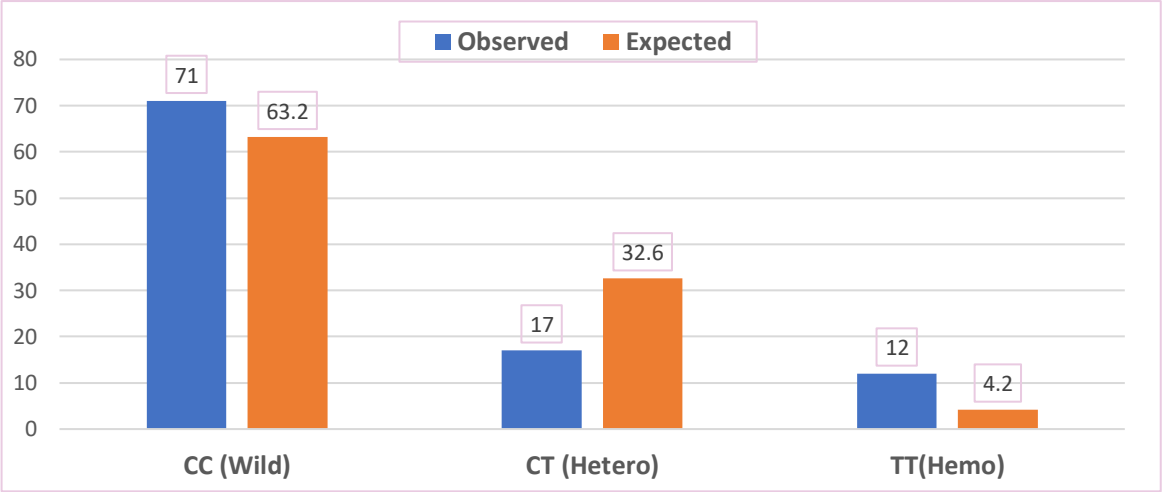
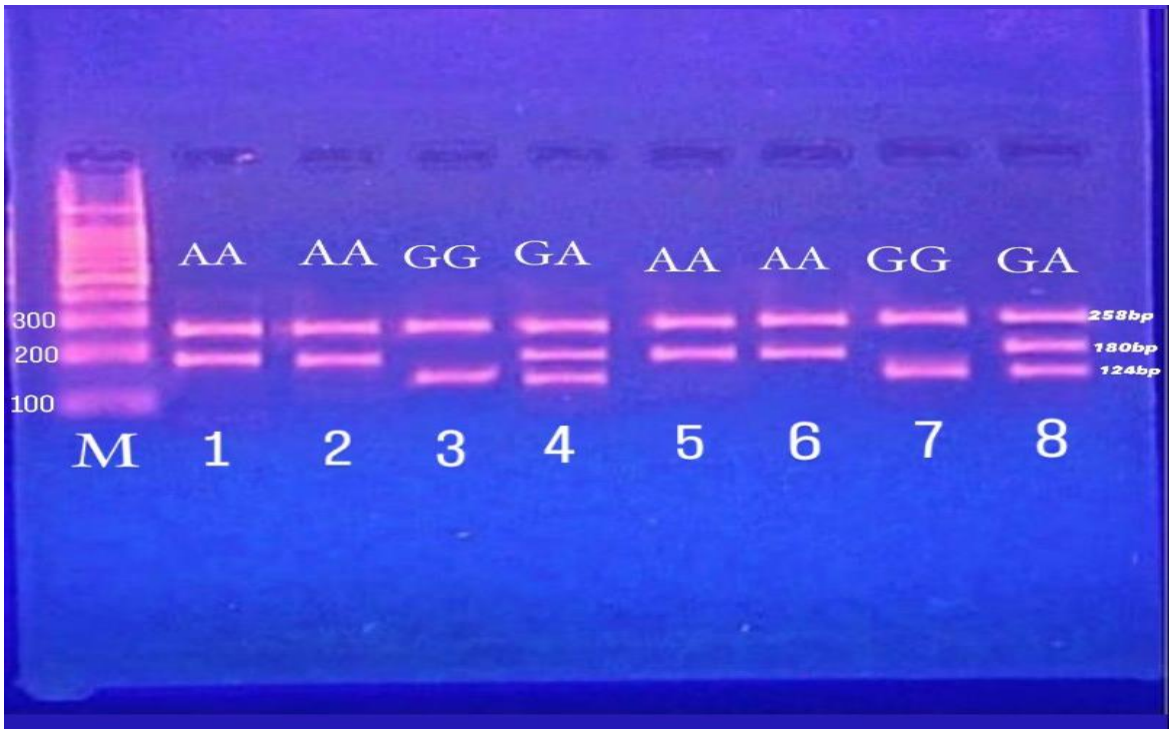


Figure (3.2): Genotype frequencies % of CYP2C19*17 (C> T, rs 11188072)among individuals' sample

3.2.2 Results of genotyping of CYP2C19*3 (G > A, rs4986893)

The results of genotype rs4986893 showed a clear band with molecular size 124 and 180 bps (Figure 3.3) .The size of amplicon was determined by comparing with DNA ladder 100 - 1500 bp.



Figure(3.3): PCR of (G>A, rs4986893) showing:line M represented the DNA ladder (100 - 1500 bp),Where lines 3,7 represented of GG (Wild) genotype, line 1,2,5,6 represented of AA (homozygous mutant) and lines 4,8 represented of GA (heterozygous mutant)

Genotyping of CYP2C19*3(G> A, rs4986893) alleles were classified into

1. The major genotype group (GG) which had frequency 20 and percentage 20% ,was the less comman among the studied BC patients

2. The homozygous genotype group (AA) which had most common frequency of 55 and percentage 55% ,.

3. The heterozygous (GA) had frequency 25 and percentage 25% .

Table (3.6) and figure (3.3) summarizes the distribution of genotyping groups of rs 4986893 in patients with breast cancer**.**

Table (3.6): Description genotype of CYP2C19*3(G > A, rs4986893) genotype in breast cancer patients.

Genotype	Group	Frequency	Percentage
rs4986893	GG (wild)	20	20%
	GA (hetero)	25	25%
	AA (homo)	55	55%
Data Presented by numbers and percentage			

The result of comparison between observed and anticipated values for CYP2C19*3 (G>A, rs4986893) the tested population were shown in figure (3.4), and table (3.7). The distribution and percentage of individuals having rs 4986893 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (20, 10.6); AA (55, 45.6); GA (25, 43.9) (goodness-of-fit $\chi 2$ for rs 93 18.507, $P < 0.001$} and therefore it was statistically significant.

Table (3.7): Hardy–Weinberg equilibrium for rs 4986893 genotype in BC patients

Genotypes			**Alleles**		**Hardy–Weinberg equilibrium X^2 test**
			G	**A**	
Genotype N= 100	Frequeny	%	0.325	0.675	18.507 P < 0.001 [S]
GG(Wild Type)	20	20			
GA(heterozygous type)	25	25			
AA(homozygous type)	55	55			

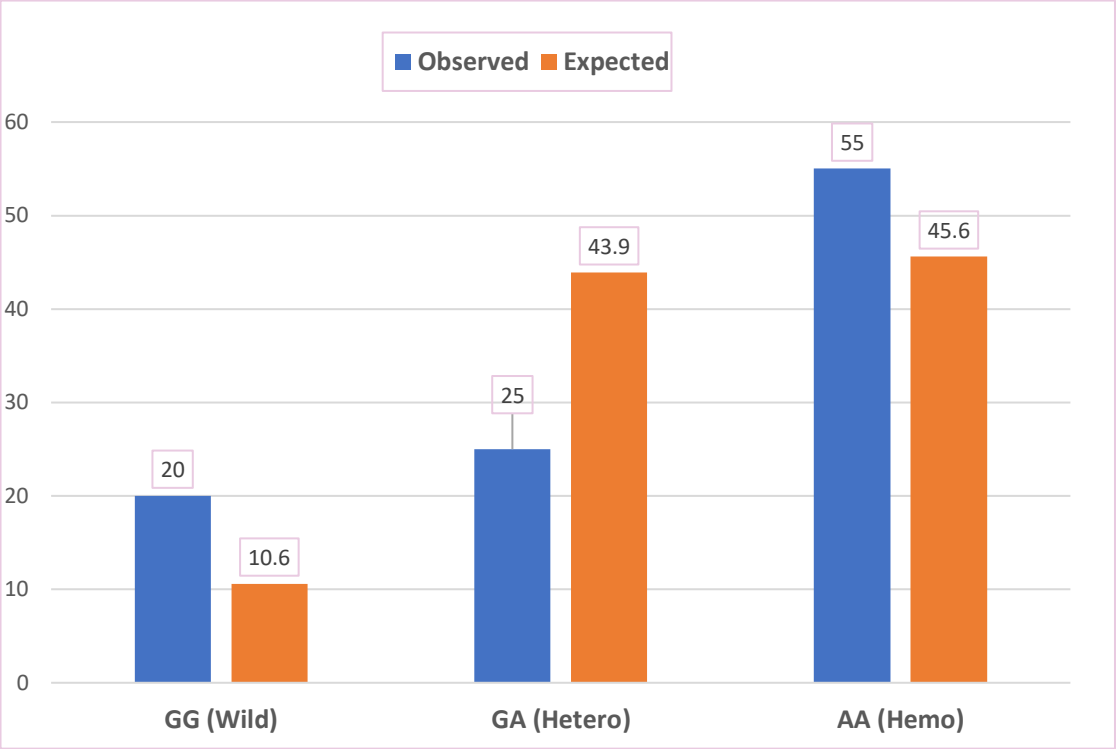


Figure (3.4): Genotype frequencies % of CYP2C19*3 (G >A, rs4986893) among individuals' sample

3.3 Relationship between demographic characteristics and CYP2C19 genotype

3.3.1.Relationship between demographic characteristics and (C > T, rs 11188072) genotypes

The difference between CYP2C19*17 (C>T, rs 11188072) genotypes with demographic characteristics (mean) was demonstrated in (table 3.8), one-way ANOVA test was performed to compare the clinical demographic characteristics included (Age, BMI, duration of treatment and diagnosis). No statistically significant difference was found among the mean values ($p > 0.05$).

A chi-square test was conducted between other categorial variables (marital status, family history, breast cancer Side, lymph node involvement, number of patients who were having (surgery, chemotherapy or radiotherapy), types of histochemical tests, types of side effect and complications) and the genotypes of the rs **11188072**, there was no statistically significant difference between them ($p > 0.05$**).**

Table (3.8): difference of demographic characteristic in CYP2C19*17 (C > T, rs 11188072)

Demogrhic parameters	Groups	Genotype (N=100)			P-Value
		CC% (N=71)	CT% (N=17)	TT% (N=12)	
Age	44 - 49 Years	32	7	6	0.88
	50 - 55 Years	27	6	5	
	56 - 65 Years	12	4	1	
BMI	Obese	46	11	4	0.11
	Non Obese	25	6	8	
Treatment duration	Less than 5 Years	55	14	12	0.18
	More than 5 Years	16	3	0	
Diagnosis duration	Less than 5 Years	49	11	10	0.52
	More than 5 Years	22	6	2	
Marital status	Married	67	16	10	0.05
	Single	4	1	2	
Family history	Yes	31	7	6	0.73
	NO	40	10	6	
Breast cancer side	Left breast	37	14	5	0.42
	Right breast	34	3	7	
Lymph nod Involvement	Positive	47	8	9	0.15

	Negative	24	9	3	
Surgery	Positive	66	16	12	0.97
	Negative	5	1	0	
Chemotherapy	Positive	64	15	12	0.55
	Negative	7	2	0	
Radiotherapy	Positive	54	14	11	0.28
	Negative	17	3	1	
HER2	Positive	24	7	2	0.27
	Negative	47	10	10	
ER \PR	Positive both side	69	17	12	0.41
	Negative ethier	2	0	0	
Side effect of tamoxifen	Hot flashes	9	2	2	0.21
	Joint pain	54	13	9	
	Both (Hot flashes &Joint pain)	7	2	0	
	Endometrial hyperplasi	1	0	1	
Recurrence	Yes	65	17	11	0.7
	No	6	0	1	

3.3.2. Relationship between demographic characteristics and CYP2C19*3 (G>A,rs 4986893).

To show the difference between demographic characteristics (mean) and rs 4986893 (table 3.9), by performing a one-way ANOVA test to compare the mean age , BMI, duration of treatment and diagnosis no statistically significant difference was found among mean of demographic characteristics ($p > 0.05$).

A chi-square test was conducted family History, breast cancer side, lymph node involvement ,surgery, chemotherpy, radiotherapy, HER2, ER PR

,side effect and recurrence between rs 4986893 genotype , there was no statistically significant difference between them ($p > 0.05$).

Table(3.9):Difference of demographic characteristic in CYP2C19*3 (G>A ,rs 4986893).

Demographic parameters		Genotype (N=100)			P value
		GG% (N=20)	GA% (N=25)	AA% (N=55)	
Age group	44 - 49 Years	12	11	22	0.62
	50 - 55 Years	6	9	23	
	56 - 65 Years	2	5	10	
BMI group	Obese	11	14	36	0.59
	No Obese	9	11	19	
Treatment duration	Less than 5 Years	17	20	44	0.87
	More than 5 Years	3	5	11	
Diagnosis duration	Less than 5 Years	14	18	38	0.96
	More than 5 Years	6	7	17	
Marital status	Married	18	23	52	0.89
	Single	2	2	3	
Family history	Yes	12	12	20	0.23
	NO	8	13	35	
Breast cancer side	Left breast	11	15	30	0.77
	Right breast	9	10	25	
Lymph node involvement	Positive	17	14	33	0.49
	Negative	3	11	22	
Surgery	Positive	20	23	51	0.07
	Negative	0	2	4	
Chemotherapy	Positive	17	23	51	0.08
	Negative	3	2	4	
Radiothepy	Positive	17	19	43	0.92

	Negative	3	6	12	
HER2	Positive	6	9	18	0.85
	Negative	14	16	37	
ER \ PR	Positive both side	20	24	54	0.23
	Negative ethier	0	1	1	
side effect of tamoxifen	Hot flashes	1	4	8	0.19
	Joint pain	18	21	37	
	Both(Hot flashes & Joint pain)	1	0	8	
	Endometrialhyperplasi	0	0	2	
Recurrence	Yes	3	1	3	0.32
	No	17	24	52	

3.4. Effect of tamoxifen therapy on Laboratory parameter according to different single nucleotide polymorphism of CYP2C19 gene in studied breast cancer patients

3.4.1 Effect of tamoxifen on laboratory parameters according to rs 11188072 genotype.

Serum levels of biomarkers estradiol, CA15-3, vitamin D3,calcium based on the genotypes of rs 11188072 SNP groups was demonstrated in (table 3.10). Data were analyzed by regardness the wild, heterozygous and homozygous variant groups. Results were found that there was no significant difference found between the measured biomarkers and CYP2C19*17 (C> T, rs 11188072) genotype, ($p > 0.05$).

Table (3.10): Diference between alleles of CYP2C19*17 (C> T, rs 11188072) with mean levels of biomarkers in breast cancer Patients who receive tamoxifen

Lab. Parameters	rs 11188072 (N= 100)			P value
	CC (N=71)	CT (N=17)	TT (N=12)	
S. E2	32.82±11.20	35.13±12.24	32.80±10.83	0.888[NS]
S. CA15.3	18.18±6.42	16.88±5.53	18.38±6.87	0.749[NS]
S. D3	11.30±5.33	11.99±5.33	14.60±7.73	0.178[NS]
S.Ca	8.65±1.16	8.83±0.85	8.64±1.10	0.470[NS]
Results are presented as mean ± SD, p > 0.05considered non significant				

The difference between mean serum levels of lipid profile markers: cholesterol, TG, HDL, LDL and rs 11188072 SNP groups was shown in (table 3.11), results were found that there was no significant difference was found, (p > 0.05).

Table (3.11): Difference between alleles ofCYP2C19*17(C>T,rs11188072) genotype with mean levels of lipid profile in breast cancer patients who receive tamoxifen.

Lipid profile	rs 11188072 (N=100)			P value
	CC (N=71)	CT (N=17)	TT (N=12)	
S. Chol	176.18±30.44	167.00±30.82	179.41±25.01	0.370[NS]
S. TG	163.07±57.79	139.29±52.47	148.90±52.15	0.243[NS]
S. HDL	44.987±11.49	48.41±8.31	46.56±12.66	0.536[NS]
S. LDL	93.73±29.79	94.84±29.68	97.39±27.31	0.861[NS]
Results are presented as mean ± SD, p > 0.05 considered non significant				

3.4.2 Effect of tamoxifen on laboratory parametrers according to rs 4986893 genotype

The difference between mean serum levels of estradiol,CA15-3, vitamin D3,calcium and genotypes of rs 4986893 SNP groups was also examined as an clinical outcome in breast cancer patients (table 3.12) , were not showing any statistical significant difference among different genotypes, ($p > 0.05$).

Table (3.12): Difference between alleles of CYP2C19*3 (G>A ,rs 4986893) with mean levels of biomarkers in breast cencer patients who receive tamoxifen

LAB parameters	rs 4986893 (N= 100)			P value
	GG (N=20)	GA (N=25)	AA (N=55)	
S. E2	34.18±10.19	33.67±10.97	32.53±11.89	0.828[NS]
S. CA15.3	18.38±7.42	17.13±5.03	18.23±6.44	0.733[NS]
S. D3	11.11±6.19	10.42±5.38	12.70±5.60	0.208[NS]
S.Ca	8.68±0.99	8.73±1.18	8.58±1.13	0.833[NS]
Results are presented as mean ± SD, p > 0.05 be non significant.				

The difference between mean serum levels of lipid profile markers: cholesterol,TG, HDL, LDL and rs 4986893 SNP groups was shown in (table 3.13), results were found that there was no significant difference was found, ($p > 0.05$).

Table(3.13) : Difference between rs 4986893genotype with mean levels of lipid profile in breast cancer patients who receive tamoxifen

Lipid Profile	rs 4986893 (N=100)			P value
	GG (N=20)	GA (N=25)	AA (N=55)	
S. Chol	173.350±26.41	182.91±26.5	172.32±28.8	0.39[NS]
S. TG	159.13±49.28	148.90±63.06	154.10±58.13	0.79[NS]
S. HDL	46.32±11.32	46.19±13.87	45.67±10.21	0.96[NS]
S. LDL	99.47±29.41	92.37±27.15	92.16±29.28	0.48[NS]

3.5 Difference between both CYP2C19 gene (rs 11188072& rs 4986893) SNPs are mutant and either rs 11188072 or rs 4986893 SNPs mutant or no mutation:

The difference between both mutant CYP2C19 genotype(rs 11188072 & rs 4986893) and either rs 11188072 SNP or rs 4986893 SNP was examined. The study group was divided into two sub groups based on:

Group 1- Patients having mutation in Both SNPs rs 11188072 & rs 4986893, Group 2- Patients who have mutation in either rs 11188072 / rs 4986893 SNPs or patients who were not showing any gene mutation. These new groups were studied based on the demographic and clinical characteristics of the patients.Serum levels of estradiol,CA15.3 and calicum were observed to be different between two patient groups as shown in (table 3.14), no significant differences were found since ($p > 0.05$) but serum vitamin D3 value increase from normal value in both rs 11188072 & rs 4986893 mutant genotype groups and considered significantly different as $p < 0.05$.

Table (3.14): Difference in mean levels of biomarkers between both rs 11188072&rs 4986893 mutant genotype and either rs 11188072 or rs 4986893 mutant or no mutation

Lab Parameters	Patient genotype (N=100)		P value
	Either rs 11188072 SNP or rs 4986893 Mutant or no mutation (N=90)	Both rs 11188072&rs 4986893 mutant (N=10)	
S. E2	32.91±11.24	40.80±10.91	0.23[NS]
S. CA15.3	17.87±6.21	21.81±9.24	0.28 [NS]
S. D3	11.63±5.62	17.70±6.15	0.04* [S]
S.Ca	8.63±1.11	8.93±0.99	0.64 [NS]
Results are presented as mean ± SD,p > 0.05 considered non significant			

lipid profile parameter decreased markedly when compared the two groups, patients who were having mutation in Both rs 11188072 & rs 4986893SNP were showing a lower levels of lipid profile, but unfortunately, theses changes were not indicated any statistically significant (p> 0.05) as shown in table 3.15.

Table (3.15): Difference in mean levels of lipid profile between both rs 11188072& rs 4986893 mutant genotype and either rs 11188072 or rs 4986893 mutant or no mutation

Lipid Profile	Genotype (N=100)		P value
	Either rs 11188072 or rs4986893 Mutant or no mutation (N=90)	Both rs 11188072& rs 4986893 mutant(N=10)	
S. Chol	176.04±29.29	160.00±14.18	0.34 [NS]
S. TG	155.91±55.79	123.00±44.64	0.31 [NS]
S. HDL	45.95±11.38	48.33±12.58	0.72 [NS]
S. LDL	95.26±29.14	86.00±16.37	0.58 [NS]

Across tabulation analysis by using a fisher's exact test was conducted between demographic data and side effect table 3.16 , indicated that, there was no statistically significant association between them (p> 0.05).

Table 3.16 : Association side effect with demographic data

Group		Hot flashes	Joint Pain	Both	Endometrial hyperplasi	P Value
Age group (Years)	44 - 49	5	33	7	0	0.252[NS]
	50 - 55	6	29	1	2	
	56 - 65	2	14	1	0	
BMI group	Obese	7	48	5	1	0.887[NS]
	No Obese	6	28	4	1	
Duration of treatment	<5 Years	10	62	7	2	0.876[NS]
	>5 Years	3	14	2	0	
Duration of diagnosis	<5 Years	8	53	7	2	0.668[NS]
	>5 Years	5	23	2	0	

Chapter Four

Discussion

4. Discussion

Many therapies have been studied in the last few years to improve the prognosis and decrease the mortality of breast cancer females. Tamoxifen is currently the cornerstone of modern therapy for hormone receptor-positive breast cancer. Tamoxifen is a selective estrogen receptor modulator that blocks the transcriptional activity of estrogen receptors by directly binding to them, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects(150).

4.1 Demographic data

Breast cancer is a complex disease and is associated with many different causes. Apart from genetic predisposition. Many other factors could impact the development of breast cancer among women including demographic characteristics, clinical, reproductive and environmental features (151).

Besides female gender, age considers the most potent risk factor for breast cancer. In this study mean age is (51.08) with more frequency occurring between the age group of (44-49) and less frequency occurs when increasing the age . This study disagree with **Thakur et al ,**who find the risk of BC increase with increasing age (152).

Body mass index (BMI) is another essential factor for an increase in breast cancer. In this study, the Iraqi BC woman's mean BMI is (28.30) and obese females with BMI greater than 25 are about (61%) . **Cecchini et al.** showed that females with greater BMI appear to be related with poor survival and at a greater risk of cancer recurrence among positive ER- than those with low BMI(153).

BMI is associated with more aggressive biological features of tumor including a higher percentage of lymph node metastasis and greater size. Obesity might be a reason for high mortality rates and a higher probability of cancer relapse especially in premenopausal women (154). Increased body fat will enhance the inflammatory state and affect circulating hormone levels, facilitating pro-carcinogenic events(155).

Marital status is another risk factor that is descriptive in this study and we found 93% of breast cancer women married and 7% unmarried. So, some studies have reported no significant correlation between marriage and breast cancer risk while other studies have shown that marriage is a protective factor for disease outcomes, also there are studies showed that married women have increased breast cancer risk compared with the unmarried (20).

In this study, more than half of the cases were not associated with a family history of BC; while several studies showed that BC risk increased by 67% among women with a first–degree relative diagnosed with the same disease and twofold in females with more than one relative affected (156).

4.2 Genetic analysis

CYP2C19 was predicted to be an essential biomarker for response to tamoxifen because it has similar in vitro activities to CYP2D6 and also can catalyze the conversion of tamoxifen to endoxifen (157).

CYP2C19 play an essential role in tamoxifen metabolism; this cross-section study will focus on CYP2C19*17 (rs11188072) and CYP2C19*3 (rs4986893) in woman with BC and their effect on tamoxifen response. There are limited numbers of studies have reported about rs11188072 and rs 4986893 genotype.

Justenhoven etal, showed that genotype frequencies of CYP2C19*17 in breast cancer women using hormone therapy for 10 years or longe is that CC (66.8%), CT(28.3%) and TT (4.9%) genotypes ,it agrees with current study by that wild type of rs11188072 is most frequency than other genotype and homozygous is less one genotype frequency (158).

The previous study in a sample of Iraqi population done by **Sahib etal,** found that the frequency of CYP2C19*17 was (19.4) and the allele frequency of CYP2C19*17 in Iraqi population was similar to that found in African Americans 21%, Danish 20.1% and Greece 19.6(126) .

Justenhoven etal, CYP2C19*17 predisposing ultrarapid metaboliser phenotype and protect from breast cancer due to decrease of estrogen levels. In particular, women who used hormone therapy for more than 10 years had an even more pronounced reduction of breast cancer recurrence of more than 40% when they carry of the CYP2C19*17 allele(158), beacuse breast cancer recurrence is low about (7%) among our patients. So current study agrees with above study.

However there are limited numbers of studies have reported CYP2C19*3 in populations other than the Asians, but Sahib etal found CYP2C19*3 frequency was 0.2% and CYP2C19*3 has been considered as an Asian mutation allele and after genotyping for CYP2C19*2 it was responsible for the remaining alleles in Asian poor metaboliser (126), in this study wild type of rs4986893 less frequency in a sample of BC patients and the homozygous is most common.

4.2.1 Hardy-Weinberg equilibrium for CYP2C19*17 (rs11188072) in breast cancer females treated with tamoxifen.

The genotype and allele frequencies for rs11188072. For both the recessive and the dominant models, the allele and genotype frequency distributions disagreed with Hardy-Weinberg equilibrium ($p < 0.05$). A significant difference was discovered for these polymorphisms of the study participants.

In this study, wild genotypes of the rs11188072 polymorphism had a genotype frequency of 71%, the heterozygous mutant had a frequency of 17% and homozygous mutants had a genotype frequency of about 12%, so that high percentage of the wild type occurred between samples of Iraqi BC female. No explanation was provided. Additional research is needed to examine the other genotypes and to compare the plasma medication concentrations in patients receiving tamoxifen therapy.

4.2.2 Hardy-Weinberg equilibrium for CYP2C19*3 (rs4986893) in breast cancer females treated with tamoxifen.

The genotype and allele frequencies for rs4986893, for both the recessive and the dominant models the allele and genotype frequency distributions disagreed with Hardy-Weinberg equilibrium ($p < 0.05$). A significant difference was discovered for these polymorphisms of the study participants.

In the study, the wild genotypes (GG) type of the rs4986893 polymorphism had a frequency of 20 %, the heterozygous (GA) type BC had a frequency of 25 % and the homozygous mutants (AA) type had a genotype frequency of about 55 % so that homozygous (GA) type had higher genotype frequency in Iraqi BC female samples.No explanation was provided.

Additional research is needed to examine the other genotypes and to compare the plasma medication concentrations in patients receiving tamoxifen therapy.

The results of CYP2C19 based on Hardy-Weinberg equilibrium is disagreement with previous study done by **sahib et al**, who found that there is no statistical difference in the actual and expected frequency distribution as ($p>0.05\%$) of genetic polymorphism of CYP2C19 in sample of Iraqi population(126). It agrees with **Moyer et al** ,who found the genotypes frequencies for Cyp2C19*17 were in Hardy –Weinberg equilibrium(159)

This cross sectional study was the first study in Iraqi breast cancer females who take tamoxifen .There is no associated results between two SNPs and drug response, this results is agree with cohort study by **Moyer et al**, who shows that neither SULT1A1 or CYP2C19 genotype was associated with clinical outcomes in early breast cancer patients receiving tamoxifen (159).

A previous study by **Damkier et al**, who found no evidence cllinicaly role of CYP2C19 genotype polymorphism and response to tamoxifen in breast cancer patients and CYP2C19 genotype status should not be included in clinical decision on tamoxifen treatment(160).

While the meta analysis study by **Bai et al**, found CYP2C19*17 genotypes carriers are associated with improved survival outcomes in BC women who were using tamoxifen (161).

4.3 Biochemichal analysis associated with genetic effects

4.3.1 Estrodiol level (E2)

Estrogen is the primary woman's sex hormone and is vital in the development and progression of breast cancer (12). It acts as a factor that accelerates the growth and /or survival of existing transformed cells (promoters) or factors that provoke genetic damage that leads to cellular transformation (initiators) (16).

The variant CYP2C19*17 has been associated with a fast metabolizer phenotype of CYP2C19 due to an increased expression of CYP2C19 while CYP2C19*2 and *3 are responsible for the vast majority of poor metabolizer phenotype(162)(163).

Detectable amount of estrogen level in BC patients but with normal rang and agrees with **Schroth et al**, showed that breast cancer woman who used tamoxifen therapy and carrier CYP2C19*17 (rs11188072), will increase tamoxifen metabolism and lead to an augmented production of 4-OH-tamoxifen (active metabolite), which may improve the treatment outcome(136).

However, long-term exposure to tamoxifen has been shown to induce a state of adaptive hypersensitivity in breast tumors to E2 and the development of tamoxifen resistance; low estrogen levels may stimulate tumor growth (164). Because tamoxifen and estrogens are both partly metabolized by the enzymes CYP2C19, 2D6, 3A5, and SULT1A1(165). Estrogen levels may be necessary during long-term tamoxifen therapy so need further investigation for this enzyme genetic polymorphism.

4.3.2 Tumor marker (CA15. 3) level

Cancer antigen (CA15.3) is used for monitoring therapy response along with other clinical features and radiological imaging . CA15.3 level in healthy individuals was with in the normal range (0-30U/ml). In contrast, its level was significantly increased in breast cancer patients and gradually decreased in patients who used hormonal therapy such as tamoxifen(166).

In this study, females with the wild type (CC) and homozygous mutant (TT) had the highest serum CA15.3 level compared with harboring heterozygous mutant type (CT) of rs11188072 , which had the lowest serum CA15.3 level. However, these results were still within the normal range and non-significant associated with CYP2C19*17 genetic polymorphism. Results of this SNP with normal limits and the differences between genotype groups were non-significant.

In this study, the highest level of serum CA15.3 occurred in the wild type (GG) and homozygous mutant type (AA) compared with the heterozygous mutant type (GA) of rs4986893 . However, these results remain with normal values .

This result is non-significant association between genotypes and tumor marker (CA15.3) in BC Iraqi women who received tamoxifen therapy. However, The level of CA15.3 does not exceed the average value and this is a good point for tamoxifen therapy response as increasing serum levels of CA 15-3 are found during therapy, disease progression may be expected but declining in the concentrations of the biological tumor marker indicate a positive treatment effect and are a minimum stable disease course. This positive correlation between clinical presentation and tumor marker behavior is mentioned by several authors (167)(168).

4.3.3 Vitamin D3 and calcium level

Vitamin D is a precursor to the hormone calcitriol (1,25-dihydroxy vitamin D3) which governs multiple functions in many human tissues (169). Vitamin D may be created endogenously in the skin by sun exposure and to a lesser extent gained through food and supplementation. Vitamin D is well-known for its involvement in calcium homeostasis and in influencing bone mineralization(170)(171).

Previous research has revealed that the link between vitamin D and breast cancer risk is higher in premenopausal women than in postmenopausal women. Two cohort studies in postmenopausal women found no connection between vitamin D level and breast cancer risk (172)(173).

According to lab tests a standard value for vitamin D3 is (30_100) ng/ml; the level of vitamin D3 had declined from the normal range in both SNPs but this difference is non-significant associated with (rs 11188072) and (rs4986893) genotype. This result is inverse to a previous study by **Kim et al.**, who showed that patients who received anti-hormonal therapy (tamoxifen) had significantly increased serum 25OHD levels at six months and 12 months compared with baseline serum 25OHD, while decreased with chemotherapy in BC patients(174).

An explanation for these results is that patients do not take regular vitamin D supplements because a previous study stated that vitamin D, combined with tamoxifen reduces cancer cells' proliferation and induces cell cycle arrest at G0/G1 phase(175).

Serum vitamin D3 level had declined from the basel line in Iraq woman with BC taking tamoxifen therapy and associated considerably with SNPs. The results are significant statistically so that by these results, suggested that

vitamin D3 ascend to be important affected factors with breast cancer women and further investigation need.

Serum calcium levels influence vital physiologic processes such as heart rate and nerve conduction and therefore are under tight physiologic control. The skeleton is the reservoir for calcium in the blood. When levels of ionized calcium in serum drop below their set point, the calcium-sensing receptor on the parathyroid glands signals parathyroid cells to manufacture and release parathyroid hormone (PTH) into the circulation. PTH acts to conserve calcium by driving the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-dihydroxy vitamin D (1,25(OH)2D) in the kidney; reducing calcium excretion in the urine and by liberating calcium from the skeleton into the circulation. The resulting increase in ionized calcium in blood restores calcium balance. It may be associated with tumor protective effects by inhibiting further release of PTH that have anti-apoptotic effects and may promote invasiveness and stimulate tumor growth(176)(177).

A prospective cohort study showed that serum calcium levels were inversely associated with breast cancer risk in premenopausal women; it also indicates that calcium levels are positively associated with breast cancer in over weight postmenopausal women (178)

In this study, founf calcium level is not difference between wild, homo, and heterozygous mutant types of both SNP's average range value (8.1-10.4)mg\dL. The results agreed with the previous study that showed no significant change in serum calcium between the test and control groups. So it did not have any unfavorable effect on bone (179).

4.3.4 Serum lipid profile level

Tamoxifen acts as a selective estrogen receptor modulator by binding to the estrogen receptors on the mammary epithelium and blocking the proliferative action of estrogen on the mammary epithelium(180).

In this study serum TG was raised from baseline in wild genotype (TT) but remained non-significant associated with (rs11188072) as ($p > 0.05$). There is an increase in the value of serum TG from baseline in wild genotype (GG) but remains non-significant associated with (rs4986893) as ($p > 0.05$).

In addition, tamoxifen has also been observed to affect the patients' biochemical profiles, including fasting lipid profile and plasma lipoprotein concentrations(181)(182).Tamoxifen therapy has favorable effects on serum lipid profile in both premenopausal and postmenopausal patients of breast cancer(183).

A previous study reported that tamoxifen increased TG and VLDL levels after initiating the drug (184). However, **Hozumi Y.et al**,found that changes of lipid profiles in Japanese postmenopausal women treated with tamoxifen were relatively favorable, while exemestane and anastrozole had no clinically significant effect on the serum lipids and tamoxifen may be a treatment choice for patients at high risk of cardiovascular events such as hyperlipidemia (185).

4.4 Association of side effects

The most common side effects of hormonal therapy (tamoxifen) are hot flush, joint pain, and endometrial hyperplasia; in this present study, joint pain (76%) , hot flush (13%) and endometrial hyperplasia (2%) of BC patient.

joint pain is the highest frequency of side effects occurring concerning the duration of treatment but it is non-significant statistically; these results were explained by several studies that specifically for treatment-related musculoskeletal adverse effects. The incidence of joint-related adverse effects were reported to be between 30% and 66% (186).

Moderate to high-grade joint-related symptoms are also associated with poor adherence to therapy or treatment discontinuation, besides interfering with the daily activities of the survivors(187). Low 25(OH)D concentrations are strongly linked with increased bone fracture rates and lower physical performance, especially in postmenopausal women(188).

Joint pain is among the most common side effects seen in patients receiving tamoxifen or aromatase inhibitors (Als) for prolonged periods as adjuvant hormonal treatment and harm their quality of daily life due to the acute reduction of sex hormones caused by hormonal treatment (189).

Most patients refused to participate in this study (either afraid of the blood being drawn or insufficient blood quantity). The small sample size was due to cases being restricted to Karbala and most patients' relatives receiving tamoxifen monthly as the patient was not come by himself . Results to be more satisfactory, so we need to overcome these limitation in the future.

Conclusion
&
Recomendation

4.5 Conclusion

Results obtained from this cross section study the following may be concluded:

1. CYP2C19*17 and CYP2C19*3 of Iraqi breast cancer patients were detected with variable frequencies of different genotype .

2. In Iraqi breast cancer samples treated with tamoxifen detected that rs11188072 wild type (CC) have highest frequency and homozygous mutant type (TT) have lowest frequency.

3. In Iraq breast cancer samples detected that rs4986893 homozygous mutant (AA) type had highest frequency and the wild type (GG) had lowest frequency by ARMS- PCR .

4. It was noted that the genetic variants of CYP2C19 gene were not significantly correlated with biochemical investigation (serum levels of estradiol ,cancer antigen CA15.3, vitamin D,calcium and lipid profile) suggested that had no effect on tamoxifen therapy response.

4.6 Recommendations and further work

1. Multicenter need for large scale and use of case-control study instead of cross-sectional observational study to compare between control and patient taking tamoxifen therapy.

2. Study genetic variant in other enzymes such as CYP2C6 that play main role in metabolism of tamoxifen could contribute to individual variability in tamoxifen response and may need further investigation

3. The plasma level of tamoxifen metabolites (endoxifene) must be measured for more accurate results of genetic polymorphism.

4.In clinical setting,we recommend that genetic tests should be developed to predict tamoxifen treatment with more safety and efficacy.

Referenences

References:

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, etal. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians. 2021;71(3):209-49.
2. Al-Alwan NAS, Mualla FHF .Promoting clinical breastexamination as A screening tool for breast cancer in Iraq. Iraqi National Journal of Nursing Specialties .2014;27(1): 30-39.
3. Al Alwan NAS. Cancer control and oncology care in Iraq. Journal Contemporary Medical Sciences. 2022; 8 (1): 82-5
4. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics 2022. CA:A Cancer Journal Clininicians . 2022 ; 72 (1): 7-33.
5. Virani S, Wetzel EC, Laohawiriyakamol S, Boonyaphiphat P, Geater A, Kleer CG, et al. Ethnic disparity in breast cancer survival in southern Thai women. Cancer epidemiology. 2018; 54: 82-9.
6. Mollah Karim S, Ali Ghalib S, Mohammad S A, Fattah FHR. The incidence of breast cancer and the age at diagnosis of breast cancer among the Iraqi Kurdish population and its comparison with some countries in the Middle East and the West. International Journal Surgery. 2015; 13:71-5.
7. El-Essawy OJ, Daccache H, Daccache MT, Obaid A, Khalil M. Breast cancer in Fallujah district (Iraq) A comparative pathological study. Medico-Legal Update. 2020; 20 (2): 502-7.
8. Chhichholiya Y, Suman P, Singh S, Munshi A. Genetic engineering of breast cancer metastasis, focus on a mechanistic 10. Aspects, signs of treatment and treatment strategies. Medical Oncology.2021; 38 (8): 1-23.

9. Pfeffer CM, Singh ATK. Programmed cell death: a target for anti-cancer treatment .International journal molecular Science. 2018; 19 (2).

10. Al-Nuaimi NMA, Muhammad A. Think nk. A study on the impact of risk factors on breast cancer and the development of breast cancer in Iraqi women. Will Reef Farm. 2020; 11 (12): 1647-54.

11. Godet I, Gilkes DM. BRCA1 and BRCA2 mutations and treatmentstrategies for breast cancer. Integrated cancer science and therapy. 2017; 4 (1).

12. Almeida Chuffa LG, Lupi-Júnior LA, Costa AB, de Arruda Amorim JP, Seiva FRF. The role of sex hormones and steroid receptors on female eproductive cancers. Steroids. 2017;118:93-108.

13. Cui J, Shen Y , Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends in Molecular Medicine, 2013;19(3):197-209.

14. Caldon CE. Estrogen signaling and the DNA damage response in hormone dependent breast cancers. Frontiers in Oncology. 2014;4:106.

15. Santen RJ, Yue W and Wang J-P. Estrogen metabolites and breast cancer.Steroids. 2015;99(A):61-66.

16. Yue W, Yager JD, Wang J-P, Jupe ER , Santen RJ. Estrogen receptor dependent and independent mechanisms of breast cancer carcinogenesis. Steroids. 2013;78(2):161-170.

17. Ferretti G, Felici A, Papaldo P, Fabi A, Cognetti F. The role of her2 / neu in breast cancer: from predictive enemy to predictive friend. CURR Gynecol Open Obstetrics. 2007; 19 (1): 56-62.

18. Zhang Y, Lv F, Yang Y, Qian X, Lang R, Fan Y, et al. Clinicopathological features and prognosis of metaplastic breast carcinoma: experience of a major Chinese cancer center. PLoS One.2015;10(6): e0131409.

19. Lacroix M . Significance, detection and markers of disseminated breast cancer cells.Endocrine-related cancer (Bioscientifica).2006;13(4):1033-67.

20. Balekouzou A, Yin P, Pamatika CM, Bekolo CE, Nambei SW, Djeintote M,et al. Reproductive risk factors associated with breast cancer in women inBangui: a case–control study. BMC Women's Health. 2017;17(1):14.

21. Key TJ, Appleby PN, Reeves GK , Travis RC, Alberg AJ, et al.Sex hormones and risk of breast cancer in premenopausal wome Acollaborative reanalysis ofseven prospective studies. Lancet Oncology. 2013; 14(10):1009–1019.

22. Folkerd E , Dowsett M. Sex hormones and breast cancer risk and prognosis. The Breast. 2013; 22:S38–S43.

23. Zhang X, Tworoger SS, Eliassen AH, Hankinson SE. Postmenopausal plasma sex hormone levels and breast cancer risk over20 years of follow-up. Breast cancer research and treatment. 2013; 137(3): 883–892.

24. Giordano SH. Breast cancer in men. The new England journal of medicine. 2018; 378, 2311–2320.

25. Siegel R , Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA :Cancer journal for clinicians. 2014; 64(1): 9–29.

26. McGuire A, Brown JAL, Malone C, McLaughlin R, Kerin M J. Effects of age on the detection and management of breast cancer. Cancers. 2015; 7: 908–929.

27. Shiyanbola OO, Arao RF, Miglioretti DL, Sprague BL, Hampton JM ,et al. Emerging trends in family history of breast cancer and associated risk. Cancer epidemiology biomarkers Prev. 2017; 26(12): 1753–1760.

28. Baglia ML, Tang M-TC, Malone KE, Porter P, Li C I. Family history and risk of second primary breast cancer after in situ breast carcinoma. Cancer Epidemiology biomarkers Prev. 2018; 27(3): 315–320.

29. Brewer HR , Jones ME , Schoemaker M J, Ashworth A , Swerdlow AJ. Family history and risk of breast cancer: an analysisaccounting for family structure.Breast cancer research and treatment . 2017; 165:193–200.

30. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. Annals of oncolology. 2015; 26: 1291–1299.

31. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, et al. Breast cancer development and progression: risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & Diseases. 2018;5(2):77-106.

32. Danaei G, Hoorn SV, Lopez AD, Murray CJ, EzzatiM. Causes of cancer in the world: comparative risk assessmentof nine behavioural and environmental risk factors.Lancet .2005; 366: 1784-1793.

33. Almutlaq BA, Almuazzi RF, Almuhayfir AA, Alfouzan AM, Alshammari BT, AlAnzi HS, et al. Breast cancer in Saudi Arabia and its possible risk factors. Journal of Cancer Policy. 2017;12:83-89.

34. Protani M, Coory M, Martin JM. Effect of obesity on survival of women with breast cancer: Systematic review and meta-analysis.Breast Cancer Reserech Treatment. 2010; 123: 627–635.

35. Iyengar NM, Arthur R, Manson JE, Chlebowski RT, Kroenke CH, Peterson L, et al. Association of body fat and risk of breast cancer in postmenopausal women with normal body mass index. JAMAOncology. 2019; 5: 155–163.

36. Kolb R, Zhang W. Obesity and Breast Cancer: A Case of inflamed adipose tissue. Cancers .2020; 12: 1686.

37. Chen X , Wang Q , Zhang Y, Xie Q ,Tan X. Physical activity and risk of breast cancer: A meta-analysis of 38 cohort studies in 45 study reports. Value in Health .2019; 22: 104–128.

38. McTiernan A, Kooperberg C, White E, Wilcox S,et al.Recreational physical activity and the risk ofbreast cancer in postmenopausal women. Journal of the American Medical Association .2003;290(10) :1331–1335.

39. Catsburg C, Miller AB, Rohan TE. Active cigarette smoking and risk of breast cancer. International Journal of Cancer .2015; 136 :2204–2209.

40. Jones M E , Schoemaker M J , Wright L B, Ashworth A , Swerdlow A J. Smoking and risk of breast cancer in the generations study cohort. Breast Cancer Research . 2017; 19:118.

41. Rachdaoui N, Sarkar D.K. Effects of alcohol on the endocrine system. Endocrinol Metab Clin North Am.2013;4(3):593–615.

42. Coronado G.D, Beasley J, Livaudais J. Alcohol consumption and the risk of breast cancer. Salud Publica Mexico. 2011; 53(5):440–447.

43. Dieci MV, Orvieto E, Dominici M, Conte P and Guarneri V. Rare breast cancer subtypes: histological, molecular, and clinical peculiarities. The Oncologist. 2014;19(8):805-813.

44. West A, Wullkopf L, Christensen A, Leijnse N, Tarp JM, Mathiesen J, et al. Division induced dynamics in non-invasive and invasive breast cancer.Biophys J. 2017;112(3):123–125.

45. Ward EM, DeSantis CE, Lin CC, Kramer JL, Jemal A, Kohler B, et al.Cancer statistics: breast cancer in situ. Cancer Journal for Clinicians 2015;65(6):481-495.

46. Inoue M, Nakagomi H, Nakada H, Furuya K, Ikegame K, Watanabe H et al.Specific sites of metastases in invasive lobular carcinoma: a

retrospective cohort study of metastatic breast cancer. Breast Cancer.2017;24(5):667–672.

47. Henry LN, Ism1aila N, Hammond EH, Andre F, Collyar DE, Kudere NM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer. Journal Clinical Oncolology.2016;34(10):1134–1150.

48. Page K, Guttery DS , Fernadez-Garcia D, Hills A, Hastings RK, Luo J, et al. Next-generation sequencing of rolling cell-free DNA for the evaluation of mutations and gene amplification in metastatic breast cancer. Clinical Chemistery. 2017:63(2):532-541.

49. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Reserche. 2004;6:149–152.

50. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A,et al. Comprehensive molecular portraits of invasive lobular breast cancer. Cell.2015;163(2):506-519.

51. Joglekar-Javadekar M, Van Laere S, Bourne M, Moalwi M, Finetti P,Vermeulen PB, et al. Characterization and targeting of platelet-derived growth factor receptor alpha (PDGFRA) in inflammatory breast cancer(IBC). Neoplasia. 2017;19:564–573

52. Leong AS-Y and Zhuang Z. The changing role of pathology in breast cancer diagnosis and treatment. Pathobiology. 2011;78(2):99-114.

53. Colomer R, Rojo F, Albanell J, García-Caballero T, Ciruelos E, López-García M, et al. Biomarkers in breast cancer: A consensus statement by the spanish society of medical oncology and the spanish society of pathology. Clinical and Translation Oncology. 2018; 20: 815–826

54. Li Y, Yang D , Yin X , Zhang X , Huang J, Wu Y, Wang M, et al. Clinicopathological Characteristics and Breast Cancer–Specific

Survival of Patients with Single Hormone Receptor–Positive Breast Cancer. JAMA Netw. Open 2020,3, e1918160.

55. Duffy M , Harbeck N, Nap M, Molina R , Nicolini A , Senkus E, Cardoso F. Clinical use of biomarkers in breast cancer:Updated guidelines from the European Group on Tumor Markers (EGTM). European Journal of Cancer. 2017;75: 284–298.

56. Nasrazadani A, Thomas RA, Oesterreich S, Lee AV. Precision medicine in hormone receptor-positive breast cancer.Frontiers in Oncology. 2018; 8: 144.

57. Tse L A, Li M, Chan W-C, Kwok C-H, Leung S-L, Wu C, Yu ITS, et al. Familial risks and estrogen receptor-positive breast cancer in Hong Kong Chinese women. PLoS ONE. 2015; 10: e0120741.

58. Konan H-P, Kassem L, Omarjee S, Surmieliova-Garnès A, Jacquemetton J, Cascales E,et al. ERa-36 regulates progesterone receptor activity in breast cancer. Breast Cancer Research. 2020; 22: 50.

59. Freelander A, Brown LJ, Parker A, Segara D, Portman N, Lau B, LimE. Molecular biomarkers for contemporary therapies in hormone receptor-positive breast cancer. Genes. 2021; 12: 285.

60. Wu J-R, Zhao Y, Zhou X-P, Qin X. Estrogen receptor 1 and progesterone receptor are distinct biomarkers and prognosticfactors in estrogen receptor-positive breast cancer: Evidence from a bioinformatic analysis. Biomedicine and Pharmacotherapy. 2019;121: 109647.

61. Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, et al. Annual report to the nation on the status of cancer,1975-2011,featuring incidence of breast cancer subtypes by Race/Ethnicity, Poverty and State. Journal of the National Cancer Institute.2015; 107(6): djv048.

62. Furrer D, Paquet C, Jacob S, Diorio C. The Human Epidermal Growth Factor Receptor 2 (HER2) as a prognostic and predictive biomarker: molecular insights into HER2 activation and diagnostic implications. Cancer Progn. 2018.

63. Kondo K, Van Schil P, Detterbeck FC, Okumura M, Stratton K, Giroux D,et al. The IASLC/ITMIG thymic epithelial tumors staging project:proposals for the N and M components for the forthcoming (8th) edition ofthe TNM classification of malignant tumors. Journal of Thoracic Oncology.2014;9(9):S73-S80.

64. Taherian-Fard A, Srihari S and Ragan MA. Breast cancer classification:linking molecular mechanisms to disease prognosis. Briefings in Bioinformatics. 2014;16(3):461-474.

65. Ulaner GA. PET/CT for Patients With Breast Cancer: Where Is the Clinical Impact? American Journal of Roentgenology. 2019:1-12.

66. Akram M, Iqbal M, Daniyal M, Khan AU. Awareness and current knowledge of breast cancer .Biological Research.2017;50(1):1-23.

67. Heng YJ, Lester SC, Tse GM, Factor RE, Allison KH, Collins LC, et al. The molecular basis of breast cancer pathological phenotypes.The Journal of pathology. 2017;241(3):375-391.

68. Kabel AM. Tumor markers of breast cancer: new prospectives. Journal of Oncological Sciences. 2017;3(1):5-11.

69. Li J, Liu L, Feng Z, Wang X, Huang Y, Dai H, et al. Tumor markers CA15-3, CA125, CEA and breast cancer survival by molecular subtype: a cohort study. Breast Cancer. 2020:1-10.

70. Araz M, Beypinar I, Kazan S, Inci F, Celiker M, Uysal M. Arepreoperative serum CA15-3 levels different in breast cancer subgroups?.Current Problems in Cancer. 2019;43(2):115-122.

71. Gaughran G, Aggarwal N, Shadbolt B , Harris R S. The utility of the tumor markers CA15. 3, CEA, CA-125 and CA19. 9 in metastatic breast cancer. Breast Cancer Management. 2020;9(4):BMT50

72. Rack B, Schindlbeck C, Jückstock J, Tesch H ,Genss EM, Lorenz R, et al. Prevalence of CA 27.29 in primary breast cancer patients before the start of systemic treatment. Anticancer Research.2010;30(5):1837-1842.

73. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. PLoS One. 2015;10(7):e0133830.

74. Gillbert FJ , Doming KP. Diagnosis and staging of breast cancer:When and How to Use Mammography, Tomosynthesis, Ultrasound,Contrast-Enhanced Mammography, and Magnetic Resonance Imaging.Diseases of the Chest, Breast, Heart and Vessels. 2019:155-66.

75. Gastounioti A, McCarthy AM, Pantalone L, Synnestvedt M. Effect of mammographic screening modality on breast density assessment. Radiology.2019;291:320-327.

76. Mann RM, Balleyguier C, Baltzer PA, Bick U, Colin C, Cornford E, et al. Breast MRI: EUSOBI recommendations for women's information.European Radiology. 2015;25(12):3669-78.

77. Kelly K, Dean J, Comulada W, Lee S. Breast cancer detection using automated whole breast ultrasound and mammography in radiographically dense breasts. European Radiology. 2010;20(3):734–742.

78. Ahmed BA. Awareness and practice of breast cancer and breast self examination among university students in Yemen.Asian Pacific Journal Cancer Prevention. 2010;11:101–105.

79. Cardoso F, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rubio I., et al. Early breast cancer:ESMO clinical practice guidelines

for diagnosis, treatment and follow-up. Annals of Oncology. 2019;30:1194–1220.

80. Chatterjee A. Long term effects of modern breast cancer surgery. Gland Surgery. 2018;7(4):366-370

81. Jones C, Lancaster R. Evolution of operative technique for mastectomy.Surgical the clinics. 2018;98(4):835-844.

82. Lester J. Local treatment of breast cancer. Seminars in Oncology Nursing. 2015 ; 31(2):122-133.

83. Masood S. Neoadjuvent chemotherapy in breast cancer .Women heal.2016;12(5):480-491

84. Tao JJ, Visvanathan K, Wolff AC. Long term side effects of adjuvant chemotherapy in patients with early breast cancer.The Breast. 2015;24(2):S149-S153.

85. Ishii K, Morii N, Yamashiro H. Pertuzumab in the treatment of HER2-positive breast cancer: An evidence-based review of its safety, efficacy, and place in therapy. Core Évidence. 2019; 14: 51–70.

86. Tremont A , Lu J, Cole JT. Endocrine therapy for early breast cancer: updated review. Ochsner Journal. 2017;17(4):405-411.

87. Mirzabeigi MN, Nelson JA, Fischer JP, Kovach SJ, Serletti JM, Wu LC and et al. Tamoxifen (selective estrogen-receptor modulators) and aromatase inhibitors as potential perioperative thrombotic risk factors in free flap breast reconstruction. Plastic and Reconstructive Surgery. 2015;135(4):670e-679e.

88. Burstein HJ, Lacchetti C, Anderson H, Buchholz TA, Davidson NE Gelmon KA, et al. Adjuvant endocrine therapy for women with hormone receptor-positive breast cancer: ASCO clinical practice guide line focused update. Journal of Clinical Oncology. 2018;37(5):423-438.

89. Nguyen X, Hooper M, Borlagdan JP, Palumbo A . A review of Fam-Trastuzumab Deruxtecan-nxki in HER2-Positive Breast Cancer. Annals Pharmacotherapy. 2021;55(11):1410-1418 .

90. Chlebowski RT, Schottinger JE, Shi J, Chung J and Haque R. Aromatase inhibitors, tamoxifen, and endometrial cancer in breast cancer survivors.Cancer. 2015;121(13):2147-2155.

91. Abraham J and Staffurth J. Hormonal therapy for cancer. Medicine.2016;44(1):30-33.

92. Dowsett M, Lønning PE, Davidson NE. Incomplete estrogen suppression with gonadotropin-releasing hormone agonists may reduce clinical efficacy in premenopausal women with early breast cancer. Journal of Clinical Oncology . 2016;34:1573,1584, and1594.

93. Kharode Y, Bodine PV, Miller CP, Lyttle CR, Komm BS. The pairing of a selective estrogen receptor modulator, bazedoxifene, with conjugated estrogens as a new paradigm for the treatment of menopausal symptoms and osteoporosis prevention. Endocrinology. 2008;149(12):6084–6091.

94. Traboulsi T, El-Ezzy M, Gleeson JL, Mader S.Antiestrogens: structure—activity relationships and use in breast cancer treatment . Journal of Molecular Endocrinology. 2017; 58(1): R15-R31.

95. Brauch H, Schroth W, Eichelbaum M, Schwab M, Harbeck N. Clinical significance of CYP2D6 genetics to tamoxifen response in breast cancer. Breast Care. 2008;3(1):43-50.

96. Li Li, Chang B, Jiang X, Fan X, Li , Li T, et al. Clinical ouctomes comparison of 10 years verus 5 year of adjuvant endocrain therapy in patients with early breast cancer. BMC cancer.2018;18(1):977.

97. Jordan VC.Tamoxifen (ICI46, 474) as a targeted therapy to treat and prevent breast cancer. British journal of pharmacology.2006;147(S1): S269- S276.

98. Riggs BL, Hartyyann LC. selective estrogen receptor modulators mechanism of action and application to clinical practice. The New England Journal of Medicine. 2003; 348 (12): 618-629.

99. Clemons M, Danson S, Howell A. Tamoxifen ("Nolvadex"):A review. Cancer Treatment Reviews 2002;28(4):165-180. 110.

100. Ilroy MM, Fleming FJ, Buggy Y, Hill A D , , Young L S. Tamoxifen - induced ER - a - SRC - 3 interaction in HER2 positive human breast cancer ; a possible mechanism for ER isoform specific recurrence . Endocrine related cancer.2006; 13(4): 1135-1145.

101. Helland T, Henne N, Bifulco E, Naume B, Borgen E, Kristensen V N, et al .Serum concentrations of active tamoxifen metabolites predict long - term survival in adjuvantly treated breast cancer patients. Breast Cancer Research.2017; 19 (1): 125.

102. Yang J, Wen L, Jiang Y, Yang B. Natural estrogen receptor modulators and their heterologous biosynthesis.Trends in Endocrinology and Metabolism .2019;30(1):66-76.

103. Desta Z, Ward BA, Soukhova NV,Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6.Journal of Pharmacology and Experimental Therapeutics .2004; 310 (3) :1062-1075.

104. Shahbaz K. Tamoxifen:Pharmacokinetics and Pharmacodynamics. Journal of pharmaceutical Research .2017;1-8.

105. Kulkoyluoglu - Cotul E, Arca A , Madak - Erdogan Z. Crosstalk between estrogen signaling and breast cancer metabolism. Trends Endocrinology & Metabolism .2018; 30(1):25-38.

106. Burstein H J, Lacchetti C, Anderson H, Buchholz T A, Davidson N E, Gelmon KA, et al. Adjuvant endocrine therapy for women with hormone receptor - positive breast cancer: American Society of Clinical

Oncology clinical practice guideline focused update on overian suppression. Journal of Clinical Oncology.2016; 34(14):1689-1701.

107. Benksim A, Addi RA, Elkhoudri N, Cherkaoui M. Medical characteristics and therapeutic approaches used to treat primary and secondary infertile women. Iranian Journal of Public Health. 2018; 47 (12): 1832-1837.

108. Derman, O., Kanbur, N. Ö. , & Kutluk, T. (2003). Tamoxifen treatment for pubertal gynecomastia . International journal of adolescent medicine and health, 15(4), 359-364. 1

109. Arya R , Rathi A K, Singh K, Srivastava A, Panda D, Parida S N, et al. Gynecomastia: A review of literature. MAMC Journal of Medical Sciences.2016; 2(2): 69-75.

110. Sonia Z, Wiem K, Ibtisam A, Salem AB, Salma A, Hayfa B, et al. Male breast cancer: case studies and review of the literature. Open Access Library Journal.2017; 4 (7): 1-4

111. Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee K-H,et al. CYP2D6 genotype, antidepressantuse, and tamoxifen metabolism during adjuvant breast cancer treatment. Journal of the National Cancer Institute.2005; 97(1): 30–39.

112. Kelly C M, Juurlink D N, Gomes T, Duong-Hua M,Pritchard K I, Austin PC, et al. Selective serotonin reuptake inhibitors and breast cancer mortality in women receiving tamoxifen: A population based cohort study. British Medical Journal.2010;340.

113. Sideras K, Ingle J N, Ames M M, Loprinzi C L, Mrazek D P, Black J L, et al. Coprescription of tamoxifen and medications that inhibit CYP2D6. Journal of Clinical Oncology.2010; 28(16): 2768–2776.

114. Slovacek L, Ansorgova V, Macingova Z, Haman L, Petera J. Tamoxifen-induced QT interval prolongation.Journal of Clinical Pharmacy and Therapeutics.2008;33(4): 453–455.

115. Golan D, Tashjian A H, Armstrong E. Principles of pharmacology: the pathophysiologic basis of drug therapy. Lippincott Williams & Wilkins .2011.

116. Westbrook K, Stearns V .Pharmacogenomics of Breast Cancer Therapy. Pharmacology Therapeutics.2013;139(1):1-11.

117. Johnson MD, Zuo H, Lee K-H, Trebley JP, Rae JM, Weatherman RV,et al.Pharmacological Characterization of 4-hydroxy-N-desmethyl tamoxifen, a Novel Active metabolite of tamoxifen. Breast Cancer Research and Treatment.2004; 85: 151–159.

118. Helland T, Naume B, Hustad S, Bifulco E, Kvaløy JT, Saetersdal AB, et al. Low Z-4OH tam concentrations are associated with adverse clinical outcome among early stage premenopausal breast cancer patients treated with adjuvant tamoxifen. Molecular Oncoogyl.2021; 15: 957–967.

119. Gaedigk A, Ingelman-Sundberg M, Miller NA, Leeder JS, Whirl-Carrillo M, Klein TE. The pharmacogene variation (PharmVar) consortium: Incorpation of the human cytochrome P450 (CYP) allele nomenclature database. Clinical Pharmacology and Therapeutics. 2018;103(3): 399–401.

120. Boocock D J,Brown K, Gibbs AH et al. Identification of human CYP forms involved in the activation of tamoxifen and irreversible binding to DNA.Carcinogenesis.2002; 23(11): 1897–1901.

121. Bailey KR, Cheng C. The great debate: genome-wide association studies in pharmacogenetics research, good or bad?. Pharmacogenomics. 2010; 11(3): 305-308.

122. Ahmed S, Zhou Z, Zhou J,Chen S-Q. Pharmacogenomics of drug metabolizing enzymes and transporters: relevance to precision medicine. Genomics Proteomics Bioinformatics. 2016; 14: 298-313.

123. Gupta PD. Pharmacogenetics, pharmacogenomics and ayurgenomics for personalized medicine: a paradigm shift. Indian Journal of Pharmaceutical Sciences. 2015; 77: 135-141.

124. Agarwal A, Ressler D, Snyder G. The current and future state of companion diagnostics. Pharmgenomics and Personalized Medicine. 2015; 8: 99-110.

125. De Vries Schultink A, Zwart W, Linn SC, Beijnen JH, Huitema ADR Effects of pharmacogenetics on the pharmacokinetics and pharmacodynamics of tamoxifen. Clinical pharmacokinet. 2015; 54 (8): 797-810.

126. Sahib H, Erhim Muhammad B, Abdul-Majid BA. Cyp2C19 gene polymorphisms in a sample of the Iraqi population. International Journal of Pharmacy Biological Sciences. 2015; 5(4) :54 -60.

127. Tan S-H, Lee S-C, Goh B-C, Wong J. Pharmacogenetics in breast Cancer treatment. Clinical Cancer Research. 2008; 14 (24): 8027-8041.

128. Gao CM, Pu Z, He C, Liang D, Jia Y, Yuan X, et al. Effect of an OATPIBI genetic polymorphism on the absorption of tamoxifen and its metabolite, endoxifene. Oncology Reports. 2017; 38 (2): 1124-1132.

129. Tulsyan S, Mittal RD, Mittal B. Effect of ABCB1 polymorphism on breast cancer treatment outcomes. Genomics Personalized Medicine. 2016; 9:47-58.

130. Karki R, Pandya D, Elston RC, Ferlini C. Defining "mutation" and "polymorphism" in the era of personal genomics. BMC Medical Genomics. 2015; 8: 37

131. Zanger UM, and Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology and Therapeutics. 2013;138(1): 103-141.

132. Kiyotan K,MushirodaT, NakamuraY, Zembutsu H. Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters. Drug Metab Pharmacokinet.2012;27,(1):122–131.

133. Morais SM, Wilkinsons GR, Blaisdell J, Nakamura K, Meyer UA, Goldstein JA. Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in humans. Mol Pharmacol. 1994; 46(4): 594-598.

134. Brauch H, Schorth W. Tamoxifen use in postmenopausal breast cancer: CYP2D6 matters. Journal of ciinical Oncology.2013; 31(2):176-180 .

135. Li-Wan-PoA,GirardT,FarndonP,CooleyC,Lithgow J. Pharmacogenetics of CYP2C19: functional and clinical implicationsof a new variant CYP2C19*17. British Journal of Clinical Pharmacolology.2010; 69(3): 222–230 .

136. Schroth W, Antoniadou L ,Fritz P ,Schwab M ,Muerdter T,Zanger U M, et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. Journal of clinical Oncology.2007; 25(33): 5187–5193 .

137. Kadri K. Polymerase chain reaction (PCR): principle and applications.Synthetic Biology-New Interdisciplinary Science: IntechOpen. 2019.

138. Thiele D. Polymerase chain reaction (PCR) and applications. Immun Infekt. 1991;19(5):138–42.

139. Lee PY, Costumbrado J, Hsu C-Y, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments. 2012;62 (e3923):1-5.

140. Abo Almaali H. Investigation of vacA genotypes of Helicobacter pylori from samples in Karbala Governorate: Ph.D.theses, genetic engineering and biotechnology, Baghdad University, Iraq; 2014.

141. Xin T-B; Chen H; Lin Z; Liang S-X and Lin J-M. A secondary antibodyformat chemiluminescence immunoassay for the determination of estradiol in human serum. Talanta. 2010;82(4):1472-1477.

142. Hilkens J, Buijs F, Hilgers J, Hageman P, Calafat J, Sonnenberg A, et al.Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. InternationalJournal of Cancer. 1984;34(2):197-206.

143. KUFE D; Inghirami G; Abe M; Haues D; Justi-wheeler H and Schlom J.Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. Hybridoma. 1984;3(3):223-32.

144. Hopkins Laboratory Procedures Manual J. NHANES, Total Cholesterol, Direct HDL, Precipitated HDL, Triglycerides, LDL. 2003

145. Hafiane A, Genest J. High-density lipoproteins: measurement techniques and potential biomarkers of cardiovascular risk. BBA Clinic. 2015; 3: 175-88.

146. Hayashi T, Koba S, Ito Y, Hirano T. A method for estimating elevated sdLDL-C by measuring triglyceride and lipoprotein B levels. Diss lipid health. 2017 Jan 26 [cited 2022-Jul 161:16(1):1-10).

147. Lapić I, Kralik Oguić S. Rogić D. Preliminary evaluation of eight less frequent endocrine assays designed for MAGLUMI 800 chemiluminescence immunoanalyzer. Scand J Clin Lab Invest. 2021;81(4):332-338.

148. Bauer PJ. Affinity and Stoichiometry of calcium binding by arsenazo III. Analytical Biochemistry.1981;110(1):61-72.

149. World Health Organization. The SuRF Report 2. Surveill Risk Factors Reports Ser. 2005;22.

150. Jahangiri R, Mosaffa F, Razavi AE, Teimoori-Toolabi L, Jamialahmadi K. Altered DNA methyltransferases promoter

methylation and mRNA expression are associated with tamoxifen response in breast tumors.Journal of Celluler Physiology. 2018;233(09):7305–7319.

151. Thun MJ, Linet MS, Cerhan JR, Haiman CA, Schottenfeld D. Cancer epidemiology and prevention (4th ed). Oxford University Press. 2018; 861–888.

152. Thakur P, Seam RK, Gupta MK, Gupta M, Sharma M , Fotedar V. Breast cancer risk factor evaluation in a Western Himalayan state: A case–control study and comparison with the Western World. South Asian Journal of Cancer. 2017;6(3):106.

153. Cecchini RS , Swain S M, Costantino JP, Rastogi P, Jeong JH, Anderson SJ, et al .Body mass index at diagnosis and breast cancer survival prognosis in clinical trial populations from NRG Oncology/NSABP B-30, B-31, B-34, and B-38. Cancer Epidemiology Biomarkers Prevention.2016;25(1):51-59.

154. Sun L, Zhu Y, Qian Q, Tang L. Body mass index and breast cancer prognosis. Medicine. 2018;97: e11220

155. James F, Wootton S, Jackson A, Wiseman M, Copson E,Cutress R. Obesity in breast cancer—What is the risk factor? European. Journal of Cancer. 2015; 51(6): 705–720.

156. Bravi F ,Decarli A, Russo AG. Risk factors for breast cancer in a cohort of mammographic screening program: a nested case–control study within the FR iCaM study. Cancer Medicine. 2018;7(5):2145-2152

157. Crewe HK, Notley LM, Wunsch RM, Lennard MS, Gillam EM. Metabolism of tamoxifen by recombinant human cytochrome p450 enzymes: formation of the 4-hydroxy, 4′-hydroxy and N-desmethyl metabolites and isomerization of trans-4-hydroxytamoxifen. Drug Metab Dispos. 2002;30:869–874.

158. Justenhoven C, Hamann U, Pierl CB, Baisch C, Harth V, Pesch B, etal.CYP2C19*17 is associated with decreased breast cancer risk.Breast Cancer Research and Treatment .2008;115(2):391-396.

159. Moyer A M, Suman VJ, Weinshilbom RM, Avula R, Black JL, Kuffel MJ, et al. SULT1A1, CYP2C19 and disease-free survival in early breast cancer patients receiving tamoxifen. Pharmacogenomics . 2011;12(11): 1535–1543

160. Damkier P, Kjærsgaard A, Barker KA, Cronin-Fenton D, Crawford A, Hellberg Y.CYP2C19*2 and CYP2C19*17 variants and effect of tamoxifen on breast cancer recurrence: Analysis of the international tamoxifen pharmacogenomics.SCIENTIFIC Reports.2017;7:7727.

161. Bai L, He J,He GH, Xu F, Xu GL. Association of CYP2C19 polymorphisms with survival of breast cancer patients using tamoxifen: results of a metaa nalysis. Asian Pacific journal of Cancer Prevention. 2014;15(19):8331–8335.

162. Baldwin RM, Ohlsson S, Pedersen RS, Mwinyi J, IngelmanSundberg M, Eliasson E, Bertilsson L (2008) Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. Br J Clin Pharmacol65:767–774.

163. Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clinical Pharmacology Therapeutics.2006; 79:103–113.

164. Berstein LM, Wang JP, Zheng H, Yue W, Conaway M, Santen RJ: Long-term exposure to tamoxifen induces hypersensitivity to estradiol.Clinical Cancer Research. 2004,;10(4):1530-1534.

165. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT. Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. Endocrinology .2003;144(8):3382-3398.

166. Munsoor MM, Algily N, Abdulrahim M. Evaluation of circulating CA15-3 tumor marker among Sudanese women with breast cancer. Journal of Science and Technology . 2013;14(1):72-76.

167. Cheng JP, Yan Y, Wang XY, Lu YL, Yuan YH, Jia J and Ren J.MUC1-positive circulating tumor cells and MUC1 protein predict chemotherapeutic efficacy in the treatment of metastatic breast cancer. Chinese Journal of Cancer.2011; 30(1): 54-61.

168. Tondini C, Hayes DF, Gelman R, Henderson IC and Kufe DW:Comparison of CA15-3 and carcinoembryonic antigen in monitoring the clinical course of patients with metastatic breast cancer. Cancer Research .1988;48(14): 4107-4112.

169. Feldman D, Krishnan A V, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nature Reviews cancer. 2014;14(5):342–357.

170. Bhamb N, Kanim L, Maldonado R, Svet M, Metzger M. Effect of modulating dietary vitamin D on the general bone health of rats during posterolateral spinal fusion.Journal of Orthopaedic Research. 2018;36(5):1435–1443.

171. Anderson PH, Atkins GJ, Turner AG, Kogawa M, Findlay DM, Morris HA. Vitamin D metabolism within bone cells: Effects on bone structure and strength. Molecular and Cellular Endocrinology.2011;347(1–2):42–47.

172. Frazier AL, Li L, Cho E, Willett WC, Colditz GA. Adolescent diet and risk of breast cancer. Cancer Causes and Control. 2004;15(1):73–82.

173. McCullough ML, Rodriguez C, Diver WR, Feigelson HS, Stevens VL, Thun MJ, et al. Dairy, calcium, and vitamin D intake and postmenopausal breast cancer risk in the cancer prevention study II nutrition cohort. Cancer Epidemiology, Biomarkers Prevention. 2005;14(12):2898–2904.

174. Kim HJ, Koh BS, Yu JH, Lee JW, Son BH, Kim SB, et al. Changes in serum hydroxyvitamin D levels of breast cancer patients during tamoxifen treatment or chemotherapy in premenopausal breast cancer patients. European journal of Cancer. 2014;50(8):1403–1411.

175. Yetkin D, Ballı E, Bayrak G, Kibar D, Türkegün M. The Investigation of the Effects of Tamoxifen and Vitamin D Combination on the Expression of P53, Bcl-2 and Bax and Cell Cycle in Mcf-7 Cell Line. Proceedings.2018;(2):1527.

176. Brown EM. Physiology of calcium homeostasis. The parathyroids. 2001;2:167–181.

177. Datta M, Schwartz GG. Calcium and vitamin D supplementation and loss of bone mineral density in women undergoing breast cancer therapy. Critical Reviews Oncology/Hematology.2013;88(3):613–624.

178. Almquist M, Manjer J, Bondeson L, Bondeson AG. Serum calcium and breast cancer risk: Results from a prospective cohort study of 7,847 women. Cancer Causes Control. 2007;18(6):595–602.

179. Gopinath M, PremalethaT K . Effects of Tamoxifen on Lipid Profile, Serum Calcium and Gonadotropin Levels in Carcinoma Breast Patients. Journal of Evolution of Medical and Dental Sciences. 2017;6(31):2507–2511.

180. Birzniece V, Barrett PHR, Ho KKY. Tamoxifen reduces hepatic VLDL production and GH secretion in women: A possible mechanism for steatosis development. European Journal Endocrinology. 2017;177(2):137–143.

181. Carlson RW, Allred DC, Anderson BO, Burstein HJ, Carter WB, Edge SB, et al. Breast cancer Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network . 2003;1(2):148–188.

182. Shien T, Doihara H, Sato N, Anan K, Komaki K, Miyauchi K, et al. Serum lipid and bone metabolism effects of Toremifene vs. Letrozole as adjuvant therapy for postmenopausal early breast cancer patients: results of a multicenter open randomized study. Cancer Chemotherapy and Pharmacology. 2018;81(2):269–275.

183. Gupta S, Tandon VR, Kapoor B, Gupta A, Gupta GD, Khajuria V. Effects of tamoxifen therapy on plasma lipid profile in patients of breast cancer. J Assoc Physicians India 2006;54:183–6.

184. Hozumi Y, Kawano M, Saito T, Miyata M. Effect of tamoxifen on serum lipid metabolism. Journal of Clinical Endocrinology and Metabolism. 1998;83(5):1633–1635.

185. Hozumi Y, Suemasu K, Takei H, Aihara T, Takehara M, Saito T, et al. The effect of exemestane, anastrozole, and tamoxifen on lipid profiles in japanese postmenopausal early breast cancer patients: Final results of national surgical adjuvant study BC 04, the TEAM Japan sub-study. Annals of Oncology. 2011;22(8):1777–1782.

186. Nyrop KA, Callahan LF, Rini C, Altpeter M, Hackney B, Wilson A ,et al. Adaptation of an evidence-basedarthritis program for breast cancer survivors on aromatase inhibitor therapy who experience joint pain. Preventing Chronic Disease. 2015;12:E91.

187. Hershman DL, Shao T, Kushi LH, Buono D, Tsai WY, KwanM, et al. Early discontinuation and non adherence to adjuvant hormonal therapy are associated with increased mortality in women with breast cancer. Breast Cancer Research and Treatment. 2011;126(2):529–537.

188. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P.Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. Journal of Clinical Endocrinology Metabolism. 2009;94(4):1244–1250.

189. Martin-Herranz A, Salinas-Hernandez P. Vitamin D supplementation review and recommendations for women diagnosed with breast or ovary cancer in the context of bone health and cancer prognosis/risk. Crit RevOncol Hematol. 2015;96(1):91–99.

الخلاصة

الخلفيـة: يعتبـر سـرطان الثـدي مشـكلة صـحية عالميـة تـؤثر علـى الثـدي. تاموكسـيفين هـو مُعـدّل مستقبلات هرمون الاستروجين الانتقائي غير الستيرويدي ، ويعتبـر علاجًـا مسـاعدًا قياسـيًا ويسـتخدم على نطاق واسع في عـلاج الغـدد الصـماء للمـريض المصـاب بسـرطان الثـدي الإيجـابي لمسـتقبلات هرمون الستيرويد يلعب CYP2C19 دورًا أساسيًا في تكوين نسبة من مستقلبات تاموكسيفين ، بما في ذلك تحويل 4--تاموكسيفين إلى إندوكسيفين.

أهـداف الدراسـة: الهـدف مـن هـذه الدراسـة هـو التعـرف علـى تعـدد الأشـكال الـوراثي لــ rs4986893 (CYP2C19 * 3 (G> A و rs11188072 (C>T) CYP2C19 * 17، في النساء العراقيات المصابات بسرطان الثدي والتحقيق فـي تـأثير تعـدد الأشـكال الجينـي CYP2C19 على استجابة العلاج بالتاموكسيفين

المرضى والطرق: هذه دراسة قائمة على الملاحظة أجريت في مركز الإمام الحسين الطبي / مركـز الأورام في كربلاء ومختبرات كلية الصـيدلة / جامعـة كـربلاء. أجريـت الدراسـة علـى إجمـالي 100 امـرأة عراقيـة تتـراوح أعمـارهن بـين 45-65 سـنة مصـابات بسـرطان الثـدي الإيجـابي لمسـتقبلات هرمون الاستروجين و/ أو البروجسترون ، تناولت أقراص تاموكسـيفين 20 ملـغ جرعـة يوميـة علـى الأقل 3 أشهر دون أي أمراض.

تم سحب حوالي 6 مل من الدم الوريدي من جميع الإناث المساهمة فـي هـذه الدراسـة ، تـم وضـع (2 مل) من الدم في أنبوب EDTA للتحليل الوراثي ، ووضع (3 مل) فـي أنبـوب هـلام ، وتـم الحصـول على مصل الدم بعد الطرد المركزي للدم عند 3000 دورة في الدقيقة لمدة 10 دقائق لفصل المصـل ، حيث تم استخدامها لقياس استراديول ، وعلامة الورم (CA15.3) ، وملف الدهون ، ومستويات مصل الكالسيوم وفيتامينD3.

النتائج: كشفت النتائج التي تم الحصول عليها من هذه الدراسـة عـن أنمـاط وراثيـة متعـددة للألـيلات (CYP2C19 * 3 (G> A، rs4986893) التـي تشـمل الـنمط الجينـي الطبيعـي (GG) ، متماثـل الزيجه متحور (AA) والنمط الجيني متغاير الزيجه (GA) ، بينما CYP2C19 * 17 (تم اكتشاف rsl1188072، C> T) الـنمط الجينـي الطبيعـي (CC) ، والـنمط الجينـي المتحـور (TT) والـنمط الجيني متغاير الزيجه (TC) أيضًا في مرضى سرطان الثدي Iraqi الذين شاركوا فـي هـذه الدراسـة. أظهرت مستويات مصل استراديول (E2) ، وعلامة الورم (CA 15.3) ، وفيتامين D3 ، والكالسيوم ، وملـف الـدهون وجـود ارتبـاط غيـر معنـوي (p> 0.05) بـين SNPs المدروسـة لــ CYP2C19. أظهرت الدراسة الحالية أن هناك دورًا مهمًا لفيتامين D3 وأنه تم تكييفه ليكون مهمًا.

الاسـتنتاجات : لخصـت الدراسـة الحاليـة أن الاخـتلاف الجينـي فـي إنـزيم اسـتقلاب الطـور CYP2C19 ربما يساهم في التباين في الاستجابة للعلاج بالتاموكسيفين في عينة من سرطان الثـدي العراقيات .

جمهورية العراق
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كلية الصيدلة
فرع الادوية والسموم

تأثير تعدد الأشكال الجينية لـ CYP2C19 على ألأستجابة لعقار تاموكسيفين في عينة من النساء العراقيات المصابات بسرطان الثدي

رسالة

مقدمة الى مجلس كلية الصيدلة / جامعة كربلاء

كجزء من متطلبات نيل شهادة الماجستير في علم الادوية والسموم

من قبل

زهراء هادي علي

بكالوريوس صيدلة / جامعة كربلاء / 2013

بإشراف

الأستاذ المساعد — **الطبيب الاستشاري**

امال عمران موسى — **كرار كاظم محسن**

2023 ميلادي — **1444 هجري**