Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Kerbala
College of Pharmacy

Study The Impact of The ABCB1 Gene Polymorphism On Clinical Outcomes of Paclitaxel Monotherapy in Women With Breast Cancer in Kerbala

A Thesis

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

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2023 A.D. **1444 A.H.**


بسم الله الرحمن الرحيم
أَلَمْ تَرَ أَنَّ اللَّهَ أَنْزَلَ مِنَ السَّمَاءِ
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Supervisor certification

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DEDICATION

To My husband and his endless love, support and encouragement and my presious son for his patientce

To my beloved father and mother for their kindness efforts and for inspiring me to be strong despite many obstacle in life.

To my brothers and lovely sister and every person in my life who helped me finish my master degree.

To those who are suffering from cancer, I wish them a-healthy life.

Best regards

Doaa Alaa 2023

Acknowledgements

First of all I thank Almighty Allah for blessing me with health, strength, and knowledge.

I owe a deep gratefulness to the College of Pharmacy at Kerbala University, Department of Pharmacology and Toxicology for giving me a chance to complete this study.

I would like to thank my first research supervisor the Dean of Pharmacy College **Prof**. **Dr. Ahmed Salih Sahib** for his continuous support, vast knowledge and patience.

I would like to thank my second supervisor **Asst**.**prof Dr. Ahmed Abbas Hasan Al-Rokan** for his support, and for sharing his profound knowledge with me.

My appreciation is also conducted to the chairman of the Pharmacology and Toxicology Department in College of Pharmacy, **Asst**. **Prof**. **Amal Umran Mousa** for her support.

I would like to thank **Dr. Mazin Hamid Ouda** for his assistance and support, my thanks are extended as well for all the staff of the Department for their generous support.

Also I would have aspecial thank for **Dr**. **Bashar Alaa Mohammed Hasan** for his continuous support.

My deep gratitude to all doctors, medical staff "pharmacists and nurses" and laboratory staff at Imam AL- Hussein Medical City /Oncology center in Kerbala for their generous help, valuable advice and kindness during the clinical study.

My deepest appreciation to the patients participating in this study and their families who helped me complete this thesis.

Table of contents

List of Abbreviations	
Abbreviation	Full form
ABC	ATP binding cassette
ADC	Antibody-drug conjugates
BSE	Breast self-examination
CYP	Cytochrome P450
CIPN	Chemotherapy-induced peripheral neuropathy"
CEA	Carcinoembryonic antigen
DRG	Dorsal root ganglia
DDI	Drug-drug interactions
deoxy-TMP	Deoxythymidine monophosphate
deoxy-UMP	Deoxyuridine monophosphate
DCIS	Ductal carcinoma in situ
EGFR	Epidermal growth factor receptor
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FNAC	Fine-needle aspiration cytology
ITGA2	Integrin alpha-2/beta-1
IBC	Inflammatory breast cancer
LCIS	Lobular carcinoma in situ
MDD	Major depressive disorder
MPV	Mean platelet volume
MCHC	Mean corpuscular hemoglobin concentration
MCH	Mean corpuscular hemoglobin
MTX	Methotrexate
NADPH	Nicotinamide adenine dinucleotide phosphate
PDW	Platelet distribution width
PN	Peripheral neuropathy

P-gp, ABCB1	P-glycoprotein
PXR	Pregnane X receptor
RDW	Red cell distribution width
RBC	Red blood cell (RBC
SHBG	Hormone-binding globulin
SNPs	Single nucleotide polymorphisms
SNV	Single-nucleotide variation
TNS	Neuropathy Score (TNS
TNBC	Triple-negative breast cancer
UICC	Union of international cancer control
WBC	White blood cell
5-FU	5-fluorouracil
6-MP	6-mercaptopurine

Abstract

Background Breast cancer is defined as a disordered growth of cells from any of the tissues of the mammary gland (ducts or lobules) with the ability to spread, It is considered the most common cancer among women in both developed and non developing countries, wo. CA15.3 is an indicator of tumor response to treatment, which allows early identification of recurrence and adjustment of Paclitaxel treatment. In this sense, pharmacogenetics plays a fundamental role in understanding the individual factors that modify the response to breast cancer treatment, particularly the study of genes involved in the of Paclitaxel chemotherapy transporting . In this case, those of the A BCB1 3435G>A (rs1045642) and 2677G>A (rs2032582) pathway. The aim of study was to investigate of ABCB1gene polymorphism with two SNPs 3435G>A (rs1045642) and 2677G>A in postmenopausal women with breast cancer in Iraq as well as ABCB1gene polymorphism on paclitaxel efficacy and association between paclitaxel induce peripheral neuropathy and response.

Methods: This study included 100 patients (45-75 years) with a clinical diagnosis of breast cancer who were going to start (paclitaxel) chemotherapy . The able to identify genetic variants and their association with main adverse effect of this drug, as well as to complete neurological response in patients with chemotherapy treated Patients at the oncology center at Imam al-Hussain medical city in Kerbala, Iraq, who sorted into groups based on their postmenopausal age, disease duration, and treatment long for this study, which was done between July 2022 and december 2022 for sample collection, The efficacy of Paclitaxel, serum concentrations of (Estradiol and CA 15.3) were measured in women with breast cancer who had been taking the drug, forms of informed consent with participants' signatures were collected from everyone. The determination of polymorphisms was performed by Allele-Specific PCR assay for two gene

polymorphism 3435G>A (rs1045642) and of 2677G> A (rs2032582), and serum concentration of paclitaxel was measured by high performance liquid chromatography.

Results: The study found that patients with CA15-3 > 30 U/mL are at risk of non-responses compared to those with CA15-3< (30 U/mL). The findings demonstrated a statistically significant low in the amount of CA 15-3 found in breast cancer patients response (22.45± 9.28) group in comparison to the level found in the non-response (86.93 ± 11.34) group for patients who received Paclitaxel chemotherapy. There is a significant decrease in CA 15-3 levels with a significant increase in neuropathy score.it found reverse negative correlation value (-0.821) between concentration of CA15.3 and grade score of peripheral neuropathy in response to paclitaxel treatment. Also we found that ABCB1 gene highly polymorphism and two snips 3435G>A (rs1045642) . Patients with allele GG in their chromosomes respond better (15.56±6.89) than those with alleles AA and GA, as well as patients with the allele GG of the 2677G>A (rs2032582)that had a greater response to treatment (195.17±14.68) compared to those with the alleles AA and GA.

Conclusion: According to the findings of the study,ABCB1is highly polymorphism and in breast cancer women in Iraq .There is a substantial connection between two SNPs ABCB1 3435G>A (rs1045642) and 2677G>A (rs2032582) with paclitaxel efficacy. The incidence of peripheral neuropathy is the main adverse effect of Paclitaxel chemotherapy in women have breast cancer that have better response according to low Ca 15.3 . continuous ,effective measurement and close follow up are required to control situation.

. Chapter one

Introduction

1.1 The Breast:

The breast is a modified sweat gland that produces milk under hormonal influence. It is composed of fatty tissue that contains the glands responsible for milk production in late pregnancy and after childbirth. Within each breast, there are about 15-25 lobes formed by group of lobules, each one consists of grape-like clusters of acini. These lobules are arranged around ducts that funnel milk to the nipples, about 15-20 ducts come together near the areola to form ampullae. Two main arteries supply the breast, a branch from axillary artery to the outer half of the breast and two or three branches from the internal mammary artery to inner half of the breast. Venous drainage follows its arterial supply. The lymphatic drainage of the entire breast is to the axilla (Cowin & Wysolmerski., 2010 and Holliday et al.,2018).

1.2 Breast Tumors:

Most types of tumors that are formed in the breast are benign. Breast cancer is a malignant tumor that has developed from cells of the breast. The disease occurs mostly in women, but does occur rarely in men (Feng et al .,2018).

Some breast cancers are called insitu, because they do not spread beyond the area where they begin, while others are invasive cancer. The majority of insitu tumors will not progress to become an invasive tumor, and at this stage nearly all of these cancers can be cured (Ward et al.,2015).

As general consideration breast cancer is the second most common type of cancer after lung cancer and the fifth most common cause of cancer death

after lung cancer, stomach cancer, liver cancer, and colon cancer(Qiu et al.,2021).

1.3 Epidimiology:

In 2020, there were 2.3 million women diagnosed with breast cancer and 685000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer, the number of cases worldwide has significantly increased since the 1970s, aphenomenon partly blames on modern lifestyles (Lei et al., 2021).

Among women, breast cancer has remained the commonest tumor in Iraq, it forms 22.3% of all malignant tumor and 37% of the registered female cancers with a sharp increase in the incidence of this tumor in younger age group. It forms 5% of those under 30 years and 75% of those older than 40 years and highest number of cases is between 40 and 50 years, and the incidence looks to be increasing (Alwan, 2016).

1.4 Types of Breast Cancer:

Malignant breast tumors account for around 25% of all cases. In the lobules (milk-producing glands), or tissues known as terminal ductolobular. The majority of these malignancies emerge from epithelied tissues (surface or lining). The following is a recognized pathological categorization of adenocarcinoma of the breast based on the tumor's cellular origin and appearance (Shockney & Tsangaris.,2008).

1.4.1Non-invasive:

A-Ductal carcinoma in situ (DCIS):

1-Papillary DCIS.

2-Non-papillary DCIS which includes: comedocarcinoma, solid carcinoma, cribriform, micropapillary, clinging, and cystic hypersecretory(Konstantinova et al.,2017).

B-Lobular carcinoma in situ (LCIS)

1.4.2 Invasive carcinoma:

1-Invasive ductal carcinoma-not otherwise specified (NOS).

2-Invasive lobular carcinoma.

3-Tubular carcinoma.

4-Medullary carcinoma.

5-Mucinous carcinoma.

6-Invasive papillary carcinoma.

7-Apocrine carcinoma.

8-Adenoid cystic carcinoma.

9-Paget's disease.

10-Invasive comedocarcinoma (Makki,2015).

1.5 Signs and Symptoms of Breast cancer:

The most important physical symptom of breast cancer is a painless mass. Up to 10% of patients, however, have breast pain and no mass. Less common symptoms include persistent changes to the breast, such as thickening, swelling, skin irritation or distortion, nipple symptom, including spontaneous discharge, erosion and inversion or tenderness. Early breast cancer, when it is most treatable, typically does not produce any symptoms. It is, therefore, very important for women to follow recommended guidelines for finding breast cancer before symptoms develop(Firmino et al .,2021 and Gursen et al., 2021).

1.6 Risk factors of breast cancer :

1.6 .1 Age:

The risk of breast cancer is higher in middle-aged and elderly women than in young women This risk increases as a woman ages rising after the age of 40 (Tarver, 2012).

1.6 .2 Family history:

A woman who has previous history of breast cancer has three to four folds at increased risk of developing a new cancer in the other breast. Women who have had benign breast problems are also at increased risk but to a lesser extent. The risk of breast cancer is higher among women who have a close blood relative (mother, sister or aunts) who have had the disease (Hemminki et al.,2012)

The increase in risk is especially high if the relative developed breast cancer before the age of 50 or in both breasts . The effect of family history on breast cancer risk is believed to be due primarily to genetic factors (Monticciolo et al.,2018).

1.6 .3 Mutation:

It has been shown that germ line mutations in the BRCA1 and BRCA2 genes account for a large proportion of cases of hereditary breast cancer (Domchek *et al*., 2010). As much as 5–10 % of all breast cancer cases are attributable to specific inherited singlegene mutations, and many other cases have some genetic component.

1.6 .4 Estrogen production:

Women who reach menarche at a relatively early age (12 or younger) and those who reach menopause at a relatively late age (55 or older) are slightly more likely than other women to develop breast cancer (Britt,2012): These relationships are believed to be mediated through estrogen production

During the reproductive years, a woman’s body produces high levels of estrogen. Women who start to menstruate at an early age and/or reach

menopause at a late age are exposed to high levels of estrogen for more years than women who have a late menarche or early menopause (Ghazanfarpour et al.,2021)

1.6 .5 Pregnancy:

Age at first pregnancy is another point of reproductive history that is associated with breast cancer risk (Nechuta *et al*., 2010). Women who have their first full-term pregnancy at a relatively early age have a lower risk of breast cancer than those who never have children or those who have their first child relatively late in life . The biologic basis for this relationship is not entirely clear. Parity (having children) and the age of the woman at the birth of her first offspring are other endogenous hormonal factors that influence breast cancer. (Kurian et al.,2021).

Woolcott et al (2012) presented that women who have never had children (nulliparous) are at greater risk for the development of breast cancer than women who have had children (parous). There is also consistent evidence that first pregnancy completed before age 30-35 lowers risk of breast cancer, and that first full-term pregnancy after age 30-35 raises risk. More limited evidence suggests that women who have many pregnancies may be less likely to develop breast cancer than those who have only one pregnancy .

In some studies, premature termination of pregnancy appears to increase breast cancer risk. In incomplete pregnancy, the breast is exposed only to the high estrogen levels of early pregnancy and thus may be responsible for the increased risk seen in these women. However, some other studies found no association between abortions and increased risk of breast cancer (COG Committee Opinion , 2009 ; Huang et al.,2014). Other studies showed that women who breast-feed their babies may be less likely to develop breast cancer than those who have children but do not breast-feed (Anstey et al.,2017) .

1.6 .6 Radiation:

Women who were exposed to high doses of radiation, especially during adolescence, have an increased risk of breast cancer ,This association has been observed both among atomic bomb survivors and among women who received high-dose radiation for medical purposes (Little et al.,2022).

1.6 .7 Diet:

A possible relationship between breast cancer and diet has been suggested due to the variation of breast cancer in societies with different national diets (the high rates in Western industrialized nations and the low rates in Asia, Latin America, and Africa) (Abe et al., 2021).

A comparison of vegetarian versus meat-eating women produced inconclusive results, The effects of fiber, fruits, and vegetables now appear to be small, at best. Diets high in fruits and vegetables and low in fat and calories are healthful for many reasons, and they may indirectly reduce the risk of breast cancer by helping to prevent obesity (Marzbani et al.,2019).

1.6 .8 Obesity:

Obesity has been consistently associated with an increased risk of breast cancer among postmenopausal women .This relationship may be mediated again by estrogen production . Fat cells produce some estrogen and obese postmenopausal women, therefore, tend to have higher blood estrogen levels than lean women (Clear & Grossmann., 2009 and Mair et al.,2020).

1.6 .9 Smoking:

There is some evidence that cigarette smoking may be associated with a small increase in breast cancer risk. However, epidemiological studies have variably shown positive, inverse, or null association . Among women who have already been diagnosed with breast cancer, smoking may be associated with an increased risk that the cancer will progress more rapidly(Bottorff et al.,2014 And Kispert & McHowat., 2017).

1.7 Diagnosis of breast cancer:

Triple assessment clinical examination, mammography, fine-needle aspiration cytology [FNAC] of a breast lump is an established algorithm and the combination of all three modalities increases diagnostic accuracy substantially. Ninety percent of women with a palpable breast cancer should be diagnosed before operation. (Karim et al.,2020).

Ultrasonography is a useful adjunct in experienced hands and, as a non-invasive technique, it is also helpful in combination with both mammography and FNAC for guidance. Diagnostic accuracy from FNAC can reach 95% in experienced hand, especially if performed by a trained cytopathologist (Health Quality Ontario., 2016 and Harada-Shoji et al., 2021).

1.8 Prognosis of breast cancer:

The prognosis of breast cancer is closely related to the stage at diagnosis; accordingly, early detection of the tumor is vital in increasing the chance of survival. To achieve these aim different methods were used including mammography, periodic examination by health personnel and breast self-examination (BSE). The latter has been adopted recently in several countries. It is an easy, low cost and highly effective procedure. In addition, several studies have revealed that breast self-examination is an effective procedure in the early detection of breast tumor.(Perry et al.,2008 And Dilaveri et al.,2019) .

1.9 Delay diagnosis of breast cancer:

Delay in the diagnosis of breast cancer is an area of increasing litigation for medical practitioners, both in primary care and in hospital practice. The doctor who wishes to reassure an anxious patient must balance the risk of missing the diagnosis against creating unnecessary anxiety. Whether delay in the diagnosis of breast cancer affects the outcome has been uncertain, but

some studies have found evidences of reduced survival (Tremblay et al., 2016).

1.10 Treatment of breast cancer:

The type of cancer, the severity of the disease, and the location of the cancer all have a role in determining how the cancer should be treated. There are three different approaches to treating cancer, and they are as the following:

1. 10.1 Surgical treatment of breast cancer:

The actual removal of the tumor, often together with part of the surrounding tissue, is one of the primary goals of the surgical treatment of breast cancer. During the operation, a biopsy of one or more lymph nodes may be conducted; more often than not, the lymph node sample is carried out via a sentinel lymph node biopsy (Bleicher et al.,2016).

Common surgical procedures are as the following:

- **Mastectomy**: Removal of the whole breast.
- **Quadrantectomy**: Removal of one-quarter of the breast.
- **Lumpectomy**: Removal of a small part of the breast.

After the tumor has been removed, the affected individual has the option of undergoing breast reconstruction surgery, which is a subspecialty of plastic surgery, in order to enhance the cosmetic look of the area that has been treated. Women also have the option of using breast prosthesis or opting to have a flat chest to provide the appearance of having breasts beneath garments. After a mastectomy, the use of a nipple prosthetic is permissible at any time (Platt et al.,2011).

1. 10.2 Radiation treatment of breast cancer:

Radiation therapy — also called radiotherapy — is a treatment that uses high-energy radiation to kill cancer cells and shrink tumors.Radiation therapy uses special high-energy X-rays or particles to damage a cancer cell's DNA. When a cancer cell's DNA is damaged, it can not divide successfully and it dies(Jaffray & Gospodarowicz.,2015).

Radiation may also harm healthy cells in the vicinity. Yet, cancer cells are more susceptible to radiation damage than healthy ones. Cancer cells are more prone to uncontrolled growth and division than regular cells. Consequently, the ability of cancer cells to recover from radiation damage is diminished. Therefore, radiation is more effective in killing cancer cells than healthy ones because the latter can more quickly repair themselves and resist the former(Baskar et al.,2012).

Radiation therapy can be used to treat all stages of breast cancer. Pregnant women should not have radiation therapy because it can cause birth defect the unborn baby (Toesca et al., 2014).

The timing of radiation treatment in your overall breast cancer treatment plan depends on your individual situation and the characteristics of the breast cancer. In many cases, radiation therapy is given after surgery. If chemotherapy is planned after surgery, radiation usually follows chemotherapy. If you're having mastectomy and have decided to have breast reconstruction, it's important to know that radiation can cause a reconstructed breast to lose volume and change color, texture, and appearance. In particular, radiation therapy is known to cause complications with implant reconstruction. Research also suggests that a reconstructed breast may interfere with radiation therapy reaching the area affected by cancer, though this can vary on a case-by-case basis. For these reasons, some surgeons advise waiting until after radiation and other treatments, such as

chemotherapy, to be completed before breast reconstruction surgery is done. Other surgeons may recommend a more staged approach, which places a tissue expander after mastectomy to preserve the shape of the breast during radiation treatments. Once radiation is completed and the tissues have recovered, the expander that was used to maintain the shape of the breast is removed and replaced with tissue from another part of the body or a breast implant(Jobsen et al.,2013).

Certain antioxidant vitamin supplements, such as vitamins C, A, D, and E, while you're having radiation therapy .These vitamins might interfere with radiation's ability to destroy cancer cells. This is because radiation works in part by creating free radicals — highly energized molecules that damage cancer cells. Free radicals in the environment can damage all cells, but in the case of radiation treatment they are focused on the cancer cells. Antioxidants help keep free radicals from forming or neutralize them if they do form(Yasueda et al.,2016 and Dochniak,2022).

The side effects of radiation therapy depend on the type of radiation therapy you're having. In general, the side effects tend to develop as treatment goes on and may be more troubling toward the end of treatment. Overall, the most common side effects are redness, swelling, and skin peeling in the area being treated(Sourati et al.,2017).

1. 10.3 Systemic treatment:

Systemic therapy refers to any type of cancer treatment that targets the entire body, and it is include:

1. 10.3.1 Hormonal drugs:

Hormone therapy is a cancer treatment that slows or stops the growth of cancer that uses hormones to grow. Hormone therapy is also called hormonal

therapy, hormone treatment, or endocrine therapy, hormone therapy is used for two main reasons:

- **Treat cancer.** Hormone therapy can stop or slow cancer's growth and reduces the chance it will return.
- **Ease cancer symptoms.** Hormone therapy may be used to reduce or prevent symptoms in men with prostate cancer who are not able to have surgery or radiation therapy (Kuhle et al., 2016).

Hormone therapy falls into two broad groups, These that block the body's ability to produce hormones and the ones that interfere with how hormones behave in the body (Santen et al.,1990).

Cases like prostate and breast cancers that use hormones to grow. Hormonal therapy is most often used along with other cancer treatments. The types of treatment that you need depend on the type of cancer, if it has spread and how far, if it uses hormones to grow, and if you have other health problems (Abdulkareem & Zurmi., 2012)

Because hormone therapy blocks body's ability to produce hormones or interferes with how hormones behave, it can cause unwanted side effects. The side effects depend on the type of hormone therapy received and how body responds to it. People respond differently to the same treatment, so not everyone gets the same side effects. Some side effects also differ if you are a man or a woman (Miller et al., 2019), some common side effects for women who receive hormone therapy for breast cancer include: hot flashes , vaginal dryness , changes in your periods if you have not yet reached menopause , loss of interest in sex , nausea , mood changes and fatigue

Hormone therapy may be given in many ways:

- **Oral.** Hormone therapy comes in pills that you swallow.

- **Injection.** The hormone therapy is given by a shot in a muscle in your arm, thigh, or hip, or right under the skin in the fatty part of your arm, leg, or belly.
- **Surgery.** You may have surgery to remove organs that produce hormones. In women, the ovaries are removed(Sioka & Kyritsis. 2009).

1. 10.3.2 Anti-human epidermal growth factor:

The epidermal growth factor receptor (EGFR) is one of the first identified important targets of these novel antitumor agents. Approximately half of cases of triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) overexpress EGFR (Masuda et al.,2012).

In normal cells, HER2 helps control cell growth. Cancer cells that make too much HER2 may grow more quickly and are more likely to spread to other parts of the body(Feng et al.,2018).

Epidermal growth factor receptor is protein involved in normal cell growth. Human epidermal growth factor receptor 2 may be made in larger than normal amounts by some types of cancer cells, including breast, ovarian, bladder, pancreatic, stomach, and esophageal cancers(Wee & Wang., 2017).

Kidneys are predominant source of EGF production. EGF/EGFR signaling promotes embryonic development and stem cell regeneration and regulates ion transport(Zeng & Harris., 2014)

Breast cancer cells with higher than normal levels of HER2 are called HER2-positive. These cancers tend to grow and spread faster than breast cancers that are HER2-negative, but they are much more likely to respond to treatment with drugs that target the HER2 protein(Yin et al.,2020).

The development of antibody-drug conjugates (ADC) that administer chemotherapy into cancer cells and are absorbed by specific targets has reduced the systemic adverse effects of the chemotherapeutic agent.

Trastuzumab and the cytotoxic substance emtansine were the components of the first authorized ADC for the treatment of HER2 (T-DM1). T-DM1 was authorized in HER2-positive breast cancer for the second-line metastatic setting or for treatment in patients with residual disease after neoadjuvant trastuzumab-containing chemotherapy due to its improved effectiveness and good risk-benefit profile(Marei et al.,2022).

1.10.3.3 Chemotherapy:

Chemotherapy is a powerful chemicals and most often used to treat cancer, since cancer cells grow and multiply much more quickly than most cells in the body.

Chemotherapy is a cancer treatment where medicine is used to kill cancer cells. There are many different types of chemotherapy medicine, but they all work in a similar way. They stop cancer cells reproducing, which prevents them from growing and spreading in the body (Chabner & Roberts., 2005).

Chemotherapy damages the genes inside the nucleus of cells. Some drugs damage cells at the point of splitting. Some damage the cells making copies of all their genes before they split. Chemotherapy is much less likely to damage cells that are at rest, such as most normal cells (Woods & Turchi.,2013).

Chemotherapy is common to use for stage four cancers. Often, a clinical trial may be an option, offering new treatments to help you fight stage 4 cancers(Schultz etal.,2002).

Chemotherapy can be administered to cancer patients in a number of situations, including:

- To treat the cancer without further therapies, chemotherapy can be used as the main or only form of cancer treatment.
- To eliminate cancer cells that are concealed after prior therapies. After previous treatments, such as surgery, chemotherapy can be used to eliminate any cancer cells that may still be present in the body.

- A tumor can be reduced by chemotherapy so that other therapies, such as radiation and surgery, are feasible. Neoadjuvant treatment is the term used by physicians.
- Help reduce symptoms and indications. Chemotherapy works by destroying certain cancer cells, which may help reduce cancer signs and symptoms. Palliative chemotherapy is the term used by doctors(DeVita., & Chu.,2008).

1.11Chemotherapy drugs used for treatement breast cancer:

1.11.1 Antimetabolite Chemotherapy:

Chemotherapeutic agents that target metabolites are classified as cell cycle-specific drugs because they only have an effect during one phase of the cell cycle. Cancer cells more rapidly divide (or cycle) than normal cells, making them an easy target for chemotherapy\s. There are several stages in the cell cycle, including G1, S, 2, and M. Inhibiting tumor cell DNA synthesis by focusing on the S phase, when DNA replication occurs, is the main mechanism by which antimetabolites work (Reuvers et al., 2020). Antifolates, pyrimidine and purine analogs, and ribonucleotide reductase inhibitors are all examples of drugs in this category. All three work by inhibiting the activity of folic acid, an essential component of DNA and RNA precursors. Since cell-cycle-specific chemotherapy drugs have no way of telling healthy cells apart from cancerous ones, they always have unintended side effects. A common side effect of treatment is myelosuppression (Lundström, 2022).

An examples of antimetabolites used in chemotherapy are:

Azacitidine,5-fluorouracil(5-FU),6-mercaptopurine(6-MP),Capecitabine (Xeloda) , Cladribine , Clofarabine , Cytarabine (Ara-C) and Decitabine.

These medications are the ones that are utilized most frequently as cytostatics because of how effective they are. The competition for the binding sites of enzymes that take part in critical biosynthetic activities,

followed by the incorporation of these macromolecules into nucleic acids, hinders the normal function of tumor cells and initiates the process of apoptosis, which is the process of cell death. Because of this mode of action, the vast majority of antimetabolites have a high level of cell cycle specificity and are able to specifically target the inhibition of DNA replication in cancer cells (Avendaño et al., 2015).

1.11.2 Antifoalte chemotharpy drugs:

In order to transfer and attach different one-carbon groups to other molecules, folic acid must first be reduced to THFA by dihydrofolate reductase . The enzyme thymidylate synthase catalyzes the conversion of deoxyuridine monophosphate (deoxy-UMP) to deoxythymidine monophosphate (deoxy-TMP), with the latter's 5,10-methylene-THFA molecule gaining a methylene group before being oxidized to dihydrofolic acid and needing to be reduced before further reactions can proceed (Kovalev et al., 2022).

Reduced folates are depleted and hazardous dihydrofolic acid polyglutamates accumulate when THFA production is inhibited by methotrexate (MTX) and other folic acid antagonists with a high affinity for dihydrofolate reductase. Production of nucleic acids and some other metabolic activities are hampered by blocking the transfer interactions of one-carbon groups required for the formation of purines and dTMP. Calcium folinate, or 5-formyl-THFA calcium salt, enters the cell via a decreased folate transporter and is metabolized into the other THFA derivatives, preventing the harmful action of methotrexate (Gonzales, 2019).

Antifolates are a type of antimetabolite drugs that antagonize, or impede, the effects of folic acid. Folic acid plays an important role in the body (vitamin B9). The primary role that folic acid plays in the body is that of a cofactor for the several methyltransferases that are involved in

the manufacture of serine, methionine, thymidine, and purines (Kovalev et al., 2022).

An examples of antifolate used in chemotherapy are:

Methotrexate,Pemetrexed,Proguanil,Pyrimethamine and Trimethoprim

1.11.3 The Taxanes Chemotherapies:

Taxanes are a group of diterpenes. They have a taxadiene center, and were first discovered in yew trees (genus Taxus). Common chemotherapy drugs include paclitaxel (Taxol) and docetaxel (Taxotere), the Food and Drug Administration (FDA) approved cabazitaxel for the treatment of hormone-resistant prostate cancer (Hagiwara & Sunada, 2014). Taxanes are a class of organic compounds that were first isolated from natural products but have since seen some limited synthetic development. The Pacific yew tree was the source of the first paclitaxel (Weaver, 2014). Taxanes, like taxol, have several chiral centers, making them challenging to synthesis, Corylus avellana (ordinary hazel) nuts and leaf have recently been reported to contain taxanes (Hoffman & Shahidi, 2009).

Albumin paclitaxel (Abraxane), Paclitaxel (Taxol) and docetaxel are examples of taxanes used in chemotherapy (Taxotere). These three chemicals can function independently or in tandem with other chemotherapeutic treatments (Zhang et al., 2019).

1.12 Paclitaxel:

Taxol, a natural diterpene alkaloid was originally isolated from the bark of Taxus brevifolia tree in the western region of the United States. When it was commercially developed by the Bristol-Myers Squibb Taxol was renamed to paclitaxel, melting point of paclitaxel is around 216°C–217°C, It has highly lipophilic, low water solubility; higher protein binding rate; and mainly disturbs the structure of the inner part of the cell membrane(Zhang et al.,2014) .

The role of paclitaxel has been the subject of study on anticancer agents for almost half a century. It is one of the most widely used anticancer drugs, and has been used for the treatment of various cancers from metastatic breast cancer, advanced ovarian cancer, non-small-cell lung cancer, to Kaposi's sarcoma Studies have demonstrated using low-dose paclitaxel to treat non-cancer human diseases, such as skin disorders, renal and hepatic fibrosis, inflammation, axon regeneration, limb salvage, and coronary artery restenosis. (Zhu& Chen.,2019 and Sharifi-Rad et al.,2021).

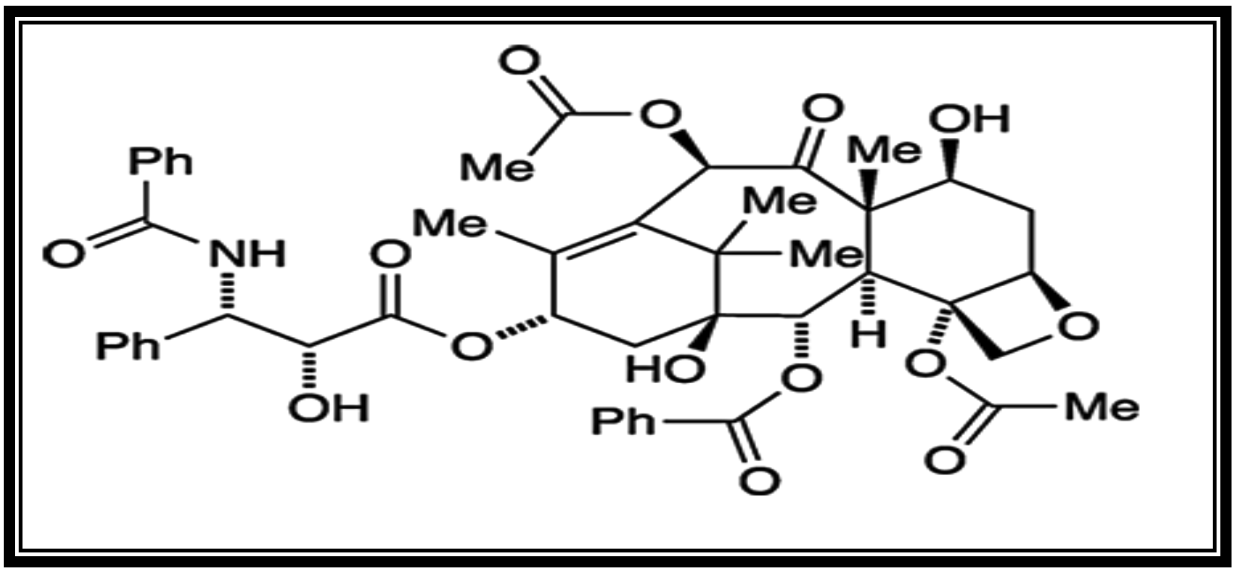


Figure (1-1) chemical structure of paclitaxel (El-Sayed et al., 2020).

1.12.1Paclitaxel mechanism as anticancer drug:

Paclitaxel belongs to the family of cytoskeletal drugs that target tubulin. As a result, paclitaxel treatment leads to abnormality of the mitotic spindle assembly, chromosome segregation, and consequently defects of cell division. By stabilizing the microtubule polymer and preventing microtubules from disassembly, paclitaxel arrests cell cycle in the G0/G1 and G2/M phases and induces cell death in cancer. It has been known that inhibition of mitotic spindle using paclitaxel usually depends on its suppression of microtubule dynamics(Weaver,2014).

However, studies demonstrated that only low-dose paclitaxel can do so, in contrast, high-dose paclitaxel might suppress microtubule detachment from the centrosomes. Paclitaxel induced reactive oxygen species generation by enhancing the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which contributed to the potent anticancer activity of paclitaxel(Kampan et al.,2015).

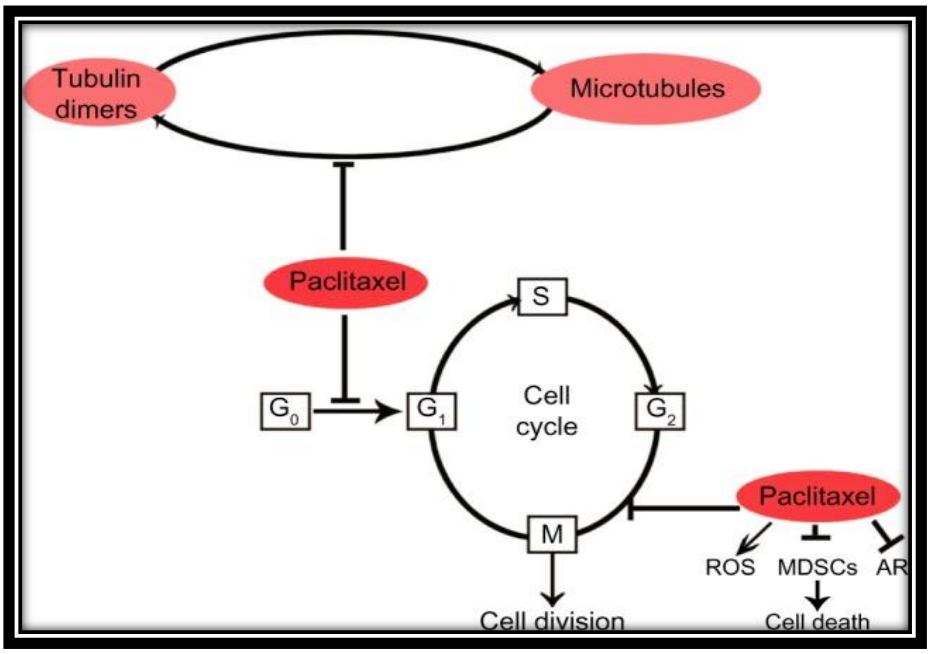


Figure (1-2) Anticancer effect of Paclitaxel (Zhang et al., 2014).

Paclitaxel is a substrate for multidrug resistance protein 1 (MDR1/P-gp, ABCB1) and other ATP-binding cassette efflux transporters (Brooks et al., 2003).

In addition, paclitaxel serves as a substrate for the organic anion transporter 2 in the kidney (OAT2, SLC22A7) (Kobayashi et al., 2005). To some extent, the pharmacokinetic heterogeneity of paclitaxel may be attributable to the activity of hepatic and renal transporters. To improve drug metabolism, paclitaxel stimulates pregnane X receptor (PXR), which in turn stimulates the expression of enzymes such cytochrome P450 3A4 (Harmsen et al., 2009).

Paclitaxel was first diluted in cremophor EL, a poly-oxy-ethylated oil combined 1:1 with ethanol, because of its hydrophobic properties. While this formulation does help with one issue, it has been linked to cremophor EL hypersensitivity in some people. Initially, this was worked around by increasing the duration of the infusions. Pretreatment with prophylactic antihistamines (both H1 and H2 receptor antagonists) and glucocorticoids. On the other hand, it has allowed for shorter infusion times while still achieving low rates of hypersensitive reactions (Harmsen et al., 2022).

The response rate to paclitaxel is highly different between the various types of cancer, with ovarian cancer typically being more sensitive than breast cancer (Oza et al., 2020). The effectiveness and safety of chemotherapy are also affected by whether or not it is combined with other chemotherapeutic medicines and by the order in which they are given. For instance, one study found that paclitaxel clearance was reduced when given after cisplatin rather than before the platinum drug, albeit this effect was not confirmed in another study (Grau et al., 2020).

Although the frequency and severity of side effects caused by paclitaxel are rather consistent among cancer types, there is a significant amount of patient-to-patient variation in their occurrence. Peripheral sensory neuropathy is the most common and serious side event, and severe neutropenia is also found in conjunction with it. Neutropenia and peripheral neuropathy are both dose-limiting side effects that can bring about decreased response rates to paclitaxel treatment. According to a database containing information with different solid tumors who were treated with paclitaxel as a single agent, severe neutropenia (less than 500 cells/cm3) occurred in 52% of the patients. Sixty percent of the patients who were treated with mixed doses (135–300 mg/m2) and infusion times (3 or 24 hours) reported having peripheral neuropathy. Three percent of those patients reported having severe neuropathy (Grade 3 or higher) (Grau et al., 2020).

While some of the findings, such as mutations in genes involved in brain repair, are consistent with expectations from a biological point of view, others demand for additional research to properly comprehend their significance. These studies that generate hypotheses have some value because they might provide new insight into the molecular mechanisms that are behind the toxicities. Nevertheless, substantial validation in multiple cohorts is required before their final interpretation and potential translation into clinical practice (Yoon et al., 2019).

It has not been possible to conduct comprehensive research on drug-drug interactions (DDI) that could impact the pharmacokinetics of paclitaxel. This is most likely due to ethical considerations around the testing of potentially dangerous DDI in cancer patients. When trying to determine how to best manage polytherapy for cancer patients, clinicians frequently extrapolate from case observations, data obtained in vitro, and limited epidemiological research. For instance, a metabolite of clopidogrel that inhibits CYP2C8 in vitro was connected to a very low clearance of paclitaxel and an increased risk of neuropathy in a patient who was being treated for ovarian cancer (ornio et al., 2022).

1.12.2 Paclitaxel's action against breast cancer:

Paclitaxel was found to promote the assembly of microtubules, which are structures that are made up of repeating subunits that are composed of /-tubulin heterodimers. In a study that was conducted in 1979, Paclitaxel lowers the critical concentration of assembled tubulin subunits and raises the percentage of those subunits that have been assembled (Zhu & Chen., 2019).

Microtubules come together to form a spindle during the prophase of the cell cycle, which is responsible for moving the chromosomes closer to the cell's poles. Later on, they will depolymerize, which will result in the dissolution of the spindle structure. The depolymerization of microtubules

can be triggered in two different ways: by exposure to cold temperatures and by exposure to calcium ions. Paclitaxel binds to microtubules and stabilizes them. Furthermore, microtubules that have been stabilized by paclitaxel are resistant to depolymerization, even when subjected to treatment with calcium ions or at low temperatures. Because of this, treatment with paclitaxel encourages the polymerization of tubulin and halts the progression of mitosis (Batran et al., 2018).

1.12.3 Pharmacokinetics and Pharmacodynamics of Paclitaxel:

With time (T1\2 of 9.9-16.0 h) and an MRT (Mean Residence Time;) of 6.47-10.24 h, the drug plasma concentration grew over the course of the 3-h infusion period and then began to decline immediately after the infusion was stopped. Both Cmax and AUC were dose-dependent (r = 0.865, P 0.001 for Cmax; r = 0.870, P 0.001 for AUC), even though the pharmacokinetic activity seemed to be nonlinear .

Over three times as much drug was absorbed at the 270 mg/m2 dose compared to the 135 mg/m2 dose (mean Cmax and AUC). As doses were increased, both CL and V were shown to be reduced. Paclitaxel was eliminated mostly via non-renal excretion, since less than 15% of the dosage was detected in the urine after 75 hours, suggesting that non-renal elimination is the predominant route of drug clearance (Wu et al., 2020).

Van Eijk *et al*., (2020) found that the duration of exposure to a paclitaxel concentration that was greater than 0.05 M was related to the development of neutropenia. In addition, it was revealed that the rate of survival for patients diagnosed with non-small cell lung cancer was found to correlate with the length of time they were exposed to 0.1 M or more of the drug paclitaxel. (Mendes *et al.*, 2020). The findings of these studies revealed that the concentration of paclitaxel in the serum was related to both the adverse effects and the clinical result. Regarding the connection between patient

characteristics and the pharmacokinetics of paclitaxel, it was shown that the level of alanine aminotransferase correlated with the amount of paclitaxel that was cleared from the body.

1.12.4 Adverse effect of paclitaxel:

The drug paclitaxel comes with a "black box" warning because it can cause hypersensitivity reactions and suppress bone marrow, before receiving an infusion. Patients should first be premedicated with corticosteroids, diphenhydramine, and H2 antagonists in order to reduce the risk of anaphylaxis and other severe hypersensitivity reactions (Horita et al., 2021; Paredes et al., 2022).

The recommended dosage of dexamethasone prior to receiving paclitaxel is 10 milligrams if the patient has advanced HIV and 20 milligrams if it is administered intravenously or orally. It is recommended that 50 mg of diphenhydramine be given intravenously thirty to sixty minutes before the dose (Beaucage-Charron et al., 2022).

Cimetidine in dosages of 300 milligrams, famotidine in dosages of 20 milligrams, or ranitidine in dosages of 50 milligrams would all be suitable options for intravenous administration 30 to 60 minutes before the dose (Mailankody. 2018).

Severe hypersensitivity reactions may include angioedema, generalized urticaria, dyspnea that requires bronchodilators, hypotension that requires treatment, and/or angioedema. In the event of a severe hypersensitivity reaction, the infusion should be stopped, and paclitaxel treatment should be discontinued. In the case of hypersensitivity reactions of a lesser severity, it is not necessary to suspend or terminate treatment. Flushing, dyspnea, hypotension, skin reactions, or tachycardia are examples of minor hypersensitivity reactions (MPharm, 2018)

Paclitaxel most frequently causes alopecia, nausea, vomiting, mucositis, neutropenia, leukopenia, anemia, hypersensitivity responses, arthralgia, myalgia, and weakness. Patients with preexisting neuropathies may be at a higher risk for developing peripheral neuropathy, another often encountered adverse effect. Patients who get significant neuropathy should have their dosage decreased by 20% (Meitasari et al., 2021).

It was also recorded that using a Paclitaxel causes Flush, edema, hypotension, skin rash, stomatitis, thrombocytopenia, hemorrhage, elevated blood alkaline phosphatase and aspartate aminotransferase, local injection site reaction, elevated serum creatinine, and many more are among the less usual adverse effects (Gur et al., 2022).

It is also illustrated that mild responses at the injection site (such as redness, soreness, discoloration, or swelling) are common, especially when the infusion is given for a longer period of time (Wildiers et al., 2021).

Patients with solid tumors and a baseline neutrophil count of less than 1500 cells/mm3, as well as patients with AIDS-related Kaposi sarcoma and a baseline neutrophil count of less than 1000 cells/mm3, should not receive paclitaxel due to the black box warning for hypersensitivity reactions and bone marrow suppression. It is important to note that suppression of bone marrow is a dose- limiting hazard. In the event of severe neutropenia, future doses should be lowered by 20% and supportive care should be considered (growth factor treatment) (Song et al., 2019).

1.12.4.1 Peripheral Neuropathy:

Peripheral neuropathy is the damage to the nerves with in extremities of the body, such as the hands, legs, and arms, can lead to the development of a condition known as peripheral neuropathy. Nerves that are affected determines which symptoms are present (Soulages et al., 2022).

Diabetes mellitus, Chemotherapy treatment, hypothyroidism, and dietary deficiencies are the most prevalent curable reasons. If the diagnosis is still unclear, further testing including electro diagn

ostic investigations or nerve biopsy may be necessary. An strategy that is systematic begins with the localisation of the damage to the nervous system, then the determination of the etio pathogenesis, and then the elimination of potentially treatable reasons(Castelli et al., 2020).

They are many symptoms for patient have peripheral neropathy which include Pain, numbness, and tingling in the extremities are common signs of peripheral neuropathy. On the other hand, symptoms of muscle weakness, most notably are in the feet, which lead to a loss of balance and coordination (Prabhakar, 2021).

One of the most prevalent side effects of many regularly used chemotherapeutic medicines is "chemotherapy-induced peripheral neuropathy" (CIPN), which can be debilitating and sometimes irreversible. Patients over the age of 65 are at increased risk for CIPN because of the prevalence of coexisting diseases that compromise peripheral nerve function dysesthesias, Paresthesias and sensory loss, muscle weakness, dysautonomia, and falls are all symptoms of chronic idiopathic painful neuropathy (CIPN) (Wasilewski, & Mohile, 2021).

1.12.4.2 Paclitaxel -induced peripheral neuropathy CIPN:

It is a typical, often limiting side effect of chemotherapeutics. At least 70% of patients receiving neurotoxic chemotherapeutic drugs have reported CIPN, Dorsal root ganglia sensory neurons are susceptible to neurodegeneration from systemic chemotherapeutic agents because they do not have a blood-brain barrier (Starobova & Vetter, 2017). A wide variety of symptoms, including difficulties with sensation, movement, and the self-regulating nervous system, can emerge from this kind of injury. Comorbid

metabolic and neurologic disorders enhance the incidence of CIPN in elderly patients undergoing treatment for cancer (Qi et al., 2019).

The chemotherapy drug paclitaxel causes axonopathy, which presents itself gradually, by interfering with microtubules, which are essential for nerve axons to function as tracks for endocytosis transport. Symptoms of paclitaxel-induced neuropathy (PIN) can include a variety of changes in motor, sensory and autonomic systems (Staff et al., 2020).

Although paclitaxel treatment is known to cause peripheral neuropathy, its exact etiology is unclear. Paclitaxel, when grown in tissue culture, causes the cytoplasm to fill with aberrant bundles of microtubules, which interferes with cell division and other normal cellular processes. Although this has the intended impact on tumors in patients, it is harmful to healthy tissue (Leen et al., 2022).

In vivo investigations using a wide variety of animal models have also been conducted to better understand the pathophysiology of neurodegeneration associated with paclitaxel chemotherapy, some of experimental studies in animal showed the side effect was appear by decreasing substance P (a neuropeptide involved in pain transmission) in the dorsal root ganglia and decreasing action potential amplitude as recorded from the caudal nerve, intraperitoneal injection of paclitaxel increases the tail-flick level in mice (Staff et al., 2020).

Patients who have preexisting diseases that may induce neuropathy (such as diabetes or kidney disease) or who have previously been given with other neurotoxic medications such as cisplatin and vincristine are at a greater risk of developing neurotoxicity after paclitaxel treatment (Banach et al., 2017).

1.12.4.3 Treatment related peripheral neuropathy (PN):

An important complication seen in patients with breast cancer chemotherapy is called treatment-related peripheral neuropathy (PN), which can be defined as the damage, inflammation, or degeneration of the peripheral nerves. This complication frequently results in a reduction or withdrawal of therapy, which has an effect on the treatment's efficacy and response, as well as a significant impact on the patient's quality of life (Hertz et al., 2021).

The National Cancer Institute's Common Toxicity Criteria score, Version 4.0 is the scale for a quantitative evaluation of peripheral neuropathy (PN) that is the most widely recognised scale in the medical community (table 1-1).

Table (1-1): Peripheral neropathy according to NCI-CT creteria

Adverse event	Grade				
	1	2	3	4	5
Peripheral sensory neuropathy	Asymptomatic, loss of deep tendon reflexes or paresthesia	Moderate symptoms, limiting instrumental ADL	Severe symptoms, limiting self-care ADL	Life-threatening consequences, urgent intervention indicated	Death
Peripheral motor neuropathy	Asymptomatic, clinical or diagnostic observations only, intervention not indicated	Moderate symptoms, limiting instrumental ADL	Severe symptoms, limiting self-care ADL, assistive device indicated	Life-threatening consequences, urgent intervention indicated	Death
Neuralgia	Mild pain	Moderate pain, limiting instrumental ADL	Severe pain, limiting self-care ADL	—	—

The first evaluates the degree to which chemotherapy causes damage to sensory and motor peripheral nerves, and the second analyzes the neuropathies and dysfunctions that are linked with sensory, motor, and hearing neurotoxicity. Both of these analyses are performed by the neurotoxicity subscale (Griffith *et al*., 2010).

The total neuropathy score, often known as TNS, is another technique of evaluation. It is a comprehensive evaluation that takes into account the patient's symptoms, electrophysiology, ability aspects, and signs The TNS may be decreased or used simply for clinical purposes (Curcio, 2016).The comprehensive version of the TNS comprises the performance of a quantitative analysis of the vibration perception threshold. The reduced TNS consists of an electrophysiological evaluation of one motor (common peroneal) nerve and one sensory (sural) nerve (Cheng, & Molassiotis, 2019).

The strictly clinical version is based on the assessment of the patient's sensory, motor, and autonomic complaints, as well as their sensitivity to pin and vibration, muscle strength, and deep tendon reflexes. However, electrophysiological examinations are not always accessible and relevant in all cases (Cavaletti et al., 2007).

1.12.5 Pharmacogenomics of Paclitaxel:

In the past, Taxol's had most common side effects which included severe hypersensitivity reactions (mostly attributable to Cremophor EL), hematologic toxicity (often manifesting as severe neutropenia), and neurotoxicity (sometimes manifesting as cumulative sensory peripheral neuropathy). The dysfunctional microtubules in dorsal root ganglia, axons, and Schwann cells have been shown to be the mechanism for the neurotoxicity (Xiong et al., 2021).

Now, some of studies reported there was a correlation between the variable pharmacokinetics of paclitaxel and the negative effects that the

medication for patient intake. It has been hypothesized that polymorphisms in genes that code for paclitaxel-metabolizing enzymes, transporters, and therapeutic targets may contribute to the interindividual heterogeneity observed in toxicity and response (Horiuchi et al., 2022).

In addition to being processed in the liver by the cytochrome P450 (CYP) 3A subfamily and CYP2C8, paclitaxel is also a substrate for the human MDR1 (ABCB1) gene, which is responsible for the production of the P-glycoprotein enzyme (Priyadarshini et al., 2021). A number of researchers have found that there is a connection between the single nucleotide polymorphisms (SNPs) of the MDR1 gene and the expression or function of MDR1. According to the findings of, persons who are homozygous for a mutation at exon 26, also known as C3435T, was associated with noticeably reduced levels in the body and plasma with the highest Paclitaxel concentrations levels after oral administration (Chen et al., 2021).

Hertz et al., (2021) found that alleles *2 and *3 had a lower clearance of paclitaxel than other variants of the CYP2C8 gene. There are just a few data available at this time concerning the connection between the pharmacokinetics of paclitaxel and the polymorphisms of, MDR1 and CYP3A5, CYP3A4.

Some of studies illustrated that some genes associated in paclitaxel clearance and drug response were further characterized, and their most important variations were singled out, polymorphisms were found in the genes that encode for paclitaxel metabolic enzymes and transports (CYP3A4, P-glycoprotein , CYP2C8, OATP1B3 and CYP3A5). This protein was found to be essential for paclitaxel hepatic absorption (Hofman et al., 2021).

1.13 ATP binding cassette (ABC) multidrug transporters:

ATP binding cassette (ABC) multidrug transporters such as P-glycoprotein (P-gp, ABCB1) and BCRP (ABCG2) confer resistance against anticancer drugs and can limit their oral availability. Thus contributing to failure of chemotherapy. Like P-gp and BCRP, another ABC transporter, MRP2 (ABCC2), is found in apical membranes of pharmacologically important epithelial barriers and in a variety of tumors. MRP2 transports several anticancer drugs and might thus have a similar impact on chemotherapy as P-gp and BCRP (Xiao et al., 2020).

Some studies have found that the ABCC10 single nucleotide polymorphism (SNP), rs2125739, impacts docetaxel cytotoxic in lungs carcinoma cell lines in addition to its clinical adverse effects, and some looked into how the rs2125739 variant affected paclitaxel (PTX) toxicity in lung tumor cell lines (Horiuchi et al., 2022).

Previously, ATP binding cassette (ABC) transporter genes were linked to progression of breast cancer and medication response. SNPs from the ABCC1, ABCC2, ABCB1, and ABCG2 genes were evaluated in carcinoma patients and healthy individuals from the Jordanian-Arab population in the current investigation (Al-Eitan et al., 2019).

1.14 ABCB1 gene Polymorphism:

ABCB1 gene encodes an adenosine 5′-triphosphate–binding cassette transporter, which not only confers multidrug resistance phenotype in malignant cells, but is also present in several nonmalignant tissues. For the last thirty years, ABCB1 expression in breast cancer has been described by many authors, but the extent of expression differs among the studies, and there is no consensus regarding its potential role in carcinogenesis or in the tumor response to antineoplastic drug.

The ABC transporters are a class of transmembrane proteins that are responsible for the transport of a wide variety of substrates across lipid external and intracellular membranes. These substrates include metabolites, carcinogens, and cytotoxic medicines, including anticancer drugs. Based on the degree of similarity between their sequences, the 48 distinct ABC transporters found in the human genome have been categorized into the seven A-G subfamilies (Fung, & Gottesman, (2009).

There are 28 introns and 28 exons in the ABCB1 gene, which can be found on chromosome 7q21.1. This gene is responsible for encoding the P-glycoprotein. The coding region of the gene ABCB1 is 120 kb in size, and the mRNA for that gene is 4.7 kb in size. About 50 different types of SNPs related to the ABCB1 gene have been found after extensive research into the gene's potential for characteristic polymorphisms (Johnatty et al., 2013).

The most widely studied variant of ABCB1 is a commonly synonymous C to T transition at nucleotide position 3435 in exon 26 (3435 C > T), an exon 26 of the MDR1 gene is responsible for the up regulation of this gene's activity, which in turn effluxes a wide variety of chemicals throughout the plasma membrane (Ozen et al., 2011). Although this transition does not change its encoded amino acid with Ile at position 114522, TT variant has been significantly associated with the decreased mRNA expression and

protein stability and may have reduced the drug transport capacity. So far the effect of synonymous polymorphisms on the protein has not been fully understood (Ozdemir et al., 2013). However it is assumed that they can affect the post-transcriptional processing of mRNA by interfering with the process of removing introns or affect the process of alternative transcript splicing. Silent polymorphisms can be important in the process of protein folding, leading to its abnormal form. In addition, (Fung, K. L., & Gottesman, 2009) indicate, replacing as a result of silent polymorphisms often used in translation of codons into rare ones can affect the rate of protein folding, and thus change its function or change its substrate specificity. On the other hand, synonymous polymorphisms can change the structure and/or function of a protein by coupling to non-synonymous polymorphisms that directly change the amino acid sequence of a protein (Kimchi-Sarfaty et al., 2007).

P-gp is expressed in the apical membranes of many tissues and can be implicated in numerous various processes like differentiation, proliferation, apoptosis and immune response regulation. In a normal lung, P-gp is expressed on the top surface of the bronchial epithelium, where it can act to remove external compounds from the lung. In lung cancer, initially low P-gp expression level, can change after exposure to chemotherapy as part of acquired drug resistance. P-gp confers resistance to cytotoxic drugs, including etoposide and cisplatin, and polymorphisms may affect the specificity of the substrate (Karthika et al., 2022). Research on the influx and efflux mechanisms of drug transporters may be useful to assess the effectiveness of therapy, Various studies have shown that the family of ATP-binding transport proteins (ABC transporters), such as ABCB1 or ABCG2, may be associated with the development of drug resistance (Halder et al., 2021).

MDR1 (ABCB1, P-glycoprotein) is a protein that pumps foreign molecules that enter the cell out of the cell. ABCB1 substrate drugs such as

phenytoin, diltiazem, cyclosporine, and clozapine are affected by the activity of this efflux pump. Among the MDR1 genetic polymorphisms, 1236C>T (rs1128503), 2677G>T/A (rs2032582), 3435C>T (rs1045642) are those that have been shown to be clinically important (Tsuchiya et al., 2017). A nonsignificant trend was also observed between placental P-gp expression and polymorphisms at position 2677 (GG>G/mut>mut/mut) by Tanabe et al. (2001) ABCB1 SNPs may boost cancer cell resistance to antineoplastic drugs or increase the rate at which these drugs are flushed out of the cells (Chang et al., 2009).

Aim of study :

1- **To investigate the distribution of genotying on 2677G<T/A and 3435C<T in breast cancer patients on paclitaxel.**
2- **To study the effect of polymorphism on paclitaxel response.**
3- **To study the effect of 2677&3435 on incidence of adverse effect on paclitaxel.**

Chapter two

Patients, Materials and Methods

Chapter Two

2. Materials and Methods

2.1. Materials:

2.1.1 Equipments:

Table (2-1): The general Equipments utilized in this study.

Apperatus	Company	Origin
Centrifuge	Thermo scientific	Japan
ELISA	Biotek	UK
Exispin centrifuge	Bioneer	Korea
Gel electrophoresis	Shandod Scientific	UK
Incubator	Mammert	German
Micropipettes 5-50, 0.5-10, 100-000µl	CYAN	Belgium
Mobile camera	Samsung	Korea
Oven	Mammert	Germany
Reflotron	Roche	USA
Refrigerator	Concord	Lebanon
Sensitive Balance	Sartorius	Germany
Thermocycler PCR	Bioneer	Korea
UV Transilluminator	ATTO	Japan
Vortex	CYAN	Belgium
Hematology Analyzers	Swelab	UK
Water Bath	Mammert	Germany

2.1.2 Chemicals and solutions:

Table (2-2): The chemicals utilized in the study.

Chemicals	Company	Country
Absolut ethanol	Bioneer	Korea
Agarose	Bioneer	Korea
Blue Master Mix	Bioneer	Korea
DNA ladder marker 100bp	Bioneer	Korea
Ethidium Bromide	Bioneer	Korea
Hinf 1 Restriction Enzyme	Bioneer	Korea
Loading dye	Bioneer	Korea
Primers	Bioneer	Korea
TBE buffer (10x) Tris-Borate	Bioneer	Korea

2.2 Study Setting:

The study was conducted in Imam Al-Hussein teaching Hospital for Oncology , which is specialized in cancer treatment and at the labs of the College of Pharmacy at the University of Kerbala as well as in Oncology Center in Kerbala during the period of July 2022 to December 2022 for sample collection only .

Patients were approved the study and randomly assigned to weekly paclitaxel intravenous injection over 1 or 3 hours. The study's primary endpoint, peripheral neuropathy (PNP), was measured by sensory symptoms, strength, musculotendinous reflexes, and vibratory sensation (range 0-12; PNP >3 points) (Mielke et al., 2003).

The clinician identified neuropathy according to total neuropathy score based on the patient's symptoms which include sensory and motor symptom, autonomic symptom, vibration sensibility, strength and tendon reflex, burning or prickling feeling in the hands, arms, legs, or feet (Paresthesia),

loss of sensation or feeling nerve ending (Numbness) and fatigue (Miah et al., 2021).

2.2.1 Ttest of peripheral neuropathy:

A test was conducted by the investigator in the hospital from which the sample of cases was decided to be taken. A neurologist will perform a physical examination and take a patient's medical history in order to make a diagnosis of peripheral neuropathy. Nerve conduction investigations are frequently used as a second method of diagnosis confirmation.

The main steps of this test were the fallowing:

1. To determine the difficulties that might emerge during the daily Nerve conduction investigations .
2. To test the information of the questionnaire form and to test it's completeness and to suggest any modification required according to Total Neuropathy Score (TNS).

Table (2-3) Pain in the upper and lower extremities, difficulty in standing (Hertz et al., 2018).

Parameter	Score				
	0	1	2	3	4
Sensory symptoms	None	Symptoms limited to finger or toes	Symptoms extends to ankle or wrist	Symptoms extends to knee or elbow	Symptoms above knees or elbows, or functionally disabling
Motor symptoms	None	Slight difficulty	Moderate difficulty	Require help/assistance	Paralysis
Autonomics symptoms	0	1	2	3	4o5
Pin sensibility	Normal	Reduced in finger/toes	Reduced up to wrist/ ankle	Reduced up to elbow/ knee	Reduced above elbow/knee
Vibration sensibility	Normal	Reduced in finger/toes	Reduced up to wrist/ ankle	Reduced up to elbow/ knee	Reduced above elbow/knee
Strength	Normal	Mild weakness	Moderate weakness	Severe weakness	Paralysis
Tendon reflex	Normal	Ankle reflex reduced	Ankle reflex absent	Ankle reflex absent, others reduced	All reflexes absent
Vibration sensation (QST vibration)	Normal to 125% of ULN	%150-126of ULN	%200-151of ULN	%300-201of ULN	%300<of ULN
Sural amplitude	Normal/reduced to <5% of LLN	%95-76of LIN	%75-51of LLN	%50-26of LLN	%25-0of LLN
Peroneal amplitude	Normal/reduced to <5% of LLN	%95-76of LLN	%75-51of LLN	%50-26of LIN	%25-0of LLN
QST=Quantitative Sensory Test; ULN - Upper Limit of Normal, LIN= Lower Limit of Normal					
Note In addition to the TMSc, parameters written in italics are used only in full-length TNS, underlined ones in TNST Adapted from the original versions.					

2.3 Selection of the Study Sample:

2.3.1 patients

Cases were postmenopausal women consulted Imama Hussein Hospital with histological confirmed diagnosis of breast cancer. They were admitted to hospital either for cytotoxic, radiotherapy or hormonal treatment.

2.3.2 Controls

Controls were women free from breast cancer and matched to cases for age ± 5 years. Breast cancer, among controls, was excluded by the investigator on chemical investigation only(CA15.3 and estradiol level).

2.4 Period of the Study:

Six months is the period which was planned for collection of data, starting in 1st of July 2022, and to be end by the end of December 2022. The study was conducted at the labs of the College of Pharmacy at the University of Kerbala as well as in Oncology Center in Kerbala

2.5 Study Design:

A cross-sectional study design was aproved to achieve the aim of the present study. To examine the possible relation of an exposure to a certain Paclitaxel medication, a group of people(100 patients) with the disease (cases) and a group of people(100 free of disease) without that disease (control), must be identified.

2.6 Sources of Data:

The main source of data was obtained directly from cases and controls by the investigator or interviewing with them and completed from the case sheets for other relevant information especially for laboratory investigations.

A detailed questionnaire form was prepared to record all the relevant information related to cases and controls in the sample. This questionnaire form includes the following:

2.6.1 General Information

This information includes: age in years, occupation, residence, marital status, level of education, and husband's occupation..

2.6.2 Specific Information:

1-Family History: Family history was considered positive if the patient gave definite history of breast cancer in her relations including mothers, sisters, daughters (first degree), grandmothers, aunts (second degree) and other cousins (third degree).

2- Menstrual History: The most important factors in menstrual history was the age at menarche , postmenopausal and age at menopause.

3-Reproductive History: Three main factors were taken in the reproductive history includes: Age at first birth, parity and history of abortion including number of abortions and whether it is spontaneous or induced.

4-Contraceptive History: This include the current type and also the previous types of contraceptive which was used by the patient with its duration. Also the history includes any hormonal replacement therapy which was taken by the patient.

5-Lactation: It includes whether the patient ever lactates her children and the average duration of lactation natural or artificial breast feeding.

6- Previous Breast History: This include history of X-ray exposure, trauma to the breast and previous breast disease with its diagnosis and treatment.

7- Current Smoking Status: This was divided into smoker, non-smoker, or ex-smoke according to the classification of the WHO (1979)

One. **A smoker:** is the one who smokes at least one cigarettes per day, or equivalent in pipe or cigar tobacco until 3 months of the onset of breast cancer.

Two. **An ex-smoker:** is the one who smoked at least one cigarettes per day or the equivalent in pipe or cigars tobacco but ceased smoking at least 3 months before the onset of the disease.

Three. **Non-smoker:** is the one who has not, at any time smoked tobacco at the level indicated above.

Number of cigarette per day, number of years of smoking, types of smoking and number of years of giving up smoking for ex-smokers was also included.

8- Type and number of birth: Knowing whether normal vaginal delivery or Cesarean section delivery.

9- Side effects of paclitaxel and duration of symptoms: This includes time for symptoms to appear after starting paclitaxel and duration for each symptoms of side effects.

10- Other disease and there treatments: Knowing other disease that patients have and there treatments.

2.7.Inclusion criteria:

1- Women with histologically confirmed breast cancer and postmenopause.
2- They were required to be non-pregnant.
3- all patients were required to have clinically or radiographically measurable disease and to have adequate renal and hepatic function normal.
4- women aged range (45-75 years)

2.8 Exclusion criteria:

1- Patients were ineligible if they had diabetic mellitus or any other underlying medical condition that would hinder study participation.

2- Those with child-bearing potential who did not implement adequate contraceptive measures were also ineligible

2.9 Serological study:

2.9.1 Principle of Estradiol assay

This analysis uses the quantitative sandwich enzyme immunoassay technique. Antibody specific for Estradiol has been pre-coated on to a micro plate. Standards and samples are pipetted into the wells and any Integrin alpha-2/beta-1 (ITGA2) present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Estradiol is added to the wells. After washing, avoid conjugated Horseradish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to wells and color develops in proportion to the amount of Estradiol bound in the initial step .The color development is stopped and the intensity of the color is measured .

Table (2-4): Kit Contents of Estradiol Pg/mL ELISA Kit.

Reagents	Quantity
Assay plate (12x8 coated Micro wells)	96 wells
Adhesive strip (for 96 wells)	4
Biotin- antibody (100x concentrate)	1x120 µl
Buffer wash (25x concentrate)	1x20ml
Biotin- Diluent antibody	1x15ml
HRP-avidin (100x concentrate)	1x120 µl
HRP-Diluent avidin	1x15ml
Sample Diluent	1x50ml
TMB Substrate	1x10ml
Stop Solution	1x10ml

Assay Requirements

1. Microplate reader capable of measuring absorbance at 450 nm,
2. An incubator which can provide stable incubation conditions up to 37˚C±0.5˚C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microliter plate.

5. 100ml and 500ml graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.

Serum samples will require a 10-fold dilution via adding 25µl from sample to 225µl of sample dilution

2.9.2 Reagent preparation

Bring all reagents to room temperature (25°C) before use for 30min:

1. Biotin-antibody 1x:centrifuged the vial before opening ,requires100-fold dilution. Suggested 100-fold dilution in 10 µl of Biotin –antibody +990 µl of Biotin-antibody Diluent
2. HRP-avidin(1x)- centrifuged the vial before opening, requires 100-fold dilution .a suggested 100-fold dilution in 10 µl of HRP-avidin+990 µl of HRP-avidin Diluent
3. Washing Buffer (1x):if crystals have formed in the concentrate, warm up to room temperature and mixed gently until the crystals have completely dissolved .Dilute 20ml of wash buffer concentrate into deionized or 480 ml distilled water
4. Standard: by centrifuged the standard vial at 6000-10000 rpm for 30s, this re-formation produces a resolved solution of 15ng/ml, mixed the standard to ensure complete re-formation and allowed the standard to remain for a least of 15 minutes. Pipette 250 µl of sample diluent in to each tube. used the resolved solution to produce a 2-fold dilution series. Mixed the solution that present in each tube thoroughly before the transfer. The undiluted standard serves as the high standard (15ng/ml).Sample diluent serves as the zero standard (0ng/ml).

2.9.3 Assay Procedure Estradiol:

Before starting the procedure Human Estradiol kit components was left at room temperature for 30 min.

1. Prepared all reagents, samples, working standards and calibrations of work according to previous section.
2. Referred to assay layout sheet to determine the number of wells to be used and put any remaining wells and the desiccant back in to the pouch and seal the Ziploc, store unused wells at 4°C.
3. Added 100µl of standard and sample per well, cover with the adhesive strip provided. Incubated for 2 hours at 37°C .A plate layout is provided to record standards and samples assayed.
4. Removed the liquid in each well, do not wash.
5. Added 100µl of Biotin-antibody (1x) to each well. Cover a new adhesive strip. Incubated for 1 hour at 37°C .Warm up to room temperature and mixed gently until solution appears uniform.
6. Aspirate each well and wash, repeating the process two times for a total of three washes by ELISA Washer. After the last wash, remove any remaining wash Buffer by aspirating or decanting. invert the plate and blot it against clean paper towels.
7. Added 100µl HPR-avidin (1x) to each well. Cover the microtiter plate with a new adhesive strip. Incubated for 1 hour at 37°C.
8. Repeat the aspiration /wash process for five times as in step6
9. Added 90µl of TMB Substrate to each well. Incubated for 15-30 minutes at 37°C. Protect from light.
10. Added50µl of stop solution to each well, gently tap the plate to ensure thorough mixing.
11. Determinate the optical density of each well within 5 minutes used a microplate reader set to 450 nm. If wavelength corrections is

available, set to 540 nm or 570 nm. Subtract reading at 540 nm or 570 nm from the reading at 450 nm.

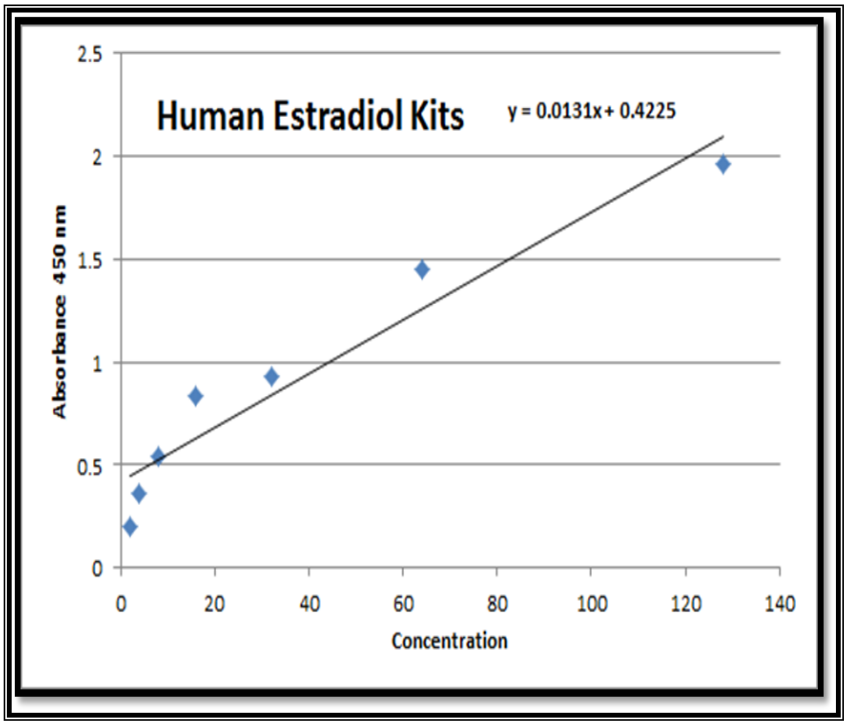


Figure (2-1): standard curve of Human Estradiol Pg/ml.

2.10 Cancer Antigen 15.3 measurement:

Cancer Antigen 15.3 was measured by using ELISA as flowing: Bring all specimens and kit reagents to room temperature (20-25 DC) and gently mix.

1. Patient samples should be diluted 10-fold before use.
2. Secure the desired number of coated wells in the holder. Dispense 25 III of CA15-3 standards, diluted samples, and diluted controls into the appropriate wells.
3. Add 100ul of Antibody-Biotin Conjugate Reagent to all wells. Gently mix for 20-30 seconds at 500-600 rpm.
4. Incubate for 60 minutes at room temperature.
5. Remove liquid from all wells. Wash each well three times with 350 III of 1X wash buffer. After each wash, sharply and firmly tap the upside down plate on absorbance paper or paper towels to remove residual droplets.
6. Dispense 100 III of Enzyme Conjugate into each well.

7. Incubate for 60 minutes at room temperature.
8. Remove the contents and wash the plate 3x as described in step 5 above.
9. Dispense 100 III ofTMB Solution into each well.
10. Incubate at room temperature for 15 minutes.
11. Stop the reaction by adding 50~11 of stop solution to each well.
12. Read the absorbance at 450nm (using a reference wavelength of 630nm) with a microtiter plate reader within 15 minutes.

2.11 Calculations of Results :

1. Calculate the average absorbance values for each set of reference standards, control. and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in U/ml on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of CA15-3 in U/ml from the standard curve.

2.11.1 Measure of drug concentration of patient serum Sample preparation and HPLC condition for measurement paclitaxel:

The HPLC model sykam_ from Germany was used to create a new method of analysis. An aliquot of human plasma, measuring 0.1 mL, was placed in the extraction tube. After that, we combined 4 mL of ethyl acetate and 1 mL of acetonitrile at a ratio of 4:1:1. The sample was frozen after being centrifuged at 3500 rpm. The organic layer was then shifted to a glass tube and evaporated in a nitrogen gas flow. After dissolution of the dry residue in 200 uL of 50% MeOH, the solution was centrifuged at 3500 rpm before being transferred to an autosampler vial. The HPLC apparatus was given a sample aliquot of 100 uL. Mixtures of acetonitrile and ultrapure water with

0.1% trifluoroacetic acid used as the mobile phase (70:30). The mobile phase was flowing at a rate of 1 mL/min. Twenty minutes of run time were achieved using a UV-Visible detector set at 227 nm and a C18-ODS column measuring 25 cm in length by 4.6 mm in diameter (Ankit Jian, 2013).

2.11.2 Complete blood count:

The CBC is usually performed by an automated hematology analyzer, which counts cells and collects information on their size and structure. The concentration of hemoglobin is measured, and the red blood cell indices are calculated from measurements red blood cell (RBC), white blood cell (WBC), hemoglobin, and platelet counts by using Hematology Analyzers according to manufacture instruction.

Blood is transferred between two electrodes through an aperture so small that only one blood cell at a time can pass through. As a cell travels through, the impedance varies. Changes in impedance are proportional to cell volume, yielding a cell count and volume measurement.

The following blood tests were used for each particicpants : Mean corpuscular hemoglobin (MCH),Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Mean platelet volume (MPV), Platelet distribution width (PDW) and White blood cell differential.

2.12 Molecular study:

2.12.1 DNA Extraction:

2.12.1.1 Blood Samples

1. Transferred up to 200µL of whole blood to a 1.5 ml. microcentrifuge tube added 20µL of proteinase K then mixed by pipetting , Incubated at 60^{o}c for 5minutes.
2. Cells were lysed by added 200 µL of GSB buffer then mixed by vortex 3-shaker placed into water path of 60c for 20m.
3. DNA Binding agent added 200 µL of ethanol absolute to each sample, and mixed immediately by shaking vigorously for 10 second by vortex it in.
4. placed a GS Column (in a 2 ml collection Tube) and transferred all of the mixture to the GS column then centrifuged (14-16.000 x g for2 minute). The Gs column was placed in 2 ml collection tube, and the tube containing the filtrate was discarded.
5. Washing : the GS column was carefully opened and added 400 µL of W1 Buffer to the GS column without wetting the rim .The cap was closed and centrifuged at 14- 16.000 x g RPM for 30 seconds. The GS column was placed in a clean 2 ml collection tube, and collection tube containing the filtrate was discarded.
6. Washing Buffer (600µL) has been added (make sure absolute ethanol was added) to the Gs column and centrifuged at 14-16.000 x g rpm for 30 seconds then discarded the flow through. Placed the Gs column back in the 2 ml collection tube, centrifuged again for 3 minutes at 14-16.000 x g to dried the column matrix.
7. Elution: transferred the dried Gs column to a clean 1.5 ml microcenterfuge tube. 50µL of elution buffer were added , TE Buffer to maintain DNA or water in to the center of column matrix, and incubated at room temp for 3 minutes to allowed Elution Buffer ,TE

Buffer or water to be completely absorbed and centrifuged at 14-16.000 x g for 30 seconds to elute purified DNA

8. The DNA was stored at 4 °C for short term and -20˚C (freezer) in the appropriate sample box for long term storage.

Table (2-5): DNA extract kit components.

Material	Company	Country
Buffer cell	Geneaid	Taiwan
Collection Tubes	Geneaid	Taiwan
Ethanol absolute (EA)	Geneaid	Taiwan
Elusion buffer (EB)	Geneaid	Taiwan
GSP	Geneaid	Taiwan
umGd Col	Geneaid	Taiwan
Proteinase K (PK)	Geneaid	Taiwan
Washing buffer (WB)	Geneaid	Taiwan

2.12.1.2. Optimization of PCR Conditions.

PCR conditions for ABCB1 3435C>T (rs1045642) and ABCB1 2677G>A/T (rs2032582) with respective amplified fragments are mentioned in table (A and B), different volumes of primer (0.5 µl, one µl, 1.5 µl) with varying volumes of template DNA (One µl, two µl, three µl, four µl, five µl, six µl) and different experiments of the reaction conditions were trailed to optimize the needs of the reaction.

PCR tube was centrifuged for 30 seconds at 2000 xg in a micro-centrifuge to mix solutions well at room temperature. Then, tubes were placed in the thermocycler to start the reaction. Programs of the PCR protocol reaction for the IL-10 gene both polymorphism

Table(2.6) PCR optimazqtion for detection of ABCB1 3435C>T (rs1045642) polymorphism

Steps	Temperature (ºC)	Time	NO. of cycles
Initial denaturation	**95**	**5 minute**	**1**
Denaturation	**95**	**60 sec**	**35**
Annealing	**85**	**30 sec**	
Extension	**72**	**60 sec**	
Final extension	**72**	**10 minute**	**1**

Table(2.7) PCR optimzation for Detection of ABCB1 2677G>A/T (rs2032582) polymorphism

Steps	Temperature (c)	Time	NO. of cycles
Initial denaturation	95	3 minute	1
Denaturation	95	60 sec	35
Annealing	64	30 sec	
Extension	72	60 sec	
Final extension	72	10 minute	1

2.12.1.3 Agarose Gel Electrophoresis:

After DNA extraction, gel electrophoresis agarose has been adopted to underline the presence and integrity of the extracted DNA.

A. Components of agarose gel electrophoresis.

1. Agarose.
2. 1X TBE Buffer.
3. Bromophenol Blue in 1% glycerol (loading buffer).
4. Ethidium Bromide.
5. DNA Ladder Marker 100bp.

B. Preparation of 1X TBE Buffer

The 1X TBE buffer was prepared from 10X TBE buffer (as stock solution) by adding 100 ml of this stock solution to 900 ml of distilled water.

C. Gel Electrophoresis protocol

1. The amount of 1 X TBE (10ml) has been taken in a beaker.
2. Agarose powder (1.5 gm) has been added to the buffer.
3. The solution has been heated to boiling using microwave until all gel particles are dissolved.
4. Added 5 µL of ethidium bromide of (10mg/ml) was added to the agarose solution.
5. The agarose has been stirred in order to be mixed and avoid making bubbles.

6. The solution was left to cool down at - (50 – 60) ^{o}C.

D. DNA Loading and Electrophoresis:

DNA (5µL) was blended with 2µL of bromophenol blue color (loading dye). Tests were loaded deliberately into the individual wells of the gel, and after that electrical power was turned on at 70 volt for 30 minutes. A while later, the DNA was moved from cathode (-) to anode (+) posts. The Ethidium Bromide recolored groups in the gel were envisioned utilizing an UV transiluminator at 350 nm.

Table (2-8) Primers sequences of BCB1 3435C>T (rs1045642) genetic polymorphism.

Allele specific	**Primer sequence(5 '->3 ')**	Product size
Reverse allele A	5-GGGTGGTGTCACAGGAAGAGATT-3	400 BP
Reverse allele G	5-GGGTGGTGTCACAGGAAGAGATC-3	

Table (2.9) Primers sequences of ABCB1 2677G>A/T (rs2032582) genetic polymorphism.

Allele specific	**Primer sequence(5 '->3 ')**	Product size
Forward allele T	5-TGAAAGATAAGAAAGAACTAGAAGGTT-3	222 BP
Forward allele G	5-TGAAAGATAAGAAAGAACTAGAAGGTG-3	
Forward allele A	5-TGAAAGATAAGAAAGAACTAGAAGGTA-3	
Common Reverse	5-AGTCCAAGAACTGGCTTTGC-3	

2.13 Data Analysis

Computer feeding, tabulation and statistical analysis was conducted with personal computer Pentium III by using StatXact under Windows and SPSS under Windows program.

Various statistical methods were used to analyze data, chi-square (χ^2) ,nova test and R value for contingency tables to find the statistical difference between cases and controls about the risk factors.

Chapter three

Results

3. Results

3.1. Demographic characteristic of patients:

A total of 100 women participated in this study, which were divided into subgroups based on Age, BMI and duration of paclitaxel treatment.The clinical demographic characteristics and laboratory parameters of patients group were summarized in table (3.1) and (3.2). Table illustrated the mean age of participants which was within a mean age of (54.36 ± 4.21) years old, number of birth (3±1) times. The descriptive table also shown an adjustment of other characteristics and risk factors which were collected through the self-reported technique. 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.

Table (3.1): Demographic characteristics of samples

Variables	Mean ±SD
Age (Years)	54.36± 4.21
Range	45-75 years
BMI (Kg/m^2)	24.22± 3.26
Duration of disease (Years)	3.49 ± 1.69
Duration of paclitaxel (Years)	2.32 ± 1.47
Number of birth	3 ± 1

BMI; body mass index

The study found Most women diagnosed with breast cancer were at the range 25-29.9 stage of body mass index, it was recorded as 63% from total of postmenopsal women who have breast cancer. The result also found the

most common breast cancer location was left side as (55%) from total of women who they have breast cancer in the right sides (45%).

Eighten cases of women not married have breast cancer, it was different from postmenopsal married women which they recorede as 82% of cases. There were 90% and 72% women have breast feeding and normal vaginal delivery, respectively

Table (3.2): Demographic characteristics of postmenopasal women

Variables		Frequency (%)
Age	54 - 45Years	45%
	55 - 64 Years	38%
	74 - 65Years	17%
BMI	< 18.5	1%
	18.5-24.5	27%
	25-29.9	63%
	>30	9%
Marital status	Married	%82
	Single/Divorced	%18
Family history	Yes	%30
	NO	%70
Breast cancer	Left breast	%55
	Right breast	45%
Lymph node involvement	Yes	77%
	No.	23%
Vaginal delivery	Yes	72%
	No.	28%
Breast feeding	Yes	90%
	No.	10%

Based on the findings of the study, The majority of women diagnosed with breast cancer were recorded at 85% of the total number of postmenopausal women having surgical involvement. The results also found that all women with breast cancer have taken chemotherapy (100%) as well as 5% of women who have breast cancer have received radiotherapy

treatment. The results also found that rare cases of postmenopausal women (6% of the total) had recurrences of breast cancer.

Table (3.3): External affect of status for women with breast cancer

Status		Percentage (%)
Surgery	Yes	85%
	No.	15%
Paclitaxel	Yes	100%
	NO	0%
Radiotherpy	Yes	5%
	No	95%
Recurrence	Yes	6%
	No	94%

The study was found increase of platelet levels in women more than normal range as 4.3 to 5.6 X 1012/L. On the other hand, the result found decrease levels of serum RBCs, WBCs and Hemoglobin in women as in the table (3.4).

Table (3.4): Description mean levels of haematological parametesr of women with breast cancer (n=100)

CBCs		Normal range
Red blood cell (RBC)	**1.41±0.082 million cells/mcL**	**4.2 to 5.4 million cells/mcL**
White blood cell (WBC)	**3.01± 2.77X 10^9/L**	**4.5 to 11.0 X 10^9/L**
Hemoglobin	**09.58± 1.49 g/dL**	**12.1 to 15.1 g/dL**

Platelet counts	**6.3 ±1.79 X10^{12}/L**	**4.3 to 5.6 X 10^{12}/L**

The present study was found patients with Ca15-3 > 30 U/mL are at risk of non-responses compared to those with Ca15-3 (< 30 U/mL). According to the figure (3.1), the findings demonstrated a statistically significant decrease in the amount of Ca 15-3 found in breast cancer patients responder (22.45 ± 9.82) group in comparison to the level found in the non-responder (86.93± 11.34) group for patient taken Paclitaxel chemotherapy.

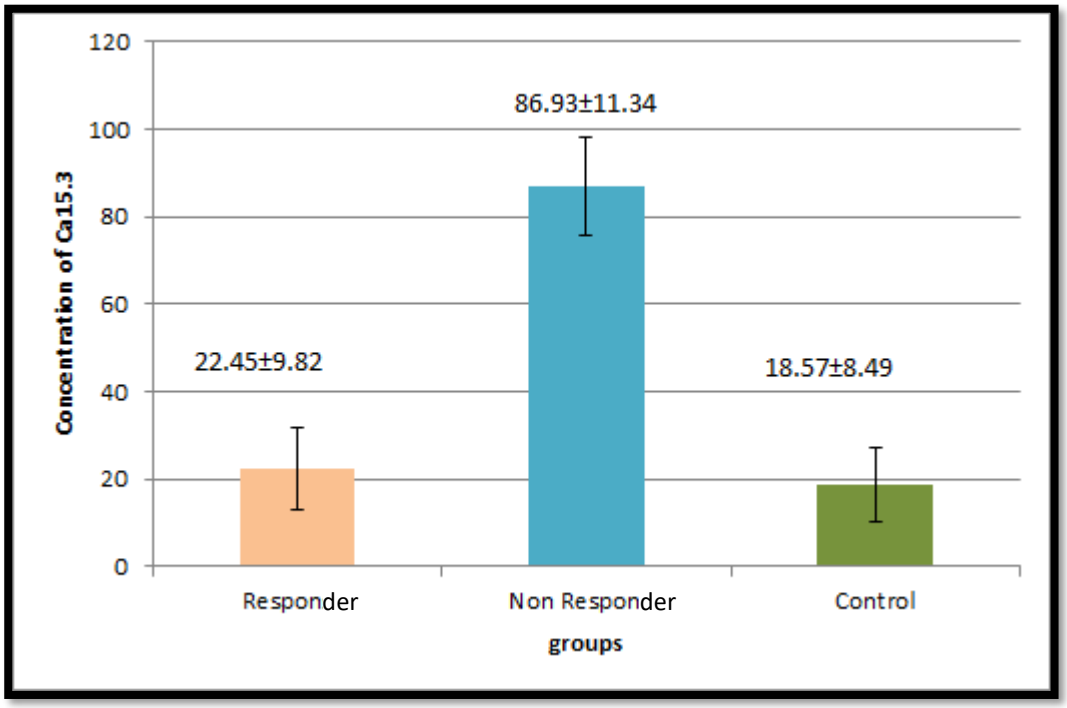


Figure 3.1: serum level of ca15_3 in breast cancer women

The present study was found patients with Estrdiol levels are at risk of non-responses compared to response and control groups. According to the figure (3.2), the findings demonstrated a statistically significant rise in the amount of Estradiol found in breast cancer patients non-response (37.29 ± 10.58) group in comparison to the level found in the response (27.88± 10.34) group for patient taken Paclitaxel chemotherapy, and (28.78±9.04) for control groups.

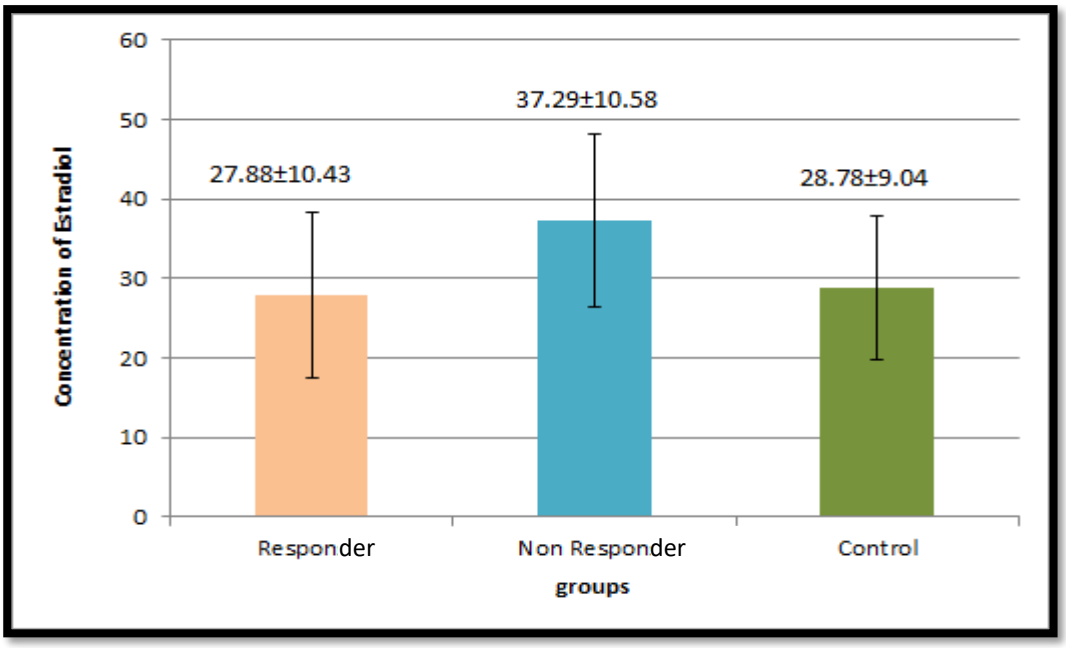


Figure 3.2: Concentration of estradiol in the patients have breast cancer

Concerning physical examination by the medical neurologist, results clarified some clinical features for patient with neuropathy as following table (3.5), 67% from patient women have parasthesia , 55% have neuropain effect, 41% have sensory motor, 37% of women have difficult movement, 28% of them have numbness and finally two women have tremor effect.

Table (3.5): Clinical feature of patient with score peripheral neuropathy

Clinical features	Percentage
Parasthesia	67%
NeuroPain	55%
Sensory motor	41%
Difficult Movement	37%

Numbness	28%
Tremors	2%

*** : Represented score of peripheral neuropathy according to (Mielke et al., 2003)**

3.2. Molcular analysis

3.2.1. Genotyping of A BCB1 3435G>A (rs1045642)

The results of genotype ABCB1 3435G>A (rs1045642) genetic polymorphism was a clear band with a molecular size 400 bps (figure 3.3) The size of amplicon was determined by compare with DNA ladder 100 - 1000 bp, genotype of rs1045642 which were classified into three genotypes:

1. The major genotype group (GG) for the allele G.
2. The homozygous genotype group (AA) for the allele A.
3. Heterozygous (GA).

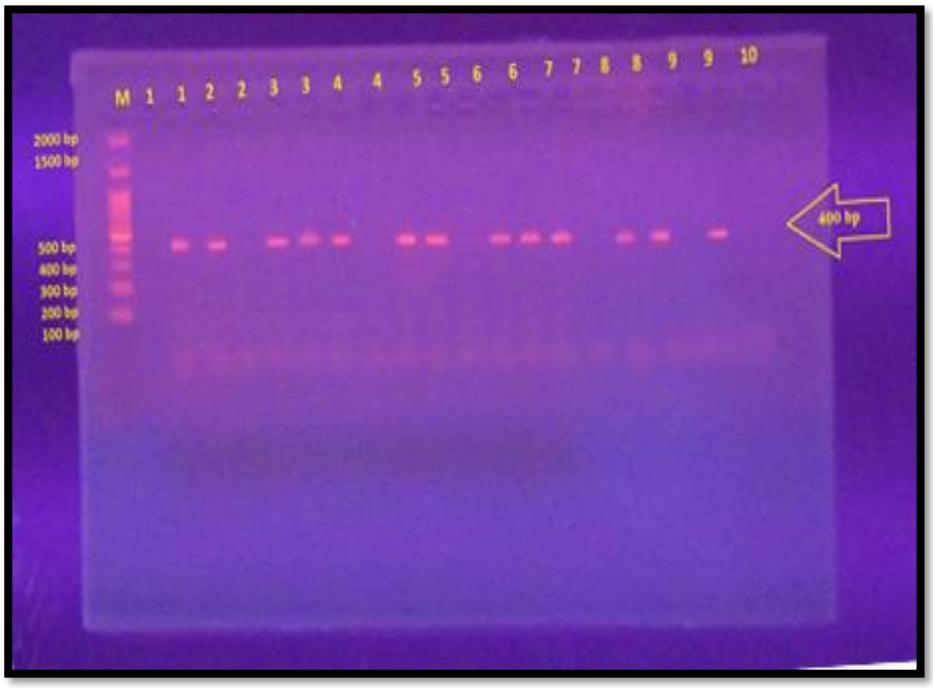


Figure (3.3):Detection ofABCB1 3435G>A (rs1045642) genetic polymorphism by Allele specific PCR with three possible genotype (GG, AA, and GA).

Table (3.6): Distribution of ABCB1 3435G>A (rs1045642) genotypes in breast cancer patients.

Variable	Group	Frequency	Percentage
Genotype	**GG (Wild)**	28	28%
	AA(Homo)	36	36%
	GA (Hetero)	36	36%
Data Presented by numbers and percentage			

The result of comparison between observed and anticipated value for ABCB1 3435G>A (rs1045642) tested population were shown in figure (3.3) and table (3.6). The distribution and percentage of individuals having rs1045642 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (28); GA (36); AA (36) (goodness-of-fit $\chi 2$ for rs1045642; 7.5824, $P < 0.001$} and therefore it was statistically significant.

Table (3.7): Hardy–Weinberg equilibrium for rs1045642 genotype in breast cancer patients.

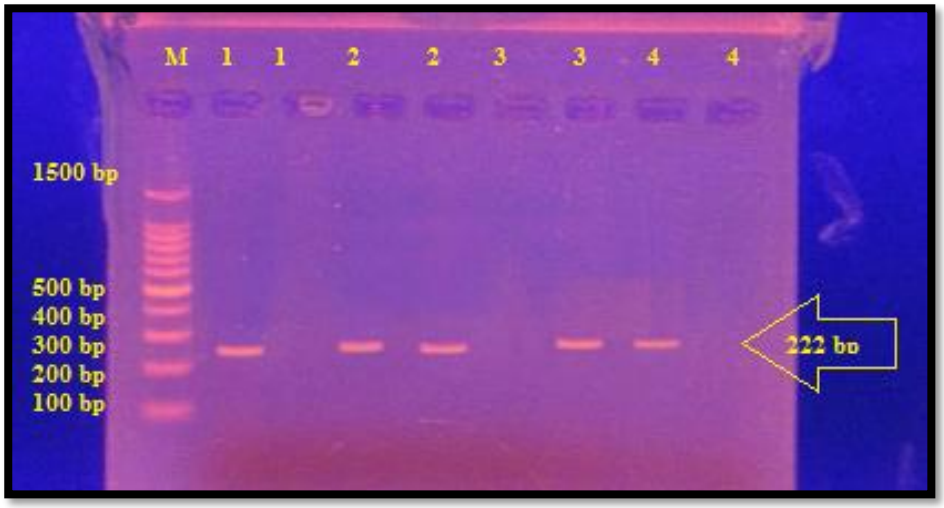
Genotypes Frequency		**Alleles**			**Hardy–Weinberg equilibrium X^2 test**
			G	A	
Genotype N= 100		%	0.46	0.54	7.5824 P < 0.001 [S]
GG (Wild Type)	**28**	**28%**			
GA Heterozygous type)	**36**	**36%**			
AA (homozygous type)	**36**	**36%**			
Total	**100**	**100%**			

3.2.2 Genotyping of ABCB1 2677G> A (rs2032582) genetic polymorphism

Genotyping of ABCB1 2677G> A (rs2032582) alleles were classified into three genotypes:

1. The major genotype group (GG) for the allele G.
2. The homozygous genotype group (AA) for the allele T.
3. The heterozygous (GA).

Table (3.8) and figure (3.4) summarize the distribution of genotyping groups of rs2032582 in women with breast cancer.



Figure(3.4):Detection of ABCB1 2677G>A (rs2032582) genetic polymorphism by allele specific PCR with three possible genotype (GG, TT , GT).

Table (3.8): Distribution of ABCB1 2677G>A (rs2032582) genetic polymorphism in breast cancer patients taking Paclitaxel therapy.

Variable	Group	Frequency	Percentage
Genotype	**GG (Wild)**	26	26%
	AA(Homo)	22	22%
	GA (Hetero)	52	52%
Data Presented by numbers and percentage			

The result of comparison between observed and anticipated values for ABCB1 2677G>A (rs2032582) the tested population were shown in figure (3.4), and table (3.8). Distribution and percentage of individuals having rs2032582 differ from those expected under Hardy–Weinberg equilibrium {number of observed vs expected were: GG (26, 10.6); AA (22, 45.6); GA (52, 43.9) (goodness-of-fit $\chi 2$ for 0.1736 , $P > 0.05$} and therefore it was statistically non significant.

Table (3.9): Hardy–Weinberg equilibrium for rs rs2032582 genotype in BC patients

Genotypes Frequency		**Alleles**			**Hardy–Weinberg equilibrium X^2 test**
			G	A	
Genotype N= 100		%	0.52	0.48	0.1736 P > 0.05 [NS]
GG (Wild Type)	**26**	**26%**			
GA (Heterozygous type)	**52**	**52%**			
AA (Homozygous type)	**22**	**22%**			
Total	**100**	**100%**			

3.3 Relationship between Tumor marker with response of paclitaxel chemotherapy.

The results of the current study showed that there is a significant decrease in the concentration of CA15-3 levels with a significant increase in the score of neuropathy. In other words, the higher the score, the greater the significant decrease in the concentration of CA15-3 We found reverse negative correlation value (-0.821) between concentration of CA15-3 and grade score of peripheral neuropathy in the response of paclitaxel treatment. The result was reflected with what was shown by patients with breast cancer and there is a response to treatment by Paclitaxel chemotherapy table (3.10).

Table (3.10): Correlation of Ca15.3 tumor marker with of peripheral neuropathy in patient response and non-response treatment.

Tumor marker		**Grade**
CA15-3 levels	Responder	-0.821
	Non-Responder	0.478

Table (3.11) showed that there is an inverse relationship in the response of chemotherapy between tumor marker and incidence of neuropathy, as we observed a response to chemotherapy with a rate of up to 82% of incidence neuropathy. In contrast, we observed a decrease in the number of patients who had a high concentration of CA15-3 tumor marker in the their blood, vice versa.

Table (3.11): Relationship between response with Tumor marker and incidence neuropathy.

	Non-Responder	Responder
Tumor Marker	77	23
Incidence neropathy	18	82
Statistical analysis	X2= 69.79 , Df= 1, P= 0.001	

X2; Chisquare test , DF; degree of freedom

On the other hand, we noticed in table (3.12) that there are some menopausal women (13%) who have signs of neurological adverse effects and at the same time they have an increase in the concentration of CA15-3 in their blood. We also noticed that there are some women who have high neurological adverse affect and at the same time it noticed a decrease in the concentration of CA 15.3 in the blood.

Table (3.12): Relationship between tumor marker with adverse effect of neuropathy.

Tumor Marker Ca15.3	Adverse effect	free-adverse effect
< 30 U/mL*	69%	31%
> 30 U/mL	13%	87%

* **Prognostic role of CA15. 3 (Sandri et al., 2012)**

3.3.1. Relationship between tumor marker of chemotherapy intake and ABCB1 3435G>A (rs1045642) genetic polymorphism

Table (3.13) shows that there is an inverse relationship between the concentration of Ca15.3 with the therapeutic response of the patient when using paclitaxel chemotherapy and when compared with the level of

genotyping frequency among ABCB1 3435G>A (rs1045642) genetic polymorphism. We noticed that patients who have allele GG in their chromosomes respond to treatment (15.56±6.89) better than patients who have alleles AA and GA, respectively. LSD value was recorded 4.67 among the genes and indicated the existence of significant differences (P< 0.05) among the three alleles.

Table (3.13): Relationship between tumor marker of chemotherapy intake and ABCB1 3435G>A (rs1045642) genetic polymorphism

Genotype/ Non-Responder Ca15.3			**Genotype/ Responder Ca15.3**		
Genotype "rs1045642"	**N**	**Tumor Marker Mean ±SD**	**Genotype "rs1045642"**	**N**	**Tumor Marker Mean ±SD**
GG	19	88.38±6.24	GG	9	15.56±6.89
GA	25	85.85±8.47	GA	11	23.78±5.36
AA	33	82.94±7.13	AA	3	24.76± 7.98
LSD		7.37	LSD		4.67

LSD; Least significant differences

3.3.2. Relationship between concentration of chemotherapy paclitaxel intake and ABCB1 3435G>A (rs1045642) genetic polymorphism

Table (3.14) shows that there is an inverse relationship between the concentration of drug with the therapeutic response of the patient when using paclitaxel chemotherapy and when compared with the level of genotyping frequency among ABCB1 3435G>A (rs1045642) genetic polymorphism. We noticed that patients who have allele GG in their chromosomes respond to treatment (192.8±16.33) better than patients who

have alleles AA and GA, respectively. LSD value was recorded 11.16 among the genes and indicated the existence of significant differences (P< 0.05) among the three alleles.

Table (3.14): Relationship between Paclitaxel serum level intake and ABCB1 3435G>A (rs1045642) genetic polymorphism

Genotype "rs1045642" Mean ±SD	N	Drug Concentration No-Responder	Drug Concentation Resposnder	P value
GG	28	176.82±14.46	192.8±16.33	0.037
GA	36	168.45±16.39	184.91±13.76	0.053
AA	36	167.36±14.8	165.26±15.7	0.247
LSD		9.37	11.16	-

LSD; Least significant differences

3.3.3. Relationship between tumor marker of paclitaxel chemotherapy intake and ABCB1 2677G>A (rs2032582) genetic polymorphism

Table (3.15) shows that there is an inverse relationship between the concentration of Ca15.3 with the therapeutic response of the patient when using paclitaxel chemotherapy and when compared with the level of genotyping frequency among ABCB1 2677G>A (rs2032582) genetic polymorphism , we noticed that patients who have allele GG in their chromosomes respond to treatment (19.18±7.08) better than patients who have alleles AA and GA, respectively. LSD value was recorded 2.67 among the genes and indicated the existence of significant differences (P< 0.05) among the three alleles.

Table (3.15): Relationship between tumor marker of chemotherapy intake and 2677G>A (rs2032582) genetic polymorphism

Genotype/ Non-Responder Ca15.3			Genotype/ Responder Ca15.3		
Genotype " rs1045642"	**N**	**Tumor Marker Mean ±SD**	**Genotype "rs1045642"**	**N**	**Tumor Marker Mean ±SD**
GG	18	84.19±8.27	GG	8	19.18±7.08
GA	42	83.74±7.39	GA	10	23.78±6.14
AA	17	85.63±9.11	AA	5	25.96± 6.38
LSD		6.45	LSD		2.67

LSD; Least significant differences

3.3.4. Relationship between concentration of chemotherapy paclitaxel intake and ABCB1 2677G>A (rs2032582) genetic polymorphism

Table (3.16) shows that there is an inverse relationship between the concentration of drug with the therapeutic response of the patient when using paclitaxel chemotherapy and when compared with the level of genotyping frequency among ABCB1 2677G>A (rs2032582) genetic polymorphism. We noticed that patients who have allele GG in their chromosomes respond to treatment (195.17±14.68) better than patients who have alleles AA and GA, respectively. LSD value was recorded 10.57 among the genes and indicated the existence of significant differences ($P< 0.05$) among the three alleles.

Table (3.16): Relationship between drug concentration of chemotherapy intake and ABCB1 2677G>A (rs2032582) genetic polymorphism

Genotype " rs2032582" Mean ±SD	N	Drug Concentration No-Response	Drug Concentration Resposne	P value
GG	26	173.62±15.17	195.17±14.68	0.037
GA	52	167.35±14.28	174.8±12.84	0.059
AA	22	171.22±13.51	156.65±11.73	0.047
LSD	100	4.51	10.75	-

LSD; Least significant differences

3.4. Correlation between tumor marker and ABCB1 gene genotype in postmenopausal women have breast cancer.

The study was found significant association between two SNPs and tumor marke. It found the odd ratio with tumor marker ca15.3 is 1.19 with non-significant differences (P=0.54). We don't found any significant differences with other biochemical markers like estradiol, the odd ratio with estradiol levels is 0.75 with non-significant differences (P=0.32)

Table (3.17) Correlation between tumor marker andABCB1 genotype

Characteristic	rs2032582 *Mean±SD*	rs1045642 *Mean±SD*	*Odd ratio 95%CI*	*P value*
Tumor marker Ca15.3 (normal range 30U/ml)				
< 30 U/ml	35	31	1.19 0.66-2.16	0.54
≥ 30 U/ml	65	69		
Estradiol (normal range 30pg/ml)				
< 30 pg/ml	49	56	0.75 0.43-1.31	0.32
≥ 30 pg/ml	51	44		

Chapter four

Discussion

4. Discussion

This study included 100 women stratified by age, BMI, and paclitaxel duration intake. The clinical demographic data and laboratory parameters of patients group were presented in table (3.1) and (3.2). the mean age of participants which was within a mean age of (54.36 ± 4.21) years old, number of birth (3±1) times. They are many adjusted other self-reported characteristics and risk factors responsible for breast cancer. These characteristics included BMI, marital status, family history, lymph node involvement, number of patients who had surgery or chemotherapy, disease duration and diagnosis, cancer location, and histochemical test results

Some of studies showed the majority of breast cancers are found in women over the age of 50 years old, and some women will develop breast cancer despite having no other known risk factors. The presence of a risk factor does not guarantee the presence of the disease, and not all risk factors have the same effect (Nelson et al., 2012).

Several studies have shown that young women typically do not worry about getting breast cancer. On the other hand, breast cancer can affect people of any age. Just 5% of women diagnosed with breast cancer are under the age of 40. Risk factors for breast cancer vary from woman to woman (Helmrich et al., 2016).

Postmenopausal women who are overweight or obese have an elevated risk of developing breast cancer (BMI), some of recent studies with meta-analysis estimated that for every 3% increase in body mass index (BMI), (Bergström et al., 2001). Obesity has been linked to an increased risk of breast cancer, but the underlying mechanism is unclear, one possible explanation is an increase in the serum level of bioavailable estradiol due to an increase in estrogen production by aromatase in adipose tissue and a

reduce in the serum concentration of sex hormone-binding globulin (SHBG)(Siiteri et al., 2007).

The result also found the most common breast cancer location was left side as (55%) from total of women who they have breast cancer in the right sides (45%). The ratio of tumors on the left side of the breast to those on the right side ranges anywhere from 1.05 to 1.26, according to a number of studies that have consistently shown that women have a slightly increased risk of being diagnosed as having cancer in the left breast compared to the right. The study found most women diagnosed with breast cancer were at the range 25-29.9 stage of body mass index, it was recorded as 63% from total of postmenopsal women who have breast cancer.

Many explanations have been proposed to illustrated explain this left-sided preponderance, such as the bigger size of the left breast, the ease with which right-handed women may detect left-sided malignancies, and the preference for right-sided breast feeding. However, further study has not confirmed these explanations, and they are not widely accepted. Breast cancer laterality may be affected by a number of factors, including, but not limited to, age, breast quadrant "the possibility that each may have a different laterality ratio", and genetics. Nonetheless, there is still a lack of clarity about this phenomenon (Abdou et al., 2022).

Eightenn cases of women not married have breast cancer, it was different from postmenopsal married women which they recorede as 82% of cases, there were 90% and 72% women have breast feeding and normal vaginal delivery, respectively.

There may be a connection between a woman's marital status and her chance of acquiring breast cancer. However, the quality of the available evidence may have been hampered by factors including poor control group selection, inadequate investigation of confounding effects, inaccurate

reporting of marital status, and/or publication bias. Therefore, more prospective robust cohort studies are needed to reach firm conclusions about whether or not marital status is an independently risk factor for breast cancer (Li et al., 2020). Some study reviewed existing literature and found that never-married women, and especially those who remained single throughout their whole lives, had a greater chance of acquiring breast cancer than married women (Ghorbani et al., 2021).

The study found Most women diagnosed with breast cancer with risk factor of external influences were recorded as 85% from total of postmenopsal women have surgical involvement. The result also found the all women with breast cancer have been taken chemotherapy (100%) and 5% from total of women who they have breast cancer have radiotherapy treatment. The results also found rare cases of postmenopasal women (6%) have recurrence of breast cancer.

The results of the current study showed that there were 30% women suffering from breast cancer with a family history, some study findings with being a first-degree family with breast tumors (daughter, sister, or mother) almost doubles a woman's risk for beat cancer. Women who have a father or brother who has had breast cancer are at a higher chance of developing the disease. It was recorded that the most cases of breast cancer come from two or more first- or second-degree relatives with breast cancer on the same side of the family (Kim et al., 2019). According to the findings of a recent study conducted in India, Mizo women who have relatives who have had breast cancer, as well as numerous other malignancies and inherited disorders, have an increased risk of developing breast cancer themselves (Zodinpuii et al., 2022).

About 77% of women screened for breast cancer had lymph nodes in our study. If breast cancer has spread to lymph nodes, the patient may be

subjected to more intensive treatment than they would have been if the cancer hadn't invaded the lymph nodes (Zhou et al., 2020). Swarnkar et al., (2021) demonstrated that the five-year survival rate for breast tumors that has not spread to adjacent lymph nodes is 99 percent, whereas the percentage drops to 86 percent after it has.

The results of the current study showed that out of a total of women, (72%) had cesarean delivery in most their children. Major risk factors for postpartum breast tumor include cesarean delivery, and women who have never given birth before had a decreased risk of developing postpartum breast cancer, other wise the Cesarean delivery is a substantial risk factor for postpartum breast cancer, and normal vaginal delivery women had a decreased risk of developing postpartum breast cancer rather than cesarean delivery (Choi et al., 2019).

On the other hand, in our study, women who were breastfed were 90% of the total, during lactation, the majority of breastfeeding mothers suffer hormonal shifts that cause a delay in the onset of their menstrual cycles. This lowers a woman's overall intake of hormones like estrogen during her entire life, which may stimulate the growth of breast cancer cells (Fortner et al., 2019).

The results of our current study showed that 85% of women with cancer had surgical intervention, breast surgery, also known as a wide local excision, is a type of operation in which the region of cancer in the breast that needs to be removed is cut out surgically. The cancerous tissue and a margin of healthy tissue all the way around it are removed by the surgeon. They do so while preserving the maximum amount of healthy breast tissue feasible (Lovelace et al., 2019). A total breast removal, sometimes known as a mastectomy, may be necessary for some women. They also have the option of undergoing this surgical procedure. The breast tissue, including

the skin and nipple, as well as the tissues that protect the chest muscles, are both removed by the surgeon during the procedure (Greenup et al., 2019). Extremely infrequently, the surgeon will also remove the muscles that make up the chest wall. This type of mastectomy is known as a radical mastectomy (Berhili et al., 2019).

The study observed five women with breast cancer who were users of radiotherapy, breast cancer radiation therapy involves the use of X-rays, protons, or other high-energy particles to eradicate cancer cells (Helm et al., 2020). Radiation therapy is more effective against rapidly dividing cells, such as cancer cells, than it is against stationary ones. There is no discomfort or visual impact from the X-rays or particles. After treatment, there are no longer radioactive and can safely be in close proximity to others, including children (Duma et al., 2019).

Breast cancer at nearly any stage may be treated with radiation treatment. After breast cancer surgery, radiation therapy is an effective method of lowering the likelihood of a recurrence. In addition, it is frequently used to alleviate the discomfort associated with metastatic breast cancer (Helm et al., 2020).

The results of the current study showed that all women (100%) had chemotherapy,and just 6% had recurence breast cancer when cells that were a part of initial breast cancer detach out of the initial cancerous growth and hide nearby in the breast tissue or in another region of the body. This might lead to recurrent breast cancer. After some time has passed, these cells start developing again (Alom et al., 2019).

The most important causes for cancer recure was depend on experience of surgeon. Some time the surgeon eliminates all detectable cancer during the initial breast cancer surgery. However, current cancer diagnostics cannot detect the presence of even a small number of cancer cells that may

survive despite surgical removal. A fraction of cells may endure post-operative radiation and chemotherapy. A tumor can form from as little as one surviving cancer cell after treatment (Fujiwara et al., 2019).

4.1. Clarification of haematological parametesr of women with breast cancer:

The study was found increase of platelets levels in the serum of women more than normal range as 4.3 to 5.6 X 1012/L, an account of circulating red blood cells may decrease as a side effect of certain chemotherapy medicines. Its side effects include fatigue, weakness, fainting, and difficulty breathing. A transfusion of blood may be necessary if your red blood cell count is dangerously low (Abdel-Razeq, & Hashem, 2020).

Anemia caused by chemotherapy is a common side effect of systemic chemotherapy. This anemia is linked to a lower functional ability as well as a lower quality of life. A sizeable number of chemotherapy-treated patients will experience hematologic toxicities such as anemia, neutropenia, and thrombocytopenia, which may lead to a delay in treatment or a dose reduction (Shaw et al., 2021).Patients who are at risk for this major toxicity may receive better supportive care and individual management if the severity of chemotherapy-induced anemia can be predicted in advance (Kilpatrick et al., 2021).

The study was found an increase of platelet levels in women more than normal range as 4.3 to 5.6 X 10^{12}/L, on the other hand, the result found decrease levels of serum RBCs, WBCs and Hemoglobin in women as in the table (3.4). It was discovered that postmenopausal women's platelet counts rise to a greater extent than those of the control groups during chemotherapy, and that increasing platelet counts during chemotherapy typically entails delaying the next dose of chemotherapy (Wang et al., 2002).

Recent studies suggest that chemotherapy may put breast cancer patients at an increased risk for developing thrombosis, an experiments study were done so that to determine whether or not the increased thrombosis was caused, at least in part, by an effect that chemotherapy had on the responsiveness of endothelial cells (Nash et al., 2002).

The results were not consistent with other study (Kuter, 2015) who was found that Cancer patients frequently have thrombocytopenia as a side effect of treatment. Some chemotherapy medicines are more likely to cause low platelet counts than others, and this can have catastrophic consequences. Bleeding and/or the necessity of delaying chemotherapy are both outcomes of a low platelet count.

Thrombocytosis has been linked to an increase in the prevalence of a number of malignancies. In addition, thrombocytosis predicts a lower likelihood of surviving a cancer diagnosis. An elevated platelet count is linked to a 7-fold greater chance of ovarian cancer , and a 1.7-fold increased incidence of mortality among ovarian cancer survivors. These relationships are particularly robust for women with ovarian cancer. Others have demonstrated that a high platelet count speeds up the development of ovarian cancer (Giannakeas, 2022).

According to the figure (3.1), breast cancer patients who received Paclitaxel chemotherapy had a statistically significant decrease in Ca15-3 mean ±SD (22.45 ± 9.82) compared to the non-responders mean ±SD (86.93± 11.34). Blood tumor markers indicate breast cancer stage. Thus, blood indicators primarily diagnose and monitor metastatic illness. CEA and one MUC1 mucin, usually CA15.3 or CA27.29, are the most often used markers. The diagnosis of symptomatic metastases now includes tumor marker testing. Biochemical assessment utilizing blood markers to evaluate endocrine and cytotoxic therapy in advanced disease correlates with

standard UICC criteria and has many advantages, making it a potentially better method. CA15.3, CEA, and ESR are best validated (Cheung et al., 2002). This decrease in the level of CA15.3 in post menopausal women indicates that it has been a response to chemotherapy, which is required under study.

Breast cancer patients with Estrdiol levels are at risk of non-responses compared to response and control groups. The non-response group had a statistically significant increase in Estradiol (37.29 ± 10.58) compared to the response group (27.88± 10.34) for Paclitaxel chemotherapy patients and the control group (28.78±9.04).

The group of women who responded to chemotherapy had a decrease in the level of estradiol in their serum as a result of the use of Palcitaxel chemotherapy. This study came in accordance with what the researcher (Septiani et al., 2022) explained about the low levels of estradiol for patient with chemotherapy, It is thought that chemotherapy lowers estradiol levels. According to Soewoto et al.,(2018) estradiol has a significant impact on breast cancer prognosis since excessive levels reduce the likelihood of cure while normal levels improve survival for breast cancer patients.

Although estrogen's function in breast cancer carcinogenesis has been demonstrated by a number of theories, no effective preventative measures are currently available, damaged ovarian granulosa cells are one of the side effects of chemotherapy used to treat breast cancer. This leads to a disruption in the ovary's ability to produce estrogen. Surgery, like oophorectomy, or medication that disrupts ovarian function can reduce circulating estradiol levels. So chemotherapy can lower estradiol levels without surgery or medicines that disrupt ovarian function (Soewoto & Agustriani, 2023). Regarding the results of the patient's physical examination by the medical neurologist, the table (3.5) below outlines some

of the clinical characteristics that are associated with neuropathy. 67% of the patient women have parasthesia, 55% of the women have the effect of neuropain, 41% of the women have sensory motor, 37% of the women have difficulty moving, 28% of them have numbness, and finally two women have the effect of tremor. The peripheral sensory neuropathy is one of the most frequently documented neurotoxic effect of paclitaxel, and it is responsible for limiting medication with high and progressive doses of paclitaxel when it is administered alone or in conjunction with other neurotoxic anticancer drugs such as cisplatin (Scripture et al., 2006).

The result was agreement with (Klein & Lehmann, 2021), who was recoded. The first signs, such as tingling, allodynia and numbness in the patient's toes and fingers, can be seen 24 to 72 hours after the injection. In a "glove and stocking" way, patients can feel tingling and numbness all the way up to their lower legs and arms.

Chen et al., (2022) Clarified that the buildup of paclitaxel in the dorsal root ganglia (DRG) is the primary cause of sensory symptoms, which can manifest in the hands and feet and include pain and numbness. According to study of (Postma et al., 1995) paclitaxel-induced neuropathy is at most somewhat reversible, according to follow-up data on 12 patients who stopped receiving paclitaxel treatment after it had been administered before. Some of studies (Staff et al., 2020) showed that paclitaxel activates ion channels that regulate reactions towards external signals and causes alterations in growth factor release, neuropeptide calcium signaling, mitochondrial damage, and reactive oxygen species generation with matrix metalloproteinase 13 (MMP-13) has recently been shown to play a role in causing neuropathy.

4.2. Genotyping of A BCB1 3435G>A (rs1045642)

According to figure 3.3 and table 3.6 display the results of a comparison between the observed value and the expected value for the ABCB1 3435G>A (rs1045642) tested population. It was statistically significant since the distribution and proportion of individuals who had rs1045642 varied from those expected under Hardy–Weinberg equilibrium. The number of observed vs expected were: GG (28); GA (36); AA (36) (goodness-of-fit 2 for rs1045642; 7.5824, P= 0.001).

In this wasy, Mutations, gene flow, and other factors can all upset the Hardy-Weinberg equilibrium, For instance, rs1045642 polymorphism introduce novel alleles into a population, which might shift the balance of existing allele frequencies (Sychev et al., 2018). This investigation concurred with the findings of (Barliana et al., 2021), who discovered the The Hardy–Weinberg equilibrium was used to conduct an investigation of the frequency of alleles for each gene. The genetic profiles of ABCB1 rs1045642 were found to be significantly different from equilibrium in the population of Indonesia.

4.3.Genotyping of ABCB1 2677G> A (rs2032582) genetic polymorphism

Figure (3.4) and table (3.8) compare observed and expected values for ABCB1 2677G>A (rs2032582) in the tested population. It was statistically non-significant since the distribution and proportion of people with rs2032582 differed from those expected under Hardy–Weinberg equilibrium: GG (26, 10.6); AA (22, 45.6); GA (52, 43.9) (goodness-of-fit, $P > 0.05$). The results did not agree with those of (Vencatto et al., 2019), who reported that the G2677 T/A variation in ABCB1 was not in agreement with Hardy-Weinberg equilibrium in breast cancer patients (P value < 0.05). He decided that statistical analysis using univariate and multivariate models

showed no evidence of a link between ABCB1 variations and recurrence (P> 0.05).

4.4. Relationship between Tumor marker with response of paclitaxel chemotherapy.

Our results found a reverse negative correlation value of (-0.821) between the concentration of Ca15.3 and grade score of peripheral neuropathy in the response to paclitaxel treatment. This result was reflected with what we know about the relationship between concentration of Ca15.3 and grade score of peripheral neuropathy. The results of the current study showed that there is a significant decrease in the concentration of Ca 15.3 levels with a significant increase in the score of neuropathy.

CA 15-3 may play a role in determining how well breast cancer patients respond to chemotherapy, as indicated by research from Kurebayashi et al., (2004). This was corroborated by the findings of Duffy et al, who concluded that the most essential role for CA 15-3 is in evaluating chemotherapy in patients who have advanced breast cancer (Duffy, 2006).

Table (3.11) showed that there is an inverse relationship in the response of chemotherapy between tumor marker and incidence of neuropathy, as high to 82% of patients with neuropathy improved after chemotherapy palictaxel treatment. Conversely, the study showed (X2= 69.79 , Df= 1, P= 0.001) a fall in the number of individuals whose blood exhibited an abundance of the tumor marker CA15-3.

However, table (3.12) shows that some menopausal women show symptoms of neurological side effects and also have an increase in their blood concentration of CA 15-3. Similarly, there are some women who do show high symptoms of neurological side effects and also show a decrease in their blood concentration of CA 15-3. This may be due some of study (Al-Azawi et al., 2006) demosstarted in large part to a high concentration of CA 15-3 indicates a poor response to chemotherapy. Additionally,

lympho-vascular invasion and HER2 status, should be need in conjunction with persistently high CA15-3 levels after chemotherapy, and predict a lower disease-free survival after treatment in locally advanced breast cancer.

4.5. Relationship between chemotherapy adverse effect and ABCB1 3435G>A (rs1045642) genetic polymorphism

According to the genotyping frequency of the ABCB1 3435G>A (rs1045642) genetic polymorphism, the study found that patients with the allele GG in their chromosomes respond to treatment better have CA15.3 concentration (15.56±6.89) than patients with the alleles AA and GA, respectively. This inverse relationship between CA15-3 concentration and therapeutic response was observed using paclitaxel chemotherapy (Table 3.13). The mean LSD for all genes was 4.67, indicating that there are statistically significant differences ($P < 0.05$) between the three genotype frequency. This indicates that patients who carry the A allele, are less responsive to treatment compared to other patients who carry the G allele.

Postmenopausal women with the allele GG in the ABCB1 3435G>A (rs1045642) genetic polymorphism have a better response to treatment in the CA15-3 levels (192.8±16.33) than patients with the alleles AA and GA, respectively, as shown in table (3.14), which also shows that there is an proportional relationship between the concentration of drug and the therapeutic response of the patient when using paclitaxel chemotherapy. The mean LSD for all genes was 11.16, indicating that there are statistically significant differences (P 0.05) among the three alleles.

The result was in agreement with the findings of (Zhang et al., 2023), who conducted a study on patients with bipolar disorder (BPD) and major depressive disorder (MDD) and Han Chinese community. He decided that G allele in rs1045642 polymorphism was a protective factor in his study of patients with MDD and BPD.

4.6. Relationship between tumor marker of chemotherapy intake and ABCB1 2677G>A (rs2032582) genetic polymorphism

2677G>A (rs2032582) genetic polymorphism in patients with the allele GG in their chromosomes respond to treatment (19.18±7.08) better than patients with the alleles AA and GA, respectively, as shown in table (3.15), which also shows that there is an inverse proportional relationship between the tumor marker and the therapeutic response of the patient when using paclitaxel chemotherapy. The LSD for all genes was 2.67, indicating that there are statistically significant differences ($P < 0.05$) among the three alleles. This study was agreement with (Shan et al., 2019) who was found the A allele was more risk factor of ABCB1 gene and therapeutic response in the local Chinese Han population who they have selective serotonin reuptake inhibitors (SSRIs)

4.7.Relationship between drug concentration and ABCB1 3435G>A (rs1045642) genetic polymorphism.

Table (3.14) shows that there is an inverse relationship between drug concentration and patient therapeutic response when using paclitaxel chemotherapy, and when compared with the level of genotyping frequency among ABCB1 3435G>A (rs1045642) genetic polymorphism, the study noticed that patients with allele GG respond to treatment (192.8±16.33) better than patients with alleles AA and GA, respectively. The LSD value was 11.16 among the genes, indicating that there were substantial differences (P 0.05) among the three alleles, The study found that the wild gene G allele played a role as a protective factor in order to maintain the concentration of the drug Pacitaxel in the patient's serum for a period of time, unlike the other two genetc polymorphism AA and GA, This study differed from what was explained by (Zhao et al., 2021) when he stated that

not all mutated genes play a role as a protective factor, as there are some mutated genes that were pathological in women with metastatic colon cancer.

4.8.Relationship between concentration of chemotherapy paclitaxel of chemotherapy intake and ABCB1 2677G>A (rs2032582) genetic polymorphism

Table (3.16) shows that there is an inverse relationship between drug concentration and patient therapeutic response when using paclitaxel chemotherapy, and when compared to the level of genotyping frequency among ABCB1 2677G>A (rs2032582) genetic polymorphism. We found that patients with allele GG in their chromosomes respond to treatment (195.17±14.68) better than patients with alleles AA and GA, respectively. The LSD value was 10.75 among the genes, indicating that there were substantial differences (P 0.05) among the three alleles.

This study coincided with what (Zhang, 2019) explained when he stated that mutated genes play a role as a risk factor, as there are some mutated genes that were protective in women with gastric peptic cancer.

4.9. Correlation between tumor marker and ABCB1 gene genotype in patient postmenopausal women have breast cancer.

The results of the current study showed that there were 35 patients who responded to treatment in patients who had rs2032582 genes than patients who had the rs1045642 gene when measuring CA15-3 concentration, but this response was not significant due to the (odd ratio 1.19 ; 95%CI 0.66-2.16 and P value =0.54).

While the results showed the opposite when measuring the level of estrogen in postmenopausal women, the result found that there are 56 postmenopausal women who carry the rs1045642 gene polymorphism and have a lower

concentration of eestradiol than women who carry the rs2032582 gene polymorphism, but this difference is also non-significant differences with (odd ratio 0.75; 95% CI 0.43-1.13 and P value= 0.32).

Conclusions and Recommendations

Conclusions

Based on the findings, the following conclusions can be reached:

1. Distribution of ABCB1 3435G>A (rs1045642) genotypes in breast cancer patients was found Mutant allele more prevelance than wild allele, while it was the opposite in patients with ABCB1 2677G>A (rs2032582) genetic polymorphism.
2. It found that patients whose chromosomes contained the allele GG responded to treatment more favorably than patients whose chromosomes contained the alleles AA and GA for ABCB1 2677G>A (rs2032582) and 3435G>A (rs1045642) genetic polymorphism.
3. There is a rise in tumor markers CA15-3 for postmenopausal women who have two mutant alelles for ABCB1 Polymorphisms.
4. There is an inverse relationship between low tumor marker CA15-3 and increased adverse effect in women with breast cancer as well as a clear increase in the concentration of the Paclitaxel drugs in serum .
5. It is found that the postmenopausal women are responding to therapy if there is a reduction in the level of a circulating tumor marker as well as have the adverse effect of neurotoxicity.

Recommenations

1. Genetic analysis is required for breast cancer patients to increase effectivness and reduce adverse effect of paclitaxel.
2. Other genetic polymorphism of transporter should be study like OAT2 and SLC22A7 which are substrate of paclitaxel with postmenopausal breast cancer women.
3. Investigating the genetic polymorphism of enzyme cyp3A4 that involve in paclitaxel metabolism.
4. Study the pathological axonal degeneration in vivo and in vitro for investigation the pharmaceutical effect of paclitaxel.
5. Further investigation by large patient cohorts and the development of objective end points.

Limitation of the study

1. Many cancer patients do not approved to participitate in this study due to their old age , fear and difficulty of take the blood sample from their veins.
2. Reduction the trust between the patients with medical stuff.

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Appendices

Questionnaire of breast cancer patients

Name: **Phone no.** **No.**

Age: **Weight:** **Height:**

Workplace: **Academic achievement:**

Address: **Marital status:**

First menarche: **Last menarche:**

Family history of breast cancer:

Date of breast cancer diagnosis:

Site (left, right):

Surgery:

Radiotherapy:

Chemotherapy:

Date of cancer recurrence: **Site of recurrence:**

Other diseases:

Dose of Paclitaxel:

Time on Paclitaxel chemotherapy

Adverse effects :

Other drugs used:

Lab. Data:

الخلاصة

خلفية الدراسة: عرَّف سرطان الثدي بأنه نمو مضطرب للخلايا في أنسجة الغدة الثديية (القنوات أو الفصيصات) مع القدرة على الانتشار ، ويعد أكثر أنواع السرطان شيوعًا بين النساء في كل من البلدان المتقدمة والنامية ، النساء في سن الخمسين و / أو كبار السن المصابون بسرطان الثدي يعتبرون حالات قديمة. CA15.3 هو مؤشر على استجابة الورم للعلاج ، مما يسمح بالتعرف المبكر على تكرار علاج باكليتاكسيل وتعديله. بهذا المعنى ، يلعب علم الوراثة الدوائية دورًا أساسيًا في فهم العوامل الفردية التي تعدل الاستجابة لعلاج سرطان الثدي ، ولا سيما دراسة الجينات المشاركة في التمثيل الغذائي للباكليتاكسيل المستخدمة في العلاج الكيميائي والعلاج الهرموني. في هذه الحالة ، مسار (rs1045642 A BCB1 3435G> A) و ABCB1 2677 (rs2032582) A <G). هدفت الدراسة الى تحديد العلاقة بين تعدد أشكال الجين ABCB1 في النساء بعد سن اليأس المصابات بسرطان الثدي 3435(rs104564) A <G) و 2677(rs2032582) A <G) الجين ABCB1 ، وتراكيزالمصل من العلاج الكيميائي باكليتاكسيل في العراق.

المرضى وطريقة العمل: تضمنت هذه الدراسة 100 مريضه (45-75 سنة) مع تشخيص سريري لسرطان الثدي والذين بدأوالعلاج الكيميائي تاكسانات (باكليتاكسيل) وتمكنا من تحديد المتغيرات الجينية المتعلقة بالسميات الرئيسية لهذه الأدوية ، وكذلك لاستكمال الاستجابة العصبية لدى المرضى الذين عولجوا بالعلاج الكيميائي ، تم تصنيف المرضى في مركز الأورام في مدينة الإمام الحسين الطبية في كربلاء ، العراق ، إلى مجموعات بناءً على عمرهم بعد سن اليأس ، ومدة المرض ، وطول فترة العلاج لهذه الدراسة ، الذي تم إجراؤه بين يوليو 2022 وأكتوبر 2022 ، تم قياس فعالية باكليتاكسيل ، وتركيزات مصل (استراديول و CA15.3) في النساء المصابات بسرطان الثدي اللائي تناولن الدواء لمدة 3 أشهر على الأقل ، وأشكال الموافقة المستنيرة مع المشاركين. تم جمع التوقيعات من الجميع. تم إجراء تحديد تعدد الأشكال بواسطة مقايسة PCR الخاصة بـ Allele-Specific PCR لاثنين من تعدد الأشكال الجيني (rs10456423435 A <G) و 2677 A <G rs2032582)) ، وتم حساب تركيز عقار باكليتاكسيل بواسطة جهاز HPLC.

النتائج: اكتشفت الدراسة الحالية أن المرضى الذين يعانون من CA15-3> 30 U / mL معرضون لخطر عدم الاستجابة مقارنة مع أولئك الذين يعانون من CA15-3 (30 U / mL) ، وأظهرت النتائج انخفاضًا مهمًا من الناحية الإحصائية في كمية المستضد السرطاني 3-15في استجابة مرضى سرطان الثدي (22.45 9.28) مقارنة بالمستوى الموجود في مجموعة عدم الاستجابة (86.93 11.34) للمرضى الذين تلقوا العلاج الكيميائي باكليتاكسيل. هناك انخفاض كبير في مستويات المستضد السرطاني 15.3 مع زيادة كبيرة في درجة الاعتلال العصبي ، أي أنه كلما زادت الدرجة

، زاد الانخفاض الكبير في المستضد السرطاني 15.3 ، وجدنا قيمة الارتباط السلبي العكسي (-0.821) بين تركيز Ca15.3 ودرجة اعتلال الأعصاب المحيطية استجابة لعلاج باكليتاكسيل. هناك علاقة عكسية بين تركيز Ca15.3 واستجابة المريض للعلاج الكيميائي paclitaxel ، وعند المقارنة بتردد التنميط الجيني ABCB1 3435G> A (rs1045642) تعدد الأشكال الوراثي ، يستجيب المرضى الذين يعانون من الأليل GG في كروموسوماتهم بشكل أفضل (15.56 ± 6.89) من أولئك الذين لديهم الأليلات AA و GA ، وكذلك المرضى الذين يعانون من الأليل GG من ABCB1 2677G> A (rs2032582) تعدد الأشكال الوراثي لديهم استجابة أكبر للعلاج (195.1714.68) مقارنة مع أولئك الذين لديهم الأليلات AA و GA.

الخلاصة: كشفت الدراسة ان الجين الناقل ABCB1 متعدد الاشكال بدرجة عالية في المريضات المصابات بسرطان الثدي وكانت الأعلى في (rs1045642) 3435 و 22677 (rs2032582)

حيث تعدد الاشكال الجينية للناقل تؤثر في فعالية دواء الباكليتاسيل وان حدوث اعتلال الاعصاب المحيطية هو التأثر الجانبي الشائع لدواء الباكليتاسيل في نساء المصابات بسرطان الثدي . حيث وجد علاقة عكسية بين المستضد السرطاني 3-15 حيث وجد ان قله هذا المستضد وزيادة العرض الجانبي هو اكثر استجابة للعلاج . المتابعة الدقيقة والفحص المستمر مطلوبان للحصول على دواء اكثر استجابة مع اقل تأثير جانبي .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

دراسة تأثير تعدد النمط الجيني لجين ABCB1 مع الاستجابه السريريه لعقار الباكليتاكسيل في النساء المصابات بسرطان الثدي في كربلاء

رسالة

مقدمة الى كلية الصيدلة – جامعة كربلاء

كجزء من متطلبات الحصول على شهادة الماجستير في علم الادوية والسموم

من قبل

دعاء علاء محمد حسن

بكالوريوس صيدلة (كلية الزهراوي الجامعه 2016)

بإشراف

الأستاذ الدكتور

احمد صالح صاحب

2023 ميلادي

الدكتور

احمد عباس حسن

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