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Effect of Genetic Polymorphisms of DPYD metabolizing enzyme on Capecitabine Efficacy in Iraqi Breast Cancer Women

A Thesis

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

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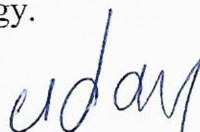

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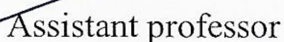

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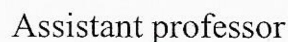

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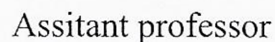

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DEDICATION

The sake of Allah, my Creator and my Master, My great teacher and messenger, Mohammed (May Allah bless and grant him),who taught us the purpose of life and to our parents who have never failed to give us moral support and financial.

To my Wife , to my beloved brothers and sisters for their kindness efforts and for inspiring me to be strong despite many obstacle in life .

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List of Abbreviations

Abbreviation	Meaning/Full form
AEs	adverse events
AIs	Aromatase inhibitors
APBI	accelerated partial breast irradiation
ASCO/CAP	American Society for Clinical Oncology and the College of American Pathologists
ASR	age-standardized incidence rate
BC	Breast cancer
BCS	Breast conserving surgery
BCS	breast-conserving surgical intervention
DCIS	ductal carcinoma in situ
DPD	dihydropyrimidine dehydrogenase
DPYD	dihydropyrimidine dehydrogenase
dThdPase	thymidine phosphorylase
ECOG	Eastern Cooperative Oncology Group
ER	estrogen receptor
FdUMP	5-fluoro-2-deoxyuridine monophosphate
FUTP	5-fluorouridine triphosphate
HDHP	Higher Deduct bling Health Plans
HER2	Human epidermal growth factor receptor 2
HFS	hand-foot syndrome
HR	Hormone receptor
HRP	Horseradish peroxidase
ITGA2	Integrin alpha-2/beta-1
MTHF	methylenetetrahydrofolate
MTHFR	methylene tetrahydrofolate reductase
PCR	polymerase chain reaction
RRM1	ribonucleotide reductase M1
SERMs	selective estrogen receptor modulators
SNPs	single nucleotide polymorphisms
SNPs	single-nucleotide polymorphisms
SNV	single-nucleotide variation
TP	thymidine phosphorylase
TS	thymidylate synthase
TSER	TS enhancer region
TYMS	thymidylate synthase
5'-DFCR	5'-deoxy-5-fluorocytidine
5-FU	5-fluorouracil

Abstract

Background : Breast cancer is the major cause of cancer death in women living in industrialized countries. In Iraq, breast cancer ranked first as a cause of cancer mortality in women and is leading cause at the same time of death in the female population aged 30 to 54 years old. Dihydropyrimidine dehydrogenase (DPYD) gene's product, a pyrimidine catabolic enzyme, is both the first step and the limiting one in the degradation of uracil and thymidine. Dihydropyrimidine dehydrogenase impairment is caused by mutations in this gene. The objective of study was to determine the association between breast cancer, the (G > A) (rs3918290) and of (rs55886062, T >G) polymorphisms of the DPYD gene, the haplotypes formed by them, and the serum concentrations of capecitabine and its active metabolite 5- flourouracil (5FU) in postmenopausal women have breast cancer in Iraq,

Methods: The study was conducted on a total of 200 women, including 100 women without breast cancer who served as a control group aged range from (40-70years) and 100 women with breast cancer whose ages ranged from (45-75years), at the oncology center at Imam al-Hussain medical city in Kerbala, Iraq, were sorted into groups based on their age, body mass index, disease duration, and treatment length for this study, which was done between July 2022 and October 2022.

The concentration of capecitabine, the plasma levels of estradiol, carcinogenic type 15.3 (CA-15.3) and calcium were measured in 100 breast cancer patients who had been taking the drug for at least 3 months, all participants gave their informed consent. The determination of polymorphisms was performed by alelle specific PCR assay for two gene polymorphism (G > A) (rs3918290) and of (rs55886062, T > G) and that of drug concentration of capecitabine and Fluorouracil were calculated by high performance liquid chromatography (HPLC). Statistical analysis was performed with the SPSS and Haploview software.

Results: Serum blood concentrations of CA15.3, Capecitabine, and 5FU in breast cancer patients showed definite and significant variations, the polymorphism (G > A) (rs3918290). Results showed that their parameters concentration were significantly higher in Capecitabine, and 5FU in patients with the TT allele than in patients with the CC and CT alleles, and that it was significantly higher in Capecitabine and 5FU in patients with the CC allele

than in patients with the AA and AC alleles in patients with DPYD*13 (rs55886062). The concentration of Fluorouracil was greater in patients with mutant alleles (p 0.001). The results showed a statistically significant increase (p 0.05) in the concentration of CA15.3 levels in women with breast cancer compared to the control group.

Conclusion: The presence of CA15.3, serum calcium, and estradiol in patient serum makes them potentially valuable novel diagnostic response biomarkers for patients with breast cancer due to their high levels of stability. Additionally, tumour markers, particularly CA15-3, may reflect the biological characteristics of tumours, and they may be useful prognostic indicators in cases of recurrent breast cancer, and genetic variation in DPYD metabolizing enzyme may be contributd to variability in efficacy to capacetabine therapy in asample of breast cancer Iraqi women besides variation in the incidence of adverse effects.

Chapter one

Introduction

Chapter one

Introduction

1. Breast cancer:

1.1. Breast cancer Preface:

A cancer; that originates in the breast tissue may be referred to as breast cancer; Some of the warning signs of breast cancer include a lump in the breast, a change in the circumference of the breast, dimpling of the skin, rejection of breast milk, fluid leaking from the nipple, a newly inverted nipple, or a patch of skin that is red or scaly (Costa *et al.*, 2020). Patients who have the disease spread to their extremities may experience symptoms such as discomfort in the bones, swollen lymph nodes, shortness of breath, or yellowing of the skin. The most prevalent symptom of breast cancer is the presence of a lump in the breast that is distinct in texture from the surrounding breast tissue (Shaikh et al., 2021). When a person feels a lump with their fingertips, more than eighty percent of the time they will uncover a cancerous tumor. Mammograms, on the other hand, are able to detect breast cancer in its earliest stages, lymph nodes located in the armpits can sometimes felt as lumps (Murthy et al., 2021).

1.2. **Epidemiology**:

Breast cancer is the most common cancer among women in developed countries, accounting for 23% of all cancers (Parkins, *et al.*, 2018), and represents the most important location of cancer in women between 20 and 79 years of age. In men, breast cancer can also be present, although it only represents 1% of all diagnosed breast cancers. Breast cancer shows a prevalence of 17.9%, followed by colorectal cancer (11.5%) and prostate cancer (9.6%) and, although its incidence (1.15 million in 2002). It is located behind lung cancer (1.35 million) when considering both sexes, it rate grow

annually by 0.5%, which justifies the importance of its study in terms of public health. Research in breast cancer, and the development of campaigns to control it and better treatment are promoting the high survival observed: 89% at five years, with an average of 75% overall survival in developed countries and a 57% in developing countries. However, despite these advances, breast cancer remains the most common cause of cancer death among women, accounting for up to 14% of deaths (Parkins, et al., 2018).

In Iraq, breast cancer 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 (Alwan, 2016). Breast cancer has been the commonest cancer in females and it accounts for 8.31, 10.5, 11.1, 11.6 and 14.3 percent for 1976-1985, 1986-1988, 1989-1991, 1992-1994 and 1995-1997 respectively, the commonest age group affected was 40-50 years with a relative increase in the incidence among younger age group in recent years (Iraqi Cancer Registry Centre, 1987; 1990; 1993; 1996; 1999; Al Khayatt et al., 2000)

With one million new cases in the world each year, breast cancer is the commonest malignancy in women and comprises 18% of all female cancers (McPherson et al., 2000). In developed countries, breast cancer is the most frequent malignancy among women. Women in their forties should give special attention to calculating their breast cancer risk before deciding on mammography, with some guidelines suggesting screening for those with a 5-year risk similar to women aged 50 (1.1%). Nonetheless, there is a lack of data on the degree of agreement across different models when estimating risk for these female patients (Schonberg et al., 2022).There has been an increase in the incidence of breast cancer all over the world. In the USA, excluding cancers of the skin, breast cancer is the most common cancer among women, accounting for nearly one of every three cancers diagnosed in American women. Between 1990 and 2017, number of newly diagnosed female breast

cancer (FBC) cases increased from 870.2 thousand to 1937.6 thousand, with the age-standardized incidence rate (ASR) significantly increased from 39.2/100,000 to 45.9/100,000 (Chen *et al.*, 2020). As reported by the surveillance, epidemiology and end results program, the incidence rate shows an increase of about 1% per year from 1973 to 1980, more rapid rise in the incidence occured in 1980, it was 84.8 per 100,000 in 1980, with an increase of about 4% per year up to 1987. Between 1987-1995, incidence rates increased by 0.5% per year, (Kelsey et al., 1997).

Similar results are also found in other developed countries. In the United Kingdom, where the age standardized incidence and mortality is the highest in the world, the incidence among women aged 50 approaches two per 1000 women per year, with a prevalence of nearly 2% (Mannu et al., 2020)

In developing countries, it comes next to carcinoma of cervix in Caribbean women, except in some Muslim countries (Fruchter, 1990).

About mortality trends, breast cancer death rate tends to be high in the developed countries, (Santucci, 2020). There has been an important reduction in death rates in recent years in USA. Between 1950 and the late 1980s, over all breast cancer mortality was relatively stable, between 1989 and 1995, death rates decreased by 1.6% annually, between 1995 and 1998, the decrease accelerated to a decline of 3.4% annually (Howe et al., 2001).

In the Eastern Mediterranean Region, the completeness coverage and quality of information available from sources of breast cancer data vary considerably from one country to another. In all these countries, breast cancer is the commonest cancer in females. In Cyprus breast cancer forms 24.2% of all female cancer, in Egypt it forms about 32.7%, in Lebanon it forms 30%, in Morocco 22.3%, in Oman 6.3%, in Qatar 33%, in Sudan 34.5%, and in Palestine 31% of all cancers in women (Onyenegecha, 2021).

1.3 Structure and histology of the mammary gland

The mammary gland is made up of three types of tissue: glandular of the tubule-alveolar type, connective tissue that connects the lobes, and adipose tissue that occupies the spaces interlobular, each mammary gland is made up of fifteen to twenty lobes that flow through a tubular system, into the lactiferous ducts that discharge milk in the nipple after childbirth (Mitchell, & Johnson, 2022). The mammary lobes are made up of numerous lobules that are joined together by connective tissue, blood vessels, lymphatic vessels and by its excretory system, the milk ducts. The lobules, in turn, are made up of ten to one hundred acini (Lawrence, 2022), each with an excretory duct, called terminal duct. The acini are structured by a set of secretory cells that produce milk secretion and form a cavity to which shed this serction. These are surrounded by myoepithelial cells and blood capillaries that actively participate in the secretion and ejection process of milk (Biswas et al., 2022).

The lobules are formed by basophilic columnar cells and myofibrils with a high sensitivity to oxytocin, and are immersed in connective tissue without elastic fibers, the ducts, on the other hand; are made up of a cylindrical or cubic epithelium surrounded by myoepithelial cells, surrounded by connective tissue and elastic fibers. In both cases the secretory epithelial cells constitute the luminal layer, while myoepithelial cells represent the basal layer (Ginter et al., 2020).

The structure of the mammary gland varies with age and is influenced by menstrual cycles, pregnancy and lactation, a stage in which it acquires its greatest development, atrophying after menopause and the absence of hormonal stimuli of estrogen and progesterone (Khachirova., 2022).

1.4 Etiological factors for breast cancer:

The degree of risk for breast cancer is not the same among the general population, although only 12% of patients with breast cancer have an identifiable risk factor. Factors related to breast cancer include the following (Britt et al., 2020; de Ruiter et al., 2021):

1.4.1. Genetic contribution: having a family history of breast cancer or being a carrier of changes in genes (BRCA1 and BRCA2) that contribute susceptibility to breast cancer.

The chance of a woman with a history of breast cancer having cancer in the other breast increases by a factor of three to four. A woman's risk is enhanced, but to a lower degree, if she has ever had breast issues, benign or otherwise. Women who have a first-degree family (a mother, sister, or aunt) with breast cancer are at a greater risk of developing the illness themselves (Hemminki et al.,2012).

1.4.2. Demographic factors: living in high socio-economic levels countries, being a woman, getting older and belonging to a low socio-economic level.

1.4.3. Endogenous factors: its had an early menarche (<12 years), reaching menopause at a late age (>54 years), not having children or having the first child at a late age, not breastfeeding and having a low physical activity. A woman's body generates a lot of estrogen during her reproductive years. Women who menstruate at a younger age and/or go through menopause later in life are subjected to higher estrogen levels for a longer period of time than their counterparts whose menstrual cycles begin later or whose menopause occurs earlier (Ghazanfarpour et al.,2021) and obese after menopause.

1.4.4. Exogenous factors: use of oral contraceptives, treatment with hormone replacement therapy, or exposure to ionizing radiation before adolescence. Cigarette smoking has been linked to a possible, modest increase in the chance of developing breast cancer. The results of

epidemiological research are conflicting, showing either a positive, negative, or no connection at all. Smoking may hasten the development of breast cancer in women who have already been diagnosed with the disease (Bottorff et al.,2014 And Kispert & McHowat., 2017) and drinking alcohol.

1.4.5. Physical characteristics: it was being elevated IGF-1 levels, having an atypical history of benign breast tumors, , low doses of folate and high doses of unsaturated fats and red meat.

Some studies carried out on immigrants from developing countries who have moved to developed countries have shown an increase in the incidence of breast cancer in this population (Chopra, & Vidya, 2021), which clearly indicates that the environmental factors have a significant contribution to the risk of breast cancer. Similarly, those factors opposed to those indicated above will be considered protective factors against breast cancer.

1.5 Classification of breast cancer:

Most tumors that occur in the breast are benign, due to fibrocystic formations that can be painful but not cancerous. However, in cases where there is abnormal and disorderly growth of breast cells, a malignant tumor originates in the breast tissue, or breast cancer, adenocarcinoma represents the most common type of invasive breast cancer, derives from the mammary parenchymal epithelium and is characterized by its ability to invade adjacent tissues and a marked tendency to metastasize to distant tissues such as bone, lung, pleura, liver, glands. adrenals, ovaries, skin and brain (Du et al., 2020).

Breast cancer is classified to various ways: depending on whether it is familial or sporadic in appearance, where in the breast abnormal cell growth occurs, the type of cell affected, the stage of the cancer, and the characteristics of the cancer (Ekici, & Jawzal, 2020).

The two types of breast cancer described according to the type of malignant cell are, ductal carcinoma, where the cells of the ducts are affected and accounts for 90% of cases of breast cancer, and lobular carcinoma, where the cells of the glandular acini or lobular cells become malignant and represents the remaining 10% of cases (Zhao, 2021).

Depending on where the carcinoma is located, two types of breast cancer can be differentiated: carcinoma in situ, which remains confined to the lumen of the ducts or acini, and invasive or infiltrating carcinoma, in which cells proliferate (Pamphlett et al., 2020). Enough to break the basement membrane and spread infiltrating the tissues surrounding the ducts and acini, such as connective and adipose tissue, and blood and lymphatic vessels (Cotran et al., 2000).

According to the cancer stage and the tumor node metastasis (TNM) staging system created by the AJCC (American Joint Committee on Cancer) in collaboration with the UICC (International Union Against Cancer) (Greene and Sobin, 2008), numerical indices are established that indicate the progressive extension of the disease: T, which indicates the extension of the tumor, N, which establishes the infiltration of the tumor to the axillary lymph nodes, adjacent to the breast, and M, which indicates the presence of metastases to other distant tissues of the breast. In addition, based on the degree of differentiation of the tumor, the following categories have been created: well differentiated (grade I) of a milder nature and with better prognosis, moderately differentiated (grade II) and poorly differentiated (grade III), which corresponds to the most aggressive type. Both elements, the TNM value and the degree of differentiation, have been recognized as powerful prognostic factors of the disease (Oldenburg et al., 2007).

A classification that is beginning to be applied in recent years follows a genetic criterion of the tumor, according to the pattern of differential gene

expression observed in a study that included nearly 25,000 genes with constitutive expression in the tissue breast cancer in a total of 261 sporadic breast cancer tumors. Hierarchical grouping analysis of tumors according to gene expression has shown the heterogeneity of breast cancer, which has been molecularly subdivided into five different types: basal tumor, tumor with over-expression of ERBB2, luminal tumor type A, luminal tumor type B and tumor with expression similar to normal breast tissue (Lee et al., 2020).

These breast tumor subtypes have differences obvious clinics. Thus, it has been observed that the majority of tumors in familial cancer patients with mutations in BRCA1 belong to the group of basal tumors, presenting a loss of expression of estrogen receptor (ER), demprogesterone receptor (PR), a total lack of ERBB2 amplification and worse prognosis (Yang et al., 2020). Others tumors have been commonly referred to as “triple-negative” tumors (Garrido et al., 2019). Luminal A subtype tumors, on the other hand, are associated with a better prognosis.

Breast cancer has sometimes been described as a hereditary disease in which a factor of great importance in the risk of developing breast cancer, This indicates a high inherited genetic component that increases the risk of suffering from breast cancer with a high probability, in what has come to be called familial breast cancer, and that it follows a Mendelian pattern of inheritance (Bradbury and Olopade, 2007).

The classification of familial breast cancer has been indicated for that which affects three or more first-degree relatives, with an early age of diagnosis and the presence of ovarian cancer in some cases, characteristics that identify high-risk families (Osorio et al., 2000). On the other hand, familial aggregation breast cancer is considered to be that which affects familial risk, it is useful to assess the risk using pedigree information by age-

at-onset of cancers age of diagnosis more advanced and absence of cases with ovarian cancer (Lalloo, & Evans, 2012).

1.6 Genomic associated with prognosis of breast cancer:

Studies carried out using high-risk families for breast cancer have led to the detection of germline mutations in breast cancer susceptibility genes of high or medium penetrance. These belong, fundamentally, to the group of tumor suppressor genes, which act as sensors of DNA damage and participate in its repair process. The most important genes related to breast cancer are BRCA1, located on chromosome 17q21 (Albertsen et al., 1994) and BRCA2, located on 13q12 (Wooster et al., 1995), the latter also identified as FANCD1 (Barroso, 2009). Classification of variants in the BRCA1 and BRCA2 genes has a major impact on the clinical management of subjects at high risk for breast and ovarian cancer (Agata et al., 2020).

BRCA1 was the first locus found to segregate with breast cancer in a group of families with multiple cases and an early age at death (Sønderstrup et al., 2019). The selection of families with male breast cancer cases led, years later, to the detection of the BRCA2/FANCD1 locus (Wooster et al. ., 1994) at the germinal level, BRCA1 and BRCA2/FANCD1 that appear mutated in 20-35% of hereditary breast cancer cases and represent less than 5% of all diagnosed breast cancer cases, establishing a high susceptibility to the disease throughout life, in combination with a lower risk for other types of cancer (Al-Dallal, 2019). BRCA1 and BRCA2 negative (BRCAX) tumors are characterized, on the other hand, in divergent groups according to the expression of a large group of genes, which indicates heterogeneity of histopathology of these tumors, reinforcing the idea that multiple genetic events are involved in their origin (Takeshita et al., 2020).

Breast cancer; is a complex disease caused by common changes in the population in a number of genes, in combination with environmental factors.

The identification and characterization of these common changes have been studies many years ago. Some of previous studies showed case-control association designing with a selection of 33 candidate genes, 19 of them involved in DNA repair functions, and 14 genes with functions in the cell cycle control, genotyping a total of 169 SNPs in 547 cases of breast cancer (Rasool *et al*., 2022).

The results of association of genotype and phenotype, as well as the functional analyzes carried out have established, mainly, the implication of two genes of the FANC and E2F families in the development of breast cancer was recoded as sporadic (Cree, 2021). A synonymous coding SNP in the FANCD2 gene was associated with the disease, and the search for other putative causal variants identified a de novo change in its region promoter that presented a tendency to be associated with breast cancer. A SNP in 3'UTR of the E2F1 gene, on the other hand, showed association with the disease and was pointed to the same in the system of regulation by microRNAs. Therefore, it has allowed a greater involvement of regulatory SNPs in the characterization of complex pathologies such as breast cancer (Wang et al., 2021).

Sporadic breast cancer, which involves up to 95% of all breast cancer patients, has been defined as a complex disease and is caused by common variants in a large and undefined number of genes, together with environmental factors (Cappetta et al., 2021).

Familial aggregation to breast cancer through linkage studies (Heikkinen et al., 2020). Linkage studies based on families with a high number of affected for a disease have been very useful for the identification of genes that intervene in monogenic disorders, as occurs with familial breast cancer (Mroueh et al., 2022). The application of these linkage studies requires the use of genetic markers with the cumulative risk of breast cancer. It was

estimated at 46% for BRCA1 mutation in women have carriers properties, while BRCA2/FANCD1 mutation carriers have a 43% risk (Mathew., 2006). However, the estimated risks indicated are conditioned by the location of the mutations in the gene by the effect of risk-modifying genes (Francies et al., 2010), and by the type of population. Thus, for example, in the Spanish population these values have been estimated at 52% for BRCA1 mutation carriers and 47% for BRCA2/FANCD1 mutation carriers, at the age of 70 years (Heikkinen et al., 2009).

As with other cancers, breast cancer has been considered a genetic disease in which mutations in DNA repair genes, controllers of apoptotic entry, cell cycle regulators and other functions of importance in the control of cell proliferation, lead to the process of cell malignization that leads to the development of cancer (Kan et al., 2018). Cancer cells arrive, through the above mutation events, at a phenotype with six characteristics that differentiate them from normal cells: they ignore proliferation stop signals, they ignore differentiation signals, they have an indefinite proliferation capacity, they evade apoptosis, have the ability to invasion and are capable of angiogenesis (Dey et al., 2021).

Likewise, breast cancer is considered a complex and heterogeneous disease, due to both a genetic and an environmental component. The genetic component is represented by an indeterminate number of changes in the genome that interact with each other and with the environment and that lead to an estimated risk of suffering from the disease according to a polygenic model (Mavaddat et al., 2019).

The breast cancer, therefore, varies enormously in its clinical behavior, morphological appearance and molecular alterations that it presents, so that different types of breast cancer will have characteristic risk profiles, which may show that they follow a different aetiology. The description of the

genetic and environmental component of the development of breast cancer has become a fundamental important studies (Garcia-Closas et al., 2008).

1.7 Factors Influence Breast Cancer Survival

1.7.1 Time Since Diagnosis

1: Based on the most recent data relative survival rates for women diagnosed with breast cancer are 86%, 76%, 58% and 53% for 5, 10, 15 and 20 years after diagnosis respectively.
2: Based on recent analysis of long-term cancer patient survival, among women who have already survived five years after diagnosis with breast cancer, 81% of white women and 76% of black women are expected to survive an additional five years. Among women who have already survived 10 years after diagnosis, 87% of white women and 85% of black women are expected to survive an additional five years. There is a higher incidence of aggressive breast cancer in African-American women, as well as a higher prevalence of obesity, compared to white women. (Abdou *et al*., 2020).

1.7.2 Age at Diagnosis:

The 5 years relative survival rates for breast cancer increase with age at diagnosis until age 75 years. It reached 82%, 86%, 87%, 88% and 84% for women aged <45, 45-54, 55-64, 65-74, ≥ 75 years respectively (Ries *et al*., 2001).

Researchers speculate that younger women have lower survival rates because their tumors may be more aggressive and less responsive to hormonal therapies (Erić *et al*., 2018).

1.7.3 Race and Ethnicity:

Black women with breast cancer are less likely than white women to survive 5 years; 72% Vs. 87% (Ries et al., 2001). Just over half of this difference can be attributed to later stage at detection and tumors that are more aggressive and less responsive to treatment. The presence of additional illnesses and various socio-demographic factors also contribute to the observed differences in survival between blacks and whites (Hunt et al., 2019).

1.7.4 Socioeconomic Factors:

A lack of health insurance is associated with lower survival among breast cancer patients. Also breast cancer patients with low incomes have lower 5 years relative survival rates than higher-income patients, and low-income black women are three times more likely than higher-income black women to be diagnosed with advanced disease (Yedjou et al., 2019).

Some studies implied that Higher Deduct bling Health Plans Experience (HDHP)-associated delays in breast cancer care are only partially connected to patients' sociodemographic characteristics and that women across the socioeconomic range may encounter high out-of-pocket expenses as a barrier to breast cancer care. The delay of around five to seven months in diagnosing early-stage breast cancer in women across the sociodemographic range may be indicative of inferior breast cancer outcomes (Wharam et al., 2019).

1.8. Protocol for breast cancer treatment:

1.8.1 Surgical treatment:

During the planning stage of surgery, both the breast and the ipsilateral axilla need to be taken into consideration. Breast conserving surgery (BCS) and mastectomy are two types of breast surgery (Maloney et al., 2018).

Surgery for the axilla consists of an Sentinel Lymph Node Biopsy (SLNB) as well as an Axillary lymph node dissection (ALND), when selecting various surgical procedures, the TNM stage of the patient as well as their current physical condition should be taken into consideration (Magnoni et al., 2020).

Patients with locally advanced breast cancer or patients with established distant metastases who are down-staged by systemic therapy may also be candidates for mastectomy, provided they do not have any contraindications to the procedure. The breast and pectoral muscles are both cut out during a Halsted mastectomy (Zhang et al., 2021). Halsted mastectomy has been replaced by modified radical mastectomy because of the high rate of complications and the considerable surgical trauma it causes. Although the fascia covering the pectoralis major muscle is routinely removed during a mastectomy, some researchers have found that it can be preserved during subsequent breast reconstruction procedures (Canturk et al., 2021).

It is important to pay close attention to the BCS indicators. In order to ensure that negative surgical margins are achieved, institutions that do BCS should be prepared with the necessary tools and procedures for performing histological examinations of the BCS specimens. In addition, the essential equipment for postoperative radiation must be obtained (Kordon et al., 2021).

1.8.2 Radiation:

In general, patients who have undergone BCS are required to undergo radiation. It is possible that radiotherapy may not be necessary in some cases, such as when the patient is over 70 years old, the tumor is less than 2 centimeters in size, the lymph nodes are negative, or the ER gene is positive (Li & Tse, 2022).

Preliminary investigations using accelerated partial breast irradiation (APBI) reveal that rates of local control in certain individuals with early-stage breast cancer may be equivalent to those treated with traditional whole breast radiation therapy. This is the conclusion drawn from the findings of the studies (Whelan et al., 2019).

Regional nodal irradiation is recommended for patients who have undergone ALND with 1-3 positive lymph nodes in order to minimize the risk of recurrence. However, regional nodal irradiation is not necessary for patients who have a low risk of recurrence. Patients who have a low risk of recurrence can avoid regional nodal irradiation (Johnson et al., 2019).

1.8.3 Chemotherapy:

Therapeutic Radiation and Chemotherapy in Advancement of Surgery Adjuvant chemotherapy may be recommended if the potential benefit outweighs the risk after a thorough evaluation of the patient's health (age, menstrual status, blood test results, vital organs' function, comorbidities, etc.), tumor characteristics (histological type, tumor grade, lymph node status, HER2 and hormone receptor status, lymphovascular invation), and potential treatment strategies (Moo et al., 2018).

Treatment of advanced breast cancer with chemotherapy treatment aims primarily at enhancing patients' quality of life and extending their vitality rather than curing them, cancer treatment often consists of chemotherapy and endocrine therapy. However surgery and radiation therapy may also be used, the initial tumor, the patient's history, the length of time they've been cancer-free, the location and rate of metastasis, the rate at which the disease is progressing, and other considerations are all taken into account when formulating a personalized treatment plan (Ferreira et al., 2019).

A number of different chemotherapy medicines, such as anthracyclines, taxanes, vinorelbine, capecitabine, gemcitabine, and platinum, are typically utilized in the treatment of advanced breast cancer. A patient should have an individualized treatment plan designed for them, with consideration given to the patient's previous treatment, the extent of the tumor, and its molecular characteristics. Patients at various stages should be given either single-agent chemotherapy or polychemotherapy, depending on what treatment option is best for them (Li et al., 2020).

1.8.4 Endocrine therapy:

Patients with Estrogen and/or Progestron-positive invasive breast cancer should be treated with adjuvant endocrine treatment. Guidelines from the American Society for Clinical Oncology and the College of American Pathologists (ASCO/CAP) state that tumors with 1%-100% ER immunohistochemical staining are ER-positive, while 1%-10% staining suggests modest ER expression. When deciding on a course of treatment, it is important to keep in mind that the biological activity of tumors with ER low expression is typically comparable to that of ER-negative breast cancer, and there is limited advantage regarding adjuvant endocrinology (Allison et al., 2020).

Oestrogen's carcinogenic effects appear to be mediated by its action on receptors, which results in cellular proliferation. Endocrine therapy is vital for women with estrogen-receptor-positive cancers (Burstein, 2020). It includes selective estrogen receptor modulators, aromatase inhibitors, and ovarian suppression medications, endocrine therapy for early-stage breast cancer lasts five years. Several studies support 10-year hormonal therapy, long-term negative effects and risks should be weighed against advantages (Fastner et al., 2020).

1.8.4.1 Selective estrogen receptor modulators (SERMs):

To reduce the effects of oestrogen, selective estrogen receptor modulators (SERMs) are used to block the receptor's binding to oestrogen and affect the receptor's activity by influencing its associated cofactors. According to their molecular makeup, SERMs are broken down into four classes: triphenylethylenes (tamoxifen), phenylindoles (bazedoxifene), benzothiophenes (raloxifene), and tetrahydronaphthalenes (lasofoxifene) (Das et al., 2022). Tamoxifen acts as a competitive inhibitor of estrogen receptors, inducing conformational changes in these receptors to decrease their transcriptional activity. A number of additional selective estrogen receptor modulators (SERMs) have been tried, but none of them has proven to be clearly superior to tamoxifen in the prevention or treatment of breast cancer (Zhou et al., 2021).

1.8.4.2 Selective estrogen receptor degraders:

They are anti-estrogens that are designed to attach to the estrogen receptor and stimulate its breakdown, so preventing the estrogen receptor from dimerizing and putting a stop to the estrogen receptor signaling cascade (Patel & Bihani, 2018). One example of a selective estrogen receptor degrader is fulvestrant; this type of drug is useful for treating hormone-sensitive forms of advanced breast cancer in women (Bushweller, 2019). It is not only effective at blocking the nuclear estrogen receptor, but also the cytoplasmic and membrane-bound estrogen receptors as well (Balaguer et al., 2019).

1.8.4.3 Aromatase inhibitors (AIs):

An inhibiting the activity of the microsomal aromatase enzyme, which is responsible for the conversion of testosterone to estrogen, is the goal of aromatase inhibitors, a class of anti-estrogen drugs (Zucchetti et al., 2019).

About 25 years ago, aminoglutethimide, an aromatase inhibitor from the first generation, became available, due to its severe toxicity and lack of selectivity for aromatase, aminoglutethimide was unable to compete with chemotherapy despite its efficacy. Formestane, an aromatase inhibitor introduced in 1993 with fewer adverse effects than aminoglutethimide, represents the second generation of anti-estrogen drugs. The middle of the 1990s saw the development of the third generation of aromatase inhibitors (Iannone et al., 2019).

The steroidal drugs, like exemestane, are irreversible competitive inhibitors of aromatase, while the nonsteroidal compounds, like anastrozole and letrozole, are reversible competitive inhibitors (Sabale et al., 2018).

1.9. Capecitabine:

Capecitabine is a one-of-a-kind treatment that is specific to the S phase of the cell cycle. It is an antimetabolic fluoropyrimidine deoxynucleoside carbamate and is administered orally (Alqahtani et al., 2022). The most probable place for the thymidine phosphorylase (dThdPase)-catalyzed conversion of capecitabine into 5-fluorouracil (5-FU) to take place in vivo is in tissues that are already carrying malignancies(Jurczyk et al.,2021) .

Capecitabine is a white to off-white crystalline powder with an aqueous solubility of 26 mg/mL at 20ºC(Cortés-Funes, 2006).The full chemical name for capecitabine is 5'-deoxy-5-fluoro-N-[(pentyloxy)carbonyl]-cytidine. Capecitabine has a molecular weight of 359.35 and is used to treat cancer(Hepokur et al.,2019) .

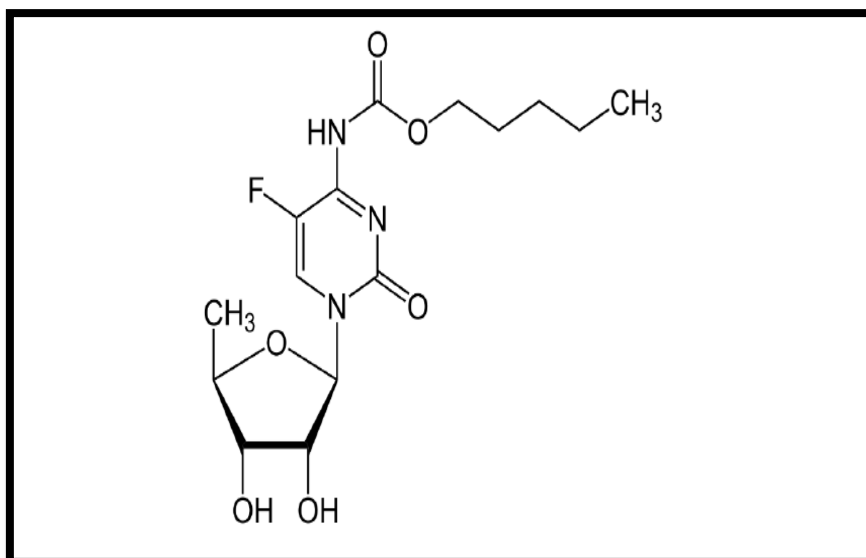


Figure (1-1): Capecitabine chemical structure(Koukourakis et al.,2010).

1.9.1 Capecitabine Pharmacokinetics:

Capecitabine peak concentrations were observed at the first observation after drug administration. First-order transit absorption model including four transit compartments most accurately described the absorption process but resulted in a slight underprediction of maximum plasma concentrations (Cmax) of capecitabine and metabolites. Increasing the number of transit compartments did not result in a significant improvement of the model fit. Visual inspection of the random-effect distribution on the transit rate constant (k tr) suggested that capecitabine absorption occurred relatively fast for the patients who previously underwent gastrectomy for advanced gastric cancer.

1.9.2 Capecitabine Pharmacodynamics:

Capecitabine is a fluoropyrimidine carbamate having anti - neoplastic action suggested for the treatment of metastatic breast cancer and colon cancer. It is an oral route systemic prodrug that has minimal pharmacologic

activity unless it is transformed to fluorouracil by enzyme which are synthesized in greater quantities in several cancers. 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate were produced after fluorouracil was oxidized in both healthy and malignant cells (FUTP) (Kalman, 2022).

The detoxifying metabolic of which is caused by the enzyme dihydropyrimidine dehydrogenase (DPD) (Wörmann et al., 2022). Individual people who are homozygous mutant or heterozygous for genetic variations within the DPD locus (DPYD) may have a partial or total DPD deficient; an approximate 0.2% of patients have absolute DPD deficiency (Lampropoulou et al., 2022). Neurotoxicity, Myelosuppression and foot-hand syndromes are just few of the toxicities associated with fluoropyrimidine treatment that are much more likely in patients with partial or total DPD deficiency (Neumann & Hoyte, 2021).

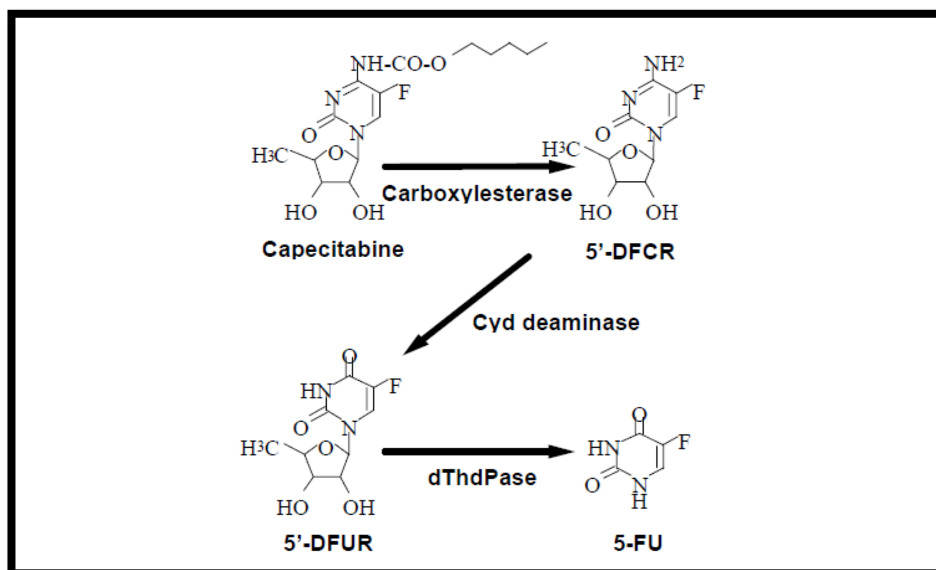
1.9.3 Bioactivation:

Capecitabine is absorbed quickly in the body once it has been given orally. The majority of the material is converted to 5'-deoxy-5-fluorocytidine (5'-DFCR) by a carboxyesterase in the liver that has a molecular weight of around 60 kilodaltons.(Brzezinski et al.,1994 and Ait Tihyaty ,2014).

According to studies, capecitabine has a high bioavailability when taken by mouth. The drug capecitabine is inactive and must undergo three additional activation processes, each of which must be catalyzed by an enzyme before it can be used. The two processes that came before it were called de- esterification, which occurred in the first step, and deamination, which occurred in the second step. However, the third step, which is the conversion of 5'-deoxy-5-fluorouridine (5'-dFdU) to 5-fluorouracil (5-FU), is carried out by the enzyme thymidine phosphorylase (TP); happens in the tissue of the tumor, making it possible for 5-FU to be selectively activated in

the region of the target. Additionally, the levels of TP are much higher in tumor tissues (Rautio et al.,2008 and Che et al.,2017).

Cytidine deaminase is an enzyme that is found in most tissues as well as cancers. It is responsible for the conversion of 5'-DFCR to 5'-deoxy-5-fluorouridine (5'-DFUR). The enzyme known as thymidine phosphorylase (dThdPase) is the one that is accountable for the conversion of 5'-DFUR into 5-FU. The enzyme thymidine phosphorylase is expressed in a broad array of tissues throughout the body. When compared to normal human tissues, the levels of expression of various carcinomas of this enzyme are much higher (Amly & Karaman.,2019).



5′dFCR : 5'-Deoxy-5-fluorocytidine ; 5'-DFUR: 5'-deoxy-5-fluorouridine ; dThdPase : thymidine Phosphorylase and 5-FU: Fluorouracil

Figure (1-2): Bioactivation of Capecitabine (Tabata et al.,2004).

1.9.4. Metabolism of Capecitabine:

To improve targeting of the drug's active moiety of Capectabine to 5-fluorouracil (5-FU), this fluoropyrimidine carbamate was developed to survive passage through the human intestinal mucosa (Shimma et al., 2000). It has been postulated that CAP activation involves three distinct enzyme processes that must occur in order to get 5fu (Bajetta et al., 1996). Carboxylesterase and cyti-dine deaminase are two enzymes mostly found in the liver that convert CAP to 5'-deoxy-5'-fluorocy-tidine (5'-DFCR) and 5'-deoxy-5'-fluorouridine (5'-DFUR), respectively (Budman et al., 1998; Miwa et al., 1998). Thymidinephosphorylase, whose activity is elevated in tumour tissue (Ishikawa et al., 1998), converts systemic 5-DFUR to 5-FU figure (1.3).

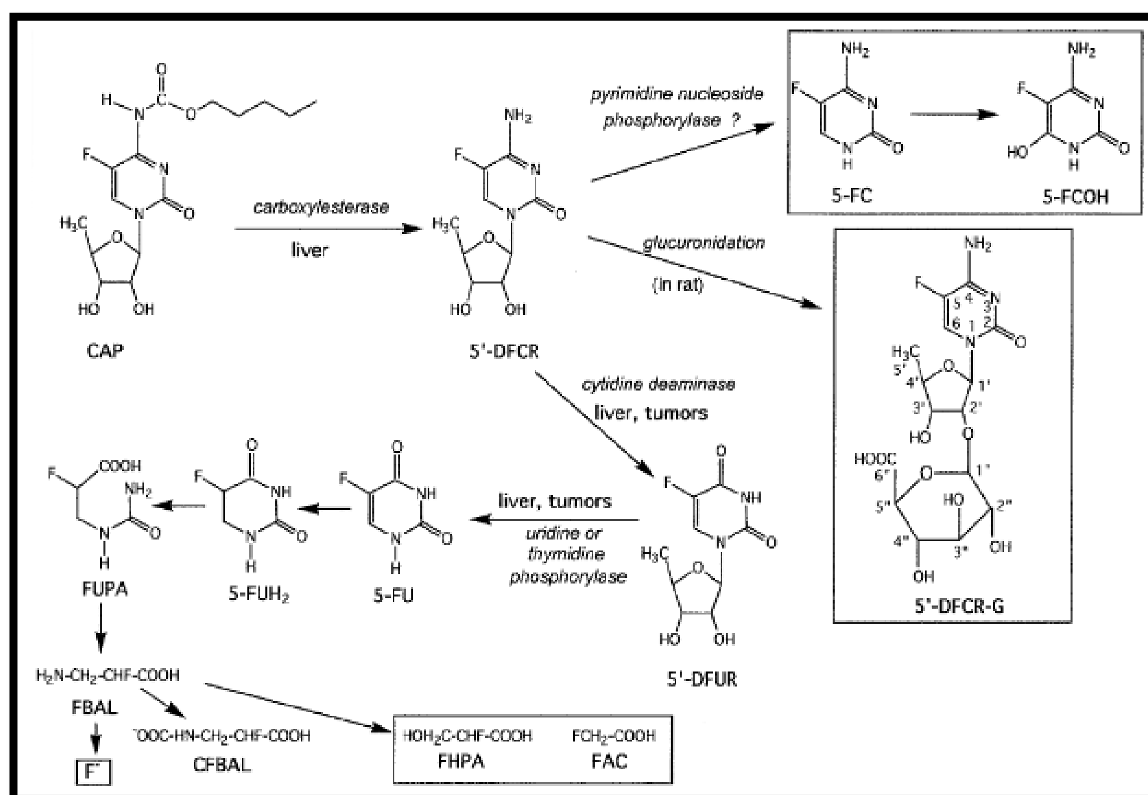


Figure (1.3): metabolism of Capectabine chemotherapy (Ishikawa et al., 1998).

However, 5-fluorouracil is broken down into two active metabolites in both healthy and cancerous cells. These metabolites are 5-fluorouridine triphosphate (FUTP) and 5-fluoro-2-deoxyuridine monophosphate. Both of these metabolites are necessary for the cell's survival (FdUMP)(Chalabi-Dchar et al.,2021).

Since FUTP is not a strong competitor in comparison to uridine triphosphate, it is responsible for the inhibition of the creation of RNA and protein, because FdUMP causes interference with thymidine synthesis(Danesh Pouya et al.,2022) , it also slows down the process of DNA synthesis. Dihydropyrimidine dehydrogenase (DPD) may convert fluorouracil into dihydrofluorouracil, which is not hazardous (Sethy & Kundu (2021).

It has been approved that Capecitabine to treat a variety of cancers, including metastatic breast cancer that has not responded to treatment with paclitaxel and an anthracycline, metastatic breast cancer that has progressed despite treatment with paclitaxel and an anthracycline, and metastatic colorectal cancer when combined with docetaxel. Other cancers have also been approved for treatment with Capecitabine (Hong & Xu.,(2022)

1.9.5. Capecitabine mechanism of action:

The end products of 5-FU metabolism include 5-fluoro-2-deoxyuridine monophosphate, also known as FdUMP, and 5-fluorouridine triphosphate (FUTP), for both normal cells and malignant cells(Clua et al.,2021). These compounds are responsible for two distinct forms of damage to cellular structures. Beginning with methylenetetrahydrofolate (MTHF), thymidylate synthase (TS) creates a ternary complex with FdUMP and the folate cofactor N5-10-covalently binding to TS (Patel et al.,2012).

This complex is then catalyzed to produce thymidylate. Because of this interaction, the production of thymidylate starting from uracil is inhibited. The process of cell reproduction may be stifled when there is insufficient thymidylate in the body. Thymidylate is a precursor to the chemical thymidine triphosphate, which is required for DNA synthesis (Niehaus et al .,2022). The second concern is that FUTP may be taken up by nucleus transcriptional enzymes during RNA synthesis instead of uridine triphosphate (Kohli & Omray ., 2022). This may cause problems for the

synthesis of RNA (UTP). If this metabolic mistake happens, there may be a problem with the processing of RNA as well as the creation of proteins.

Because capecitabine and other fluoropyrimidines contain active metabolites, they may be affected by dihydropyrimidine dehydrogenase (DPD) insufficiency(Sukkarieh et al.,2022) . DPD insufficiency is a disorder that is caused by mutations in the DPD gene. These mutations result in greatly decreased or absent activity of the enzyme that is responsible for metabolizing these compounds (eg, fluorouracil [5-FU]). It is estimated that between 3 and 8 percent of the population has a DPD deficit. However, the prevalence of this condition varies greatly across various ethnic groups. Patients who have low levels of dihydropyrimidine dehydrogenase (DPYD) have an increased risk of experiencing major adverse effects from capecitabine, including mucositis (Del Pozzo-Magaña & Liy-Wong.,2022). Other adverse effect in some patients who have DPD deficiency should not get treatment with fluoropyrimidines, including capecitabine, testing for this issue should be explored in patients who experience unusually severe toxicity from capecitabine(Diasio & Offer.,2022).

1.9.6. Side effect of Capecitabine:

The dosage get with capecitabine determines whether or not will have a diarrhea. may include: nausea, vomiting, diarrhea, stomach pain; feeling weak or tired; hand and foot syndrome; or jaundice, despite the fact that it is occasionally necessary to treat it with anti-motility medicines such as loperamide (Dao et al.,2019)

The therapy with capecitabine often only causes minor cases of nausea and vomiting. Patients are often prescribed anti-nausea medication to take prior to beginning chemotherapy in order to reduce the likelihood of experiencing adverse side effects. If the patient has nausea or vomiting after taking the preventative drug, the doctor may prescribe additional medication for the patient to take (Kumar et al.,2022.)

The palmar-plantar erythrodysesthesia, which is also known as hand-foot syndrome (HFS), is characterized by erythema, oedema, and dysesthesia. It also has the potential to progress into blistering and ulceration. This is a side effect of the chemotherapy medication capecitabine that is widely reported by patients.It has been documented that either capecitabine alone or capecitabine coupled with lapatinib may induce hemolysis, and each time it does so by a mechanism that is not immune-related. In this particular instance of autoimmune haemolytic anemia, capecitabine is the suspect that has the highest probability of being the causative agent (Kwakman et al.,2020 and Lee & Zheng., 2021).

1.10 Effect of Genetic variation on patient with breast cancer:

A single-nucleotide polymorphism is a type of genetic variation that occurs when there is a change in the germline sequence of a single nucleotide at a particular location in the genome. Many publications (Venter et al., 2001 and Consortium et al., 2015) do not apply such a frequency requirement, despite the fact that certain definitions need the substitution to be present in a sufficiently significant fraction of the population (for example, 1% or more) (Yi & Ju, 2018).

For instance, at a certain base location in the human genome, the G nucleotide may be present in the majority of individuals, but an A may be present in a minority of individuals. This phenomenon is known as allelic diversity. This indicates that there is an SNP located at this particular place, and the two possible changes in the nucleotide sequence - either G or A -are referred to as the alleles for this particular position (Monga et al., 2017).

An alteration in the DNA sequence that only involves one nucleotide is referred to as a single-nucleotide variation, or SNV for short. Therefore, an SNV can be a common SNP or a rare mutation; it can be germline or somatic; it can be induced by cancer; nonetheless, an SNP must segregate in the

population of organisms that make up a species in order to be considered valid. SNVs also frequently occur in molecular diagnostics, such as the process of generating PCR primers to identify viruses, in which the viral RNA or DNA sample may contain SNVs. This is one example of where SNVs commonly occur (Katsonis et al., 2014).

It is possible for single-nucleotide polymorphisms to occur in the coding sequences of genes, the non-coding portions of genes, or the intergenic regions between genes (regions between genes), due to the degeneracy of the genetic code, single nucleotide polymorphisms (SNPs) that occur inside a coding sequence may not necessarily change the amino acid sequence of the protein that is generated (Minotti et al., 2018).

There are two different kinds of SNPs that can be found in the coding region: synonymous SNPs and nonsynonymous SNPs. Synonymous single-nucleotide polymorphisms (SNPs) have no effect on the protein sequence, but nonsynonymous SNPs alter the order of the amino acids in the protein (Hunt et al., 2009).

1.10.1. Dihydropyrimidine dehydrogenase gene Polymorphism:

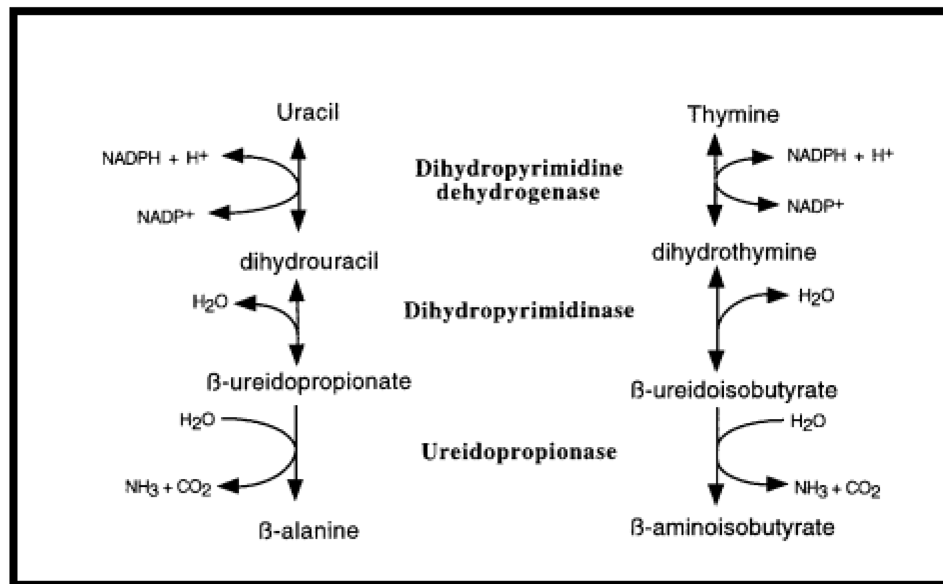
Dihydropyrimidine dehydrogenase is an enzyme that helps break down uracil and thymine when they are no longer needed in the cell, and it is encoded by the DPYD gene. Pyrimidines like uracil and thymine are a class of nucleotides. The nucleotide base is the fundamental unit of all nucleic acids, including DNA, RNA, and the energy-transfer molecules ATP and GTP (Sharma et al., 2019).

In the initial stage of the decomposition of pyrimidines, the enzyme dihydropyrimidine dehydrogenase plays an important role. This enzyme changes the structure of uracil so that it becomes another molecule known as 5,6-dihydrouracil. It also changes the structure of thymine so that it becomes 5,6-dihydrothymine. The body either eliminates the molecules that are

produced as a byproduct of the breakdown of pyrimidines or uses them in other cellular processes (Farinango et al., 2022).

The mechanism for the degradation of pyrimidine is present in the liver of human, Dihydropyrimidine dehydrogenase is the initial enzyme and rate-limiting enzyme in the catabolism of the pyrimidine bases; it catalyzes the reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine, respectively. The reduction of uracil and thymine to 5,6-dihydrouracil and 5,6-dihydrothymine occurs in three steps In the second phase, a hydrolytic ringopening is performed, which is mediated by dihydropyrimidinase. In the last step, -ureidopropionic acid (N-carbamyl-alanine) or -ureidoisobutyric acid (N-carbamyl—amin-oisobutyric acid) is transformed to -alanine or –aminoiso) (Van Kuilenburg et al., 1999) figure 2.

In children, a deficiency of DPYD is frequently accompanied with a neurological illness; however, great diversity in the clinical presentation of this condition has been recorded among the patients who suffer from it, as well as congenital thymine-uraciluria in juvenile patients and severe 5-fluorouracil toxicity in cancer patients have both been linked to a deficit in human DPD (Wei et al., 1998).



Figure(1-4): Catabolic pathway of the pyrimidines uracil and thymine (Al Khallaf *et al.,* 2013)

Dihydropyrimidine dehydrogenase (DPD), the first and rate-limiting enzyme in the pyrimidine degradation pathway, degrades fluoropyrimidines. The increased exposure to active metabolites caused by DPD deficiency might cause serious or even lethal poisoning (Sharma et al., 2019).

According to the previous studies, between 3 and 5 percent of the Caucasian population is DPD deficient, most occurrences of this deficit can be traced back to inherited forms of dihydropyrimidine dehydrogenase (DPYD) (Amstutz et al., 2011).

Only a small number of the variations that found in the DPYD gene have been investigated in relation to decreased DPD enzyme function and/or toxicity. The DPYD gene is located on chromosome 1p22 and contains 23 exons. Only three variants have been identified as being consistently related with toxicity and decreased DPD enzyme activity in patients who were treated with a fluoropyrimidine drug (Caudle *et al*., 2013).

Multiple techniques have been described for either directly or indirectly identifying DPD deficiency in a patient. There are benefits and drawbacks to

each of these approaches, and no single assay has been declared to be the best successful at predicting toxicity while also being highly sensitive and specific and cost-efficient (Van Staveren et al., 2013).

It is becoming increasingly important to identify polymorphisms in the dihydropyrimidine dehydrogenase (DPYD) genes as predictors of fluoropyrimidine-associated toxicity. It is possible that the recommendation of dose adjustment for chemotherapy that is guided by the presence of polymorphisms of the DPYD gene can potentially improve treatment safety for a large number of patients, potentially saving lives, avoiding complications, and reducing the costs of medical care (Donadio et al., 2022).

The fluoropyrimidine toxicity results from a system that is intricate and multi-step, and it is responsible for the drug's metabolism as well as the metabolism and excretion of its compounds. The dihydropyrimidine dehydrogenase (DPD) enzyme, which is called for by the DPYD gene, is involved in one of the most important steps in the cascade of 5-FU metabolism. Polymorphisms have the potential to eventually alter drug metabolism, which can lead to increased drug buildup and toxicity. The genetic factor is the primary factor responsible for this enzyme activity (Meulendijks et al., 2017).

Six DPYD variants were examined in a retrospective pharmacogenetic analysis for correlations with grades fluoropyrimidine-related adverse events: (*2A rs3918290 G>A, *13 rs55886062 T>G, rs67376798 A>T, *4 rs1801158 G>A, *5 rs1801159 A>G, *6 rs1801160 G>A) (Ruzzo et al., 2017).

1.11 Genetic polymorphism studies associated with Capecitabine metabolism:

Capecitabine is a medication that calls for the sequential activity of three: enzymes carboxylesterase 2 (CES 2), cytidine deaminase (CDD), and thymidine phosphorylase (TP). The breakdown of 5-fluorouracil (5-FU) is dependent on the activity of a number of enzymes, including dihydropyrimidine dehydrogenase (DPD) and thymidylate synthase (TS) (Ribelles et al., 2008).

In order to better understand and predict inter-individual diversity in response and extreme toxicities during treatments, pharmacogenomics has emerged as a promising field of study. There is a growing body of literature on 5-fluorouracil (5-FU) pharmacogenomics (lam *et al*., 2016), and at least two large prospective genetic screening studies (Offer et al., 2013) have focused on polymorphisms in DPYD, which encodes for dihydropyrimidine dehydrogenase, the enzyme that catabolizes 5-fluorouracil. Clinical guidelines have been developed that suggest patients with known deleterious polymorphisms in DPYD receive preventative dose reduction or, in extremely rare cases, avoidance of fuoropyrimidines altogether (Amstutz et al., 2018). Despite this, most doctors do not check patients for pharmacogenomic risk factors like 5-fluorouracil (5-FUR) before prescribing fuoropyrimidines.

Accurately predicting which patients will respond to capecitabine and which will experience adverse events (AEs) is preferable because it would allow for more efficient selection of responders and the avoidance of potentially life-threatening AEs, both of which would increase clinical benefit. The way an individual reacts to a drug can be affected by host-related genetic variations, most commonly single-nucleotide polymorphisms (SNPs). More specifically, single-nucleotide polymorphisms (SNPs) in enzymes responsible in capecitabine oxidation may impact the rate of transformation of capecitabine to 5-FU and reflect possible candidates for the predictive model of therapeutic efficacy and toxicity (Lam et al., 2018).

Different cytostatic agents vary in effectiveness and toxicity, the variability is attributed to genetic and non-genetic patient (age, sex, major bodily function status, and concurrent medication) and cancer variables. Genetic variables account for 20-95% of pharmacokinetic and pharmacodynamic variability. Most cytotoxic drugs' target or metabolic enzymes contain genetic polymorphisms, which could explain some of these variances (Narendra et al., 2022).

The two most investigated enzymes from a pharmacogenomic perspective are TS (thymidylate synthase polymorphism) and DPD (dihydropyrimidine dehydrogenase polymorphism). The TS gene is polymorphic due to a varied number of copies of a 28-bp tandem repeat in the TS enhancer region (TSER). The most common variants have TSER2 and TSER3 repeats.

Some of studies demonstrated that a correlation between the existence of the TSER3/3 genotyping polymorphism in tumor cells and a reduction in the efficacy of therapy with 5FU, according to research conducted on patients suffering from colorectal cancer (Aghabozorgi et al., 2020).

The importance of DPD deficiency in the emergence of severe 5-fluorouracil-related toxicity has been highlighted by a number of studies (Ezzeldin, & Diasio, 2014). It has been shown that germline polymorphisms are responsible, at least in part, for DPD abnormalities (van Kuilenburg et al., 2017). According to studies of the frequency of DPYD gene mutations (19, 20), the G>A mutation is the most common functional mutation, though its frequency is surprisingly low, at only 0.9% in the general Caucasian population, homozygosity for this mutation, which causes the entire exon 14 to be skipped, results in an absence of DPD activity (Ezzeldin, & Diasio, 2014).

There is a substantial association between the upregulating of the thymidylate synthase (TS) peptide and a decline in the efficacy of

Capecitabine, which has been described by (Hamal et al., 2018). It has been demonstrated that people who lack the DPD enzyme have a significantly increased likelihood of experiencing severe toxicity as a result of taking Capecitabine in morocco (Zouine et al., 2022).

On the other hand, three genes critical to the metabloism of capecitabine chmotherapy, and checked for variations in gene polymorphism. In one study (Lin et al., 2019) involving 342 patients with Metastasis Breast Cancer who were given capecitabine-based chemotherapy, 22 SNPs were genotyped in the genes encoding thymidylate synthase (TYMS), methylene tetrahydrofolate reductase (MTHFR), and ribonucleotide reductase M1 (RRM1). Genotype frequencies at each SNP among patients were distributed as predictor for MTHFR rs3737964, MTHFR rs4846048, and TYMS rs2606241 polymorphism to capecitabine chmotherapy response.

1.12 Aim of study:

1- To investigate the distribution of genotypes association of metabolizing enzymes of capacetabine in Iraqi breast cancer women
2- To investigate the effect of genetic polymorphism of metabolizing enzyme DPYD on the efficiacy of capactabine
3- To correlate the chemical efficacy of capactabine with genetic polymorphism of metabolizing enzymes and plasma drug concentration
4- To detect the association of DPYD genetic polymorphism with incidence of adverse effect specially hand foot syndrome.

Chapter two

Patients, Materials and Methods

Chapter two

2. Materials and Methods

2.1. Materials:

The instruments and equipment used in this study with their remarks were listed below.

2.1.1 Equipments:

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

Equipment	Company	Country
0.2ml pcr strips and caps	Bioneer	Korea
100-1000 Sterile Tap rackc Ml	Bioneer	Korea
EDTA (3ML)	Orisn	China
Eppendorf tubes 1.5ml and 200 μL	Ataco	China
Gel tube (6ml)	Fcovaca	Jordan
Gloves	Adwic	Egypt
Graduated cylinder	Bioneer	Korea
Labrotrary spoon	Bioneer	Korea
Micropipette	Biobasic	Canada
Natural 10-200mL Tips strile	Bioneer	Korea
Rack	Medeco	China
Tips	Medeco,	China

Table (2-2): The devices which used in the study.

Devices	Company	Origin
Deep freezer (-80°C)	Nuair	Japan
Electronic Balance	mettlerTdedom	USA
Electrophorises Gel	Bioneer	Korea
ELISA printer	Epson	USA
ELISA Reader & washer	Bio tech	UAS
Freezer (-20°C)	Samsung	Korea
Gel decuiment	Bioneer	Korea
Microwave	showinc	Thailand
PCR	Bioneer	Korea
Microcentrifuge Tube	Daiham	Korea
Vortex	smAClay	Germany
Water Bath	Daiham	Korea

2.1.2 Chemicals and solutions

Table (2-3): The chemicals utilized in the study

Chemicals	Company	Country
Absolut ethanol (99%)	Bioneer	Korea
Agarose	Bioneer	Korea
Blue Master Mix	Bioneer	Korea
DNAladder 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 Methods

2.2.1 Samples collection:

2.2.1.1 Patients:

The study was approved by the medical ethics committee of Iraqi Ministry of Health and was done on 100 Iraqi patient postmenopausal women (diagnosed with breast cancer). The patients attended to tumor unit at Al-Hussein Medical Oncology Hospital in Kerbala for diagnosis and treatment during the period July/2022 to October /2022. In addition to that, one handered healthy women aged matched were included in this study and named as control groups.

The disease was diagnosed by consultant medical staff at the hospital by using diagnostic criteria, One hundred patients (average age 45–75 years) and one hundred apparently healthy controls (age 40–70 years) were enrolled in the study, and the diagnosis was made based on clinical, mammographic, and histological findings, chemotherapy (capecitabine) was administered to patients after early dignosed of their condition.

2.2.1.2 Sample collection

The serological study was carried out by using ELISA technique, while molecular study was carried out by using PCR technique ,5ml of peripheral blood had been drawn from each patients. Then blood samples were divided in two part (3 ml) in Gel tube for serological study and (2 ml) in EDTA tube for genotyping test and tubes placed in deep freeze -20˚C until used for estimation of estradiol, calcium and Ca15.3 tumor marker.

2.2.1.3. Inclusion Creteria :

Postmenopausal women > 45 years; just get capacetabine chemotherapy in therir course treatment

2.2.1.4 Exclusion criteria:

The exclusion criteria included: other cancer, previous treatment chemotherapy, preganacy and breast feeding, contraceptive drugs, women had started Capecitabine therapy simultaneously either with adjuvant chemotherapy or adjuvant radiation therapy (or both). Also, women were taking drugs that affect the activity of dihydropyrimidine dehydrogenase (DPD) drugs inducers like 5-fluorouracil or inhibitors like Dihydrofluorouracil, Tegafur and Gimeracil ethynyluracil were also excluded.

2.2.1.3 Data collection

During the time of blood sample collection, each patient was questioned whether she had used one or more drugs that may interfere with Capecitabine metabolism or may affect the activity of DPYD dehydrogenase, to make sure that all the potentially interacting drugs were captured in the database.

The data were obtained from the medical records of consenting females and from the patients themselves and these included: age, weight, academic achievement, workplace, marital status, breast feeding, dates of first menarche and last menopause, family history of breast cancer and number, date of first birth –full pregnancy, Date of breast cancer diagnosis, Site (left, right, or both), type of breast cancer, stage and grading, , surgery, type of chemotherapy drugs, radiation, or other side effects, liver disease or any other diseases, time on Capecitabine therapy and duration, and any other drugs used.

2.2.2 Biochemical study:

2.2.2.1 Principle of Estradiol assay:

This analyze 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, avidin 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

2.2.2.2 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.

2.2.2.3. 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 .a 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 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).

2.2.2.4. 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- Remaining wash Buffer by aspirating or decanting .Inverte 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- Added 50µl 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.

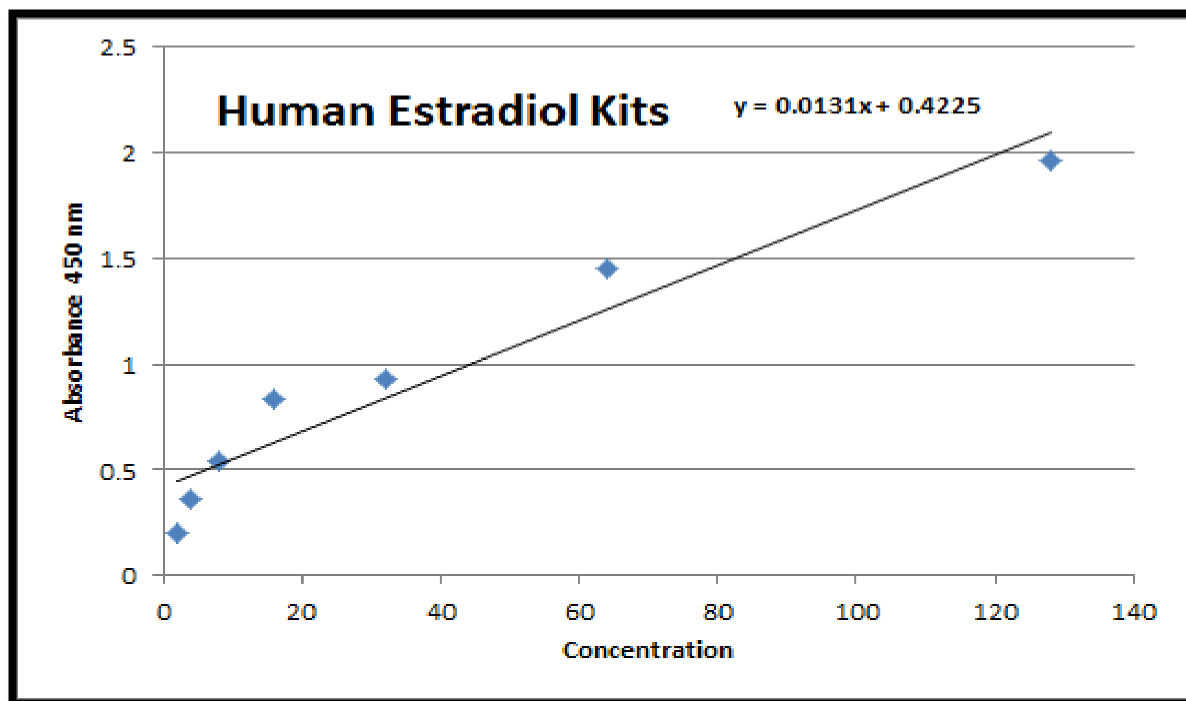


Figure (2-1): standard curve of Human Estradiol Pg/ml.

2.2.3 Total Calcium calculation mg/dl Kit:

2.2.3.1 Reagents:

1- **R1 reagent**: CAPSO:1 557 mmol/L; NM-BAPTA: 2 mmol/L, pH 10.0; non-reactive surfactant; preservative.

2- **R2 reagent**: EDTA: 7.5 mmol/L, pH 7.3; non-reactive surfactant; preservative.

2.2.3.2 Reagents preparation:

Added 2 ml of reagent B to a vial of reagent A. Reagents until expiration on label, stored at 2-8 ◦C .work reagent is stable 15 days at 2-8 ◦C.

Procedure:

Kind of analysis	End point
Reading time	5 minutes
Color stability	30 minutes
Wavelength	578nm (520-570)
Temperature	20-25◦C
Light path	1 cm
Blank reagent	Zero

2.2.4 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.

2.2.4.1 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.

2.2.5 Measurement of drug concentration in patient serum:

2.2.5.1 Sample preparation and HPLC condition for measurement Capacetabine:

A 0.1 mL aliquot of a human plasma sample was transferred to the extraction tube .Then 3 mL of ethylacetate /acetonitrile (4:1, v/v) was added and mixed. The sample was centrifuged at 3500 rpm and frozen. Then, the organic layer was transferred to a glass tube and evaporated under the stream of nitrogen. The dry residue was dissolved in 200 uL of 50% MeOH, the solution was centrifuged at 3500 rpm and transferred to an autosampler vial. A 100 uL aliquot of sample was injected into the HPLC system . The mobile phase consisted of 0.1% formic acid : MeOH (45 : 55 v/v) . The flow-rate of the mobile phase was 1.1 mL/min. The column was C18 - ODS (25 cm x 4.6 mm) < the detector was UV - Vis at 305 nm (Piórkowska et al., 2014).

2.2.5.2 Sample preparation and HPLC condition for measurement 5-Fu:

AgNO3 (20%, 600 µL) was added into a mixture containing human serum (0.1 mL) and the resulting mixture was subjected to vortex for 3 min and placed to stand for 5 min. NaCl (20%, 700 µL) was added and the mixture was subjected to vortex for another 3 min. After centrifugation at 13,000 rpm for 12 min, the supernatant (0.5 mL) was diluted with water to 1 mL in centrifuge tube and filtered through a 0.22-µm membrane. A HPLC model SYKAM - German with UV detector was used for the analysis. a C18 - ODS column (250 cm , 4.6 µm) was used for separation. The column temperature was maintained at 25°C. The standards and samples were determined using a mobile phase consisting of 5 mM KH2PO4 solution (pH = 6.0) and

methanol (96 : 4) at the flow rate of 1 mL/min. The injection volume was 100 µL. the 5-FU was detected at the wavelength of 254 nm (Zhu et al., 2012).

2.2.6 Molecular study:

2.2.6.1 DNA Extraction:

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°c for 5minutes.

2- Cells were lysed by added 200 µL of GSB buffer then mixed by vortex 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 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 for 2 minute).The Gs column was placed in 2 ml collection tube, and the tube containing the filtrate was discarded.

4- 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.

5- Six hundered microliter of washing Buffer 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.

6- 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**.**

7- 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

2.2.6.2 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 P.B. 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.

5-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 and shot.

Table (2-6) Primers sequences of DPYD*2A (G > A) (rs3918290) genetic polymorphism.

Allele specific	**Primer sequence(5 ’->3 ’)**	Product size
Reverse allele C	5-CTAAAGGCTGACTTTCCAGAACCCC-3	411 bp
Reverse allele T	5-CTAAAGGCTGACTTTCCAGAACCCT-3	
Forward Common	5-GATATGCTGCTTCTGCCTCAGGT-3	

Table (2-7) Primers sequences of DPYD*13 (rs55886062, T >G) genetic polymorphism.

Allele specific	**Primer sequence(5 ’->3 ’)**	Product size
Forward allele T	5-AGCCACCAGCACATCAATGATT-3	400 BP
Forward allele G	5-AGCCACCAGCACATCAATGATG-3	
Forward common	5-TGTTCCGCACCAGCTCTGGAT-3	

Lyophilized primers were dissolved with a certain volume of nuclease free water according to instruction of manufacture to give concentration of 100 pmol/µl (represent a stock solution).) represent the volumes of nuclease free water added to each primer to obtain 100 pmol/µl.

2.2.6.3 PCR components and programs :

PCR reaction is carried out after several attempts to detect the best annealing temperature with a total volume of 20 µl. The reaction components is described in table (2-8) and PCR amplification program is described in table (2-9).

Table (2-8) PCR reaction components for amplification

Component	Quantity (µl)
Forward primer	2
Reverse primer	2
DNA template	4
D.W	12
Master mix	5
Final volume	25

Table (2-9) PCR amplification program Polymorphism DPYD gene.

Steps	Temperature (c)	Time	NO. of cycles
Initial denaturation	95	3 minute	1
Denaturation	95	30 sec	35
Annealing	60	45 sec	
Extension	72	30 sec	
Final extension	72	5 minute	1

2.2.6.4 Analysis of PCR Products:

The PCR products and the ladder marker have been dissolved by electrophoresis. 3 µl of loading buffer in addition to 5 µl of the product were loaded on 1.5 % agarose gel (1.5g agarose/100 ml 1X TBE support) and keep running at 100 volt for 35 min. The gel was recolored with ethidium bromide (0.5 µg/ml) arrangement (0.5 µg/ml). What's more, bandes were pictured on UV trans illuminator and afterward shot .DNA ladder (100 bp) has been utilized to gauge the molecular size of the bands.

2.2.7 Statistical Analysis

In order to analyze the results, the Statistical Package for the Social Sciences SPSS (2012) program and Hapolview software were used to study the effect of different factor in study parameters. Mean ± standard error, Anova test and t test used to significant compare between means in this study . Alleles genotyping were presented as a percentage frequencies, and significant differences between their distributions in breast cancer patients and controls, were considered significant differences at $P < 0.05$.

Chapter three

Results

3. Results

3.1. Demographic Data

The studied population included 100 female patients with breast cancer. Participants' average age at study entry was 55.36 ± 10.85 years (range: 45-75). Eighty six percent were married and only (14%) single. There was a 86% disparity between the women who had a family history of breast Cancer and those who didn't (14%) among those who were diagnosed. Cancer patients who had the disease on their left sides numbered 33%, while those on their right sides numbered 67%. It is also recorded that 94% of patients have already had surgery, 88% of patients already have radiation therapy, and 92% of patients have had chemotherapy table (3-1) figure (3-1).

In addition to that one hundred healthy women with age rang (40-70) years were included in this study as control group .

Table (3-1) Patients' demographic groups and breast cancer's unique features

Characters		Percentage %
Age (Years)		55.36 ± 10.85
Duration of disease (Years)		4.31 ± 1.22
Duration of capecitabine (Years)		3.29 ± 1.95
Family History (%)	Yes	86%
	NO	14%
Marital status (%)	Married	86%
	Single	14%
Lymph node Involvement (%)	Yes	39%
	NO	61%
Breast cancer Side (%)	Left breast	33%
	Right breast	67%
History of breast cancer chemotherapy (%)	Yes	92%
	No	8%
	Yes	90%

History of breast cancer surgery (%)		
	No	**10%**
History of breast cancer radiotherapy (%)	Yes	**88%**
	No	**12%**
Hand and foot syndrome	Positive	**66%**
	Negative	**34%**
Positive for Estrogen receptor (ER)		**94%**
Progesterone receptor (PR)		**6%**

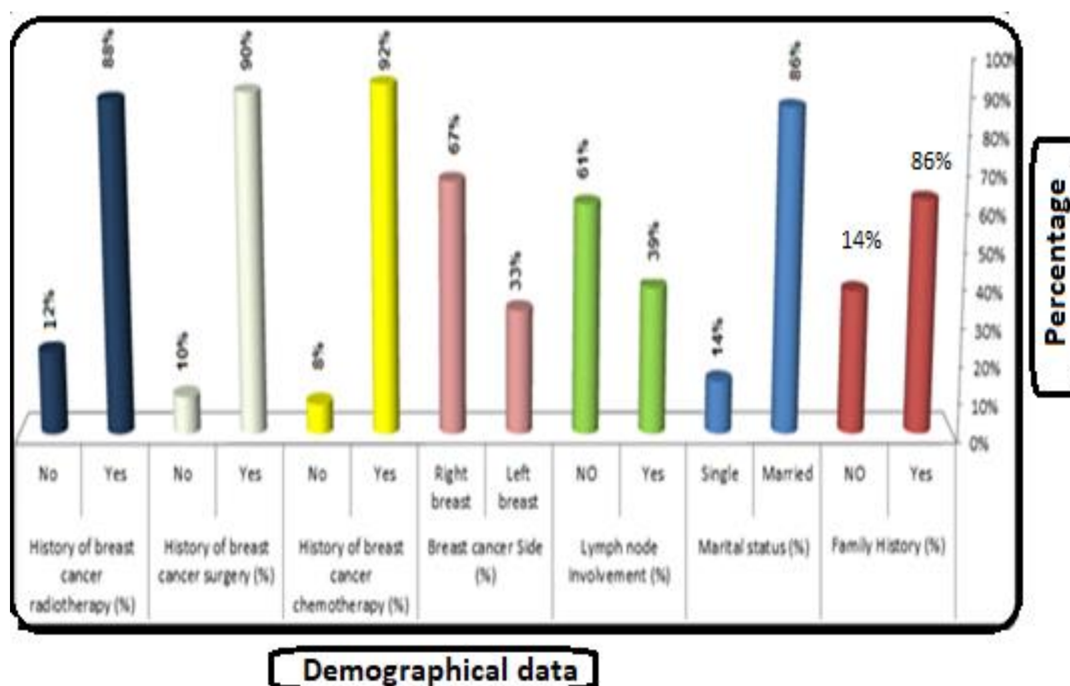


Figure (3-1): Demographic data for all patient suffering from breast cancer

3.2.Measurment of serum levels of tumor marker, calcium and estradiol:

3.2.1. Serum levels of tumor marker (CA 15.3), serum calcium and estradiol in Patient and control groups:

Table 3-2 shows that the average serum calcium and estradiol levels of patients undergoing chemotherapy for breast cancer were higher than the normal ranges of (9.35±3.5) ng/ml and (89.55± 14.7) mg/ml, respectively, while the corresponding values for controls were within the reference ranges,

it was recorded as (9.02±6.1) and (22.37± 9.67) for serum calcium and estradiol levels, respectively .

The findings demonstrated a statistically significant rise in the amount of CA15-3 found in breast cancer patients (136.4±36.7 U/ml) in comparison to the level found in the control group (31.68±11.32 U/ml).

Table (3-2): Serum levels of CA15.3, estradiol and Calicum of participant (patient and control).

Characteristic Mean ± SD	Patients *n* = 100	Control n =100	*P value*
Ca 15-3 U/ml	136.4±36.7	31.68±11.32	< 0.001 S
Serum Calcium mg/ml	9.35±3.5	9.02 ±4.1	0.05 S
Estradiol ng/ml	89.55± 14.7	22.37 ±9.67	0.001 S

3.2.2. Comparsion of serum levels of tumor marker, serum calcium and estradiol between Response and Non-Response groups:

Table (3-3) shows that the average estradiol and serum calcium levels of patients undergoing chemotherapy for breast cancer were higher in the non-responser patient for chemotherapy drug. it was measured as (87.31 ± 12.37) ng/ml and (9.44 ± 2.53) mg/ml respectively, while the corresponding values for, response activity for chemotherapy were were recorded as (76.43± 10.48) and (9.26 ± 4.5) for estradiol and serum calcium levels, respectively .

The findings demonstrated a statistically significant rise in the amount of CA 15-3 found in breast cancer of non-response patients (128.48 ±17.82 U/ml) in comparison to the level found in the response group (89.66±10.38

U/ml). The results fund a statistically significant rise in the concentration of flurouracil in breast cancer response (331.57± 34.69 ng/ml) in comparison to the level found in the non-response group (288.38± 66.52 ng/ml).

Table (3-3): Serum levels of CA15.3, Estradiol drug concentration and Calcium of patient between response and non-response.

Characteristic Mean ± SD	Responder N=61	Non-Responder N=39	*P value*
Ca 15-3 U/ml	89.66±10.38	128.48 ±17.82	<0.01 S
Estradiol ng/ml	76.43± 10.48	87.31 ±37.12	0.063
Serum Calcium mg/ml	9.26 ± 4.5	9.44±2.53	0.082
Drug concentration ng/ml (5fu)	331.57± 34.69	288.38± 66.52	0.042

Result represented as mean ± SD

3.3 Molecular analysis:

3.3.1 Results of amplification reaction "DPYD Polymorphism" (rs3918290):

The amplification of SNPs of DPYD gene: rs3918290 was shown in figure (3-2); The presence of PCR bands with identity sizes in the agarose gel indicated the genotype of the samples as positive result. Each reaction in different SNPs and genotypes are shown in detail in table 3.4. The PCR amplifications, fragment size 400 bp indicated that patient have specific alleles, it was required the use of two separate tubes for the amplification of wild-type and variant-type allele.

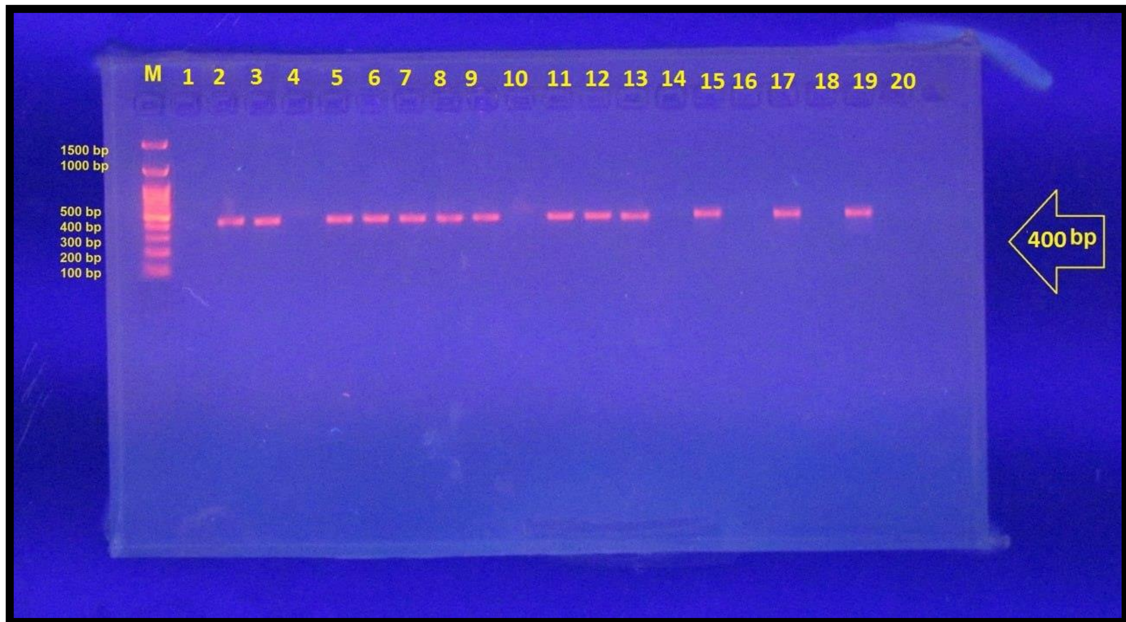


Figure (3-2) PCR amplification of rs3918290 gene showed: Line M: Represented DNA marker (ladder) 100 – 1500 bp, Line 1,2; : Represented TT genotype (Mutation), Lines 5, 6, 7, 8;11,12: Represented CT genotype (Hetrozygoite) were showed in 400 bp, Lines 3,4; 9,10;13,14; 15,16;17,18;19,20 : Represented CC genotype (wild) were shown in 400 bp.

3.3.2.Frequency of genotypes of rs3918290 gene polymorphisms in patients with breast cancer women

The frequency and percentage of rs3918290 genotype that detected in the breast cancer patients are shown in table (3-4).

The most frequent genotype in 100 breast cancer patients recruited in this study was the wild type (CC) with frequency and percentage 58 and 58% respectively, while the heterozygote type (CT) represent the modest frequent type with frequency and percentage of 28 and 28% respectively. The Mutation type of rs3918290, which carry TT genotype have been identified in frequency and lowest percentage of 14 and 14% respectively.

Table (3-4): Distribution of genotype and allele frequency of DPYD (rs3918290) polymorphism

DPYD (rs3918290)	Genotype	Patients n = 100 n (%)	(X^2)	HWE
Genotype Frequency	CC	58 (58)	(9.3364)	0.001
	C/T	28 (28)		
	TT	14 (14)		
Allele Frequency	C	72 (72)	(13.5)	0.001
	T	28 (28)		

HWE; Hardy Weinberg equlibrium

3.3.3. Results of amplification reaction "DPYD Polymorphism" (rs55886062):

The amplification of SNPs of DPYD gene: rs55886062 was shown in in figure (3-3); The presence of PCR bands with identity sizes in the agarose gel indicated the genotype of the samples as positive result. Each reaction in different SNPs and genotypes are shown in detail in table 3.5. The PCR amplifications, fragment size 410 bp indicated that patient have specific alleles, it was required the use of two separate tubes for the amplification of wild-type and variant-type allele.

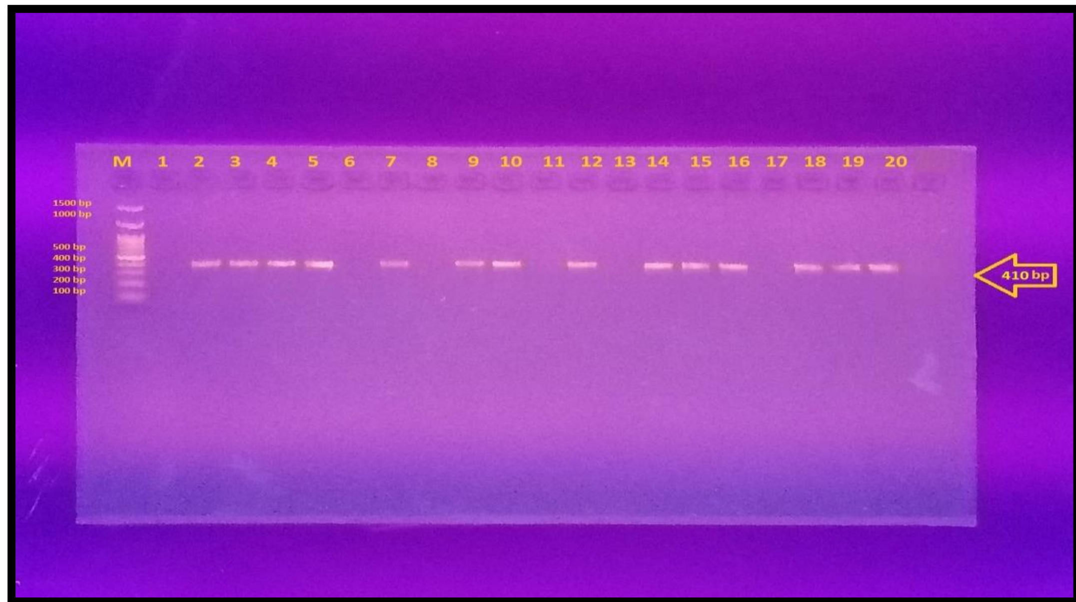


Figure (3-3) PCR amplification of rs55886062 gene showed: Line M: Represented DNA marker (ladder) 100 – 1500 bp, Line 1,2;11,12;13,14;17,18 : Represented CC genotype (Mutation), Lines 3, 3;9,10;15,16;19,20: Represented CT genotype (Hetrozygoite) were showed in 4100 bp, Lines 5,6;7,8 : Represented AA genotype (wild) were shown in 410 bp.

3.3.4 The frequency of genotype rs55886062 gene polymorphisms in patients with breast cancer women:

The frequency and percentage of rs55886062 genotype that detected in the breast cancer patients are shown in table (3-5).

The number genotype in 100 breast cancer patients recruited in this study was the mutant type (CC) with frequency and percentage 7 and 7% respectively, while the heterozygote type (CA) represent the frequent type with frequency and percentage of 40 and 40% respectively. The wild type of rs55886062 which carries AA genotype have been identified in frequency and percentage of 53 and 53% respectively.

Table (3-5) distribution of genotype oand alleleic frequency of DPYD rs55886062 gene polymorphisms.

DPYD (rs55886062)	Genotype	Patients n = 100 n (%)	(X^2) *HWE*	HWE
Genotype Frequency	AA	53 (53)	(9.3364)	0.001
	C/A	40 (40)		
	CC	7 (7)		
Allele Frequency	A	73 (73)	(13.5)	0.001
	C	27 (27)		

3.3.5Distribution of allelic frequency DPYD gene polymorphisms in patients with breast cancer among demographic data

Table (3-6) shows that there are significant differences between the mutation and the normal gene with other characteristics of female patients We found significant association between DPYD gene polymorphisms as major allele and minor allele with Family history and history of breast cancer Surgery, probability value was recorded as 0.03 and 0.05, respectivelly. We do not found any association between alleleic frequency with Lymphnode involvement, history breast cancer chemotherapy, history breast cancer radiotherapy(P> 0.05).

Table (3-6): Distribution of allelic frequency DPYD gene polymorphisms in patients with breast cancer among demographic data

Characteristic	rs3918290 *n* (%)	rs55886062 *n* (%)	*p*
Family History n= 86			
Major allele	52 (60.4 %)	65 (75.5 %)	0.03 S
Minor allele	34 (39.6 %)	21 (24.5 %)	
Lymph node involvement n= 39			
Major allele	22 (86 %)	23 (0 %)	0.78 NS
Minor allele	17 (14 %)	16 (0 %)	
History of breast cancer chemotherapy n= 92			
Major allele	56 (60.8 %)	64 (69.5 %)	0.7 NS
Minor allele	36 (39.2 %)	28 (30.5 %)	
History of breast cancer Radiotherapy ; N=88			
Major allele	51 (57.9 %)	48 (54.5 %)	0.64 NS
Minor allele	37 (42.1 %)	40 (45.5 %)	
History of breast cancer Surgery; N= 90			
Major allele	54 (60 %)	66 (73.3 %)	0.05 S
Minor allele	36 (40 %)	24 (26.7 %)	

3.3.6 Distribution of allelic frequency DPYD gene polymorphisms rs3918290 in patients with breast cancer among Tumor markers:

The study was found significant differences $P< 0.05$ between tumor markers CA15.3, estradiol and serum calcium among patient have breast cancer and genotype frequencies. The results were recorded as (85.87±7.68) in the CA15.3 with patient have TT genotype frequency in their chromosome "rs3918290", otherwise wild and heterozygote patient they are not found any significant differences between them. It was recorded as (99.52± 6.95) and (97.11±6.91) for CC and CT genotype frequency and table (3-7) shows these results.

Table (3-7) comparisom of tumor marker value between responder and nonresponder patiet according to genotype disturbution

Responder N=61			Non-responder N=39		
Genotype "rs3918290"	N	Tumor Marker	Genotype "rs3918290"	N	Tumor Marker
CC	29	99.52± 6.95	CC	29	119.11±5.36
CT	20	97.11±6.91	CT	8	118.79±6.48
TT	12	*85.87∓ 7.68	TT	2	*92.08∓6.48
LSD		9.183	LSD		8.73

Results apperar as mean ±SD ; * significant diffrences

3.3.7 Distribution of allelic frequency DPYD gene polymorphisms rs55886062 in patients with breast cancer among Tumor markers

The study was found significant differences P< 0.05 between tumor markers CA15.3, estradiol and serum calcium among patient have breast cancer and genotype frequencies. The results were recorded as (88.47±6.09) in the CA15.3 with patient have CC genotype frequency in their chromosome " rs55886062", otherwise wild and heterozygote patient they are not found any significant differences between them. It was recorded as (97.62±7.15) and (93.53± 7.39) for AA and CA genotype frequency and table (3-8) shows these results.

Table (3-8) shows Distribution of allelic frequency DPYD gene polymorphisms rs55886062 in patients with responser and non responder.

Responder			**Non-Responder**		
Genotype " rs55886062"	**N**	Tumor Marker	Genotype " rs55886062"	N	Tumor Marker
AA	26	97.62±7.15	**AA**	27	139.11±5.36
CA	29	93.53± 7.39	**CA**	11	138.73±7.51
CC	**6**	88.4.7±6.09 *	**CC**	1	101.37±4.32 *
LSD		4.32	**LSD**		7.44

Results apperar as mean ±SD ; * significant diffrences

3.3.8 Distribution of allelic frequency DPYD gene polymorphisms allele frequencies in patients with breast cancer among Tumor markers, estradiol and calcium.

Table (3-9) shows Distribution of allelic frequency DPYD gene polymorphisms in allele frequencies in patients with breast cancer among biochemical markers.

Characteristic	rs3918290	rs55886062	*p*
Tumor marker CA15.3			
Major allele	131.18±16.85	138.36±28.81	0.093 NS
Minor allele	122.45±26.7	148.11±17.81	0.02 S
Estradiol			
Major allele	79.45±13.27	88.67±17.89	0.076 NS
Minor allele	73.14±19.78	82.83±15.6	0.049 S
Serum calcium			
Major allele	8.78±2.67	8.46±2.21	0.061 NS
Minor allele	6.83±26.7	8.11±1.98	0.041 S

3.3.9 Correlation between biochemical markers and DPYD genotype in patient postmenopausal women have breast cancer.

The study found significantly association between two SNPs and tumor marker. We found the odd ratio with tumor marker CA15.3 is 2.23 with significant differences ($P < 0.05$), and we do not found any significant differences with other biochemical markers like estradiol and calcium and hand foot syndrome and table (3-10).

Table (3-10) Correlation between biochemical marker and DPYD genotype and side effect Hand foot syndrome

Characteristic	rs3918290	rs55886062	*Odd ratio 95%CI*	*P value*
Tumor marker CA15.3 (normal range 30U/ml)				
< 30 U/ml	65	49	2.23	0.021
≥ 30 U/ml	35	51		
Estradiol (normal range 30pg/ml)				
< 30 pg/ml	57	62	0.81	0.47
≥ 30 pg/ml	43	38		
Serum calcium (normal range 8.5-10 milimol/l)				
< 8.5-10 mm/l	44	40	1.17	0.56
≥8.5-10 mm/l	56	60		
Hand and foot syndrome				
Positive	67	65	1.09	0.76
Negative	33	35		

Table (3-11) showed that there were clear significant differences in each of the following concentrations of Capecitabine and 5FU in women with breast cancer. As the results, recorded a significant higher concentration of them was recorded in each of Capecitabine and 5FU in patients with the TT allele rather than in patients with the CC and CT alleles in patient with DPYD (rs3918290) characteristics, on the other hand. It was found significant decrease of estradiol and CA 15.3 concentration in patient with TT allele rather than CC and CT alleles, we do not found any significant differences (P>0.05)between three alleles with Serum calcium levels.

Table (3-11): The biochemical markers with genotype frequencies of DPYD (rs3918290) .

Characteristic biomarkers DPYD (rs3918290)	CC $n = 58$	CT $n = 28$	TT $n = 14$	p
CA 15-3 U/ml	109.32 ±22.05	107.95 ±31.69	88.98±27.42	0.02 S
Estradiol ng/ml	95.38±9.34	88.62 ±8.61	84 ±8.41	0.031 S
Serum Calcium mg/ml	9.92 ±1.76	9.85 ±1.8	9.08± 1.76	0.23 NS
Capecitabine Concentration ng/ml	27.32 ±6.49	36.87 ±5.39	42.22 ±5.19	0.028 S
5FU Concentration ng/ml	259.33 ±14.36	301.77 ±21.77	341.53 ±16.21	0.019 S

Data represented as Mean ± SD

Table (3-12) showed that there were clear significant differences in each of the following concentrations of Capecitabine and 5FU in women with breast cancer. As the results recorded a significant higher concentration of them was recorded in each of Capecitabine and 5FU in patients with the CC allele rather than in patients with the AA and AC alleles in patient with DPYD*13 (rs55886062) characteristics, on the other hand, we do not found any significant differences (P> 0.05) between three alleles with CA 15.3, Serum estradiol and serum calcium levels.

Table (3-12): The biochemical markers with genotype frequencies of DPYD (rs55886062).

Characteristic DPYD*13 (rs55886062)	AA *n* = 53	AC *n* = 40	CC *n* = 7	*p*
Ca 15-3 U/ml	118.37 ±5.32	116.13 ±11.49	94.92 ± 9.58	0.053 NS
Estradiol ng/ml	84.69 ± 5.85	82.77 ± 6.72	80.23 ±7.98	0. 68 NS
Serum Calcium mg/ml	9.38 ±1.19	8.69 ±1.25	8.46 ±1.23	0.37 NS
Capecitabine Concentration ng/ml	26.15 ±3.95	30.23 ±4.34	38.31 ±6.38	0.047 S
5FU Concentration ng/ml	277.4 ±14.21	283.77 ±23.98	375.92 ±18.59	0.038 S

Data represented as Mean ± SD

Chapter four

Discussion

4. Discussion

Capecitabine is an anti metabolite, a class of chemotherapeutic agent. Capecitabine is converted in the body to fluorouracil, which is used in a variety of chemotherapy treatments. he majority of cancers result from random mutations arising during DNA replication in the normal stem cells required during development and tissue maintenance, making and repairing DNA is essential for cancer cell proliferation (Murthy et al., 2020). Chemotherapy with the oral fluorouracil prodrug capecitabine has been found to be efficacious for breast cancer, yet, some of studies was usefulness in treating breast tumors is still up for debate. After anthracycline and taxane failure, capecitabine is typically used as a second-line chemotherapy option for patients with metastatic breast cancer (Ayala et al., 2021).

4.1 Demographic data:

The group under study consisted of one hundred breast cancer patients who were female. the average age of the participants was 55.36 ± 10.85 years (range: 45-75). When breast cancer is discovered in the lymph nodes, it indicates that the disease has already spread from the main tumor and is at least stage two in its progression, the involvement of lymph nodes is an essential component of the staging process and plays a significant role in identifying which treatments have the best chance of being successful (Han et al., 2019).

The reason for the increase in surgeries among women is in differences and failure to consult a specialist, mammary pathologists is a frequent reason for consultation in the female population, the vast majority being a benign disease, due to the little knowledge imparted in programs regarding this subject. A high percentage of patients are unnecessarily referred to mastology services, generating opportunity problems for patients with breast

cancer or benign breast disease that do require assessment by a specialist (Russo et al., 2016).

There are socio-ethical, religious, and cultural misunderstandings held by Iraqi muslim women about both the cause of breast cancer as well as the practices and behaviors that are considered to be healthy (Moey et al., 2022).

A study is conducted on the incidence of left and right breast cancer in the Iraqi women. Many novel case series from cancer registries and hospitals are also provided in some countries. A data study reveals that breast cancer in women is more prevalent in the right breast than in the left side. This study is agreement in other nations than in the United States by study was recorded the incidence of breast cancer was increased in right side rather than left side (Koto et al., 2019).

This result was agreement with (Tulinius et al., 2017) who was found 81 women were found to have original breast cancer for both breasts, with 35 cases being found in the left breast first and 46 cases being found in the right breast first.

Eighty six percent (86%) were married and only (14%) single was infected with breast cancer, Some epidemiological study have found that women who have never been married have a higher risk of developing breast cancer (Carlsen et al., 2008; Kvikstad et al., 2010). while other studies contend that a person's marital status may have no bearing on their likelihood of developing this malignancy diseases (Melchior et al., 2005). In spite of the inconsistent nature of the findings from the past, no comprehensive investigation has been conducted.

This study illustrated the diagnosis of patients with breast symptoms or signs, including basic elements screening and early or late detection of breast cancer; and the treatment of patients with capecitabine chemotherapy. In

addition, its general objective is to standardize the approach diagnostic and therapeutic of malignancy mammary pathology.

Ninety one form all postmenopausal women have been taken capecitabine chemotherapy with duration of treatment ranged (3.29 ± 1.95) years, breast cancer cells multiply and spread at abnormally high rates. Rapid dividing cells, are particularly vulnerable to chemotherapy. Yet, chemotherapy drugs are potent and have the potential to harm healthy cells, particularly rapidly dividing cells (Hassan et al., 2010).

Recurrences and metastases account for the vast majority of breast cancer-related fatalities, some of study noted around 6% of newly diagnosed cases of breast cancer have metastasized (or "metastatic") disease, about 30% of women who are diagnosed with early-stage breast cancer will go on to develop recurring advanced or metastatic illness, despite improvements in therapy.

Regarding the choice of chemotherapy in patient treatment, this depends on several factors, as in the first-line treatment: KRAS status, patient age, Eastern Cooperative Oncology Group (ECOG), and comorbidities, toxicity and drug availability. different regim (Akbıyık et al., 2022).

Our result was found 39% of patient have lymph node involvement in their armpit are called axillary lymph nodes, Immune cells in bean-shaped lymph nodes fight infection. Lymphatic vessels link them. Lymph nodes remove toxins from fluid, these cells usually reach the nearest lymph nodes in breast cancer.

Lymph nodes cluster under the arm and near the collarbone and breastbone. Sentinel lymph nodes are closest to our breast one of the most important for lymph node measurement in breast cancer is Axillary lymph nodes was armpit nodes (Vasquez et al., 2021).

The pharmacological treatment of breast cancer (BC) has evolved greatly in recent years. Regarding adjuvant therapy for patients diagnosed with BC in stages II and III, 5-fluorouracil (5-FU) has been the mainstay of treatment and has been gradually combined with other agents with the aim of increasing patient survival or monotherapy (Zhang et al., 2020); As for the future, promising therapies are being developed for the breast cancer. Several drugs are currently being evaluated in different clinical trials including (Fedele et al., 2022):combined Chemotherapy agents: like topotecan (topoisomerase I inhibitor), or targeted therapies: trastuzumab (targeted at the growth factor receptor, human epidermal [HER2], erlotinib (targets overexpressed HER1), lapatinib (double HER1/2 inhibitor), onartuzumab (inhibitor of hepatocyte growth), mapatumumab (receptor ligand inhibitor) or 5fu monotherapy.

Some of recent studies noted that another drug used in these patients is capecitabine, a non-cytotoxic fluoropyrimidine carbamate that, when administered orally, acts as a precursor of the cytotoxic agent 5-FU and is activated through several enzymatic steps, with thymidine phosphorylase being the enzyme responsible for the final conversion to 5-FU, which is found in tumor tissues (Huo et al., 2021).

Numerous studies have shown that when capecitabine is used in patients with stage III RCC, it is an alternative that is at least as effective and well-tolerated as 5-FU/LV monotherapy (Banna et al., 2022); so intravenous 5-FU can be replaced by capecitabine with the advantage of being administered orally. The association of capecitabine and oxaliplatin (XELOX scheme) has also been shown to improve survival when compared with the administration of 5-FU/LV in monotherapy(Lindgaard et al., 2019).

4.2 Biochemical analysis:

The study was recorded the concentration of CA15.3 tumor markers in postmenopausal Iraqi women, antigen 15-3 for cancer, also known as CA 15-3, is a protein that is produced by cells as a reaction to changes that take place in the body. Because elevated levels of the CA 15-3 antigen have been linked to a variety of malignancies, including breast cancer, this protein is studied as a potential tumor marker (Li et al., 2020).

Our result was found a statistically significant increase in the levels of Ca 15-3 found in breast cancer patients (136.4±36.7 U/ml) in comparison to the level found in the control group (31.68±11.32 U/ml). The CA 15-3 test is not used to detect cancer; rather, it is used to evaluate how well treatment is working against cancer, so variations in CA 15-3 levels allow oncologists to identify whether a tumor is maintaining its size, expanding, shrinking, or has returned (Nam et al., 2019).

This findings of the current study are in agreement with the findings of the previous study (De Cock et al., 2021), which indicated an increase in the level of CA 15.3 in breast cancer women who had metastatic disease. Additionally, in the histological study, a high level of microRNAs have an effect on a wide variety of oncogenes and suppressor genes, all of which play a role in the development, progression, and spread of cancer (Bartel, 2004). These findings are consistent with the findings of the present study . Tumor markers (CA15.3) concentration in the studied population, the result show no significant differences in dihydropyrimidine dehydrogenase according to difference in genotype. Studies have shown that the traditional factors of infection such as age, hormonal factors, and reproductive factors are not present in many cases, especially in women who develop BC at a young age (Jagarlamudi, & Shaw, 2018).

Regarding to the level of estradiol (E2) in the serum, the present study showed that a significant association ($p<0.05$) between the studied patient with control groups, Estrogen receptor (ER) is one of nuclear receptor family which has been activated by 17-β-estradiol and there are two isoform of ER i.e. ERα and ERβ that encoded by genes located in different chromosome (Al-Bader et al.,2011). About 65% of breast cancer women less than 50 years old and 75% of older women with BC are estrogen receptor positive (Braithwaite et al., 2018).

Estrogen is essential for the normal growth and growth of breast tissue and it also considered a major risk factor for breast cancer when exposed to high level of estrogen (Dall & Britt ., 2017), Estrogen signals through its two receptors (ERα and ERβ),but only ERα is essential for the development of breast and activates proliferative signaling in the normal and breast cancer, whereas ERβ generally antagonize ERα in the breast (Girgert et al.,2019).

Approximately seventy percent of women have a sensitivity to the oestrogen that is found in female sexual hormones. These forms of cancer have receptor sites on their cells that allow them to bind to oestrogen, which in turn encourages their growth and spread (Burstein, 2020).

Tumor cell growth and proliferation are boosted by estrogen's ability to bind to the estrogen receptor (ER) and activate receptor-regulated transcription, hormone therapies for ER-positive cancers either reduce estrogen synthesis, block ER signaling, degrade ER, or modify ER-regulated signaling or proliferation pathways (Parida, & Sharma, 2019).

The levels of serum biomarkers were also investigated dependent on the length of time that patients were treated with capecitabine. In general, patients with breast cancer were shown to have a decreasing range of calcium levels when comparing those whose treatment duration was less than 5 years to those whose treatment duration was more than 5 years. Hypercalcemia

is a frequent complication of breast cancer which causes significant morbidity and mortality. Most commonly, it occurs in patients with multiple skeletal metastases. However, in a significant minority of patients, calcium levels become elevated in the absence of skeletal disease (Stewart ,2005).

An increased level of calcium in the bloodstream is most often a complication of cancer and is referred to as hypercalcemia of malignancy. In its severe form, hypercalcemia may be a life-threatening emergency (Zagzag et al.,2018).Up to 30% of all people with cancer will develop a high calcium level as a side effect. A high calcium level can be treated, they are many types of cancers that are most commonly associated with high blood calcium are: myeloma – about 30 in 100 people (about 30%) have high calcium when they are first diagnosed of breast cancer (Goldner,2016).

The chance of developing breast cancer grows in tandem with endogenous estrogen levels, according to prospective research. Tamoxifen and raloxifene, two selective estrogen receptor modulators, inhibit the actions of endogenous estrogen in the breast, hence lowering the risk of breast cancer. It follows that endogenous estrogen levels may moderate the effects of selective estrogen receptor modulators(Branigan et al.,2020).

The risk of breast cancer and the effect of it depend on serum concentrations of estradiol. Specifically, hypothesized that women with undetectable serum concentrations of estradiol would have a very low breast cancer risk that would not be further reduced (Rojas & Stuckey.,2016).

About 80% of all breast cancers are “ER-positive.” That means the cancer cells grow in response to the hormone estrogen. About 65% of these are also “PR-positive.” They grow in response to another hormone (American Cancer Society. (2019).

4.3 Molecular analysis:

4.3.1 The frequency of rs3918290 (G>A) (DPYD) within breast cancer women:

Figure 3-2 shows that the DPYD gene SNP rs3918290 is amplified, a positive results for the samples' genotype were shown by the appearance of PCR bands of same sizes on the agarose gel. Table (3.4) displays the detailed responses for each SNP and genotype combination, patients were found to have particular alleles based on the results of polymerase chain reaction (PCR) amplifications, with fragment sizes of 400 bp.

In this study of 100 breast cancer patients, the wild type (CC) was shown to be the most common genotype (frequency = 58. 58%), whereas the heterozygote type (CT) was found to be the least common genotype (frequency = 28, 28%) rs3918290 TT genotype mutations have been shown to occur at a frequency and percentage of 14% and 14%, respectively.

We found these alleles out of hardy Weinberg equilibrium ($P< 0.05$), In addition to mutations and "natural selection, nonrandom mating, genetic drift, and gene flow" are also capable of upsetting the Hardy-Weinberg equilibrium. For instance, mutations introduce novel alleles into a population, which shifts the balance of existing allele frequencies (Sun et al., 2021).

The DPYD activity of patients with the CC genotype in the rs3918290 frequencies may be higher than that of patients with the CT or TT genotypes. Nonetheless, there have been reports of contradictory evidence. The catalytic activity of DPYD may also be affected by other hereditary and clinical variables. This result was agreement with (Olivera et al., 2019) who was clarified that DPYD activity is reduced in TT genotype patients compared to CC genotype patients. Nonetheless, there have been reports of contradictory

evidence. The catalytic activity of DPYD may also be affected by other hereditary and clinical variables.

Some of study noted the DPYD deficiency in some patient, which can cause seizures and developmental delays, manifests in the body. In addition, this deficit might cause serious side effects for cancer patients taking 5-FU after treatment. The prevalence of DPYD deficiency was estimated to be between 3% and 8%. Five DPYD polymorphisms were associated with higher toxicity to 5FU in a population-based study: DPYD*2A, (rs67376798), c.2846A>T, c.1679T>G, and c.1236G>A. Toxic effects from capecitabine and 5-fluorouracil (5-FU) were avoided to genotype-guided dosing (Stavraka et al., 2019).

4.3.2 The frequency of *rs55886062* (A>C) (DPYD) within breast cancer women:

Single nuclotidw polymorphism (SNP) rs55886062 in the DPYD gene was observed to be amplified figure (3-3), Positive results for the samples' genotype were shown by the appearance of PCR bands of same sizes on the agarose gel. Table 3.5 displays the detailed responses for each SNP and genotype combination. Patient had specific alleles as shown by PCR amplifications of fragments of 410 bp in size; amplification of wild-type and variant-type alleles required use of two different tubes.

The mutant type (CC) was the most common genotype among the 100 breast cancer patients recruited for this investigation, with a frequency and percentage of 7 and 7%, respectively, while the heterozygote type (CA) was the least common, with a frequency and percentage of 40 and 40%, respectively. The percentage of the AA genotype found in the rs5588062 wild type are 53%.

4.4 Distribution of allelic frequency major and minor alleles DPYD gene polymorphisms in patients with breast cancer among demographic data.

By studying the SNPs responsible for a site DPYD genes using allelic Specific PCR techniques. We found that the major allele represents the highest amount of the minor allele in women who have family history, with significant differences (P< 0.05),Otherwise all demographic data were note significant differences between two SNPs, where we did not find any differences in Lymph node involvement, History of breast cancer chemotherapy and History of breast cancer Radiotherapy, except History of breast cancer surgery. We found that most surgical intervention patients carry the large allele compared with minor alleles for both SNPs DPYD gene.

Many studies (Wellisch et al., 2012; Narod et al., 2015) have looked at how breast cancer surgery affects how patients feel about their bodies. In treating ductal carcinoma in situ (DCIS) and early-stage invasive breast cancer, breast-conserving surgical intervention (BCS) with radiation therapy is just as successful as mastectomy in terms of long-term survival.

4.5 Distribution of allelic frequency DPYD gene polymorphisms in patients with breast cancer among Tumor markers.

CA15.3, estradiol, and serum calcium were found to differ significantly among patients with breast cancer, and the results were recorded as (122.45±26.7, 148.11±17.81) in the CA15.3 with patients who had the rs3918290 and rs55886062 minor allele, respectivelly.

The reason for the high concentration of the capacetabin chemotherapy agent is the inability of the body to metabolize it well and therefore maintains

its presence in the body as a preventive mutation in order to maintain the existence of the treatment and the complete elimination of cancer cells via capicitabine. This result was agreement (Van Kuilenburg, 2014) who was found patients who have two partially functional alleles, one non-functional and one partially functional allele leading to a partially functional allele, should have their DPD activity determined or avoid fluorouracil/capecitabine. Tegafur is not a fluorouracil substitute because it is also metabolized by DPD. The DPYD genotyping as "critical" and recommends DPYD testing before starting fluoropyrimidines.

The result also found significant differences in the concentration of and calcium in postmenopasal women have minor allele frequency. They were recorded as 6.83±26.7 and 8.11±1.98 for rs3918290 and rs55886062 minor allele, respectively.

Estrogen, the main female sex hormone, has a crucial role in the genesis and progression of breast and other cancers, acting as promoters or genetic damage-causing agents (initiators) (Fernandez, & Russo, 2010). Minor alleles were responsible for blocking any step in the conversion pathway of androgens to estrogen, notably the final step (aromatase step) that is unique to estrogen biosynthesis, decreases estrogen production and serum estradiol levels (Abd-Allateef et al., 2016).

4.6 Correlation between tumor marker and DPYD genotype in patient postmenopausal women have breast cancer:

The study was found significant association between two SNPs and tumor marker. We found the odd ratio with tumor marker CA15.3 is 2.23and 95% interval ranged between (1.45-43.67) with significant differences (P < 0.05), and we don’t found any significant differences with other biochemical markers like estradiol and calcium.

In this study, the women with mutant genotype(rs55886062) had highest serum CA15.3 level compared with women harboring homozygous mutant (rs3918290) which had low serum CA15.3 level but still all the results within normal range and non-significant association with (CA15.3).

Table (3-11) showed that there were clear significant differences in each of the following concentrations of CA15.3, Capecitabine and 5FU in women with breast cancer, As the results recorded a significant higher concentration of them was recorded in each of Capecitabine and 5FU in patients with the TT allele rather than in patients with the CC and CT alleles in patient with DPYD (rs3918290) characteristics. On the other hand, it was found significant decrease of estradiol concentration in patient with TT allele rather than CC and CT alleles, we do not found any significant differences (P>0.05) between three alleles with Serum calcium levels. The result was agreement with (Olivera et al., 2019) who was found The T allele of rs3918290 is assigned no function by CPIC. Patients with the CC genotype may have increased activity of DPYD as compared to patients with the CT or TT genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence catalytic activity of DPYD.

Table (3-12) displays statistically significant differences between the following Capecitabine and 5FU concentrations in breast cancer patients: Patients with the CC allele of DPYD*13 (rs55886062) had significantly greater concentrations of Capecitabine and 5FU than those with the AA and AC alleles. Nevertheless, we did not find any significant differences (P> 0.05) between the three genotypes with CA15.3, estradiol, or serum calcium levels. The result was agreement with (Lunenburg et al., 2020) who was found the C allele of this variant is assigned a no function allele by CPIC. Patients with the AA genotype and cancer who are treated with fluorouracil, a fluoropyrimidine-based chemotherapy, may have decreased, but not absent, risk of drug toxicity as compared to patients with the AC or CC genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence risk of drug toxicity.

Conclusions and Recommendations

Conclusions

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

1- Different frequencies of the (G > A) (rs3918290) and of (rs55886062, T >G) polymorphisms of the DPYD gene homozygous wild, homozygous mutant, and heterozygous genotypewere discovered using Allele specififc-PCR in Iraqi breast cancer women who were treated with Capacetabine.

2- There was a significant relationship between the presence of 5FU and capacetabine drug concentration and varying serum levels of tumor marker (CA 15.3) in Iraqi breast cancer patients, indicating that (G > A) and (T >G) genotype status affected the level of these parameters.

3- Capacetabine-treated Iraqi breast cancer women did not show any correlation between (G > A) and (T >G) polymorphisms and serum calcium levels.

Recommenations and Future works

1- Large-scale and multicenter studies will be required to assess the impact of genetic polymorphism of DPYD on Capecitabine response in Iraqi breast cancer women.

2- In the clinical setting, we recommend that genetic tests should be developed to expect an individual's response to Capecitabine therapy, and that personalized drugs with greater efficacy and safety should be developed.

3-Studying genetic variants in other enzymes involved in metabolism of Capecitabine such as thymidylate synthase, methylene tetra hydro folate reductase and ribonucleotid reductase could contribute to inter – individual variability in Capecitabine response.

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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:

Si te (left, right):

Surgery:

Radiotherapy:

Chemotherapy:

Date of cancer recurrence: Site of recurrence:

Other diseases:

Dose of Capacetabine:

Time on capacetabine Therapy:

Presence of hand foot syndrom & other Side effects:

Other drugs used:

Lab. Data:

الخلاصة

الخلفية: سرطان الثدي هو السبب الرئيسي للوفاة بسبب السرطان لدى النساء اللاتي يعشن في البلدان الصناعية. في العراق ، احتل سرطان الثدي المرتبة الأولى كسبب لوفيات النساء بسبب السرطان ، وهو في الوقت نفسه السبب الرئيسي للوفاة بين النساء اللاتي تتراوح أعمارهن بين 30 و 54 عامًا. يعد منتج جين DPYD ، وهو إنزيم تقويضي بيريميدين ، الخطوة الأولى والخطوة المحددة في تحلل اليوراسيل والثيميدين. يحدث ضعف ديهيدروبيريميدين ديهيدروجينيز بسبب طفرات في هذا الجين. كان الهدف من الدراسة هو تحديد العلاقة بين سرطان الثدي (rs3918290) (G > A) وتعدد الأشكال(rs55886062) ، (T> G) لجين DPYD ، والأنماط الفردانية التي تشكلها ، وتركيزات المصل من capecitabine في النساء بعد سن اليأس مصابات بسرطان الثدي في العراق ،

طرائق العمل: أجريت الدراسة على 200 سيدة منهن 100 امرأة غير مصابة بسرطان الثدي خدمن كمجموعة ضابطة تتراوح أعمارهن بين (45-75 سنة) و 100 امرأة مصابة بسرطان الثدي تراوحت أعمارهن بين (40-70 سنة). تم تصنيف مرضى مركز الأورام في مدينة الإمام الحسين الطبية في كربلاء بالعراق إلى مجموعات بناءً على أعمارهم ومؤشر كتلة الجسم ومدة المرض وطول العلاج لهذه الدراسة ، والتي أجريت بين يوليو 2022 وأكتوبر 2022 ، تم قياس تركيز الكابسيتابين ومستويات البلازما من (استراديول ، كا 15.3 ، والكالسيوم) في 100 مريض بسرطان الثدي ممن تناولوا الدواء لمدة 3 أشهر على الأقل ، أعطى جميع المشاركين موافقتهم المستنيرة. تم إجراء تحديد تعدد الأشكال بواسطة اختبار Alelle Specific PCR لاثنين من تعدد الأشكال الجيني (G> A) (rs3918290)و(rs55886062 ، (T> G وتم حساب تركيز عقار كابسيتابين وفلورويوراسيل بواسطة .HPLC تم إجراء التحليل الإحصائي باستخدام برنامجي SPSS و.Haploview

النتائج: أظهرت تراكيز CA15.3 و Capecitabine و FU 5في مرضى سرطان الثدي اختلافات واضحة ومعنوية ، وأظهرت نتائج تعدد الأشكال (rs3918290) (G> A) أن تركيزهم كان أعلى بشكل ملحوظ في Capecitabine و FU 5في المرضى الذين يعانون من أليل TT مقارنة بالمرضى الذين يعانون من أليلات CC و CT ، وأنه كان أعلى بشكل ملحوظ في Capecitabine و FU 5 في المرضى الذين يعانون من أليل CC مقارنة بالمرضى الذين يعانون من أليلات AA و AC في المرضى الذين يعانون من . (rs55886062) DPYD * 13 كان تركيز Capecitabineأكبر في المرضى الذين يعانون من الأليلات الطافرة .(P 0.001) وأظهرت النتائج زيادة ذات دلالة إحصائية (P 0.05) في تركيز مستويات CA15.3 في النساء المصابات بسرطان الثدي مقارنة بالمجموعة الضابطة. سرطان الثدي (BC) هو الشكل الأكثر شيوعًا من السرطانات

الناتجة ؛ وكان متوسط مستويات الكالسيوم والإستراديول في الدم أعلى في المرضى الذين عولجوا بالعلاج الكيميائي لسرطان الثدي.

الخلاصة: إن وجودCA15.3 ، والكالسيوم المصل ، والإستراديول في مصل المريض يجعلهم مؤشرات بيولوجية تشخيصية جديدة ذات قيمة محتملة لمرضى سرطان الثدي بسبب مستوياتهم العالية من الاستقرار. بالإضافة إلى ذلك ، قد تعكس علامات الورم ، وخاصةCA15-3 ، الخصائص البيولوجية للأورام ، مثل الكالسيوم في الدم والإستراديول ، وقد تكون مؤشرات تنبؤية مفيدة في حالات سرطان الثدي المتكرر.

وقد يساهم التباين الجيني في انزيم استقلاب DPYD في التباين في فعالية العلاج بالكابيسيتابابين في مثال على سرطان الثدي لدى النساء العراقيات بالإضافة الى التباين في حدوث التفاعلات الدوائية الضارة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

تأثير تعدد الأشكال الجينية لإنزيم استقلاب DPYD على فعالية عقار الـ Capecitabine في النساء المصابات بسرطان الثدي في العراق

رسالة

مقدمة الى كلية الصيدلة – جامعة كربلاء

جزءا" من متطلبات الحصول على شهادة الماجستير في علم الادوية والسموم

من قبل

رائد فضل عباس

بكالوريوس صيدلة (جامعة بغداد 2005)

بإشراف

الأستاذ الدكتور	الاستاذ المساعد الدكتور
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