Impact of Cytidine Deaminase

Gene Polymorphism on the Capecitabine Monotherapy Response in Iraqi Breast Cancer Women

A Thesis

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صدق الله العلي العظيم

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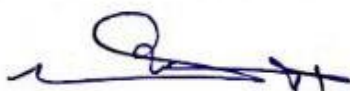
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Dedication

To my

Father & Mother

To my family

To my wife

To My Friends

I dedicate my work

Ali

Acknowledgment

First and foremost, I appreciate Almighty Allah for giving me the ability to complete this job.

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Abbreviation

Abbreviation	Meaning
5-DFCR	5'-deoxy-5-fluoro cytidine
5-DFUR	5'-deoxy-5-fluorouridine
5-FU	5-fluorouracil
B.C	Breast cancer
b.p	Base pair
BCS	Breast cancer surgery
BCT	Breast-conserving therapy
BLBC	Basal-like breast cancer
BRCA1	Breast Cancer gene 1
BRCA2	Breast Cancer gene 2
Ca	Calcium
CA 15-3	Cancer antigen 15-3
CDA	Cytidine deaminase
CEA	Carcinoembryonic antigen
Cmax	Maximum concentration
CT	Computerized tomography
DCIS	Ductal carcinoma in situ
DNA	Deoxyribonucleic acid
DPD	Dihydropyrimidine dehydrogenase
dUMP	Deoxy uridine monophosphate
E_2	Estradiol
EDTA	Ethylenediamine tetra acetic acid
ER	Estrogen receptor
ERBB	erythroblastosis oncogene B

ET	Endocrine therapy
EtOH	Ethyl alcohol
FBAL	Fluoro-beta-alanine
FdUMP	fluorodeoxyuridine monophosphate
FUH$_2$	Dihydro fluorouracil
FUTP	Fluorouridine tri-phosphate
h	hours
HER2	human epidermal growth factor receptor 2
HFS	hand-foot syndrome
HPLC	High-performance liquid chromatography
L	Liter
LCIS	Lobular carcinoma in situ
LRR	Locoregional recurrence
ml	Milliliter
MRI	Magnetic resonance image
MUC-1	Mucin-1
NFB	Neurofibromatosis
°C	The degree Celsius
PAI-1	Plasminogen activator inhibitor-1
PCR	Polymerase chain reaction
PET	Positron emission tomography
Pg/ml	Pecogram per milliliter
PI	Pneumatosis intestinalis
P-gp	P-glycoprotein
PM	Partial mastectomy
PR	Progesterone receptor

PTHrP	Parathyroid hormone-related protein
r.p.m	Revolutions per minute
RBC	Red blood cell
RNA	Ribonucleic acid
rs	reference SNP
SHS	Second hand smoking
SNPs	Single nucleotide polymorphisms
Tmax	Maximum time
TNBC	Triple-negative Breast Cancer
TP	Thymidine phosphorylase
uPA	urokinase plasminogen activator

ABSTRACT

Background: Cancer is a group of diseases characterized by uncontrolled and abnormal cell proliferation. Breast cancer is the most prevalent malignancy among women worldwide, with a 25% cancer-related mortality rate, breast cancer has become a significant threat to female health in Iraq, where it is the major leading cause of death among women after cardiovascular disease. Carboxy esterase, cytidine deaminase, and thymidine phosphorylase are the three enzymes that are required for the activation of the oral prodrug capecitabine.

Aim of study: To investigate the relationship between breast cancer, the (rs207267) cytidine deaminase genetic polymorphism, and (rs532545) cytidine deaminase genetic polymorphism on the efficacy and toxicity of the drug capecitabine and the influence of the cytidine deaminase gene polymorphism on the efficacy and toxicity of capecitabine.

Methods: At the oncology center at Imam al-Hussein Medical City in Kerbala, Iraq, the research was done on a total of 200 women between 1 July 2022 to 1 January 2023. This included 100 healthy women who acted as a control group and were older than 45 years old, as well as 100 women who had been diagnosed with breast cancer and were older than 45 years old. The research was carried out on both groups.

Estradiol, cancer antigen 15-3, and calcium levels in the blood plasma of both healthy women and women with breast cancer have been evaluated. Plasma drug concentration was determined for 100 women who had been taking capecitabine for at least three months. Using allele-specific PCR, genetic polymorphism was conducted on both genes (rs207267 and rs532545). The SPSS program was utilized for statistical analysis.

Result: In this study both alleles (rs207267 and rs532545) genetic analysis shows heterozygotes are more prevalent than homozygotes and mutant types.

ABSTRACT

Plasma drug concentration in both genes (rs207267 and rs532545) was higher in mutant types than in heterozygotes and homozygotes, respectively.

In this study, there were statistically significant differences between the control groups and the cancer patients regarding estradiol and the cancer antigen CA 15-3 and there is no statistically significant difference in calcium levels between the two groups.

Conclusion: Estradiol and cancer antigen 15-3 can be used to assess the treatment response of breast cancer patients. The genetic polymorphism of cytidine deaminase may influence the efficacy and toxicity of capecitabine in breast cancer patients.

Chapter One

Introduction

1. Breast Cancer

1.1. Background

Cancer denotes a group of diseases characterized by uncontrolled and abnormal cell proliferation. A tightly regulated process of cell division, growth, and death ensures the correct functioning of tissues and organs in a healthy body. This control mechanism malfunctions in cancer, resulting in the formation of a mass of cells known as a tumor.

Cancers can be benign or malignant. Benign tumors are noncancerous and typically do not metastasize. They can still pose a health risk if they continue to grow and exert pressure on the surrounding tissues. Malignant tumors, on the other hand, are cancerous and have the ability to spread to distant regions of the body via the bloodstream or lymphatic system, a process known as metastasis. Cancer may have multiple causes, including genetic, environmental, and lifestyle variables. Tobacco use, exposure to toxic chemicals, radiation, certain infections, and genetic predisposition are all cancer risk factors (Hausman et al., 2019).

Typically, cancers are named after the body part from which they originated; therefore, breast cancer refers to the abnormal growth and proliferation of breast tissue-originating cells (Khawaja et al., 2004).

There are two main categories of tissue in the breast: glandular tissue and stromal (supportive) tissues. Glandular tissues contain the milk-producing glands (lobules) and ducts (the milk passages), whereas stromal tissues consist of the breast's fatty and fibrous connective tissues. Lymphatic tissue-immune system tissue removes cellular fluids and waste from the breast (Sing-Huang Tan et al., 2008).

Numerous varieties of tumors can develop in several breast regions. The majority of breast tumors result from benign (noncancerous) breast changes. In

a fibrocystic change, as an example, women develop cysts (fluid-filled sacs), fibrosis (formation of scar-like connective tissue), lumpiness, and areas of enlargement, tenderness, or breast pain (Feng et al., 2018).

Breast cancers (ductal cancers) originate in the cells lining the ducts. The origin of some cancers may be in the cells that line the lobules (lobular cancers), while others may originate in other tissues (Henry et al., 2020).

Cancer cells have DNA and RNA that are similar (but not identical) to the DNA and RNA of the cells from which they originated. This is why they are rarely detected by the immune system, especially when it is compromised. Normal cells are transformed into cancer cells as a result of a DNA or RNA mutation. These modifications/mutations can occur spontaneously or they may be induced by other factors such as; nuclear radiation, electromagnetic radiation (microwaves, X-rays, Gamma-rays, Ultraviolet-rays, etc.), viruses, bacteria and fungi, parasites due to tissue inflammation/irritation, heat, chemicals in the air, water and food, mechanical cell-level injury, free radicals, evolution and aging of DNA and RNA, etc. All of these can result in mutations that can cause cancer. Cancer can be termed "Entropic Disease" Due to the reason that it causes the entropy of the organism to increase to a level where the organism is unable to repair it. The return of an organism to a state of stable entropy requires external intervention (Mieszkowski et al., 2006) (Sharma GN et al., 2010).

1.2. Epidemiology of Breast Cancer

Breast cancer is the most prevalent malignancy among women worldwide, accounting for 25% of all cancers and an estimated 1.57 million new cases as of the most recent report. It is also the leading cause of cancer-related death for women although survival rates from this disease have improved dramatically in countries with abundant resources, the risk continues to rise, resulting in high mortality rates in low- and middle-income countries (Anderson et al., 2007).

Worldwide, breast cancer is one of the leading causes of cancer morbidity and mortality. According to the status report on the GLOBOCAN 2018 estimates of cancer incidence and mortality, breast cancer was the second most commonly diagnosed malignancy, accounting for more than 11.6% of all female cancers. It ranked as the fifth most common cause of cancer deaths, leading to 6.6% of all cancer mortality worldwide (Huang J, 2021).

EMR consists of 21 member nations in the Middle East, North Africa, and Central Asia, according to the WHO classification. Among the nations participating are Iran, Bahrain, Djibouti, Egypt, Jordan, Kuwait, Lebanon, Libya, Morocco, Palestinian territories, Oman, Pakistan, Saudi Arabia, Somalia, Sudan, Syria, Tunisia and the United Arab Emirates. During the past decade, the International Agency for Research on Cancer (IARC) estimated that 292,677 new cases of cancer had been diagnosed among the female population of EMR, and 176,139 women had died of cancer. In females, the five most prevalent malignancies are breast, colorectum, cervix, ovary, and non-Hodgkin lymphoma. Approximately 99,000 cases of breast cancer have been reported in this region. Breast cancer is the most common cancer, with an exponential increase in incidence and an increasing number of patients presenting with advanced stages of the disease in the EMR. In many countries of the region, breast cancer survival rates at five years are considerably lower than in high-income countries due to these factors (Al Alwan et al., 2000).

Breast cancer has become a significant threat to female health in Iraq, where it is the primary cause of death among women after cardiovascular disease, with a mortality rate of 23%. It has been the most prevalent cancer in the Iraqi population since 1986. The Iraqi Cancer Registry recorded a total of 21,101 new cancer cases among an estimated population of 32,500,000 in the previous decade; 9,268 were in men and 11,833 were in women (Iraqi Cancer Board, 2012).

Several studies have indicated that there is a propensity for the disease to be detected early on, with a probable prevalence of poorly differentiated tumors as evidenced by substantial rates of nuclear aneuploidy, resulting in approximately 60% mortality rates (Alwan et al., 2014) (Alwan N.A.S et al., 2016).

1.3. Pathogenesis

Breast cancer is a multifactorial disease with a complex pathogenesis. Breast cancer development and progression are influenced by genetic, environmental, age, family history, diet, alcohol, obesity, lifestyle, physical inactivity, and endogenous and exogenous endocrine factors (Ali S et al., 2002).

Triple-negative breast cancer has a higher recurrence rate, a faster growth rate, and an inferior prognosis, and has recently been identified in certain patient subgroups. Other breast cancer risk factors include mammographic density and a history of benign disease. However, it is still unclear which of the factors plays the most important role in the pathogenesis of breast cancer (Abdulkareem IH et al., 2013).

1.4. Risk factors of Breast Cancer

1.4 .1. Hormonal factors

Significant roles are played by hormonal factors in the development and progression of breast cancer. Estrogen and progesterone are the two key hormones associated with breast cancer. Hormone-receptor-positive breast cancer refers to breast cancer that is influenced by these hormones. Thus, hormonal factors that affect the maturation of the mammary gland, such as earlier menarche and later menopause, increase the risk of breast cancer. Childbearing reduces the risk of developing breast cancer later in life, and breastfeeding further reduces the risk. As lactation continues, the mother's risk of developing breast cancer drops even further. In a reanalysis of data from

fifty-one epidemiological investigations, the exposure to hormonal contraceptives was assessed. There is a brief rise in the likelihood of breast cancer among oral contraceptive users; however, because oral contraceptive use typically occurs in young women at a time when breast cancer is uncommon, this increase would have little impact on the overall incidence rate of breast cancer. The impact of the hormonal status on breast tissue is well known to clinicians since it is a major biological parameter with important implications for prognosis and treatment. The hormonal status (i.e., expression of the estrogen receptor -ER- and progesterone receptor -PR) as well as the HER2 oncogene status, are routinely evaluated to orient clinicians in making decision therapy. It is important to note that estrogen and progestogen exposure takes place at a specific time when breast cancer risk is highest, and epidemiological studies and randomized controlled trials consistently demonstrate that estrogen and progestogen exposure is associated with an increased risk of breast cancer. It is believed that women with a first-degree family history of breast cancer have a greater risk of developing breast cancer, however, there is no evidence to indicate that oral contraceptives or hormone therapy during menopause affect this risk. A selective estrogen receptor modulator may be useful in treating or preventing breast cancer, depending on the specific agonist or antagonist effects on estrogen-targeted tissues (ESHRE Capri Workshop Group, 2004).

1.4.2. Alcohol

Even at modest levels of consumption (15 to 30 grams per day or 1 1/4 to 2 1/2 drinks), serum concentrations of endogenous estrogens are increased by alcohol consumption. EtOH increases ER-dependent gene expression in ER-positive human breast cancer cells, resulting in cell proliferation. This hypothesis of EtOH carcinogenesis is supported by epidemiological evidence; women with ER-positive breast tumors have a higher risk of developing breast cancer if they consume alcohol than women with ER-negative tumors. The primary

mechanism of alcohol-induced carcinogenesis is the Role of Ethanol Metabolism (EtOH). Alcohol dehydrogenase (ADH) metabolizes EtOH into acetaldehyde, its primary carcinogenic metabolite in breast cancer cells where it accumulates within mammary tissues following alcohol consumption (Shield et al., 2016).

1.4.3. Genetic factors

Breast cancer poses a significant threat to public health, and it is anticipated that identifying the genetic factors that contribute to the development of breast cancer will improve prevention efforts. Two breast cancer susceptibility genes breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) have been identified, and it is believed that between 5% and 10% of breast cancer cases are due to germline mutations in these genes. Current evidence suggests that mutations in other highly penetrant genes may play a significant role in breast cancer susceptibility, and efforts are currently underway to isolate these genes. In addition, it is believed that common variants in several gene classes operate as low-penetrance susceptibility alleles, and efforts are underway to identify and characterize these variants (Martin et al., 2000).

1.4.4. Age

Women diagnosed with breast cancer in their twenties and thirties appear to have a worse prognosis than middle-aged women. The cause of this peculiar pattern is unknown. Young women with breast cancer are more likely to have lymph nodes affected, be negative for estrogen receptors, and have large, anaplastic tumors (Kroman et al., 2000).

Nonetheless, over forty percent of breast cancer patients are over the age of sixty-five, and this age group accounts for almost sixty percent of all breast cancer fatalities. Before the age of 49, the estimated risk of developing breast cancer is 1 in 53; however, this risk increases to 1 in 23 between the ages of 50

and 59, and again to 1 in 23 between the ages of 60 and 69. Significantly, this risk is highest for women aged >70, with a 1/15 probability of developing breast cancer (McGuire et al., 2015).

1.4.5. Family history

Women with a family history of breast cancer account for 28% of all cases. A woman's chance of having breast cancer increases by a factor of 2 to 4 if she has a family history of the disease, depending on the number of affected relatives and the age at which they were diagnosed (Brewer *et al.*, 2017).

Several well-known genes are enriched in families with breast or ovarian cancers. For instance, 50–85% of women with mutations of breast cancer type 1 and type 2 genes (BRCA1 and BRCA2) will develop breast and/or ovarian cancer in their lifetime. Breast/ovarian cancers caused by mutations in these common genes are defined as Hereditary Breast and Ovarian Cancer (HBOC), as mutations can be inherited in family members and cancers are enriched in these families (Liu *et al.*, 2021).

1.4.6. Lifestyle factors

Survival after breast cancer diagnosis greatly depends on tumor characteristics (*i.e.*, tumor size, grade, receptor, and lymph node status) and appropriateness of treatment. However, an effect of a few risk factors for breast cancer onset on cancer prognosis has been reported and some of such factors are potentially modifiable (*i.e.*, postmenopausal obesity, alcohol use, physical activity) (Dal Maso *et al.*, 2008).

The association between body composition, including body weight and breast cancer risk, is well known. High Body Mass Index (BMI) has been positively associated with postmenopausal breast cancer, but most studies report that excess body weight is inversely related to premenopausal breast cancer risk.

Obesity has also been associated with reduced breast cancer survival. A physically active lifestyle compared with a sedentary lifestyle has been observed to reduce both pre- and postmenopausal breast cancer risk overall. The association between physical activity and breast cancer survival is still being debated (Lofterød et al., 2020).

1.4.7. Obesity

Obesity is associated with reduced survival in patients with breast cancer (Porter et al., 2006) a hormone that has multiple biological functions, leptin is produced primarily by adipose tissue. In humans, leptin levels correlate with total body fat, and obese women have particularly high levels. Several actions of leptin, such as the promotion of growth, migration, and invasion of normal and tumor cells, as well as the enhancement of angiogenesis, indicate that this hormone may promote an estrogen-independent aggressive breast cancer phenotype. This effect may be caused by the activation of the NFB transcription factor. Also, leptin can stimulate the activity of aromatase, which increases the production of estrogen from androstenedione in adipose tissue, thereby contributing to the progression of estrogen-dependent breast cancer. Therefore, it is hypothesized that leptin, possibly in conjunction with insulin, whose plasma concentration is correlated with that of leptin, may contribute to the well-documented negative influence of adiposity on breast cancer (Rose et al., 2002).

1.4.8. Cigarette smoking

For decades, tobacco use has been causally linked to numerous types of cancer, particularly lung cancer, and has been blamed for nearly one-third of cancer-related deaths. Malignancies associated with smoking, such as those of the stomach, pancreas, bladder, kidney, liver, uterine cervix, ovary, and colon, do

not directly involve inhalation pathways, supporting the concept of systemic effects (Kispert & McHowat, 2017).

As a result of active and secondhand smoking, tobacco smoke contains hundreds of toxic chemicals (SHS) (United States Department of Health and Human Services, 2010). There are two categories of secondhand smoke: (side stream) smoke produced by tobacco combustion and (mainstream) smoke exhaled by smokers' tobacco smoke's carcinogenic properties, specifically its polycyclic aromatic hydrocarbon content, were first identified in 1964 by the first report of the surgeon general on smoking and health. Cigarette smoke, whether mainstream or side stream, contains over 7,000 chemicals, 69 of which are known carcinogens, including over 20 known breast carcinogens (Reynolds et al., 2013).

1.5. Locational Classification of Breast Cancer

Breast cancer can be classified into different types based on the location of the cancer cells within the breast. The two primary kinds are: (Jayasinghe et al., 2009).

The term breast carcinoma in situ was coined long ago to describe lesions comprised of abnormal epithelial cells that are completely confined within breast lobules and/or ducts but that look very similar to cells of invasive carcinoma when viewed under a microscope. For many years, it was assumed that these cells were potentially able to invade the adjacent mammary stroma and that, in the absence of treatment, they would eventually progress to invasive cancer. However, it is now understood that in situ breast cancers lack or incompletely express several of the hallmarks of invasive cancers and that the molecular changes involved in progression to invasive cancer do not always occur. Among the two major types of breast carcinoma in situ, ductal carcinoma in situ (DCIS) is considered a true (nonobligatory) cancer precursor, and its

treatment is often similar to that for small, lymph node-negative breast cancer; whereas lobular carcinoma in situ (LCIS), which is also known as lobular neoplasia, is primarily viewed as an indicator of increased breast cancer risk(Ward *et al.*, 2015).

Invasive breast cancer, also known as infiltrating breast cancer, is a form of breast cancer in which cancer cells have invaded the surrounding breast tissue from the layer of tissue where they originated (ducts or lobules) (Matsumoto *et al.*, 2016).

Breast cancer cells that infiltrate and spread outside of the typical lobules and ducts of the breast, spreading into the surrounding breast stromal tissue, are known as invasive breast cancers. Two-thirds of women diagnosed with invasive breast cancer are 55 or older. Invasive carcinomas can metastasize, or spread to other organs or tissues inside the body, and fall under the category of metastatic breast cancers. The following two forms of invasive breast cancer are further classified based on the types of tissue and cells involved (Feng et al., 2018).

The stage of invasive breast cancer is determined by the size of the tumor, the extent of lymph node involvement, and the presence of metastatic disease. The staging system assists in determining the optimal treatment strategy and prognosis. Depending on the specific instance, invasive breast cancer may be treated with a combination of surgery, radiation therapy, chemotherapy, hormone therapy, and targeted therapy (Sinn et al., 2013).

1.6. Molecular Classification of Breast Cancer

One of the most novel findings regarding the molecular classification of basal-like breast malignancies. Breast tumors' genomic heterogeneity has been directly demonstrated by high-throughput technologies; Breast tumors were

categorized by global gene expression profile studies into five intrinsic subtypes using hierarchical clustering.

In addition to the traditional hormone receptor-positive and hormone receptor-negative subtypes of breast cancer, gene expression profiling has identified several additional significant subtypes. The luminal A and luminal B categories are the most reliably identified molecular subtypes among hormone receptor-positive cancers. Among hormone receptor-negative breast cancers, the main molecular subtypes identified are HER2 and basal-like. Other molecular subtypes, including luminal C and normal breast-like groups, have been identified in some studies, but they are less well-defined than the luminal A, luminal B, HER2, and basal-like subtypes (T Sorlie et al., 2001).

Breast cancer molecular subtypes differ in their patterns of gene expression, clinical characteristics, treatment response, and prognosis (Schnitt et al., 2010).

Luminal A high expression of ER-related genes and luminal epithelial genes with poor survival.

Luminal B has lower luminal expression ER-related and epithelium but a higher level of genetic multiplicity and HER2-associated genes rather than luminal A with intermediate survivals.

HER2-Over expression (OE) HER2-related genes are expressed more frequently than ER-related genes, and vice versa with intermediate survivals.

Basal-like high basal expression ectodermal and low proliferative genes a description of HER2- and ER-gene with poor survivals.

1.7. Histopathology of Breast Cancer

The histopathology describes the pattern of tumor proliferation. Pathologists have long been intrigued by the histological diversity of breast adenocarcinomas; they have identified specific morphological and cytological

patterns that are consistently associated with distinct clinical presentations and/or outcomes. These patterns are known as 'histological types (Weigelt et al., 2010).

The most prevalent type of breast carcinoma is invasive ductal carcinomas not otherwise specified (IDC-NOS) or of no special type (IDC-NST), which is a diagnosis of exclusion and consists of adenocarcinomas that lack sufficient characteristics to justify their classification in one of the special types. The most recent edition of the World Health Organization classification recognizes the existence of at least 17 distinct histological subtypes of breast cancer, which account for up to 25% of all breast malignancies.

Noteworthy is the fact that grade and type provide complementary information, although grade identifies prognostic subgroups among breast cancer subtypes, some organisms with a high histological grade (e.g., medullary carcinomas) have a relatively favorable prognosis (P. Ellis et al., 2003).

1.8. Hormone receptors in the treatment of Breast Cancer

The majority of human breast tumors express hormone receptors (HRs) - estrogen receptors (ERs) and/or progesterone receptors (PRs); these transcription factors are the primary drivers of oncogenesis in HR-positive (HR+) breast cancers. Both are potential targets and predictors of the response to anti-estrogen therapy (Elgene Lim et al., 2012).

An epigenetic mechanism that restricts the recruitment of ER to a subset of potential binding sites in response to estrogen stimulation recruits ER to specific sites across the genome in an organized manner. ER signaling can be effectively inhibited by inhibiting estrogen binding to the ER with tamoxifen, blocking estrogen biosynthesis with aromatase inhibitors (AIs) and luteinizing hormone-releasing hormone (LHRH) agonists, and downregulating ER with fulvestrant . Despite adjuvant anti-estrogen therapy, a significant proportion of patients

experience relapse. The majority of patients with metastatic disease eventually develop anti-estrogen therapy resistance. It is important to note that HR+ tumors exhibit substantial molecular and clinical heterogeneity, and that they do not represent a single disease entity. HR+ breast cancer has a higher risk of recurrence following adjuvant anti-estrogen therapy than other subtypes of the disease, with a risk ranging from 1% to 4%, depending on the extent of the primary tumor (Elgene Lim et al., 2012).

1.9. Diagnosis of Breast Cancer

Over the long term, it is possible to reduce the mortality rate associated with breast cancer by substantially detecting and treating it at an early stage.

1.9.1. Mammography The gold standard for breast cancer screening is mammography, but it is less effective in women under 40 and in those with dense breasts. Mammography is less sensitive to small lesions (less than 1 mm in diameter, approximately 100,000 cells in size), and provides no indication of a patient's prognosis (Gøtzsche & Jørgensen, 2013).

1.9.2. Contrast-enhanced (CE) digital mammography provides greater diagnostic accuracy than mammography and ultrasound in dense breasts; however, it is not broadly available due to its high cost and radiation exposure (Lewis et al., 2017). Ultrasound has been utilized as a supplement to mammography as a medical imaging instrument. It is possible to detect small lesions using magnetic resonance imaging (MRI) that cannot be detected by mammography. It is expensive and has a low specificity, which may lead to overdiagnosis (Hassan et al., 2011).

1.9.3. Positron emission tomography is the best method for visualizing the spread of malignancies, or their response to treatment (Xu et al., 2015).

1.9.4. Microwave imaging (MI) It has been recommended that microwave imaging (MI) techniques may be used as an alternative to mammography for the

diagnosis of breast cancer as a safe and inexpensive method. Recent research has focused on the development of MI theory and the development of implementation systems for laboratory settings. There have been several MI methods devised and evaluated both numerically and experimentally. Recent clinical studies suggested that researchers may want to focus more on the development of MI prototypes for clinical environments, with a particular emphasis on high-sensitivity radio frequency (RF) sensors and sensor arrays (Lee et al., 2012).

In addition to screening methods, breast biopsies are typically conducted to differentiate between cancerous and benign tissues, but this method is costly and requires trained personnel. Several biomarker-based diagnostic techniques are available, such as radioimmunoassay, immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and fluorine immunoassay. In spite of their sensitive and selective nature, biomarkers are expensive, time consuming, require trained personnel, and require complex labeling procedures. As a result, it is imperative that a highly sensitive, label-free method be developed for the rapid diagnosis of breast cancer (Wang et al., 2018).

1.10. Biomarker of Breast Cancer

There are numerous breast cancer detection markers (Tang et al., 2006). The low concentrations of cancer markers in DNA biomarkers make them ineffective for early detection of cancer. However, they provide valuable information on tumor progression. Breast cancer is primarily diagnosed by protein biomarkers, which are both predictive and prognostic. Predictive protein markers provide information regarding a specific therapeutic intervention, whereas prognostic protein markers provide information regarding the subjects as a whole.

1.10.1. Proteomic Biomarkers

Several protein biomarkers have been evaluated for clinical applications, including RS/DJ-1, p53, heat shock protein 60 (HSP60), HSP90, mucin 1 (MUC1), and human epidermal growth factor receptor 2 (HER2) (Le et al., 2001). A previous study found that the serum RS/DJ-1 levels of newly diagnosed breast cancer patients are substantially higher than those of healthy subjects. However, it is difficult to infer that RS/DJ-1 is specific to breast cancer because this study did not investigate other types of breast tumors.

It has been observed that approximately 15% of breast cancer patients have p53, although this is not unique to breast cancer, as it has also been found in cases of other malignancies and inflammatory conditions. Although p53 autoantibodies are associated with a poor prognosis, HSP60 and HSP90 autoantibodies are also used to diagnose breast cancer, both of which are associated with a poor outcome (Kuli et al., 2010).

It is a traditional biomarker for advanced breast cancer with limited sensitivity for early-stage breast cancer. CA15-3, which detects mucin MUC1, has been extensively used in the diagnosis and monitoring of metastatic breast cancer recurrences (Cui et al., 2005).

An abundant amount of MUC1 is found on the exterior surface of normal secretory epithelium's apical membrane. There is evidence that MUC1 is expressed both in normal and malignant breast epithelium, however, the clinical utility of MUC1 measurements is limited to measurements of CA15-3, which is released from the surface of the cell by proteolytic cleavage (Chen et al., 2021).

Approximately 30% of breast cancer patients had substantially higher HER2 levels than healthy subjects. Human blood samples have been used to detect HER2 antigens associated with breast cancer. Breast cancer patients have HER2 levels between 15 and 75 ng/mL, whereas healthy subjects have HER2 levels

between 2 and 15 ng/mL. Researchers found that circulating HER2 levels may be useful in monitoring disease relapse, and cancer progression, and selecting appropriate treatments, such as prescribing trastuzumab to patients with HER2-positive breast cancers (Molina et al., 2012). HER2 serum levels, tumor size, lymph node involvement, and tumor markers are independent prognostic factors for both disease-free survival and overall survival (Swain et al., 2023).

1.10.2. Gene Biomarkers

Breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) are two genes associated with breast cancer susceptibility. The genes are tumor suppressors that repair DNA double-strand breaks caused by breast cancer. The instability of the human genome caused by gene mutations increased the likelihood of breast cancer by approximately 21-40% for inherited breast cancers. On a graphene-modified glassy carbon electrode, hybridization of the capture probe and reporter probe DNAs is performed in a sandwich arrangement. The sensor was stable, reproducible, and sensitive enough to detect BRCA1 gene concentrations as low as 1 femtomole (Nyberg et al., 2022).

P53 mutations are present in 30–35% of breast malignancies. The p53 gene has been analyzed using a DNA biosensor (Yang et al., 2016). To characterize the affinity properties of response elements (REs) and the p53 gene, active oligonucleotide probes are injected serially above the response elements (REs). These tests disclose differences in affinity between each ligand and REs. In a previous study (Chase et al., 1984), a single-strand binding protein biosensor was designed to detect p53 mutations in breast cancers (Hu et al., 2021).

DNA damage caused by apoptotic and necrotic cells is correlated with the development of breast cancer. Quantitative estimation of cell-free tumor DNA (cfDNA) provides a new method of noninvasive breast cancer diagnosis and therapeutic information to determine the relationship between breast cancer

progression and cfDNA concentration, cfDNA has been studied as a breast cancer indicator (Schwarzenbach et al., 2015), but the method is not yet fully developed. Using the hybridization concept and guanine oxidation, microRNAs (miRNAs) are emerging as reliable markers. (Matamala et al., 2015) investigated the target miRNAs using a variety of electrochemical nano biosensors. As one of these miRNA markers, miR-21 is the most stable and has the highest sensitivity and specificity, but it does have some drawbacks, such as sequence homology with related RNAs, occurrence in other malignancies, and low serum concentration.

1.11. Management of Breast Cancer

Each treatment plan is individualized and should be made together by a patient and a cancer doctor. For patients with stage, I to III breast cancer, the goal of treatment is to cure the breast cancer. Usual treatment consists of surgery to remove the breast tumor, medicines, and possibly radiation therapy to the breast. For patients with stage IV breast cancer, which has spread to a distant part of the body, the goal of treatment is to control the breast cancer for as long as possible. Treatment for stage IV breast cancer consists mainly of medicines.

Different types of breast cancer respond to different medicines. Hormone receptor–positive breast cancer is best treated with anti-estrogen pills. Some patients need intravenous chemotherapy infusions as well. ERBB2-positive breast cancer is treated with intravenous medicines that specifically attack the abnormal ERBB2 protein along with intravenous chemotherapy. Triple-negative breast cancer is treated with intravenous chemotherapy(Waks and Winer, 2019).

1.11.1. Surgery

Depending on the stage and nature of the tumor, a lumpectomy (removal of only the tumor) or mastectomy (removal of the entire breast) may be required. Typically, a surgeon must confirm that the margins of the removed tissue are

cancer-free, indicating that the cancer has been completely eradicated. If the removed tissue does not have distinct margins, additional procedures may be required to remove more tissue. Sometimes, a portion of the pectoralis major muscle, the primary muscle in the anterior chest wall, may be excised. There has recently been a trend towards dissecting sentinel lymph nodes (SLNs) since this requires the removal of significantly fewer lymph nodes, resulting in fewer adverse effects. Over the past decade, advancements in sentinel lymph node imaging have increased the accuracy of sentinel lymph node detection from 80% using blue dye alone to between 92% and 98% using combined modalities (Breast cancer treatment - Manipal Hospitals India, 2010).

A lumpectomy involves the removal of a tiny amount of surrounding normal tissue. Wide excision: Also known as a partial mastectomy, this procedure refers to the removal of a relatively larger quantity of the surrounding normal tissue.

A quadrantectomy is a breast-conserving technique including wide resection and tumorectomy. A quadrantectomy is designed to remove an anatomic segment of breast tissue (duct-lobular system) (Veronesi and Zurrida, 2000).

1.11.2. Radiotherapy

Radiation therapy involves the use of high-energy X-rays or gamma beams to treat a tumor or a postoperative tumor. These radiations are extremely effective at destroying cancer cells that may persist after surgery or recur in the area where the tumor was removed. In addition to this treatment, radioactive catheters (brachytherapy) can be implanted, similar to those used to treat prostate cancer. Nevertheless, this treatment option has been replaced by radiotherapy in which an electron beam is focused on the breast incision an integral part of breast-conserving treatment is radiation therapy administered following surgery for cancer cells to be eradicated, the radiation dose must be

sufficient. Treatments are typically administered five days a week for five to seven weeks. Approximately fifteen minutes are required for each treatment (Rath G. et al., 2010) (American Cancer Society, 2009).

1.11.3. Nanotechnology for Treating Breast Cancer

There are currently more than 150 clinical trials investigating the efficacy of nanotechnology-based cancer drug delivery carriers. This demonstrates the rapid development of nanotechnology. Different formulations of liposomal doxorubicin have been developed to improve the therapeutic index of conventional doxorubicin chemotherapy while preserving its antitumor properties. Three liposomal doxorubicins are currently being evaluated for efficacy: liposomal daunorubicin, liposomal doxorubicin, and pegylated liposomal doxorubicin. In addition to liposomal doxorubicin, albumin-bound paclitaxel is another example of a nanovector-based application for breast cancer chemotherapy based on EPR. Paclitaxel is a highly hydrophobic compound that is dissolved in cremophor to prevent precipitation. However, cremophor-associated toxicity is substantial (hypersensitivity reaction and neurotoxicity) and poses a challenge to paclitaxel administration. Albumin-bound paclitaxel was developed to enhance paclitaxel's solubility (Tanaka T et al., 2009).

1.11.4. Recent approaches in the management of BC

Gene Therapy

Cancer is widely recognized as the result of multiple molecular genetic defects, which produce a cellular phenotype characterized by uncontrolled growth. Based on this knowledge, numerous gene therapy strategies have been devised as potential new cancer therapies. Currently, it is believed that proto-oncogenes and tumor suppressor genes play an important role in the development of cancer, which has led to the development of gene therapy techniques designed

to eradicate or restore these genes. Alternative strategies involve using cancer cells to convert a systemically administered prodrug into a toxic metabolite or a target for destruction using replicating viral vectors. In contrast, the transfer of drug-resistance genes into normal cells may provide chemoprotection during high-dose anticancer treatment. Anticancer drug defense mechanisms can be activated through modulation of the immune system (Osborne C et al., 2004).

Oncogenes Inactivation

Several oncogenic proteins have been identified and linked to numerous types of cancer. Until now, antisense strategies have been the method most frequently utilized in clinical trials. Using the adenoviral gene E1A, which interferes with the transcription of erbB-2, it is possible to inhibit the transcription of oncogenes, which is an effective method for treating cancers that overexpress this oncogenic protein, such as breast and ovarian cancer (Osborne C et al., 2004).

Augmentation of Tumor Suppressor Genes

More than twenty-four tumor suppressor genes have been identified, and mutations in these genes have been linked to a wide range of malignancies. Several clinical trials utilizing adenoviral vectors to deliver p53 to a variety of malignancies are currently underway. A retinoblastoma gene was introduced into bladder cancer using viral vectors, and the breast cancer gene BRCA1 was introduced into ovarian cancer using viral vectors. This approach may fail in some instances because the mutant gene has negative effects on the dominant genes. In order to resolve this issue with p53 gene therapy, a genetic restoration strategy may be more effective than gene augmentation (Osborne C et al., 2004).

Cell-Target Suicide

It is possible to artificially distinguish between normal and malignant tissue by genetically engineering tumor cells to convert prodrugs into toxic metabolites. This can be accomplished through the expression of a gene that confers a dominant, negatively selectable phenotype on cancer cells, such as cell death conferred by the expression of a prodrug-metabolism enzyme. By transferring a gene that is not ordinarily found in humans (e.g., HSV-thymidine kinase) rather than by overexpressing an endogenous gene, it will be possible to kill malignant cells with greater selectivity. The prototype of this method combines HSV-1 thymidine kinase gene expression with the production of ganciclovir in a manner that is distinct from mammalian thymidine kinase. DNA synthesis and transcription are inhibited by the incorporation of phosphorylated ganciclovir into DNA. Several clinical trials have been conducted involving multiple malignancies, and the efficacy and safety of this method are being evaluated (Jin-Hui W et al., 2003).

Chemoprotection Approach

The MDR-1 gene, which encodes the multidrug therapy transporter protein (also known as P-P-glycoprotein), has received considerable attention in this regard. A transmembrane protein transports various chemotherapeutic agents (such as doxorubicin, vinca alkaloids, epipodophyllotoxins, and paclitaxel) out of cells, protecting them from their toxic effects (Arango-Varela et al., 2022).

Virus-mediated oncolysis

Adenoviruses and HSV-1 can infect lysed tumor cells. An addition to multidimensional cancer treatment is the combination of oncolytic viruses with other gene-based anticancer therapies. In tumor cells, selective viral replication results in cell lysis and local dissemination of infectious viral progeny. The

majority of research applications of this strategy have involved replication-competent adenovirus and HSV-1 (Ma et al., 2020).

Immunomodulation

A variety of cytokines can enhance immunity against cancer cells, and this discovery has led to the development of gene-based approaches to modulate immune responses in cancer patients.

In tumor cells or their microenvironment, several cytokines have been shown to inhibit tumor growth when expressed ectopically. Some immunostimulatory agents do not initially affect the growth rate of the tumor, but when the animal is subsequently challenged with wild-type tumor cells, they induce immunity against tumor growth.

Immune improvement: One such strategy is to express immunogenic molecules on the surface of cancer cells, such as allotype MHC antigens. To activate T cells, additional "costimulatory" pathways are required that are distinct from those of the T-cell. One such pathway is stimulated by B7-1 (CD 80) and B7-2 (CD 86) molecules. In conjunction with antigen binding to the T-cell receptor, B7s interact with specific receptors on the surface of T cells, whose expression is typically restricted to antigen-presenting cells and other specialized immune effector cells (Jin-Hui W et al., 2003).

1.11.5. Chemotherapy

Chemotherapy is the treatment of cancerous cells with anti-cancer medicines. Depending on the patient's overall health, medical history, age (whether menstruation is present or not), type and stage of cancer, and tolerance to specific medications and procedures, specific treatments will be prescribed most chemotherapy treatments are administered in cycles, with one treatment followed by a recovery period and then another treatment. When chemotherapy

is administered before surgery, the size of the tumor can be reduced, which can sometimes result in a breast-conserving procedure rather than a mastectomy. After surgery, it is commonly administered every three weeks or every two weeks in a dose-dense regimen (Wells B. G. et al., 2004).

1.12. Capecitabine

An oral precursor to the cytotoxic component of 5-fluorouracil is the non-cytotoxic fluoropyrimidine carbamate known as capecitabine (5-FU) (S. W. Lam et al., 2016). Both alone and in combination with other medications, capecitabine is appropriate in advanced or metastatic stages of gastrointestinal or breast cancer. Capecitabine is a prodrug that is completely absorbed from the gastrointestinal tract after oral absorption (Quinney et al., 2005).

1.12.1. Activation of capecitabine

Through three metabolic stages, capecitabine, a novel oral fluoropyrimidine carbamate, is preferentially converted to the cytotoxic moiety fluorouracil (5-fluorouracil; 5-FU) in target tumor tissue (Awrich et al., 1979).

It is first converted to 5-deoxy-5-fluorocytidine (DFCR) by hepatic carboxylesterase and then to 5-deoxy-5-fluorouridine (DFUR) by cytidine deaminase (CDA) after absorption, found in significant concentrations in many human tumor tissues as well as healthy liver tissue (Schüller et al., 2002).

The 5-DFUR is then converted to 5-FU by the tumor-associated angiogenic factor thymidine phosphorylase (TP), which is more prevalent in tumors than in healthy tissues, this reduces 5-FU's exposure to healthy tissues (Nicholl & Dunlop, 1999).

It is widely accepted that treatment of cells with 5-FU causes DNA damage during the S phase, specifically double-strand (and single-strand) breaks, owing to the improper incorporation of FdUTP into DNA (De Angelis et al., 2006).
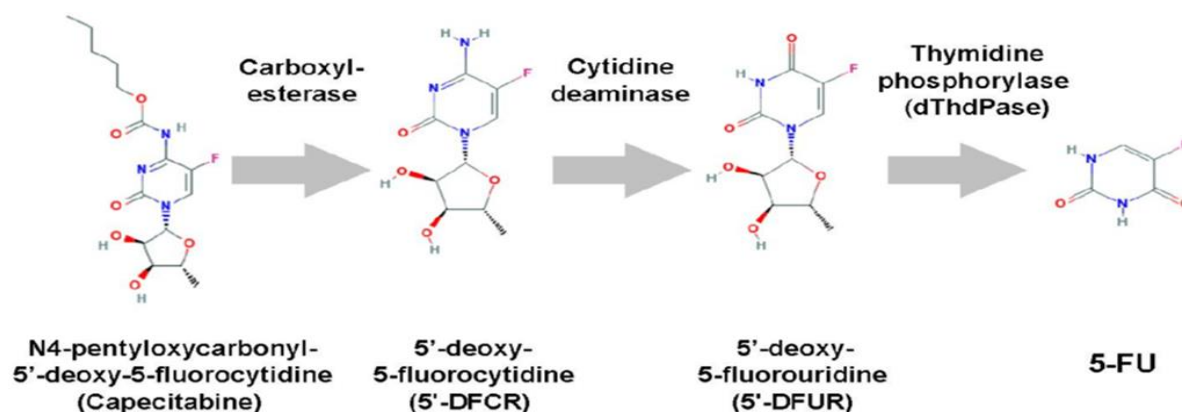
Figure (1-1): Activation of capecitabine (Kim et al., 2002).

1.12.2. Mechanism of action of capecitabine

Dihydropyrimidine dehydrogenase (DPD) is responsible for the catabolism of capecitabine. Dihydropyrimidine dehydrogenase also has cytotoxic activity (Figure 1-2). Fluoro-beta-alanine (FBAL) is converted into dihydrofluorouracil (FUH2) during the degradation of FU, which is mediated by DPD. FBAL is then eliminated through the urine. These two metabolites have no antiproliferative properties. Patients with DPD deficiency, which accounts for 2% to 4% of the population, are at higher risk for FU toxicity because of the role of DPD in detoxifying FU. Fluorodeoxyuridine monophosphate is a mediator of the first cytotoxic mechanism (Walko & Lindley, 2005).

A product of thymidine kinase's enzymatic conversion of FU. Thymidylate synthase is inhibited in the rate-limiting stage of de novo thymidine synthesis by FdUMP, which competes with dUMP to form a stable tertiary complex with folic acid and thymidylate synthase. DNA synthesis is slowed down in the absence of thymidine, which causes cellular death. Additionally, thymidine phosphorylase converts FU into fluorouridine triphosphate, the desired triphosphate form, to prepare it for inclusion into RNA. Additionally, this fake

nucleotide's inclusion prevents DNA synthesis, which leads to apoptosis (Mayer et al., 2015).
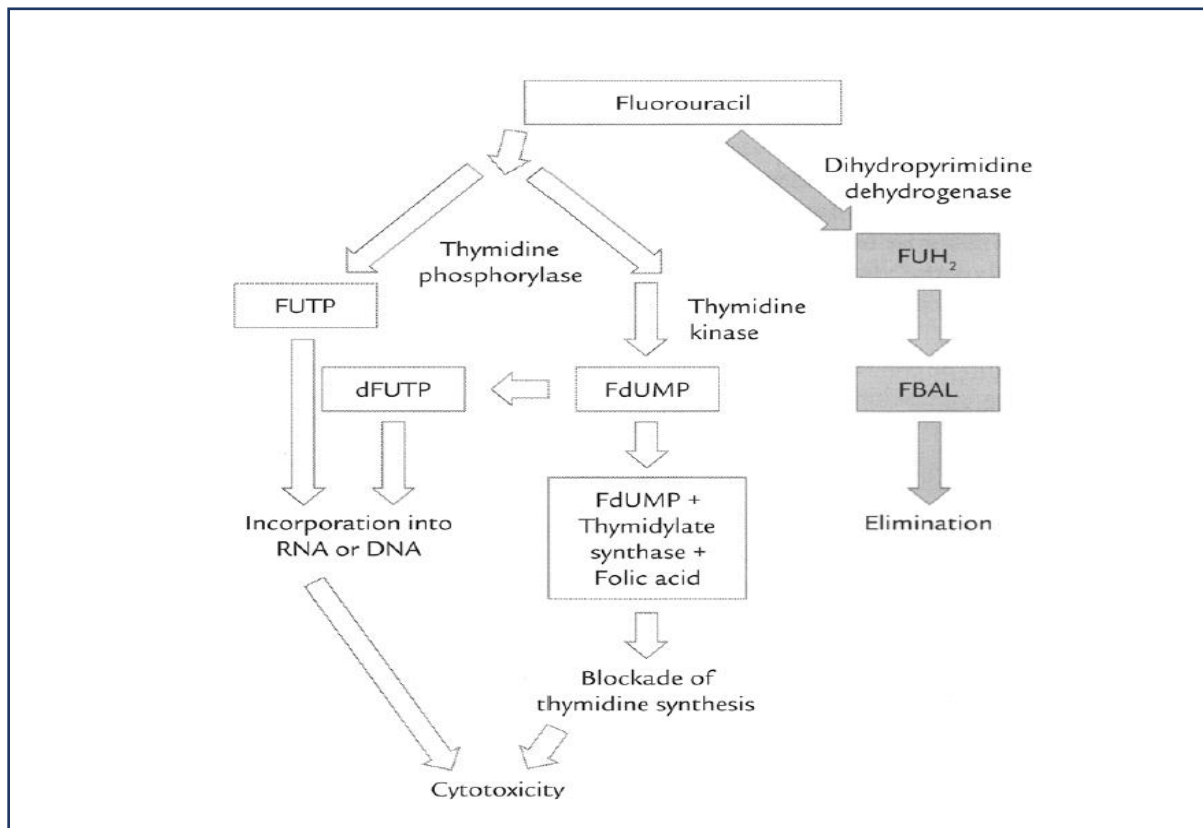
Figure (1-2): The catabolic and anabolic pathways of fluorouracil before elimination or cytotoxic effect, respectively. FUTP=fluorouridine triphosphate; FUH 2 = dihydrofluorouracil; dFUTP =deoxyfluorouridine triphosphate; FdUMP = fluorodeoxyuridine monophosphate; FBAL = fluoro-beta-alanine.

1.12.3. Adverse effect of capecitabine

Several cancers are treated with capecitabine, which has adverse effects such as diarrhea, nausea, and/or vomiting, as well as hand-foot syndrome. It is more feasible and preferred for many patients to administer capecitabine twice daily orally rather than intravenously, as this offers multiple opportunities for dose adjustment and prompt treatment adaptation (Leonard et al., 2011).

1.12.3.1. Diarrhea

In response to cancer treatments like chemotherapy or radiation, gastrointestinal problems, in particular diarrhea, are frequently caused. Chemotherapeutic drugs taken orally can cause diarrhea by harming the lining of the intestines (Abd El-Atti et al., 2009).

Although it has been shown that diarrhea may persist for up to five days after treatment has been stopped, capecitabine usually presents little risk of prolonged harm past this point. To our knowledge, it is the first to link capecitabine to PI (pneumatosis intestinalis) and breast cancer and the third instance of intestinal perforation in a breast cancer patient linked to capecitabine (Khan et al., 2019).

Cytotoxic drugs cause PI by inhibiting homeostasis and can be interfered with by several elements, such as cytostatic drugs, leading to alterations in epithelial structure and cell functions, resulting in mucosal damage. Indeed, cytotoxic agents act on proliferating cells, inhibiting specific cellular processes according to their mechanisms of action, without any distinction between cells (Gazzaniga *et al.*, 2022) .

1.12.3.2. Hand-foot syndrome (HFS)

The most serious side effect of capecitabine is hand-foot syndrome (HFS) (Lassere & Hoff, 2004). Burning pain, together with visible symmetric edema and erythema, may develop in 3–4 days from this. The hands are typically more frequently affected than the feet, and in some instances, they may even be the sole affected part. HFS can disrupt normal daily activities, particularly when blistering, moist erosions, excruciating pain, or ulceration appear. HFS is controllable, but if ignored, it can progress. However, capecitabine dose reduction and cessation typically result in an improvement of signs and symptoms without negative long-term effects. Hand-foot syndrome is

frequently brought on by inhibitors of several kinases and capecitabine (Harder et al., 2012).

1.12.4. Pharmacokinetic of capecitabine

Capecitabine is an oral fluoropyrimidine carbamate that preferentially changes in target tumor tissue through a sequence of three metabolic stages into the cytotoxic moiety fluorouracil (5-fluorouracil; 5-FU). Capecitabine has a relatively short elimination half-life (t1/2) (33 to 53 min) after oral administration of 1250 mg/m^2 and is rapidly and extensively absorbed from the gastrointestinal tract (with a time to reach peak concentration (Tmax) of 2 hours and peak plasma drug concentration (Cmax) of 3 to 4 mg/L). Nearly 100% of drug-related material is recovered from urine and feces (Awrich et al., 1979). Hepatic metabolism is a major route of elimination for the novel oral fluoropyrimidine carbamate capecitabine (Reigner et al., 2003).

1.12.5. Dosage and Administration

Capecitabine should be taken orally, after meals, twice a day for 14 days and one week off at a dosage of 1250 mg/m^2. The treatment is contraindicated in individuals who are pregnant, have significant renal impairment [creatinine clearance of 30 ml/min (1.8 L/h)], or have a known intolerance to fluorouracil. The dosage should be modified according to tolerability. In patients with moderate renal impairment (creatinine clearance 30 to 50 ml/min (1.8 to 3 L/h)), the beginning dose should also be decreased to 940 mg/m^2 (McGavin & Goa, 2001).

1.12.6. Pharmacogenetic of capecitabine

The pro-drug capecitabine is activated by three enzymatic activities to -DFCR and subsequently -DFUR. Thymidine phosphorylase (TP), the final enzyme in the process of converting thymidine to 5-FU, is specific to tumors and is

selectively upregulated by capecitabine. In contrast, 5-FU is inactivated by dihydropyrimidine dehydrogenase (DPYD) (Rudek et al., 2013). Different polymorphisms of the enzymes involved in the metabolism of capecitabine may be responsible for the varying efficacy and toxicity in patients receiving the drug (Roberto et al., 2017).

1.12.6.1. Dihydropyrimidine dehydrogenase (DPYD)

Dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme for the breakdown of pyrimidine bases, plays a critical role in the pharmacogenetic syndrome of 5-fluorouracil (5-FU). Lack of DPD action following 5-FU administration leads to severe toxicities, including death. Numerous studies have shown that a lack of dihydropyrimidine dehydrogenase (DPD) activity is the cause of this pharmacogenetic disease. This deficiency is caused by molecular abnormalities in the DPYD gene (SAIF, 2013).

1.12.6.2. Cytidine deaminase

CDA expression is heterogeneous between tissues. CDA is most commonly expressed in bone marrow and liver, and more moderately or even undetectable in other tissues. More generally, the activity of CDA is higher in mature hematopoietic cells than in immature cells. In cancer cells, the CDA gene is not lost, nor amplified, meaning that the regulation of CDA expression is mainly at the transcriptional level. Several binding sites to transcription factors have been described as CDA promoter or enhancer regions(Frances and Cordelier, 2020).

The CDA gene, which has four exons, is found on the first pair of chromosomes and codes for the human CDA enzyme (1p36.2-p35). An active site of CDA is composed of four identical subunits, each of which must contain zinc. In addition to the liver and placenta, mature neutrophils also contain significant amounts of CDA. Cytidine and 2-deoxycytidine are physiologically deaminated into uridine and 2-deoxyuridine, respectively, by this enzyme. Due to the

significant inter-individual variation in CDA's period of action, it is of great interest (Serdjebi et al., 2015).

The first exon includes the 5′ untranslated region (5′ UTR) and parts of the coding region, whereas the fourth exon encodes for the C-terminus of the protein and the 3′ UTR of the respective mRNA. In several studies, this may have been due to the insufficient numbers of individual patients investigated, whereas other studies have demonstrated a correlation between low CDD activity levels caused by genetic CDD variants with therapy-associated side effects (Lachmann, Brennig and Moritz, 2013).

As nucleoside analogs, these compounds act as antimetabolites, either inhibiting the ability of cancer cells to synthesize precursors of nucleic acids required to ensure sustained growth or directly interfering with DNA or RNA synthesis. Besides their structural similarity, some of these molecules such as gemcitabine, capecitabine, cytarabine, or azacytidine also share a common metabolic pathway, in which cytidine deaminase (CDA) plays a major role. Indeed, this enzyme, produced in the liver but also expressed in other tissues, catalyzes the deamination of pyrimidine analogs, thus allowing either their deactivation (gemcitabine, cytarabine, and azacytidine) or activation of capecitabine (Cohen *et al.*, 2019).

In terms of CDA, the promoter region mutations CDA c.-451C>T (rs532545) and CDA. -33delC (rs2072671, also known as c-943del/insC) have been linked to altered enzyme activity. Those who carry the CDA T-allele. -451C>T showed increased CDA enzymatic activity and a higher incidence of grade 2 diarrhea, Similarly, grade 3 HFS was more likely to occur in these patients. Reduced mRNA expression was due to CDA c.-33delC, closely related to CDA c.-451C>T, and carriers of the C-insertion variant had a lower risk of grade 3 HFS. In addition to genotyping CDA, the enzymatic activity of CDA in serum has been recommended as a reasonable biomarker for the metabolic conversion

rate of capecitabine. A condition known as ultra-metabolizing has been linked to higher capecitabine efficacy or extreme toxicity (Siu W. Lam et al., 2018).

Aims of study

1. Investigate the distribution of genotypes associated with capecitabine's activating enzyme in Iraqi breast cancer patients.

2. To study how the genetic variation of the CDA activation enzyme affects the effectiveness of capecitabine treatment.

3. To study the relationship between biomarkers and the concentration of drugs in plasma, as well as the effect of genetic polymorphism activation on the toxicity and effectiveness of capecitabine.

4. Investigate the association between the CDA genetic polymorphism and the occurrence of adverse effects, particularly hand-foot syndrome.

Chapter Two

Patients, Materials, & Methods

2. Patients, Materials, and Methods

2.1. Materials

2.1.1. Instruments

This table lists the capecitabine-related research instruments used in the current study.

Table (2-1): instrument used in this study procedures of drug capecitabine.

Instrument	Manufacture
Sykam HPLC	German
Hood	LabTech / Korea
High-speed centrifuge	Germany
Freezer (-20 ˚C)	Lebanon
Stirrer for hot plates	LabTech / Korea
Mini-VIDAS	France
Nano-drop	England
Polymerase chain reaction -thermocycler	England
Lab Refrigerator	Lebanon
Ultra violet-transilluminator	England
Vortex	Germany
Cl-900	Mindray/China
Bs-430	Mindray/China

2.1.2. Chemicals and kits

The characteristics of the chemicals and kits utilized in polymerase chain reaction (PCR), DNA extraction, and high-performance liquid chromatography (HPLC) are detailed in Table (2-2).

Table (2-2): Specifications of chemical and kit used in PCR, DNA extraction, and high-performance liquid chromatography.

Kits and Chemicals	Manufacture / Origin
Ethyl acetate/acetonitrile	France
Methanol hydroxide	France
Formic acid	France
Buffer. Solution (Mgcl$_2$)	Genaid/Taiwan
Washing 1 solution	Genaid/Taiwan
Washing 2 solution	Genaid/Taiwan
Proteinase k	Genaid/Taiwan
Elution solution	Genaid/Taiwan
Ethanol 95%	Genaid/Taiwan

2.2. Patients

2.2.1. Population studies

This research was done in Kerbala's Imam Hussein Medical/Oncology Center. The study is cross-sectional observational research that will be conducted between 1 July 2022 to 1 January 2023. The Scientific Ethical Committee of the College of Pharmacy at Kerbala University approved the study's protocol, and all participants signed a consent form indicating they understood the research's purpose and nature.

This investigation included 200 women, 100 of whom were diagnosed with breast cancer and taking capecitabine as monotherapy for more than three months. One hundred of them served as the control group and were analyzed for biochemical markers estradiol, CA 15-3, and calcium.

2.2.2. Inclusion criteria

The inclusion criteria involved:

1. Postmenopausal women from (45-75) years old taking capecitabine for more than three months
2. With adequate organ function (kidney and liver function)
3. Take capecitabine as monotherapy for treating breast cancer

2.2.3. Exclusion criteria

The exclusion criteria involved:

1. Pregnancy or lactation
2. Serious infections such as sepsis
3. Females who taking capecitabine as a combination or adjuvant therapy with another chemotherapy

2.2.4. Sample collection and analysis

After the Scientific and Ethical Committee has given their approval. Takes 5 ml of blood samples from patients and puts 2 ml in an EDTA tube for genetics analysis. Put 3ml in gel tube then centrifuge with speed 4000 r.p.m for 10 minutes for biochemical analysis such as Calcium, Estradiol E_2, tumor marker CA 15-3.

Asked each patient about drugs used in chemotherapy that may interfere with cytidine deaminase, the enzyme responsible for the second activation step of capecitabine to 5-FU. Also, exclude any patients taking another chemotherapy drug with capecitabine. Information was obtained from the medical record which contain date of birth, age, marital status, family history, breastfeeding, if the patient had reconstruction surgery, if the patient had radiotherapy when breast cancer was diagnosed, CT scan information about the tumor, and if metastasis to another organ.

A special form had been prepared to ask the patient about the drug such

as compliance, and any adverse effects experienced during treatment with capecitabine, period of using capecitabine.

2.3. Methods

2.3.1. Biochemical analysis

2.3.1.1. Calcium (Ca)

Principle of measurement of calcium level

The mineral calcium is the most abundant in the human body. Although the fact that the majority of the body's calcium is stored in the skeleton, the free, hydrated cation in solution functions as a critical physiological mediator in a variety of metabolic and regulatory processes. Soluble calcium remains in the extracellular fluid in the body.

Specimen

Specimen stored at frozen temperature -15c to -20c.

PROCEDURE

1. In an acidic solution, Arsenazo-III dye reacts with calcium to form a blue-purple complex. The sample's calcium concentration is proportional to the chroma measured at 660 nm. To dilute the sample, use 0.85 to 0.9% NaCl solution.

2. The dilution factor must be input on the screen for the patient or control order.

3. Using this dilution factor, the system automatically adjusts the concentration by multiplying the result by the inputted factor.

4. If the operator fails to input the dilution factor, the result must be multiplied by the appropriate dilution factor before being reported.

Reference Range

These ranges were obtained from the scientific literature and represent the CPC (O-cresol phthalein complex one) and AAS (atomic absorption spectrophotometry) techniques. Refer to the method comparison section of this product insert for a comparison between CPC and the AEROSET Calcium assay (Arsenazo III method).

2.3.1.2 Estradiol

Estradiol levels for postmenopausal women normally range between 0 and 30 pg/ml (Richardson et al., 2020).

Principle of measurement of estradiol level

1. Estradiol levels can be measured with the Chemiluminescent (CL) series E2 assessment, which uses a competitive binding immunoenzymatically technique.

2. A sample paramagnetic microparticle covered with goat anti-rabbit IgG, sample treatment solution, and rabbit polyclonal anti-estradiol antibody was put into a reacting jar. After incubation, the anti-estradiol antibody in the sample will be related to the estradiol in the sample.

3. In the second step, the reaction vessel was then filled with an estradiol alkaline phosphatase conjugate. Estradiol in the sample serum competes with estradiol alkaline phosphatase conjugate for binding sites on the anti-estradiol antibody. On the microplate, antigen: antibody complexes were bound to goat anti-rabbit IgG, which was magnetically collected, while unbound compounds were eliminated.

4. In the third step, the substrate solution was added to the reaction vessel. It was activated in the immunocomplex retained on the microplate by estradiol-alkaline phosphatase conjugate.

5. Within the apparatus, a photomultiplier measured the resulting chemiluminescent reaction as relative light units (RLUs). The correlation between the quantity of estradiol in the sample and the number of relative light units produced by the reaction was negative.

6. The estrogen, level is determined using a calibration curve.

Table (2-3): Estradiol level in males and females.

Gender	Estradiol level pg/ml
Males	10–50 pg/mL
postmenopausal phase	0–30 pg/mL
ovulating	30–400 pg/mL
early follicular	30–100 pg/mL
late follicular	100–400 pg/mL
luteal phase	50–200 pg/mL
prepubertal children, normal	Less than 10 pg/mL

2.3.1.3. Cancer antigen 15-3 (CA 15-3)

Tumour markers CA 15–3 and CEA were captured at diagnosis and retrieved from the database. Laboratory assays were standardized throughout the study period using an automatic electrochemistry luminescence immunoassay system.

The assay reference values of CA 15–3 and CEA were below 25U/mL and 5.0 ng/mL respectively, and the value was considered elevated or within normal limits for the marker if the level was above or below the cut-off value respectively (Hing *et al.*, 2020).

Principle of measurement of CA (15-3) cancer antigen

1. The 115D8 and DF3 monoclonal antibodies are used to define the CA 15-3 assay values.

2. Monoclonal antibodies 115D8, produced against human milk-fat globule membranes, and DF3, produced against a membrane-enriched fraction of metastatic human breast cancer, react with epitopes expressed by polymorphic epithelial mucins, a family of high molecular weight glycoproteins.

3. The Microparticle Enzyme Immunoassay (MEIA) technology is employed in the CA 15-3 assay.

4. The MEIA technique detects analytes using a solution of suspended, submicron-sized latex particles.

5. The particles are coated with a capturing molecule that is specific to the analyte under analysis.

6. Sample and reactant are transported to a reaction vessel (RV).

7. In the reaction vessel sample and reagent are combined allowing them to react with temperature.

8. In the inert fiberglass, the immunological complex is retained by the glass fibers due to the irreversible binding of the microparticles, while the reaction fluid flows swiftly through the matrix's wide pores.

9. In this step 4-Methylumbelliferyl Phosphate, an Alkaline Phosphatase-labeled conjugate is added to the glass fiber matrix (MUP).

10. Conjugate reacts to hydrolysis MUP to Methylumbelliferone (MU).

11. The fluorescence MU as it is produced on the matrix is proportional to the analyte concentration in the test sample.

12. Levels above 31.3 U/mL were considered high.

2.3.1.4. Measurement of concentration 5-FU

High-performance liquid chromatography (HPLC) is used to measure the concentration of 5-FU (Reigner *et al.*, 2003). High-Performance Liquid Chromatography (HPLC) is a type of chromatography and one of the most widely used analytical procedures. The chromatographic process is a separation technique that involves substance transfer between the stationary

and mobile phases. To separate the components of a mixture, HPLC employs a liquid mobile phase. The stationary phase might be either liquid or solid. These components are first dissolved in a solvent before being passed through a chromatographic column under high pressure. The mixture separates into its constituents in the column. The level of resolution is significant and is determined by the degree of interaction between the solute components and the stationary phase. The static packing material in the column is referred to as the stationary phase (Patil *et al.*, 2011).

Principle of high-performance liquid chromatography

The concentration of active metabolites of drug (5-FU) was measured in the Ministry of Science and Technology. Take 5 ml of blood from the patient and out in an EDTA tube. Then centrifuge the blood and separate the serum. 500 µL of 20% AgNO3 was added to a mixture containing 1 mL of human serum, which was then vortexed for 3 minutes and allowed to stand for 5 minutes. 650 µL of 20% NaCl was added, and the mixture was vortexed for an additional 3 minutes. After 12 minutes of centrifugation at 13,000 rpm, the supernatant (0.5 mL) was diluted to 1 mL with water and filtered through a 0.22-m membrane. For the analysis, a SYKAM - German HPLC model equipped with a UV detector was utilized. For separation, a C18 - ODS column (250 cm, 4.6 m) was utilized. The column temperature was kept at 25 degrees Celsius. At a flow rate of 1 mL/min, standards and samples were determined using a mobile phase of 5 mL M KH2PO4 solution (pH = 6.0) and methanol (96:4). The volume of the injection was 100 µl. The 5-FU was detected at 254 nm wavelength.

2.3.2. Molecular-Analysis

2.3.2.1. DNA Extraction

1. Fill a 1.5 ml microcentrifuge tube with 900 µl of RBC Lysis Buffer and 300 µl of whole blood.

2. For the production of a leukocyte (white blood cell), incubate at room temperature for 5 minutes, followed by centrifugation at 3,000 x g for 5 minutes.

3. Remove the precipitate with care, retaining approximately 50 µl of buffer and leukocytes.

4. In a tube, add 300 µl of Cell Lysis Buffer and vortex it thoroughly.

5. Ensure the sample lysate is clear and homogeneous by incubating at 60°C for at least 10 minutes.

6. Rotate the tube every three minutes during incubation.

7. After incubation at 60°C, add 1.5 mL of RNase A (10 mg/ml) to the sample lysate and vortex it. Incubate at ambient temperature for five minutes.

8. Mix the sample lysate with 100 µl of Protein Removal Buffer and vortex it for 10 seconds.

9. Centrifuge at 14-16,000 x g for 3 minutes to produce a compact, dark brown protein particle.

10. Add 300 µl of isopropanol to the precipitate, and gently invert the tube 20 times to thoroughly blend it.

11. Remove the precipitate with caution and wash the particles with 300 µl of 70% ethanol after centrifuging for 5 minutes at 14-16,000 x g.

12. For three minutes, centrifuge the particle at 14,000-16,000 x g, then carefully remove the filtrate and air-dry it for ten minutes.

13. DNA precipitate should be kept at -20°C to -40°C.

2.3.2.2 Primer's design

Table (2-4) shows the forward primer and reverse primer sequence used for CDA K27Q (rs2072671) genotyping.

Table (2-4): Primer sequences used for CDA K27Q genotyping (rs2072671).

Primer	Sequence	Tm (°C)
(A) Forward	5′-CCTTCCAGTAGCGTGGCACCAC-3	72
(B)Reverse (K*-specific)	5′GACTGTAGGGGCAGTAGGCTGACTT-3′	68
(C)Reverse Control	5-CAGCCAGCAGAGGAAGTCAGAGC-3	70.1

Table (2-5) show shows forward primer and reverse primer sequences used for CDA rs532545 (451 A>G).

Table (2-5): Primer sequences used for CDA rs532545 (451 A>G).

Primer	Sequence	Tm (°C)
(A) Forward (common)	F: GAA GGG CTG AGG CTG AAA	73
(B)Reverse (Specific)	R: TGG GCT AGG GCA AAG AGA	65.36
(C) Reverse	CTG CAG CTT GTT CAT GCC TCC TGC CT	70

2.3.2.3. Polymerase-Chain-Reaction (PCR)

In this, study allele-specific PCR technique was used to detect SNIPK27Q (rs2072671) and SNIP rs532545 (451 A>G) variation of cytidine deaminase enzyme according to a previous study (Lam, Guchelaar, and Boven, 2016).

2.3.2.4 Optimization of the PCR conditions

PCR adjustment was completed after numerous trials to get the optimal primer concentration and annealing temperature.

2.3.2.5 PCR Principle

1. The first procedure involves denaturing the double-stranded DNA (ds-DNA) obtained from the sample under investigation (target DNA). This includes raising the temperature to 95-98°C for 60 seconds, which results in two single strands of DNA (ss-DNA). These ss-DNA patterns or templates are used to bind synthetic primers.

2. The next step is the annealing step, in which the temperature is decreased to 37-50°C for 60-120 seconds, and primers are added in excess to the reaction mixture (primers are synthetic oligonucleotides, short bits of single-stranded DNA consisting of nucleotide base pairs long). The lower temperature allows the primers to bind to their complementary ss-DNA sequences (templates), one to each of the two single strands created in the previous phase.

3. The third and last stage is an extension reaction performed by an additional thermostable DNA polymerase, an enzyme capable of generating a complimentary copy of the original single strands by extending the 3' (but not the 5') ends of the annealed primers. This third stage, normally performed at 72°C for 60-120 seconds, signifies the end of the first cycle of the method, in which two double strands of DNA

were produced, resulting in a doubling of the initial target DNA molecules.

Table (2-6) shows the component of the solution used for the Polymerase chain reaction.

Table (2-6): Components of PCR working solution.

Component	Volume (µl)
Primer 1 (outer)	1.5
Primer 2 (outer)	1.5
Primer 3 (inner)	1.5
Primer 4 (inner)	1.5
DNA sample	4
Nuclease free water	8
Master mix	10
Total PCR premix	25

In the current study, the thermal program for detecting rs532545 (451 A>G) is demonstrated in Table (2-7).

Table (2-7): Steps of PCR working procedure for SNIP rs532545 (451 A>G).

Steps	Temperature (ºC)	Times (Min: Sec)	Cycles
Initial denaturation	94	4:00	1
Denaturation	94	0:30	35
Annealing	60	0:30	
Extension	72	00:55	
Final extension	72	6:00	1

Table (2-8): Steps of PCR working procedure for SNIP K27Q rs2072671.

Steps	Temperature (ºC)	Times (Min: Sec)	Cycles
Initial denaturation	94	4:00	1
Denaturation	94	0:30	35
Annealing	60	0:30	
Extension	72	00:55	
Final extension	72	06:00	1

2.3.3. Gel Extraction Protocol

1. Gel Dissociation

       Remove the agarose gel slice containing the required DNA fragments and any excess agarose to reduce the size of the gel slice. Fill a 1.5 ml microcentrifuge tube with up to 300 mg of gel slice. Mix with 500 μl of QG Buffer using a vortex. Incubate at 55-60ºC for 10-15 minutes to ensure the gel slice has been thoroughly dissolved. During incubation, invert the tube every 2-3 minutes. If the liquid has gone purple, add 10 of 3M Sodium Acetate (pH5.0) and thoroughly mix. Let the dissolved sample mixture return to room temperature.

2. DNA Binding

       In a 2 ml Collecting Tube, place a DFH Column. 800 μl of the sample mixture should be transferred to the DFH Column. For 30 seconds, centrifuge at 14-16,000 x g. Remove the flow-through and re-insert the DFH Column into the 2 ml Collecting Tube. Repeat the DNA Binding stage if the sample combination is more than 800 μl.

3. Wash Fill the DFH Column with 600 μl of Wash Buffer (ensure ethanol is added) and let stand for 1 minute. Centrifuge for 30 seconds at 14-16,000 x g, then discard the flow-through. Return the DFH Column to the 2 ml Collecting Tube. Fill the DFH Column with 600 μl of Wash Buffer

(ensure ethanol is added) and let stand for 1 minute. Centrifuge for 30 seconds at 14-16,000 x g, then discard the flow-through. Return the DFH Column to the 2 ml Collecting Tube. Centrifuge the column matrix for 3 minutes at 14-16,000 x g.

4. DNA Elution Pass the dried DFH Column to a1.5 ml microcentrifuge tube. Fill the middle of the column matrix with 20-50 μl l of pre-heated Elution Buffer or TE (60-70). Let for at least 2 minutes to ensure that the Elution Buffer is thoroughly absorbed. To elute the pure DNA, centrifuge it for 2 minutes at 14-16,000 x g.

2.3.3.1.   Component of Gel Extraction

Table (2-9): Components used in gel extraction.

Component	volume
QG Buffer	3 ml
W1 Buffer	2 ml
Wash Buffer (Add Ethanol)	1 ml (4 ml)
Elution Buffer	1 ml
DFH Columns	4
2 ml Collection Tubes	4

2.4. Hand-foot syndrome (HFS)

Specialized forms that are designed to identify and categorize hand-foot disorders according to the symptoms that appear in patients. Tablets (2-10) illustrate the various grades of hand-foot syndrome as well as the symptoms that are associated with each grade of hand-foot syndrome.

Table (2-10): Graded of hand-foot syndrome with symptoms(Leonard *et al.*, 2011).

	Symptoms
Grade 1	Palm and sole paresthesia or dysesthesia
Grade 2	In addition to painless redness or swelling, individuals suffer difficulty when walking or holding objects.
Grade 3	Entails uncomfortable swelling and erythema that inhibits daily activity.
Grade 4	characterized by skin desquamation, ulceration, and blistering together with severe discomfort.

2.5. Statistical Analysis

IBM's version 25 of Statistical Package for the Social Sciences (SPSS) was used for statistical analysis. The genetic groupings were expressed as a percentage and in frequency. The biochemical parameters were presented as means of standard deviations. The mean differences in biochemical markers measured among genotype groups in breast cancer patients were examined using a t-test and single-factor ANOVA test. Pearson's correlation coefficient was used to compare breast cancer patients' genotype groups and parameter levels. Genotype groups and tumor marker levels were examined using the Chi-square test (x_2). These genotypes were examined about tumor marker CA15.3 elevation and hand-foot syndrome incidence using the odds ratio (OR) and 95% confidence interval (CI-95). In all statistical analyses in this study, p-values less than 0.05 indicate a significant difference.

Chapter Three

Results

3.1. Patients' demographic data

Table (3-1) displays the demographic information of the participants in the present study. The age range for breast cancer diagnosis was (55.36 ± 10.85) with a range (45-70) years; approximately 62% of individuals had a family history of breast cancer, while 86% of married women and 14% of unmarried women had cancer.

Approximately 33% of women have left-sided breast cancer, while 67% have right-sided breast cancer.

The proportion of patients who underwent surgery (mastectomy, lumpectomy with or without lymph node removal) was 90%; the proportion of individuals with estrogen receptor positivity was 94%; progesterone positivity was only 6%; and 39% of patients in this study had lymph node involvement.

The percentage of females who received radiation therapy was 88%, while the percentage of females who did not receive radiation therapy was 12%; the percentage of patients who received chemotherapy was 92%, while 8% did not receive chemotherapy; and 53% of patients suffered from hand-foot syndrome, a serious side effect of capecitabine.

Table (3-1): The demographic data for the individuals who participated in this study.

Characteristics		Percentage
Marital status	Married	86%
	Single	14%
Family history	Positive	62%
	Negative	38%
location of breast cancer	Left	33 %
	Right	67%
Receptor status	Estrogen receptor-positive	94%
	Progesterone receptor-positive	6%
Lymph node involvement	yes	39%
	no	61%
Radiation therapy	yes	88%
	no	12%
Surgery	yes	90%
	no	10%
Hand foot syndrome	yes	53%
	no	47%
Chemotherapy	yes	92%
	no	8%
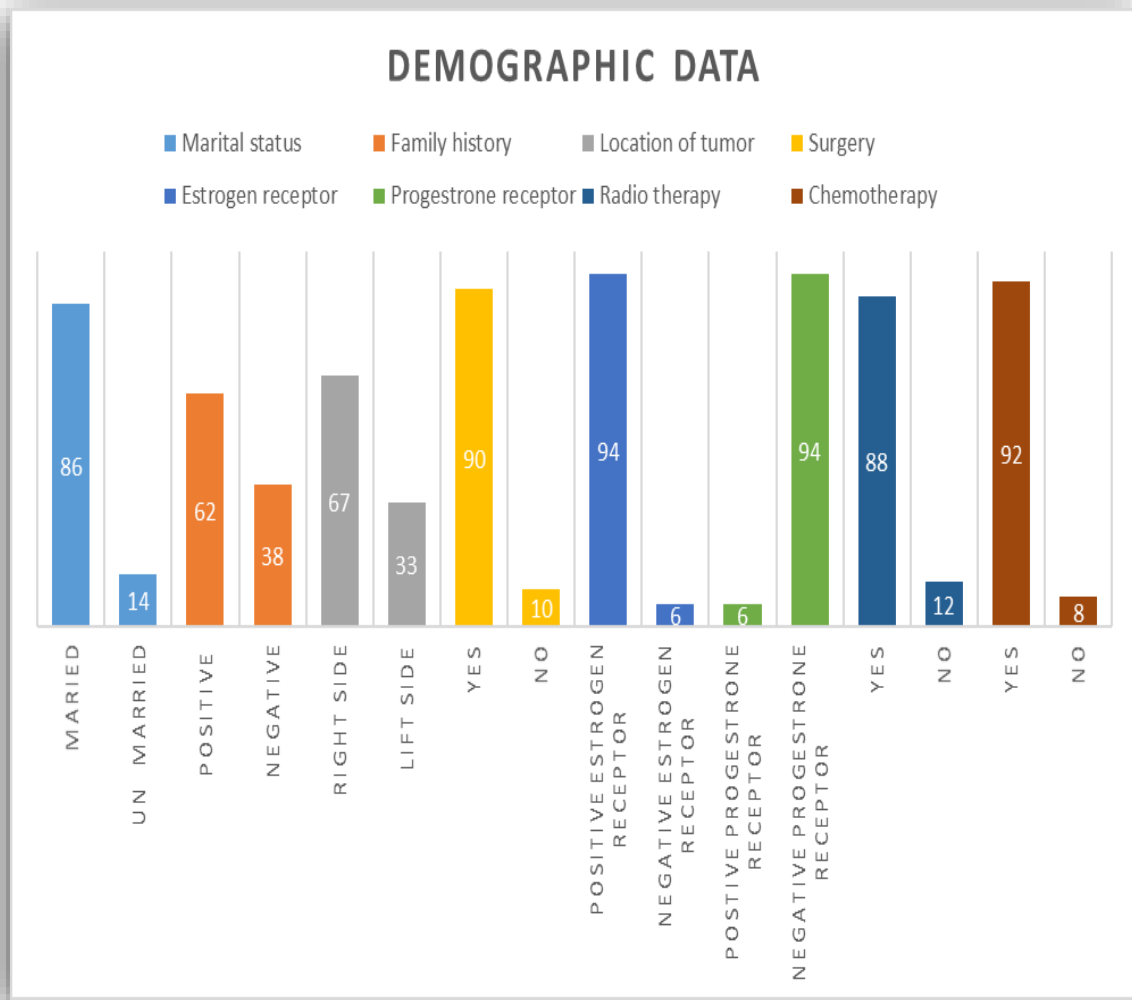
Figure (3-1) Demographic data of breast cancer patients.

3.2. Comparative evaluation of healthy people and individuals with breast cancer.

Comparing the healthy control group with breast cancer patients, table (3-2) shows that there is a significant difference in the levels of tumor marker CA15.3 for the healthy group (29.97 ± 11.4) and breast cancer patients (88.18 ± 12.90) as well as estradiol for the healthy group (21.8 ± 9.07) and breast cancer patients (88.06 ± 15.33).

Calcium level (8.64 ± 3.659) for the healthy group as well as for breast cancer patients (8.96 ± 3.542), there is no significant difference between the two groups.

Table (3-2): Comparative table of biomarkers (CA15-3, estradiol, and calcium) between the control group and patients' group.

Parameter	Healthy group	Breast cancer patients	*P value
Tumor marker(U/mL)	29.97±11.4	88.18 ±12.90	0.02 S
Estradiol(pg/mL)	21.8 ± 9.07	88.06 ± 15.33	0.019S
Calcium level(mg/dl)	8.64 ± 3.659	8.96 ± 3.542	0.2 NS

* t-test P-value if 0.05< P significant difference, P>0.05 non-significant difference, the result represented as mean and St deviation and NS=non-significant while N=significant.

3.3. Comparison between responder and non-responder

	Tumor Marker	
	rs2072671	rs532545
■ Response +	67	46
■ Response -	33	54

X2= 8.97
P= 0.002
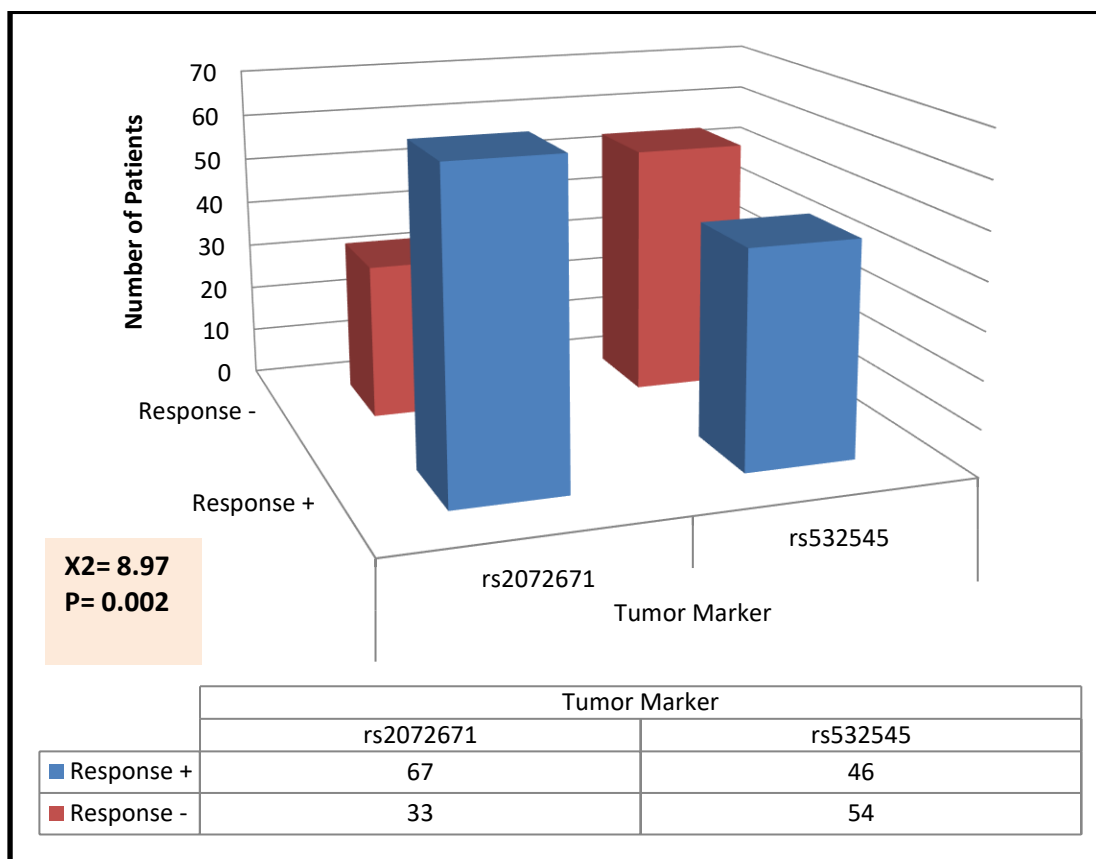
Figure 3-2: The number of patients who have Ca15-3 more than 30 U/ml is 67 patients responding and 33 non-responders for rs2072671 polymorphism while 46 patients have responded for chemotherapy for rs532545 polymorphism.

Tables (3–3) represent the distribution bio-marker for major alleles (Homozygotes) and minor alleles (Heterozygotes and mutants). The mean and standard deviation of response patients for tumor marker CA 15-3 about SNIP rs2072671 major allele (128.33±14.29) and minor allele (121.62±14.3) whereas SNIP rs532545 major allele (136.77±19.25) and minor allele (137.75±16.24) show a no difference with non-response patients in major allele in two SNIP rs532545 and rs2072671 and significant difference in minor allele. Concerning estradiol mean and standard division of response patients for estradiol regarding SNIP rs2072671 major allele (75.27±11.37) and minor allele (69.19±15.44) whereas SNIP rs532545 major allele (78.53±15.63) and a minor allele (88.27±14.9) and there is a significant difference with non-response patients in

major allele in both SNIP rs532545 and rs2072671, also a significant difference in the minor allele. For serum calcium regarding SNIP rs2072671 major allele (8.49±1.48) and minor allele (7.52±22.1) whereas SNIP rs532545 major allele (7.62±1.95) and minor allele (8.35±1.19), there is a no-difference with non-response patients in the major allele in both SNIP Rs532545 and rs2072671, as well as the minor allele

Table (3–3): Distribution of biomarkers (CA15-3, estradiol, and calcium) in response and non-response breast cancer patients according to major and minor allele genotyping.

Characteristic	rs2072671	rs532545	*P value*
Tumor marker CA (15-3)			
Major allele	128.33±14.29	136.77±19.25	0.12 NS
Minor allele	121.62±14.3	137.75±16.24	0.024 S
Estradiol			
Major allele	75.27±11.37	78.53±15.63	0.068 NS
Minor allele	69.19±15.44	88.27±14.9	0.031 S
Serum calcium			
Major allele	8.49±1.48	7.62±1.95	0.17 NS
Minor allele	7.52±22.1	8.35±1.19	0.14 NS

* t-test P-value if P <0.05 significant difference, P>0.05 non-significant difference, the result represented as mean and St deviation and NS=non-significant while N=significant.

Table (3-4) compares biomarkers (estradiol, CA15-3, and calcium) and drug concentration (5-FU) between breast cancer patients who responded and non-responder patients.

There is a statistically significant difference between responder (59.13±14.35) and non-responder (77.44±14.15) estradiol levels.

There is a statistically significant difference between CA15-3 level respondents (43.46±22.57) and non-respondents (99.16± 13.56).

There is no statistically significant difference between the calcium levels of those who responded to the treatments (8.15±2.67 vs. 9.01± 3.31) and non-responder patients.

Regarding to concentration of (5FU) there is a statistically significant difference between responders (with drug concentrations of (349.35±56.78) and non-responders (with drug concentrations of 301.24±82.69).

Table (3–4): Biomarker parameters and drug concentration for response patients and non-responders.

Parameters	Responder	Non-Responder	P value
Estradiol(pg/mL)	59.13±14.35	77.44±14.15	**0.034 S**
CA 15-3 (U/mL)	43.46±22.57	99.16± 13.56	**0.026 S**
Calcium (mg/dl)	8.15±2.67	9.01± 3.31	**0.146 NS**
Drug Concentration 5FU (ng/ml)	349.35±56.78	301.24±82.69	**0.047 S**

** t-test p-value if P <0.05 significant difference, P>0.05 non-significant difference, the result represented as mean and St deviation and NS=non-significant while N=significant.

3.4. Biomarker

3.4.1.1. Biomarker concentration (CA15-3, estradiol, and calcium) in breast cancer patients with   cytidine deaminase gene polymorphism

The patient's blood CA 15-3 levels and the cytidine deaminase gene polymorphism are shown in Table (3-5). Most of the patients (around 55%) have tumor marker levels that are higher than the normal range (less than 30 U/mL).

Table (3-5): Percentage of responder and non-responder patients according to tumor marker level.

	Responder CA 15-3<30 U/ml	Non-Responder CA 15-30> U/ml
Cancer patients	45%	55%

Regarding biomarkers, as shown in table (3–6) for both SNIP rs532545 and rs207267.

For the first SNIP rs207267, the tumor marker for the homozygous (AA) type with a value of (93.09± 30.57) while heterozygotes (AC) have a CA15-3 (mean ± SD) of (65.89± 13.97), which exceeds the normal range, followed by, and the mutant type (CC) with a value of (117.77± 36.41), and there is a statistically significant difference between homozygous, heterozygous, and mutant, respectively.

The estradiol level for SNIP rs207267, homozygous (AA) has a value of (51.89± 10.42) while the heterozygous (AC) has an elevated estradiol level (mean ± SD) of (53.98± 10.96) and the mutant type (CC) has a value of (41.01± 10.78), with a statistically significant difference between the three groups.

For calcium level, the wild type homozygous (AA) with a value of (8.75 ± 0.12) while the heterozygous type (AC) has calcium (mean ± SD) of (9.11± 0.15), followed by the mutant (CC) with a value of (8.99± 0.08) and the, and there is significance difference between the three genotypes.

Concerning rs532545, for the CA 15-3 level, the homozygous (AA) with a value of (71.83± 21.22) while the heterozygotes (AG) have a CA15-3 (mean ± SD) of (106.76±31.33), which exceeds the normal range, followed by the, and the mutant type (GG) with a value of (66.08± 19.02), and there is a statistically significant difference between homozygous, heterozygous and mutant, respectively.

For the estradiol level, the wild type (AA) homozygous has a value of an elevated estradiol level (mean ±SD) of (74.43± 14.86), the heterozygous (AG) has a value of (33.08± 3.72), and the mutant type (GG) has a value of (37.98± 3), with significant differences between homozygous, heterozygous and mutant genotypes.

For calcium level, the homozygous (AA) has a calcium level (mean ± SD) of (9.09± 0.16), followed by the heterozygous (AG) with a value of (8.93 ± 0.12), and the mutant type (GG) with a value of (8.82± 0.1); three groups are significant difference.

Table (3-6): Distribution of bio-marker (CA15-3, estradiol, and calcium) in breast cancer patients according to genotyping.

Alleles Rs2072671	AA Homozygote wild type	AC Heterozygote type	CC homozygote Mutant type	P value
No.	30	48	22	-
CA 15.3	93.09± 30.57	65.89± 13.97	117.77± 36.41	0.014 S
E2	51.89± 10.42	53.98± 10.96	41.01± 10.78	0.032 S
Calcium	8.75 ± 0.12	9.11± 0.15	8.99± 0.08	0.007 S
Alleles Rs532545	AA Homozygote wild type	AG Heterozygote type	GG homozygote Mutant type	P value
No.	40	48	12	-
CA 15.3	71.83± 21.22	106.76±31.33	66.08± 19.02	0.005 S
E2	74.43± 14.86	33.08± 3.72	37.98± 3	0.018 S
Calcium	9.09± 0.16	8.93 ± 0.12	8.82± 0.1	0.028 S

* ANOVA test P-value if P <0.05 significant difference, P>0.05 non-significant difference, the result represented as mean and St deviation and NS=non-significant while N=significant.

3.4.1.2. CA 15-3 level and CDA enzyme polymorphisms in breast cancer patients: a correlation analysis

Tables (3-7): Illustrate that in this study's patients, there was no correlation between rs2072671 and CA15-3, and the p-value was non-significant. Also, there was no correlation between rs532545 and CA15-3, and the p-value was non-significant.

Table (3-7): Correlation coefficient between enzyme polymorphisms and CA15-3 levels.

SNIP	Correlation Coefficient	P value
rs2072671	0.589	0.381 NS
rs532545	0.216	0.764 NS

* Correlation coefficient test if P <0.05 significant difference, P>0.05 non-significant and NS=non-significant while N=significant.

3.4.1.3. The odds ratios of the detected genotypes of CDA enzyme polymorphisms in the elevation of serum CA15.3 in the BC patients treated with Capecitabine

Table (3-8): Shows that rs532545 has a non-significant effect on the elevation of serum CA15-3 level (odds ratio 2.46, p> 0.05) for rs2072671 and (odds ratio 1.78, p > 0.05) for rs532545.

Table (3-8): the odds ratios of CDA gene polymorphisms (rs2072671, rs532545) with elevation of CA15-3 levels.

SNIP	Odd ratio 95% CI	P value
rs2072671	2.46	0.17 NS
rs532545	1.78	0.22 NS

* Odd ratio test P <0.05 significant difference, P>0.05 non-significant and NS=non-significant while N=significant.

3.5. Concentration of active drug 5-fluorouracil

Table (3-9) shows (mean ± SD) of the concentration of 5-FU for both alleles rs207267 and rs532545.

Regarding SNIP rs207267, the wild-type homozygous (AA) (338.4± 38.28) whereas the heterozygous (AC) (341.54± 39.22) and mutant type (CC) show mean and St deviation (346.9± 26.54). Although there is a significant difference between homozygous type and heterozygous and, mutant type respectively.

Regarding SNIP rs532545 the wild type homozygous (AA) (335.45± 47.18) whereas the heterozygous (AG) (343.95± 28.6) and mutant type (GG) show mean and St deviation (350.77± 38.19). However, there is a significant difference between homozygous, and heterozygous mutant types respectively.

Table (3-9): Plasma concentration 5-FU in breast cancer patients for SNIP rs207267.

Genotype \ SNIP	Homozygotes (AA)	Heterozygotes (AC)	Mutant Homozygotes (CC)	P value
rs207267	338.4± 38.28	341.54± 39.22	346.9± 26.54	0.012 S

*t-test p-value if P <0.05 significant difference, P>0.05 non-significant and NS=non-significant while N=significant, the result represented as mean and St deviation and NS=non-significant while N=significant.

Table (3-10): Plasma concentration 5-FU in breast cancer patients for SNIP rs532545.

Genotype SNIP	Homozygotes (AA)	Heterozygotes (AC)	Mutant Homozygotes (CC)	P value
rs532545	335.45± 47.18	343.95± 28.6	350.77± 38.19	0.033 S

*t-test p-value if P <0.05 significant difference, P>0.05 non-significant and NS=non-significant while N=significant, the result represented as mean and St deviation and NS=non-significant while N=significant.

3.6. Genetic Analysis

3.6.1. Result from polymerase chain reaction (PCR) amplification

Figure (3-3) shows the amplification of SNIP of cytidine deaminase enzyme: rs2072671 and figure (3-4) shows the amplification of SNIP of cytidine deaminase: rs532545 (451 A>G).
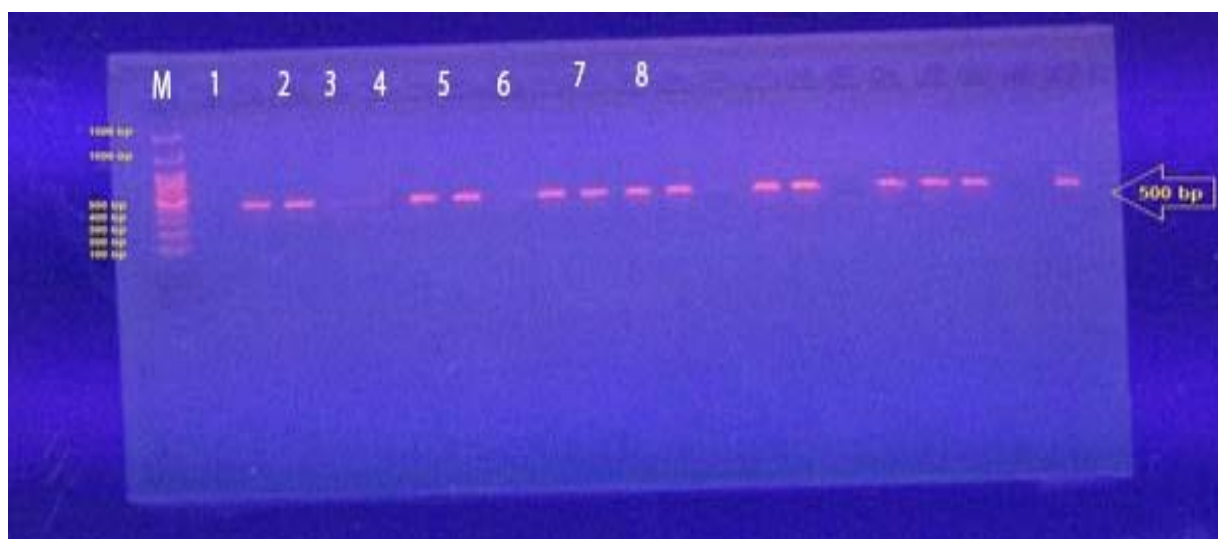
Figure (3-3) represents the DNA amplification of SNIP rs2072671 where line M represents DNA marker 1 represents mutant type CC and 2,3 homozygous type AA,5, 7,8 represent heterozygous AC.

Figure (3-4) represents DNA amplification of SNIP rs532545 where line M represents a DNA marker with 405bp and 1,6,7,8,9,10 represent mutant type GG and 2,11,4 homozygous type AA,3,5 represent heterozygous AG.
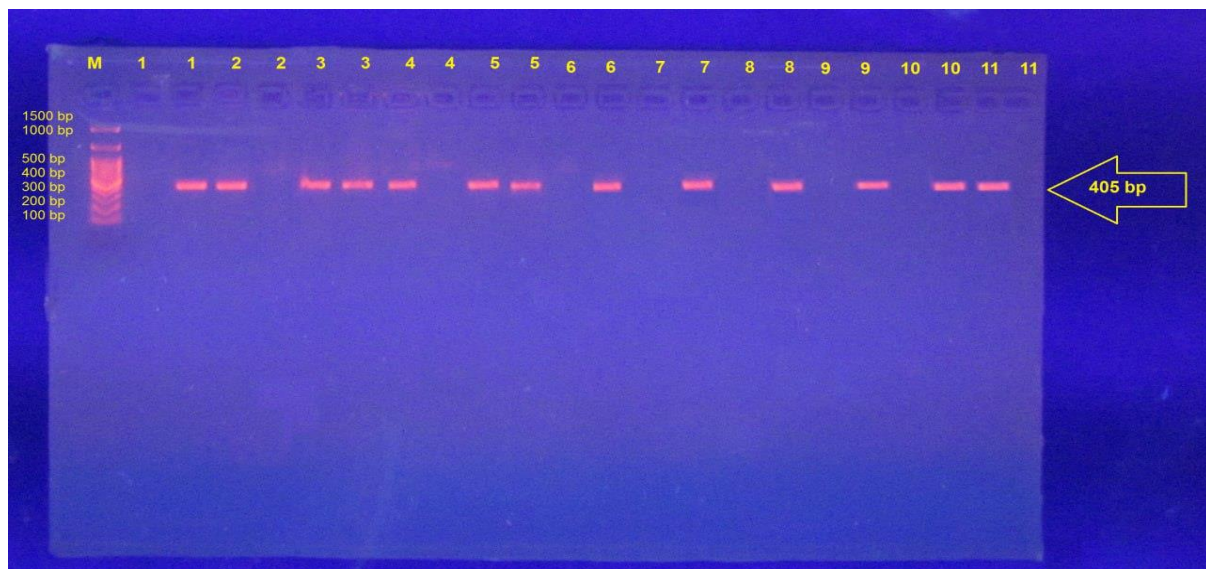
3.6.2. The genetic polymorphism of cytidine deaminase enzyme in breast cancer patients

Tables (3–11) Show the percentage and number of SNIP rs532545 and genotypes rs2072671 found in patients with breast cancer on capecitabine.

For this study most common genotype among the 100 breast cancer patients regarding SNIP rs532545 was (AG) heterozygous with 42 numbers of patients and 42% percentage and had higher frequency; while the wild type homozygous (AA) was with frequent numbers of 40 and a percentage of 40%, and the mutant type, which has a lower frequency and percentage, was 18% percentage and frequently 18.

For rs207267 was (AC) heterozygous, with 48 numbers of patients, and 48% percentage had a higher frequency, while the wild type homozygous (AA) was, with frequent 30 and a percentage of 30%, and the mutant type (CC), which has a lower frequency and percentage, was 22% and frequently 22.

Table (3-11): Percentage and frequency of cytidine deaminase enzyme SNIP (rs532545) and SNIP (rs2072671) genotype in breast cancer patients.

SNIP	Genotype	Frequency	Percentage
rs532545	AA (Homozygote, wild type)	40	40%
	AG (Heterozygote type)	42	**42%**
	GG (homozygote, mutant type)	18	18%
rs2072671	AA (Homozygote, wild type)	30	30%
	AC (Heterozygote type)	48	**48%**
	CC (homozygote, mutant type)	22	22%

3.7. Hand-foot syndrome

In this study 53% of patients who participated suffered from hand-foot syndrome, compared to 37% of them with grade 1 hand-foot syndrome with symptoms of palm and sole paresthesia, 12% of patients with grade 2 hand-foot syndrome with symptoms of painless redness or swelling, and individuals suffering difficulty when walking or holding objects. Four percent of the patients suffered from grade 3 hand-foot syndrome with symptoms that entail uncomfortable swelling and erythema that inhibits daily activity, and no patients had grade 4 hand-foot syndrome.

In this study, homozygotes (AA) for SNIP rs2072671 had a higher percentage of hand-foot syndrome a percentage of 20% higher than heterozygotes (AC) at 13% and mutants (CC) at 10%.

Tables (3-12) Show the effect of the genetic polymorphism of SNIP rs2072671 on hand-foot syndrome, and the frequency as well as percentage of hand-foot syndrome for each genotype.

Table (3-12): Percentage and frequency of hand-foot syndrome according to SNIP Rs2072671 genotyping.

SNIP	Genotyping	Frequency of HFS	Percentage
rs2072671	AA (Homozygote, wild type)	20	**20%**
	AC (Heterozygote type)	13	13%
	CC (homozygote, mutant type)	10	10%
	Total	10	43%

Similarly, to SNIP rs2072671, SNIP rs532545 Homozygote, wild type (AA) had a higher incidence of hand-foot syndrome a percentage of 26% higher than heterozygotes (AG) at16% and mutants (GG) at 13%.

Table (3-13) shows the effect of genetic polymorphism of SNIP rs532545 on hand-foot syndrome. and the frequency and percentage of hand-foot syndrome for each genotype.

Table (3-13): The effect of genetic polymorphism of SNIP Rs532545 on hand-foot syndrome.

SNIP	Genotyping	Frequency of HFS	percentage
rs532545	AA (Homozygote, wild type)	26	**26%**
	AG (Heterozygote type)	16	16%
	GG (homozygote, mutant type)	13	13%
	Total	53	53%

Chapter Four

Discussion

4. Discussion

Capecitabine is an anti-cancer drug that works by inhibiting the metabolism of fluoropyrimidine nucleosides used either alone in patients with metastatic breast cancer who have developed resistance to both paclitaxel and an anthracycline-containing regimen or in combination with docetaxel after anthracycline-based treatment has failed (Alqahtani et al., 2022). Capecitabine is a prodrug that is rapidly absorbed and activated in the body. Capecitabine is first converted to 5′-deoxy-5′-fluorocytidine (5′- 5′-DFCR) by hydrolysis by hepatic carboxyl esterase (Reigner et al., 1998). Then, cytidine deaminase, an enzyme present in both tumor cells and healthy liver cells, breaks down 5′-DFCR to 5′-deoxy-5-fluorouridine (5′-DFUR) (Miwa et al., 1998). The enzyme thymidine phosphorylase (TP) then converts 5′-DFUR into 5-fluorouracil, 5-fluorouracil is a therapeutic agent (Umar, 2018).

Siu W. Lam's studies show an association between single nucleotide polymorphisms (SNPs) in the CDA enzymatic activity and prognosis, response to capecitabine-containing treatment, and adverse effect (Lam et al., 2018; Dahan et al., 2012).

Consequently, it is crucial to study the influence of CDA enzyme polymorphisms on the response to chemotherapy for breast cancer to establish an effective therapeutic response and reduce capecitabine side effects.

To the extent that we know, this is the first study to examine how CDA enzyme genetic variants affect therapeutic response to capecitabine and the occurrence of capecitabine side effects in breast cancer patients from Iraq.

4.1. Demographic data in breast cancer woman

The most common form of cancer in women, breast cancer has several causes, including genetics, family history, age, and lifestyle choices (Di Sibio et al., 2016).

The demographic information of the patients who precipitated in this study shows that women in this study had age (55.36 ± 10.85) with a range (45-70) years, which agrees with the study (Lin et al., 2019) and is incompatible with academic research women under the age of 40 had an increased likelihood of developing breast cancer (Azamjah et al., 2019). Some women develop breast cancer at a younger age (below 40 years), but this only accounts for a small percentage of the overall incidence; however, because these cases are combined with more aggressive subtypes, it was anticipated that they would be diagnosed at a more advanced stage, which would result in a worse prognosis. This finding may be because early menarche will cause early progression of the breast and therefore early exposure to estrogen, which results in an increase in the risk of developing breast cancer. On the other hand, an older age at menarche will result in a lower risk of breast cancer in premenopausal females (Derks et al., 2018).

Several studies have found a correlation between a woman's marital status and her chance of developing breast cancer. Unmarried women and women who had lived their entire lives without ever marrying had a significantly higher chance of developing breast cancer (Hinyard et al., 2017). On the other hand, a study that looked at how marriage affected the stage of cancer at diagnosis found that unmarried female cancer patients had a significantly higher probability of presenting with the disease at a more advanced stage than married female cancer patients did (Yuan et al., 2021).

In several different ways, marriage can benefit female patients diagnosed with women's cancer and improve their prognosis of the disease's

progression. The first influence is on the individual's behavior. The presence of a spouse or partner in a woman's life has a beneficial impact on the lifestyle choices and health behaviors that she engages in throughout her life. In addition, marriage has been shown to decrease risk-taking behavior and exert social control on behaviors such as maintaining a healthy diet and exercise routine (Liu et al., 2019).

According to the findings of this study, breast cancer patients with a positive family history were more likely to have the disease than those with a negative family history, which is in agreement with the research (Brewer et al., 2017). A positive family history practically doubles the chance that a woman with in situ breast cancer will develop invasive breast cancer (Sackey et al., 2016).

4.2. Comparison of biomarker estradiol, tumor marker CA 15-3, and calcium levels between normal volunteers and breast cancer patients

This study, compared healthy volunteers and breast cancer patients in three parameters: estradiol, tumor marker, and calcium.

However, this study found a substantial difference between the estradiol levels of breast cancer patients and healthy females. The concept that is the most frequently accepted and is supported by a significant amount of experimental evidence is that breast cancer is associated with an increase in estradiol and that estradiol promotes cell proliferation and initiates mutations that occur as a result of errors that occur during DNA replication. According to Yue et al.'s (2010) findings, estradiol promotes the growth of cells that already carry mutations, which leads to a buildup of these cells, which eventually leads to the development of cancer.

The tumor marker in breast cancer patients rises because healthy cells and malignant tumors both release substances in response to carcinogenesis and other illnesses. The majority of these indicators are produced by both normal and cancer cells, although tumor cells do so in significantly higher amounts than normal cells (Zhang et al., 2022).

For calcium level, which is in the normal range of less than 10.5 mg/dl (Tanasijevic et al., 1995). In this study, both healthy and breast cancer patients were in the normal range.

4.3. Impact of cytidine deaminase gene polymorphisms on the estradiol levels in breast cancer patients

Estradiol is an important estrogen in humans that regulates reproductive function. In women secreted from the ovaries, while in men is secreted from the testis, and the adrenal cortex secretes estradiol in men and women (Abbott Laboratories, 2009).

Many studies have examined the correlation between rising serum estrogen levels and the onset of breast cancer (Mahdi et al., 2021). Most human breast cancers begin as estrogen-dependent, suggesting a role for estrogen signaling and the estrogen receptor (ER) in cancer growth (Saha Roy & Vadlamudi, 2012).

Hormone receptor-positive tumor cells have estrogen or progesterone receptors (Ameer Abdul Ridha et al., 2022). Estrogens are thought to play a significant role in encouraging the proliferation of both normal and malignant breast epithelium. Epidemiological studies have now proved what has been suspected for a long time: that they are breast carcinogens. Three main pathways are thought to be implicated in their carcinogenic effects stimulation of cellular proliferation via their receptor-mediated hormone action; direct

genotoxic effects via boosting mutation rates via cytochrome P450-mediated metabolic activation; and production of aneuploidy (Russo & Russo, 2006).

In this study, the estradiol level for patients receiving capecitabine was higher than other studies in Asian countries (Soewoto & Agustriani, 2023), that mean must patient not respond to treatment.

In this study, for SNIP rs2072671 there is a significant difference in estradiol levels between the mutant type (CC), heterozygotes (AC), and homozygotes (AA). Estradiol levels for homozygote AA are the same as those for heterozygote (AC). However, the mutation type of SNIP rs2072671 enzyme CDA lowers estradiol levels, which is in contrast with the study effect of chemotherapy that lowers estradiol levels (Septiani et al., 2022).

4.4. Impact of cytidine deaminase gene polymorphisms on the tumor marker CA15-3 in breast cancer patients

Cancer antigen CA 15-3 was monitored in patients with breast cancer, and serum levels of CA 15-3 increased during treatment(Fakhari et al., 2019). CA 15-3 levels are raised in just 3% of individuals with cancer without metastatic cancer but up to 70% of patients with metastatic cancer. CA 15-3 is not a useful indicator for screening a high-risk population for cancer, but it may be beneficial as a prognostic marker in breast cancer patients (Daniele et al., 2013)

Serum levels of cancer antigen 15-3 (CA15-3) are monitored periodically in breast cancer patients throughout the course of treatment (Fejzi et al., 2015). CA15-3 may be useful in estimating the extent to which breast cancer has spread. An elevated level of CA15-3 would suggest the presence of metastases, particularly bone metastases. Patients with bone metastases may find it helpful to examine CA15-3 as a prognostic factor (Steinauer et al.,

2014). However, no research has linked CA15-3, a tumor marker, to CDA enzyme genetic variation.

Higher mean and St. Deviation were found in this study for the SNIP rs2072671 mutation type compared to homozygotes and heterozygotes. In contrast SNIP rs532545 higher tumor marker CA 15-3 found in heterozygotes compared to mutant and homozygotes. All genetic polymorphisms that reduce CDA's ability to convert the prodrug capecitabine into the active drug 5-FU through acetylation in the liver reduce the medication's activity and its effect on tumor markers.

According to the findings of the Grau study (Grau et al., 2008), a genetic variation of the CDA enzyme affects the activity of capecitabine and results in a poor prognosis for cancer.

4.5. Impact of cytidine deaminase gene polymorphisms on the calcium levels in breast cancer patients

Hypercalcemia is dangerous and life-threatening for patients with breast cancer. In hypercalcemia, the concentration of calcium in serum is higher than 10.5 mg/dl. Hypercalcemia in breast cancer patients accompanied with or without bone metastasis significantly affects mortality and morbidity(Hassan et al., 2012).

Hypercalcemia is a common consequence of breast cancer and a major source of morbidity and mortality from the disease. Patients with multiple skeletal metastases are especially at risk. However, in a sizeable subset of patients, elevated calcium levels develop even in the bones and joints (DeMauro & Wysolmerski, 2005).

When serum calcium levels are over 10.5 mg/dl and albumin levels are under 4 g/dl, a condition known as hypercalcemia develops (Brighton et al., 2005).

When chemotherapy is given to patients with hypercalcemia due to breast or lung cancer, the calcium levels decrease, most likely due to a decrease in PTHrP levels (Hassan et al., 2012).

In the current study for SNIP rs2072671, homozygotes (AA), heterozygotes (AC), and mutant homozygotes (CC) all of them have calcium levels that are within the normal range.

Regarding SNIP rs532545, the same scenario applies: all homozygotes are (AA), heterozygotes are (AG), and mutant homozygotes are (GG), and all of them have calcium levels that are within the normal range.

4.6. Impact of cytidine deaminase gene polymorphisms on the concentration of capecitabine in breast cancer patients

The fluoropyrimidine carbamate known as capecitabine is a non-cytotoxic fluoropyrimidine that is used as a precursor to the cytotoxic component known as 5-fluorouracil (5-FU) and is taken orally (Bakkes et al., 2022).

The cytotoxic component fluorouracil can be found in plasma at extremely low doses (with a Cmax ranging from 0.22 to 0.31 mg/L) (Awrich et al., 1979).

Capecitabine, when taken orally, is quickly absorbed and then converted to 5'-deoxy-5-fluorocytidine (5'-DFCR) in the liver by the action of carboxylesterase. In the liver and tumor tissues, cytidine deaminase (CDA) converts 5'-DFCR to 5'-deoxy-5-fluorouridine (5'-DFUR). Thymidine phosphorylase, highly expressed in tumors, catalysis is the last step of the process, converting thymidine to 5-fluorouracil (Schüller et al., 2000).

In this study found that the plasma concentration of 5-FU was greater in SNIP rs2072671 mutant homozygotes (CC) compared to homozygotes (AA) and heterozygotes (AC).

Additionally, the plasma concentration of 5-FU is greater in SNIP rs532545 mutant homozygotes (GG) compared to homozygotes (AA) and heterozygotes (AG).

This was because plasma CDA activity was independent of the metabolic ratio (AUC5'-DFUR+5-FU/AUC5'-DFCR) (Inaishi et al., 2020).

4.7. Frequency of the detected genotypes of CDA gene within breast cancer patients

The percentage and frequency of CDA enzyme polymorphism in breast cancer patients were determined in this study.

About SNIP rs2072671 This study finds that the findings of a previous one (Xandra et al., 2015) are consistent with what they found. That study indicated that heterozygotes of type (AC) were more common than those of type (AA) or (CC) (mutant homozygotes), respectively.

Xandra's research reveals that 239 individuals in Spain were genotyped using SNIP rs2072671. Of those patients, 106 have heterozygotes (AC), 37 have mutant homozygotes (CC), and 96 have homozygotes (AA).

In contrast to the findings of a prior study (Cohen et al., 2019), the current study on SNIP rs532545 demonstrates that heterozygotes AG are more prevalent than homozygotes AA and mutant heterozygotes GG.

Cohen studies done in Paris show that for SNIP rs532545, 47% of patients contain homozygotes (AA), 42% of patients contain heterozygotes (AG), and 11% of patients contain mutant homozygotes. These findings were based on an analysis of 181 patients.

4.8. Impact of CDA gene polymorphisms on the development of hand-foot syndrome in Iraqi BC women treated with capecitabine

Some systemic chemotherapy drugs can cause a cutaneous side effect known as hand-foot syndrome. Especially noticeable on the palms and soles of the feet, erythema, swelling, and a painful burning sensation are all indications (Wu et al., 2014).

This study found that 53 percent of patients participating in this study suffered from hand and foot syndromes of varying degrees, either as a side effect of the medication capecitabine or as a result of a genetic polymorphism involving SNIP rs2072671 and SNIP rs532545.

These findings agree with those of a study (de With et al., 2023), which shows an influence of the genetic polymorphism of CDA on the occurrence of hand-foot syndrome. This study was carried out in the Netherlands.

In this study, for SNIP rs2072671 homozygotes show a higher incidence of hand-foot syndrome than heterozygotes and mutant homozygotes, respectively.

This finding matches the results of recent research (Pellicer et al., 2017) that found those who were homozygous had a 1.89-fold increased risk of toxicity. Additionally, this patient population presented a higher risk of having severe HFS.

In this study, for SNIP rs532545 homozygotes show a higher incidence of hand-foot syndrome than heterozygotes and mutant homozygotes, respectively.

This finding agrees with a study in the British Journal of Cancer for 430 patients showing that homozygotes suffer more from hand-foot syndrome with different grades (Loganayagam et al., 2013).

Changes in CDA enzyme activity have been linked to a promoter variant, c.-451C >T (rs532545). CDA c.-451C > T carriers exhibited a higher frequency of grade 2 diarrhea and higher CDA enzymatic activity. In addition, such patients were at a higher risk of developing HFS of grade 3 (Lam et al., 2018).

Conclusions & Recommendations

Conclusions

1. In Iraqi women who had breast cancer, the cytidine deaminase enzyme was found to be highly polymorphic. It was also found to have different genotypes and variable frequencies.

2. Homozygotes and mutant homozygotes were less common than heterozygotes (AG) and (AC) for both SNIP rs532545 and rs2072671.

3. An impact of CDA enzyme genetic polymorphism on the levels of these parameters, and thus possibly on capecitabine response, was observed, and a significant correlation was found between highly polymorphic CDA enzyme and variable serum levels of estradiol hormone and cancer antigen CA15-3.

4. In contrast to CA15-3 and estradiol, calcium is non-significantly related to the genetic polymorphism of CDA enzyme.

5. There is no relationship between CDA enzyme activity and the concentration of the active drug 5-FU.

6. Existing data indicated a link between CDA enzyme genotype and the development of hand-foot syndrome in Iraqi breast cancer patients.

7. Hand-foot syndrome in both SNIP rs532545 and rs2072671 is more common in homozygotes than heterozygotes and mutant homozygotes.

Recommendations

1. The influence of CDA enzyme SNPs on capecitabine responsiveness should be studied in conjunction with an adequate number of BC patients.

2. Patients using capecitabine should have their genetic variants in the cytidine enzyme (which activates the drug) studied, and the results should be correlated with their clinical response.

3. Examine the possibility that genetic differences in the enzymes responsible for activating capecitabine may explain the broad variety of responses seen in patients taking this medicine.

4. Look into the possibility that additional genes may play a role in hand-foot syndrome.

5. Additional research into the association between breastfeeding and breast cancer risk in Iraqi women is necessary.

6. More research is needed to confirm the relationship between CDA enzyme polymorphisms, the prevalence of hand-foot syndrome, and capecitabine's effectiveness in treating BC.

7. Both carboxy esterase and thymidine phosphorylase enzymes, should be studied in the future because of the role of these enzymes in the activation of capecitabine because of possibility effect on toxicity and efficacy.

Future work

1. Biomolecular research that relies on CDA enzyme sequencing is needed.

2. The role of CDA enzyme polymorphisms on the toxicity and efficacy of capecitabine can be studied by examining its effect on tumor markers, estradiol levels, and cell concentrations of the active medication.

Research Restrictions

1. It was difficult to question some patients about side effects because they didn't realize they had cancer.

2. Number of patients taking capecitabine alone for treating breast cancer.

3. Size of the Sample: Due to the fact that many patients did not come personally to receive their treatment from the oncology hospital, this study comprised a rather small sample size.

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Appendices

Questionnaire of breast cancer Iraqi patients

Name of patient…………………...

Age…………………………………

Phone number……………………...

Address……………………….......

Weight………………………….....

Date of Diagnosis…………………...

Family history   Yes. ☐   No. ☐

Location of breast cancer Right ☐   left ☐   Both ☐

Metastasis   Yes. ☐   No. ☐

If yes write the organ that cancer metastasized……………………...

Breast feeding   Yes. ☐   No. ☐

Marital Status   married. ☐   Single. ☐

Capecitabine treatment duration……………………………….

Unless capecitabine is another medicine used …………………...

Adverse effect of hand-foot syndrome   Yes. ☐   No. ☐

Symptoms of hand-foot syndrome

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ABSTRACT

المقدمة :يصنف السرطان هو مجموعة من الأمراض التي تتميز بتكاثر الخلايا غير المنضبط وغير الطبيعي. سرطان الثدي هو الورم الخبيث الأكثر انتشارا بين النساء في جميع أنحاء العالم ، مع معدل وفيات مرتبط بالسرطان بنسبة 25 ٪ ، أصبح سرطان الثدي تهديدا كبيرا لصحة المرأة في العراق ، حيث هو السبب الرئيسي للوفاة بين النساء بعد أمراض القلب والأوعية الدموية.

هنالك ثلاثة انزيمات لازمة لتنشيط عقار كابيستابين استريز كاربوكسي ، سيتيدين ديميناز ، وفوسفوريلاز ثيميدين عن طريق الفم.

الهدف من الدراسة: للتحقق في العلاقة بين سرطان الثدي ، تعدد الأشكال الوراثي (rs532545) للسيتيدين دياميناز ، و تعدد الأشكال الوراثي (rs207267) للسيتيدين دياميناز على فعالية وسمية عقار الكابسيتابين.

الطرق :اجري البحث في مركز الأورام في مدينة الإمام الحسين الطبية في كربلاء ، العراق ، على ما مجموعه 200 امرأة بين شهري يوليو2022 ويناير 2023. وشمل ذلك 100 امرأة سليمة عملن كمجموعة تحكم وكن أكبر من 45 عاما ، بالإضافة إلى 100 امرأة تم تشخيص إصابتهن بسرطان الثدي وكن أكبر من 45 عاما. تم إجراء البحث على كلا المجموعتين.

تم تقييم استراديول ، مستضد السرطان 15-3 ، ومستويات الكالسيوم في بلازما الدم لكل من الأشخاص الأصحاء والأشخاص المصابين بسرطان الثدي. تم تحديد كمية دواء البلازما ل 100 شخص كانوا يتناولون كابسيتابين لمدة ثلاثة أشهر على الأقل. باستخدام تفاعل البوليميراز المتسلسل الخاص بالأليل ، تم إجراء تعدد الأشكال الجيني على كلا الجينين (rs207267 و rs532545). تم استخدام برنامج SPSS للتحليل الإحصائي.

النتيجة : في هذه الدراسة ، يظهر التحليل الجيني لكلا الجينين (rs207267) و (rs532545) أن الزيجوتات غير المتجانسة أكثر انتشارا من الزيجوت المتماثل والأنواع المتنحية.كان تركيز دواء البلازما في كلا الجينين (rs207267 و rs532545) أعلى في الأنواع الطافرة منه في الزيجوتات غير المتجانسة والزيجوت المتماثل ، على التوالي.

في هذه الدراسة ، كانت هناك فروق ذات دلالة إحصائية بين المجموعات الضابطة ومرضى السرطان فيما يتعلق باستراديول ومستضد السرطان CA 15-3. ولا يوجد فرق ذو دلالة إحصائية في مستويات الكالسيوم بين المجموعتين.

ABSTRACT

الاستنتاج : يمكن استخدام استراديول ومستضد السرطان 15-3 لتقييم استجابة العلاج لمرضى سرطان الثدي. قد يؤثر تعدد الأشكال الجيني لسيتيدين دياميناز على فعالية وسمية الكابيسيتابين في مرضى سرطان الثدي.

جمهورية العراق

وزارة التعليم العالي و البحث العلمي

جامعة كربلاء

كلية الصيدلة

تاثير تعدد الاشكال الجينية CDA جين على فعالية عقار ال Capecitabine الاحادي في النساء العراقيات المصابات بسرطان الثدي

رسالة مقدمة الى مجلس كلية الصيدلة ـ جامعة كربلاء

استيفاءا لشروط الحصول على شهادة الماجستير في علم الادوية و السموم

من قبل

علي امال الدين مجيد

(بكالوريوس صيدلة ٢٠١٤)

بأشراف

<table>
<tr><td>الأستاذ المساعد الدكتور</td><td>الأستاذ الدكتور</td></tr>
<tr><td>حسنين شاكر محمود</td><td>احمد صالح صاحب</td></tr>
</table>