Impact of gene polymorphism on toxicity and clinical outcome in breast cancer patients treated with doxorubicin, cyclophosphamide and taxane-based chemotherapy

A dissertation submitted to the
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DOCTOR OF PHILOSOPHY

Submitted By
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Session: 2021-2022

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Year: 2026

Declaration

I hereby declare that the thesis entitled **"Impact of gene polymorphism on toxicity and clinical outcome in breast cancer patients treated with doxorubicin, cyclophosphamide and taxane-based chemotherapy"** has been prepared for submission to the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh, for partial fulfillment of the requirements for the degree of Doctor of Philosophy. I confirm that I am the sole author of this thesis. To the best of my knowledge, this thesis contains no material previously published by any other person, except where due acknowledgements have been made. Furthermore, this thesis contains no material that has been accepted as part of the requirements for any other academic degree or non-degree program, in English or any other language.

Signature of the Candidate

Md. Shalahuddin Millat

Certificate

A PhD Thesis Work

This is to certify that the thesis entitled **"Impact of gene polymorphism on toxicity and clinical outcome in breast cancer patients treated with doxorubicin, cyclophosphamide and taxane-based chemotherapy"** is an original research work carried out by Md. Shalahuddin Millat (Registration No: 58, Session: 2021-2022) in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, under our supervision. The thesis contains no material previously published by any other person except where proper reference is made in the text.

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Publications

1. **Md. Shalahuddin Millat**, Md. Saddam Hussain, Arafat Miah, Md. Abdul Barek, Mohammad Sarowar Uddin, Mahmuda Ferdous, Md. Abdul Aziz, Md. Mahmudul Hoque, Md. Saiful Islam* and Mohammad Safiqul Islam*. Exploring the toxicity and clinical outcomes of doxorubicin, cyclophosphamide, and taxane-based chemotherapies in women with breast cancer in Bangladesh. ***Journal of Oncology Pharmacy Practice***. 2025; pp: 1–17. DOI: 10.1177/10781552251377418 (**SAGE publisher; Scopus and Web of Science Indexed; Impact Factor: 0.9, Q3 journal**)

2. **Md. Shalahuddin Millat,** Md. Saiful Islam, Mohammad Safiqul Islam*. Impact of SLCO1B3 rs4149117, CYP2C8 rs11572080, and ABCC2 rs17222723 Polymorphisms on Toxicity and Clinical Outcomes in AC-T Treated Breast Cancer Patients in Bangladesh. ***Scientific Reports***. (Under Revision) (**Nature Portfolio Publisher; Scopus and Web of Science Indexed; Impact Factor: 3.9; Q1 Journal**)

3. **Md. Shalahuddin Millat**, Md. Saiful Islam* and Mohammad Safiqul Islam*. Exploring the Impact of ABCB1 (rs1128503, rs2032582, rs3213619, rs1045642) Gene Polymorphisms on Toxicity and Clinical Outcomes in Bangladeshi Breast Cancer Patients undergoing AC-T Regimen. (Manuscript under preparation)

List of Abbreviations

BC: Breast Cancer
ER: Estrogen Receptor
PR: Progesterone Receptor
ERBB2: Erb-B2 Receptor Tyrosine Kinase 2
GLOBOCAN: Global Cancer Observatory
GWASs: Genome-Wide Association Studies
SNPs: Single Nucleotide Polymorphisms
HER2: Human Epidermal Growth Factor Receptor 2
LCIS: Lobular Carcinoma in Situ
DCIS: Ductal Carcinoma in Situ
ILC: Infiltrating Lobular Carcinoma
IDC: Infiltrating Ductal Carcinoma
HRT: Hormone Replacement Therapy
CT scan: Computed Tomography scan
PET: Positron Emission Tomography
MRI: Magnetic Resonance Imaging
MG: Mammography
US: Ultrasound
SPECT: Single-Photon Emission Computed Tomography
DBT: Digital Breast Tomosynthesis
CEM: Contrast-Enhanced Mammograph
DWMRI: Diffusion-weighted MRI
CTCs: Circulating Tumour Cells
CtDNA: Circulating Tumour DNA
LncRNAs: Long Noncoding RNAs
CircRNAs: Circular RNAs
FISH: Fluorescence in Situ Hybridisation
APH: Aptamer Probe Hybridisation
NGS: Next-Generation Sequencing
P-gp: P-glycoprotein
TNBC: Triple-Negative Breast Cancer
DFS: Disease Free Survival
OS: Overall Survival
PFS: Progression Free Survival
QoL: Quality of Life
RECIST: Response Evaluation Criteria in Solid Tumours
CR: Complete Response
SD: Stable Disease
PD: Progressive Disease
CTCAE: Common Terminology Criteria for Adverse Events
DNA: Deoxyribonucleic Acid
RNA: Ribonucleic Acid
Indels: Insertion or Deletion Polymorphisms
STRs: Short Tandem Repeats
CNVs: Copy Number Variations
UTRs: Untranslated Regions
CBR: Carbonyl Reductase
SLCs: Solute Carriers
SLCO1B3: Solute Carrier Organic Anion Transporter Family Member 1B3
OATP1B3: Organic Anion-Transporting Polypeptide 1B3
OATP1A2: Organic Anion-Transporting Polypeptide 1A2
OATP1B1: Organic Anion-Transporting Polypeptide 1B1
ABCB1: Adenosine Triphosphate (ATP)-Binding Cassette (ABC) Subfamily B Member 1
MDR1: Multi-Drug Resistance Gene 1
MRP2: Multidrug Resistance-Associated Protein 2

RT-qPCR: Real-Time Fluorescence Quantitative PCR
FCM: Flow Cytometry
CNB: Core Needle Biopsies
FNAC: Fine Needle Aspirational Cytology
ACT: Adjuvant Chemotherapy
NACT: Neoadjuvant Chemotherapy
AC regimen: Doxorubicin and Cyclophosphamide
AC-T regimen: Doxorubicin, Cyclophosphamide, and Taxane
TAC regimen: Docetaxel/Paclitaxel, Doxorubicin, and Cyclophosphamide
5-FU: 5-Fluorouracil
NICRH: National Institute of Cancer Research and Hospital
BMI: Body Mass Index
IHS: Immunohistochemistry
OR: Odds Ratio
GI: Gastrointestinal
FI: Forward Inner
RI: Reverse Inner
FO: Forward Outer
RO: Reverse Outer
PCR: Polymerase Chain Reaction
Tetra-primer ARMS-PCR: Tetra-primer Amplification Refractory Mutation System-PCR
PCR-RFLP: Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
ABCC2: ATP-Binding Cassette, Subfamily C, Member 2
CYP2C8: Cytochrome P450 2C8
CIPN: Chemotherapy-Induced Peripheral Neuropathy
ALP: Alkaline Phosphatase
TNM: Tumour, Node, Metastasis
ORR: Overall Response Rate
BRCA1: Breast Cancer Gene 1
BRCA2: Breast Cancer Gene 2
CINV: Chemotherapy-Induced Nausea and Vomiting
ADL: Activities of Daily Living
LLN: Lower Limit of Normal
ULN: Upper Limit of Normal
ANC: Absolute Neutrophil Count
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
LVEF: Left Ventricular Ejection Fraction
MI: Myocardial Infarction
TAE: Tris-Acetate-EDTA
bp: Base Pair
CI: Confidence Interval
HWE: Hardy-Weinberg Equilibrium
EtBr: Ethidium Bromide
UV: Ultra Violet
Tm: Melting Temperature
EDTA: Ethylenediaminetetraacetate

Abstract

Background: Women with breast cancer receiving doxorubicin, cyclophosphamide, and taxane-based (AC-T) chemotherapy show considerable differences in both clinical response and toxicity, and clinical factors alone do not fully explain this variation. Genetic differences in drug transport and metabolism may contribute, yet data from South Asian populations, including Bangladesh, are limited. This study focuses on *SLCO1B3* rs4149117, *ABCB1* (rs2032582, rs1128503, rs3213619, rs1045642), *ABCC2* rs17222723, and *CYP2C8* rs11572080 variants and their association with breast cancer risk, response to therapy, and AC-T-related toxicities in women with breast cancer in Bangladesh. **Methods**: A total of 249 patients receiving doxorubicin, cyclophosphamide, and subsequently either paclitaxel or docetaxel, along with 251 controls, were included. Clinical information and toxicity outcomes were obtained from interviews and medical records. Genotyping was carried out using tetra-primer ARMS-PCR for rs4149117, whereas PCR-RFLP was used for rs2032582, rs1128503, rs3213619, rs1045642, rs17222723, and rs11572080. Tumor response was evaluated following RECIST v1.1, and treatment-related toxicities were graded according to CTCAE v5.0. **Results**: Patients who were underweight or had triple-negative tumors tended to respond poorly to AC-paclitaxel, whereas patients diagnosed with infiltrating or metastatic ductal carcinoma demonstrated a more favorable response to AC-paclitaxel. Dysgeusia (78.71%) was the most common non-hematological toxicity, anemia (61.45%) was the most common hematological toxicity, and hepatotoxicity (30.92%) was the most common organ toxicity. Furthermore, 10.84% died within 5 years of their cancer diagnosis, 7.23% died after 5 years of diagnosis, and 81.93% were still living at the time of our last follow-up. *SLCO1B3* rs4149117 showed a consistent protective association with breast cancer. Whereas *ABCB1* rs1128503 and *ABCB1* rs1045642 exhibited an elevated risk for breast cancer, along with poorer responses to AC-paclitaxel. In addition, *ABCB1* rs2032582, *ABCB1* rs3213619, *ABCC2* rs17222723, and *CYP2C8* rs11572080 variants showed no risk associations and were not associated with treatment response. Distinct genotype-toxicity patterns emerged during AC-paclitaxel therapy, and no meaningful associations were observed with AC-docetaxel. **Conclusion**: These findings suggest a potential role for pharmacogenetic markers and support more personalized approaches for breast cancer patients in South Asia, including Bangladesh.

Keywords: *SLCO1B3*; *ABCB1*; *ABCC2*; *CYP2C8*; breast cancer; AC-T; clinical response; toxicity

Aims and Objectives of the Study

The **primary aim** of this study was to investigate the influence of clinical characteristics and genetic polymorphisms on treatment response and chemotherapy-related toxicities among Bangladeshi women with breast cancer receiving the AC-T chemotherapy regimen.

The **specific objectives** of the study were:

• **To analyze the demographic and clinicopathological characteristics** of Bangladeshi women with breast cancer undergoing AC-T chemotherapy.

• **To evaluate the clinical response to AC-T chemotherapy and the extent of chemotherapy-induced toxicities** among breast cancer patients.

• **To assess survival and mortality patterns** among breast cancer patients treated with the AC-T regimen.

• **To identify potential pharmacogenetic biomarkers associated with treatment response and chemotherapy-related toxicities** in Bangladeshi breast cancer patients.

• **To investigate the association between selected genetic polymorphisms and the response as well as toxicity profiles of AC-T chemotherapy** among women with breast cancer.

• **To provide evidence that may support the development of personalized treatment strategies for breast cancer patients in Bangladesh.**

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Introduction

1. Introduction

1.1 Breast Cancer

Breast cancer (BC) has become one of the most predominant malignancies that causes death in females. Generally, it refers to the abnormal and atypical proliferation of breast tissue, resulting in the formation of a tumor-like structure. Based on the rate of growth, it can either be harmless or cancerous. Tumors that are harmless progress gradually and are not as hazardous, whereas tumors with malignant cells progress fast and are capable of spreading to other regions of the body. The prevalence and death rates of BC have been rising due to getting older, lifestyle modifications, environmental or occupational exposures, and hereditary factors. Detecting BC early is crucial for its treatment. When BC is identified early, the probabilities of survival are better. If a breast tumour is detected early, there is a 90% chance that it can be treated successfully. However, if malignancy expands to the lymph nodes or other parts of the body, the likelihood of recovery declines significantly. The probability of survival following the detection of a malignant tumor is relatively low (Sahu et al., 2024). BC is a complex illness that may be categorized into distinct subgroups according to its molecular as well as histological traits. According to histology, malignancies can be divided into ductal and lobular cancers based on where they are found and into pre-invasive and invasive subtypes based on how far they have spread. Ductal cancer originates in the ducts while lobular cancers arise from the lobule-lining cells, but a tiny percentage come from other breast regions (Xiao et al., 2020; Lei et al., 2021). The concentrations of hormone receptors like ER (estrogen receptor) or PR (progesterone receptor), the amplification of ERBB2 (which encodes HER2), and alterations in tumor suppressor genes like BRCA1 or BRCA2, are the most relevant molecular factors (Xiao et al., 2020). Men may develop BC, but most incidences are in women (Alkareem, 2023).

The Global Cancer Observatory (GLOBOCAN) 2022 reported that BC was the most frequently detected malignancy among women globally, with around 2.3 million cases newly identified. It also served as the principal reason for death due to malignancy in women, resulting in around 0.67 million deaths across the globe (Bray et al., 2024). Even though BC happens less often in poorer countries than in Western countries, the death instances are far greater. This distinction is mostly because of late-stage screening, which is typically caused by a lack of awareness of techniques for early identification as well as a lack of access to timely and efficient medical attention (Hossain, 2014; Choulli et al., 2024). Close to half of the BC patients (45.4%) were diagnosed in Asia (Lim et al., 2022). Although the region's BC landscape is characterized by notable variations in different nations of Asia, South-Central Asia recorded the highest

incidence of BC fatalities, accompanied by an age-standardized mortality rate of 13.41 per 100,000 individuals (Bray et al., 2024). Similarly, the incidence as well as mortality rates of BC are escalating quickly in South Asian countries (Lim et al., 2022). In Asia, the highest frequency of BC is seen in premenopausal women within their forties, while in Western countries, it is seen in postmenopausal women in their sixties. There could be many reasons for the difference in incidence, such as variations in location, racial or ethnic background, family history, way of life, the environment, financial status, the presence of identifiable risk factors, the use of diagnostic mammography, the stage of the illness at diagnosis, as well as the affordability of adequate treatment (Leong et al., 2010). BC is the most frequent cancer form among women in Bangladesh, accounting for 69% of females' deaths in the nation (Bray et al., 2024). Bangladesh documented 12,989 new instances of BC, ranking first in incidence among women and fourth within the country, along with a 5-year mean prevalence of 42.4 for every 100,000 women. It caused 6,162 deaths, making the disease the sixth most deadly in the country (Bray et al., 2024), with projections suggesting it will become a major health threat by 2040 (Islam et al., 2022). Consequently, the identification of early risk assessment approach and prognostic markers for this type of cancer is a major focus of oncology research.

Although the precise etiology of BC remains uncertain, it is widely believed that hereditary factors exert a significant influence. About 5% to 10% of all cases of BC are thought to be caused by genetics. Numerous single nucleotide polymorphisms (SNPs) have been identified by genome-wide association studies (GWASs) as being connected to the onset and spread of BC (Hossen et al., 2023). The incidence of BC represents one of the world's health crises (Yedjou et al., 2019), impacting women of all levels and all ethnicities (Banerjee, 2022). The number of patients suffering from BC continues to rise, and the age of patients continues to decrease. Because of this, early BC identification is crucial and directly related to patient (and overall) survival (He et al., 2020). The main modalities of BC treatment are (1) radiotherapy, (2) surgery, and (3) systemic treatment using various drugs. In several types of BC, chemotherapy is a key treatment. It involves several classes of cytotoxic agents (Al-Mahayri et al., 2020). The main classes of agents used are antimetabolites (5-fluorouracil and others), cisplatin and other platinum compounds, alkylating agents (Cyclophosphamide), antimicrotubule agents (Taxanes), and anthracyclines (Doxorubicin). The combination therapies have had much more superior and safer effects than monotherapy (González-Neira, 2012). Although treatment targeting molecular characteristics instead of total tumor burden has significantly enhanced patient outcomes, drug resistance continues to be a primary obstacle in BC therapy, especially in advanced stages of the illness (Xiao et al., 2020). Individual

heterogeneity in response to antitumor medications is prevalent throughout therapy, and the consumption of an equivalent dose may lead to a broad spectrum of toxicities along with varying antitumor activity; thus, the customization of therapy is a critical concern (Longo et al., 2010; Hong & Oh, 2010). The growing need for customized medicine has led to a great deal of research on the hereditary drug metabolism pathways as well as the function of biomarkers to enhance the choice of treatment for every single patient (Olopade et al., 2008).

1.2 Types of Breast Cancer

There are multiple ways to classify BC, but the most common is by whether the cancer is still at the original site or has progressed to neighbouring tissues (Sharma et al., 2010).

I. Based on site of origin

- **Non-invasive BC:** Cancer cells stay in milk-producing ducts as well as do not spread to nearby fatty or connective tissue.
- **Invasive BC:** Cancer cells enter the breast tissue adjacent to the duct or lobule after bursting via the wall. It's important to note that a malignancy might be invasive even if it doesn't always spread to distant organs or lymph nodes.

II. Frequently occurring types

- **Lobular carcinoma in situ (LCIS):** A non-invasive carcinoma characterised by an abnormal proliferation of cells inside the lobules (milk glands).
- **Ductal carcinoma in situ (DCIS):** The most common kind of non-invasive BC, limited to the ducts (e.g., ductal comedocarcinoma).
- **Infiltrating lobular carcinoma (ILC):** This form of cancer develops in the lobules as well as spreads into various regions of the body. It makes up approximately 10–15% of BC diagnoses.
- **Infiltrating ductal carcinoma (IDC):** About 80% of instances of invasive BC are of this form, which originates in the ducts and spreads to adjacent fatty tissue and other regions throughout the body.

III. Less common types

- **Medullary carcinoma:** This is an uncommon form of invasive cancer with distinct boundaries, making up around 5% of all BC cases.
- **Mucinous (colloid) carcinoma:** A rare type of cancer that makes mucus and is typically linked to a better outcome.

- **Tubular carcinoma:** This form of invasive carcinoma is extremely uncommon, accounting for only around 2% of BC cases, and typically results in a good outcome.

1.3 Symptoms of Breast Cancer

BC may present with the following symptoms (Alkareem, 2023):

- In the early stage, when the tumor is small and most treatable, BC often has no symptoms, which makes screening essential for early detection.
- The most common physical sign is a breast lump.
- Before the primary tumor becomes palpable, BC may spread to the axillary lymph nodes, presenting as a mass under the arm.
- Less frequent symptoms include:
    - Breast discomfort or heaviness
    - Persistent changes in the nipple, such as:
        - Spontaneous discharge (especially if bloody)
        - Nipple scaliness
        - Nipple retraction

Any persistent breast change should be evaluated by a physician.

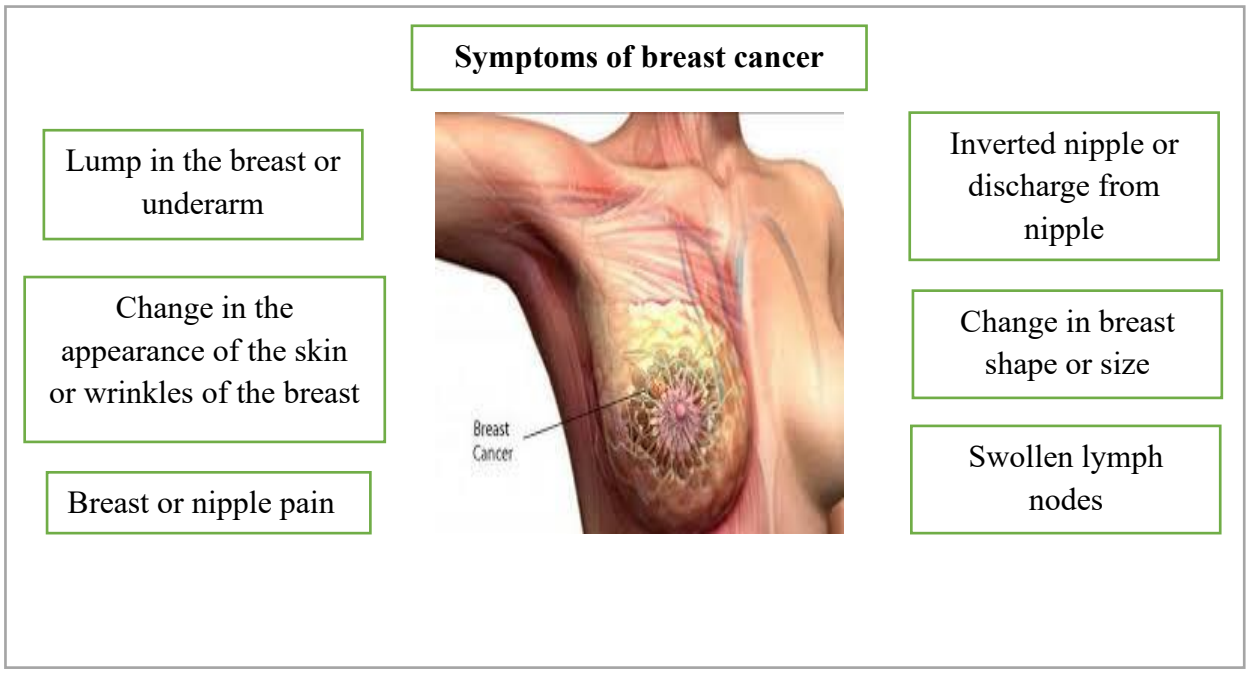

Fig. 1: Common clinical symptoms of BC (Haelle, 2024).

1.4 Risk Factors for Breast Cancer

Increased susceptibility to BC has been associated with the following factors (Leong et al., 2010; Sharma et al., 2010):

Risk variables that cannot be altered:

- Age - advanced age increases the risk
- Sex - being a woman is the biggest risk factor
- A family background of BC, especially among immediate family members.
- Inherited genetic mutations (e.g., BRCA1, BRCA2, TP53)
- A prior instance of ovarian or breast cancer
- Fertility history: premature menarche (before age 12) or post-menopause (after age 55)
- Breast tissue density: A larger density of breast tissue contributes to risk
- Origins or ethnicity: Some ethnic communities have a greater likelihood of getting sick or passing away

Risk variables that can be altered:

- Hormone exposure - prolonged use of hormone replacement therapy (HRT) or oral contraceptives
- Radiation exposure - particularly during adolescence or early adulthood
- Obesity and overweight - especially after menopause
- Physical inactivity
- Alcohol consumption

1.5 Diagnosis of Breast Cancer

A large number of imaging as well as molecular biotechnology-based diagnostic approaches have been developed to promptly and accurately screen BC (He et al., 2020).

I. Imaging Diagnosis: He *et al*. (2020) stated that imaging approaches mostly include computed tomography (CT), positron emission computed tomography (PET), magnetic resonance imaging (MRI), mammography (MG), ultrasound (US), and single-photon emission computed tomography (SPECT).

- **Mammography**: The principal tool for screening and diagnosing BC is still mammography (MG), a low-dose X-ray procedure that lowers mortality by 30–40% through early detection (Haelle, 2024). Conventional MG is widely used, but its

diagnostic sensitivity is low; about 4-10% of individuals have identifiable abnormalities, like microcalcifications. Novel techniques like digital breast tomosynthesis (DBT) along with contrast-enhanced mammography (CEM) were developed to improve diagnostic accuracy in clinical practice and overcome these limitations (He et al., 2020).

- **Ultrasound**: To analyze the form, structure, and location of tumors while maintaining patient safety, ultrasound (US) uses sound waves to image breast tissue (Haelle, 2024; He et al., 2020). Advanced methods that improve diagnostic accuracy and enable assessment of vascularity, axillary lymph nodes, and tumor staging include color Doppler, elastography, and three-dimensional ultrasound (Yang & Fried, 2017; Shi et al., 2018; Signore et al., 2010; Wang et al., 2017).

- **Magnetic Resonance Imaging**: Haelle (2024) claimed that magnetic resonance imaging (MRI) applies strong magnetic fields to generate very detailed pictures of breast tissue. It is extremely valuable to recognise familial BC early, no matter how old the patient is, how dense their breasts are, or how susceptible they are (Riedl et al., 2015). In an effort to improve diagnostic precision, highly specialised MRI methodologies, including diffusion-weighted MRI (DW-MRI), which gauges the fluctuations of water molecules dispersed throughout bodily tissues, are progressively being utilised in the evaluation of BC (He et al., 2020).

- **Positron emission computed tomography**: PET/MRI, or positron emission tomography/magnetic resonance imaging, works with the provision of molecular detail along with the ability to perform soft tissue imaging. This approach works best with the assessment of metastases, which greatly aids the positive predictive value when diagnosing BC (He et al., 2020).

II. Molecular Biotechnology Approaches: While molecular biotech tests are mainly considered as adjunct tools for diagnosis, they are able to find BC earlier than other imaging modalities. These tests analyse proteins, cells, tissues, and nucleic acids to obtain important details about the tumour at the molecular level. Some modern methods are nucleic acid and protein hybridisation, flow cytometry, needle biopsy, real-time quantitative PCR, immunohistochemistry, and some of the methods aimed at the detection of new biomarkers (He et al., 2020).

- **Novel specific biomarkers**: In attempts to differentiate between individuals with BC and those with benign breast conditions, as well as those who are otherwise healthy, the application of circulating biomarkers including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), exosomes, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) has proven to be of value as diagnostic indicators. The above-mentioned biomarkers are of great value for the purposes of cancer staging, as well as the detection of cancer at an early stage (He et al., 2020).
- **Nucleic acid hybridization system**: Fluorescence in situ hybridisation (FISH) and aptamer probe hybridisation (APH) are homologous types of nucleic acid hybridisation approaches capable of locating particular fragments of neoplastic origin, which can help to determine certain biomarkers in BC (He et al., 2020). Gene chips, or high-density oligonucleotide microarrays, allow for the simultaneous interrogation of multiple nucleic acid sequences and enable the large-scale screening of diagnostic BC biomarkers (Hao et al., 2018). Next-generation sequencing (NGS), developed by Metzker (2010), further advances BC diagnosis by enabling comprehensive detection of gene mutations and complete genomic profiling (He et al., 2020).
- **Real-time fluorescence quantitative PCR system**: RT-qPCR (real-time fluorescence quantitative PCR) allows researchers to precisely monitor the amplification of nucleic acids and the levels of transcribed genes. Some biomarkers, such as cfDNA, ctDNA, lncRNA, circRNA, and microRNA, especially those in BC, reach such low abundance levels that conventional measurements often fail to detect them. RT-qPCR can overcome this limitation by quantifying mRNA expression at a high sensitivity level, which is especially useful in assessing the risk and progression of BC (He et al., 2020).
- **Protein hybridization system**: Cancer cells contain both proteins and nucleic acids. Of these two, proteins are the main type of molecule that is utilised for the diagnosis of various cancers. According to the central dogma, while changes in nucleic acids may or may not lead to changes in malignancy, protein levels remain constant. However, in BC, altered levels of protein expression are indicators of the progression of the disease and are frequently assessed via the use of immunohistochemistry, Western blotting, or RT-qPCR. RT-qPCR specifically assesses the expression of a particular gene at the mRNA level, while Western blotting and immunohistochemistry measure the levels of a particular protein (He et al., 2020).
- **Flow cytometer**: Flow cytometry (FCM) is a powerful diagnostic tool for BC because it enables the acquisition of several attributes of each cell within a stream of fluid.

Throughout the years, starting from the 1960s, FCM has received innovations from a variety of fields, including cytochemistry, immunology, materials optics, spectroscopy, molecular biology, laser technology, computer science, and fluidics (He et al., 2020).

- **Puncture biopsy system**: Needle biopsies are among the primary methods for sampling and acquiring malignant cells and tissue for cancer diagnosis. The most common methods are vacuum-assisted breast biopsies (VABB), core needle biopsies (CNB), and fine needle aspirational cytologies (FNAC) (Zhong et al. 2018; Donaldson et al. 2018).

1.6 Treatment of Breast Cancer

There are two main approaches for the treatment of BC:

- **Local treatment** - focuses on treating the tumor in a specific part of the breast.
    - Surgery
    - Radiation therapy
- **Systemic treatment** - works throughout the whole body to target malignant cells that may have spread.
    - Chemotherapy
    - Hormonal therapy
    - Targeted therapy
    - Immunotherapy

1.7 Chemotherapy for Breast Cancer

Chemotherapy for BC employs medications to detect and kill malignant cells in the breast. People commonly obtain these medicines through a needle into a vein or by mouth as pills (Yao et al., 2010). Chemotherapy is the primary aspect of systemic therapy for advanced BC and is required for a variety of early breast carcinomas, including luminal B and HER2-enhanced, as well as triple-negative cases (Al-Mahayri et al., 2020). Cytotoxic chemotherapy is advised to halt the growth as well as proliferation of malignant cells. Adjuvant chemotherapy (ACT) is administered after surgery to eradicate any malignant cells that persist, which lessens the probability of the cancer returning. Neoadjuvant chemotherapy (NACT), on the contrary, is administered prior to surgery to make the tumour smaller and easier to remove (Yao et al., 2010). The frequency of treatments is contingent upon patient-specific criteria, including age and medical history, as well as the cancer's features, which include stage and subtype (Banerjee, 2022). Anthracyclines, antimicrotubule agents (taxanes), alkylating agents (cyclophosphamide), antimetabolites (5-fluorouracil, capecitabine), and platinum compounds

(cisplatin), along with additional medications, are the main categories of chemotherapy used. Most of the time, these medications are administered in a combination regimen (González-Neira, 2012). Typical chemotherapeutic regimens comprise anthracyclines, cyclophosphamide, and taxanes (Xiao et al., 2020). Patients can receive chemotherapy at regular intervals, either alone or in combination. During the treatment phase, the patient may get either single-agent chemotherapy or combined chemotherapy concurrently (Adeel et al., 2019). Combination therapies work better than single agent therapy in BC management (Wang, 2022).

1.7.1. Why Combination Chemotherapy Works Better Than Monotherapy in Breast Cancer?

Combination chemotherapy is generally more efficient than monotherapy in BC because it offers several biological and clinical advantages:

1. Synergistic Effects: Different medications work on different pathways, which makes them work better together. Anthracyclines destroy DNA, while taxanes stop cells from dividing. Collectively, they target cancer at distinct points in the cell cycle.

2. Reduced Drug Resistance: Malignant cells may acquire resistance to a single therapy, but they are significantly less likely to be resistant to many medicines simultaneously.

3. Broader Cancer Cell Kill: As breast carcinoma is complex, employing a combination of medicines enhances the probability of destroying different types of cells that are malignant.

4. Enhanced Tumor Shrinkage & Response Rate: Combination therapies lead to elevated rates of response as well as tumour shrinkage, which is particularly crucial prior to surgery to facilitate breast-saving approaches.

5. Improved Survival: Clinical trials demonstrate enhanced disease-free survival as well as overall survival with combination treatment, especially in aggressive tumour types like HER2-positive as well as triple-negative BC (TNBC).

1.7.2 Common Adjuvant and Neoadjuvant Chemotherapy Drugs for Breast Cancer

Here are some frequently used adjuvant as well as neoadjuvant chemotherapy medications for BC (https://www.cancer.org/cancer/types/breast-cancer/treatment/chemotherapy-for-breast-cancer.html):

- Anthracyclines, for example, doxorubicin, and epirubicin
- Taxanes, for example, paclitaxel and docetaxel
- 5-fluorouracil (5-FU)
- Cyclophosphamide
- Carboplatin

1.7.3 Common Chemotherapy Regimens for Early-Stage Breast Cancer

The following are the most typical chemotherapy treatments for early-stage BC (https://www.lbbc.org/about-breast-cancer/treatments/chemotherapy/common-regimens/acth-doxorubicin):

- AC : Doxorubicin and cyclophosphamide
- AC-T : Doxorubicin, cyclophosphamide, and taxane
- AC-TH : Doxorubicin and cyclophosphamide, then paclitaxel and trastuzumab
- CAF : Cyclophosphamide, doxorubicin, and 5-fluorouracil
- CMF : Cyclophosphamide, methotrexate, and 5-fluorouracil
- FAC : 5-fluorouracil, doxorubicin, and cyclophosphamide
- TAC : Docetaxel, doxorubicin, and cyclophosphamide
- TC : Docetaxel and cyclophosphamide
- TCH : Docetaxel, carboplatin, and trastuzumab
- TCHP : Docetaxel, carboplatin, trastuzumab, and pertuzumab
- TH : Paclitaxel and trastuzumab
- THP : Paclitaxel, trastuzumab, and pertuzumab
- AC-THP: Doxorubicin and cyclophosphamide, then paclitaxel, trastuzumab, and pertuzumab

1.7.4 Doxorubicin, Cyclophosphamide, and Taxane-based (AC-T) Chemotherapy Regimen

The most common chemotherapeutic regimens for BC are primarily based on anthracyclines, cyclophosphamide, and taxanes (AC-T regimen) (Harbeck & Gnant, 2017). The AC-T regimen consists of:

- Doxorubicin - an anthracycline which affects the DNA of cancer cells
- Cyclophosphamide - an alkylating substance that stops DNA from copying itself
- Paclitaxel/docetaxel - a taxane that blocks cell division

Doxorubicin is the most frequently employed anthracycline. It was first used in BC after studies showed it was effective for patients with metastatic disease. Multiple research studies in early-stage BC have since confirmed that doxorubicin is a crucial component of chemotherapy (Kaklamani and Gradishar, 2003). Anthracyclines disrupt DNA replication, inducing DNA damage that leads to cell death (Choulli et al., 2024). Another key drug in these regimens is cyclophosphamide, an alkylating agent and cell cycle-nonspecific prodrug. Cytochrome P450 enzymes metabolically activate cyclophosphamide in order to yield active metabolites that induce DNA cross-linking, thereby inhibiting DNA replication and transcription as well as leading to cell death. The addition of cyclophosphamide to doxorubicin produces synergistic effects and is therefore fundamental to BC treatment protocols, including the AC regimen (Jones et al., 2006). The treatment of BC also depends heavily on the taxane-class agents, docetaxel and paclitaxel (Lai et al., 2022). Docetaxel and paclitaxel are both cytotoxic and cell cycle inhibitors that induce cell death by stabilizing the mitotic spindle and preventing normal cell cycle progression (Samaan et al., 2019). These agents are the standard of care in both the adjuvant (ACT) and neoadjuvant (NACT) chemotherapy of BC, especially in locally advanced BC. The response to taxane-based therapy is heterogeneous in BC, and many tumors are taxane resistant (Tulsyan et al., 2014). The combination of doxorubicin, cyclophosphamide, and a taxane in adjuvant therapy for early BC reduces recurrence as well as death, but the legacy of effective therapy is also a burden (Sucheston et al., 2011). Clinical results from Diéras *et al*. (2004) show that doxorubicin, in combination with either cyclophosphamide or paclitaxel, is effective in neoadjuvant chemotherapy. The AC-T regimen, comprising doxorubicin, cyclophosphamide (AC), and then paclitaxel (T), is administered in a dose-controlled manner to maximise therapeutic efficacy while minimising adverse effects (Fornier et al., 2001). More recently, a combination of doxorubicin, cyclophosphamide, and nab-paclitaxel has proven

successful and well-tolerated for BC in stages II–III and triple-negative BC, where treatment options are limited (Werner et al., 2017). The first combination to show docetaxel's effectiveness in the adjuvant setting was the TAC regimen (docetaxel, doxorubicin, and cyclophosphamide). This combination is approved globally for node-positive BC (Martin, 2006). Two US trials found that adding trastuzumab to standard AC-T (doxorubicin, cyclophosphamide, then paclitaxel) improved survival in HER2/neu-overexpressing tumors (Romond et al., 2005).

1.7.5 Clinical Responses of Breast Cancer Patients to AC-T Chemotherapy

The AC-T regimen, combining Anthracycline (doxorubicin), Cyclophosphamide, then Taxane (paclitaxel or docetaxel), is widely used as adjuvant as well as neoadjuvant therapy in BC management. Its primary goal is to lessen the likelihood of the tumour coming back and achieve tumour shrinkage, as well as enhance overall survival (Gadisa et al., 2021). Standard combinational chemotherapy showed better efficacy and good overall survival (OS) with few drug-related adversities such as anemia, neutropenia, fever, diarrhea, constipation, alopecia, and arthralgia. These toxicities affect patients' quality of life (QoL) along with having the potential to impact treatment efficacy, treatment duration, and overall clinical outcomes (Mistry et al., 2024). Clinical outcome is increasingly recognised as a crucial endpoint in cancer management, and it is influenced by multiple aspects, including lymph node involvement, tumour grade, tumour stage, and the expression of Erb-B2 receptor tyrosine kinase 2 (ERBB2), progesterone receptor (PR), and oestrogen receptor (ER) (Fujii et al., 2015). However, advances in early detection and care have been shown to enhance survival rates, particularly in the case of early-stage breast cancer (Clarke et al., 2013). Doxorubicin, along with cyclophosphamide (AC), is a conventional adjuvant treatment for early-onset BC. This treatment substantially improves overall and disease-free survival, especially when paclitaxel is used as the last agent in a sequential regimen (AC-T) (Perez et al., 2008). In recent years, the rates of survival among women with BC have improved considerably, with 98.9% survival for cases diagnosed and treated at stages I–II, and 85.2% for those diagnosed and treated at stage III (DeSantis et al., 2015).

As stated by the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1, there were four distinct categories of tumour responses in BC management (Eisenhauer et al., 2009).

• **Complete Response (CR)**: Removal of each target lesion.

• **Partial Response (PR)**: The total diameters of the target tumors must be decreased by at least 30%.

• **Progressive Disease (PD)**: A minimum 20% increase in the target lesions' total diameters or the formation of new tumours.

• **Stable Disease (SD)**: There isn't enough shrinking for PR or enough growth for PD.

Individuals who responded completely or partially were designated as responders, while individuals with a progressive or stable condition were designated as non-responders (Mistry et al., 2024). There are multiple factors that determine the effectiveness of the AC-T regimen. There are certain subtypes of the tumour that may perform worse than others, depending on the different stages they are at upon diagnosis. Age, general well-being, and comorbidities are examples of patient circumstances that also matter. There are many different ways to assess response to various clinical treatments, including imaging, physical examination, and, if necessary, pathology. Knowing the response to a treatment also helps to determine the appropriate next steps. In addition to minimizing clinical toxicity, they provide a layer of safety for the overall management of BC.

1.7.6 AC-T Chemotherapy Induced Toxicity in Breast Cancer Patients

One of the most effective chemotherapy approaches for BC management is the AC-T regimen. It offers the most benefits in decreasing recurrence and increasing the survival of patients in the early stages and high-risk categories. However, the regimen is one that causes numerous complications and may have an influence on the patient's quality of life as well as their compliance, which could ultimately impact the overall success of the treatment (Mustian et al., 2011). Many individuals with BC do not begin or conclude their therapy because of negative consequences (Puts et al., 2015; Hamelinck et al., 2016). Therefore, there is an imperative necessity to regulate these instances, as the rate of survival falls from 84.7% to 46.2% in individuals who decline treatment (Joseph et al., 2012). Combination treatments cause fewer complications to individuals than single therapies (Goto et al., 2016). Adverse effects linked to anthracyclines are regarded as a major constraint in the application of potent cytotoxic medications.

Adverse reactions of anthracyclines that are dose-limiting include cardiotoxicity, haematological toxicity, and gastrointestinal toxicity, as well as febrile neutropenia (Al-Mahayri et al., 2020). Haematological toxicity is the primary adverse reaction of cyclophosphamide that

needs to be carefully monitored. Other typical adverse effects involve nausea and vomiting, as well as reversible hair loss (Emadi et al., 2009). Taxanes are associated with a number of side effects, the most relevant of which are haematologic and neurologic adverse effects. Among haematologic damages, docetaxel is the most predominant. For neurological damage, paclitaxel is more predominant. Further negative consequences include allergic responses and toxic effects on the gastrointestinal tract (Bosó et al., 2014; Frederiks et al., 2015). However, complications involving the kidneys and liver and other symptoms, such as high creatinine, abnormal proteins in the urine, elevated enzymes of the liver, and so on, are becoming an increasing concern in the AC-T chemotherapy management (Tecza et al., 2018). In most cases, the impact of the therapy is determined by the need for dose adjustment, treatment alteration, or, in some cases, therapy withdrawal due to persistent side effects. To assist in the alleviation of therapy impact, management of the AC-T-related pattern, severity, and toxicity is of the essence. For an appropriate estimation of treatment to be individualized, toxicity prediction, and management of treatment, pharmacogenomics is helpful. Common Terminology Criteria for Adverse Events (CTCAE) V.5.0 is useful for structuring toxicity classification and grading for the sake of standard reporting in both clinical as well as research settings (Freites-Martinez et al., 2021). The most frequently encountered non-hematological and hematological toxicities of the AC-T regimen and their degrees of severity are described in **Table 1** as per the CTCAE v.5.0.

Table 1: Summary of common non-hematological and hematological toxicities of the AC-T regimen with their toxicity grade based on CTCAE version 5.0.

Toxicity (Parameter)	Baseline/ Normal	Grade 1	Grade 2	Grade 3	Grade 4
Non-hematological					
Nausea	Normal appetite	Appetite loss without intake change	The intake of food reduces without concomitant weight loss or loss of hydration.	Inadequate intake; IV fluids or hospitalization	Consequences that threaten life
Vomiting	None	1-2 episodes/24h	3-5 episodes/24h	≥ 6 episodes/24h; IV fluids/hospitalization	Consequences that threaten life
Diarrhea	Normal bowel pattern	< 4 stools/day	4-6 stools/day	≥ 7 stools/day; incontinence; hospitalization	Consequences that threaten life; immediate action
Constipation	Normal bowel function	Occasional or mild symptoms; no intervention required	The symptoms are modest; medical care is required (for instance, laxatives or enemas).	Severe symptoms; recommended hospitalization; diminished personal care ADL	Consequences that threaten life; immediate action
Fever	Afebrile / normal body temperature	38.0-39.0 °C (100.4-102.2 °F)	39.1-40.0 °C (102.3-104 °F)	> 40.0 °C (>104 °F); recommended admission to the hospital	Consequences that threaten life; immediate action

Edema	No swelling	Mild swelling, pitting or non-pitting; asymptomatic; no limitation of activity	Moderate swelling; discomfort; limiting instrumental ADL (e.g., walking, daily tasks)	Extreme swelling; diminished personal care ADL; recommended hospitalization	Consequences that threaten life; immediate action
Dry mouth	Normal salivary function; no dryness	Symptomatic; able to eat a normal diet	Moderate symptoms; altered oral intake (soft foods, fluids)	extremely unpleasant symptoms; inadequate oral intake; tube feeding or hospitalization recommended	Consequences that threaten life; immediate action
Mouth sores	Normal oral mucosa; no sores	Asymptomatic or mild soreness; erythema only	Medium discomfort; not affecting oral consumption; dietary modifications are recommended	Extreme discomfort; affecting oral consumption; necessitating feeding through a tube; intravenous fluids; or being hospitalized	Consequences that threaten life; immediate action
Fatigue	Normal activity level	Mild fatigue over baseline, not limiting activity	Moderate fatigue, limiting instrumental ADL (shopping, preparing meals, etc.)	Extreme fatigue, diminished personal care ADL (e.g., bathing, dressing)	Consequences that threaten life; immediate action
Dysgeusia	Normal taste perception	Mild alteration in taste (e.g., metallic, salty, or sour taste) without dietary change	Moderate alteration; alteration in eating habits (e.g., avoidance of certain foods)	Severe alteration; major change in eating habits; inadequate oral intake	Consequences that threaten life (Extremely uncommon; immediate action)
Skin hyperpigmentation	Normal skin pigmentation	Mild; focal or diffuse hyperpigmentation, not cosmetically noticeable	Moderate; patchy or diffuse hyperpigmentation, cosmetically noticeable	Severe; extensive or disfiguring hyperpigmentation	Consequences that threaten life (Extremely uncommon; immediate action)

Gastritis	No gastric inflammation; asymptomatic	Mild symptoms; no intervention required	Modest symptoms; recommended hospital care (e.g., antacids, H2 blockers, PPIs)	Severe symptoms; intensive treatment or hospitalization recommended	Consequences that threaten life; immediate action
Peripheral neuropathy	Normal sensation and motor function	No symptoms; impairment of profound tendon reflexes or tingling that does not affect movement	The symptoms are moderate; diminished fundamental ADL	Extreme symptoms; diminished personal care ADL	Consequences that threaten life (for example, paralysis)
Myalgia/arthralgia	No muscle or joint pain	Mild pain; no limitation of activity	Medium pain; diminished fundamental ADL (e.g., walking, cooking)	Extreme pain; diminished personal care ADL (e.g., bathing, dressing)	Consequences that threaten life; immediate action
Hematological					
Anemia (Hemoglobin, g/dL)	LLN (12-16 female, 13-17 male)	< LLN-10.0	< 10.0-8.0	< 8.0; transfusion indicated	Consequences that threaten life; immediate action
Leukopenia (WBC $\times 10^9$/L)	≥ 4.0	< LLN-3.0	< 3.0-2.0	< 2.0-1.0	< 1.0
Leukocytosis (WBC $\times 10^9$/L)	≤ ULN (usually ≤ 10 $\times 10^9$/L)	> ULN-15	> 15-25	> 25-40	> 40
Neutropenia (ANC $\times 10^9$/L)	≥ 2.0	< LLN-1.5	< 1.5-1.0	< 1.0-0.5	< 0.5
Febrile neutropenia	Normal neutrophil count and afebrile	Not defined (usually not applicable)	Not defined	Neutropenia (ANC <1.0 $\times 10^9$/L) with fever ≥38.3 °C (101 °F)	Consequences that threaten life; immediate action

Neutrophilia (ANC $\times 10^9$/L)	≤ ULN (usually ≤ 7.5 $\times 10^9$/L)	> ULN-10	> 10-25	> 25-50	> 50
Lymphocytosis ($\times 10^9$/L)	≤ ULN (usually ≤ 4.0 $\times 10^9$/L)	> ULN-10	> 10-25	> 25-50	> 50
Lymphocytopenia ($\times 10^9$/L)	≥ 1.0	< LLN-0.8	< 0.8-0.5	< 0.5-0.2	< 0.2
Thrombocytopenia (Platelets $\times 10^9$/L)	≥ 150	< LLN-75	< 75-50	< 50-25	< 25
Thrombocytosis (Platelets $\times 10^9$/L)	≤ ULN (usually ≤ 450)	> ULN-600	> 600-800	> 800-1,000	> 1,000

Here, ADL: Activities of Daily Living; LLN: Lower Limit of Normal; ULN: Upper Limit of Normal; ANC: Absolute Neutrophil Count

1.8 Pharmacogenetics & Pharmacogenomics

Today's drug evolution heavily relies on individualized treatment as well as customized therapy. Hippocrates, the founder of modern healthcare, was the first to propose this idea. He claimed that doctors should contemplate aspects like the individual's age and state of health when recommending drugs, because not everyone reacts to medication therapy in an identical way (Sykiotis et al., 2005; Roden et al., 2011). An individual's general health profile and genetic makeup, along with physiological factors and environmental aspects, play a role in how they respond to medication therapy (Ma and Lu, 2011). These days, it's fairly simple to create a personalized medicine regimen that reduces adverse effects. This method is feasible because cellular biology and genetics have advanced very far, and we can now trace the pathophysiology of numerous ailments to find out how DNA varies (Cortese, 2007).

Pharmacogenetics deals with inherited genetic variations in drug metabolic processes that influence individual responses to medications, affecting both therapeutic outcomes as well as unfavorable consequences (Klotz, 2007). Pharmacogenetics is frequently used synonymously with pharmacogenomics, which examines the impact of acquired as well as inherited genetic variations on drug response and actions via a systematic analysis of genes, DNA products, and both inter- and intra-individual differences in expression of genes as well as functioning. Personalized therapy enhances the quality of medical care by enabling clinicians to administer the appropriate treatment to the individual (Cortese, 2007). The research that has linked the genetic makeup to the pathology of illness and its management has resulted in the development of pharmacogenetics as well as pharmacogenomics, which elucidate the genetic factors influencing variability in response to drugs among individual patients (Shin et al., 2009).

Pharmacogenomics is a scientific discipline focused on the systematic determination of every person's gene, their products, and the differences in expression as well as functioning both across and within individuals. The extent of its application is what makes pharmacogenomics different from pharmacogenetics. The first is for a group of people, while the second is more individualized. Pharmacogenetics looks at an unexpected drug reaction and tries to figure out what genetic factors caused it. Conversely, pharmacogenomics searches for variations in genetics within a population that may account for why some people respond to a therapeutic medicine in a certain way. Pharmacogenetics looks at how one medicine interacts with one gene, while pharmacogenomics looks at how many genes affect drug response across the whole genome (Shin et al., 2009; Sheffield and Phillimore, 2009). There are several factors that make

it challenging to employ pharmacogenetic as well as genomic biomarkers in the clinical setting. Patients are very different from each other; therefore, a single biomarker is able to estimate a tiny part of the risk of getting sick or the consequences of treatment (Freeman, 2023).

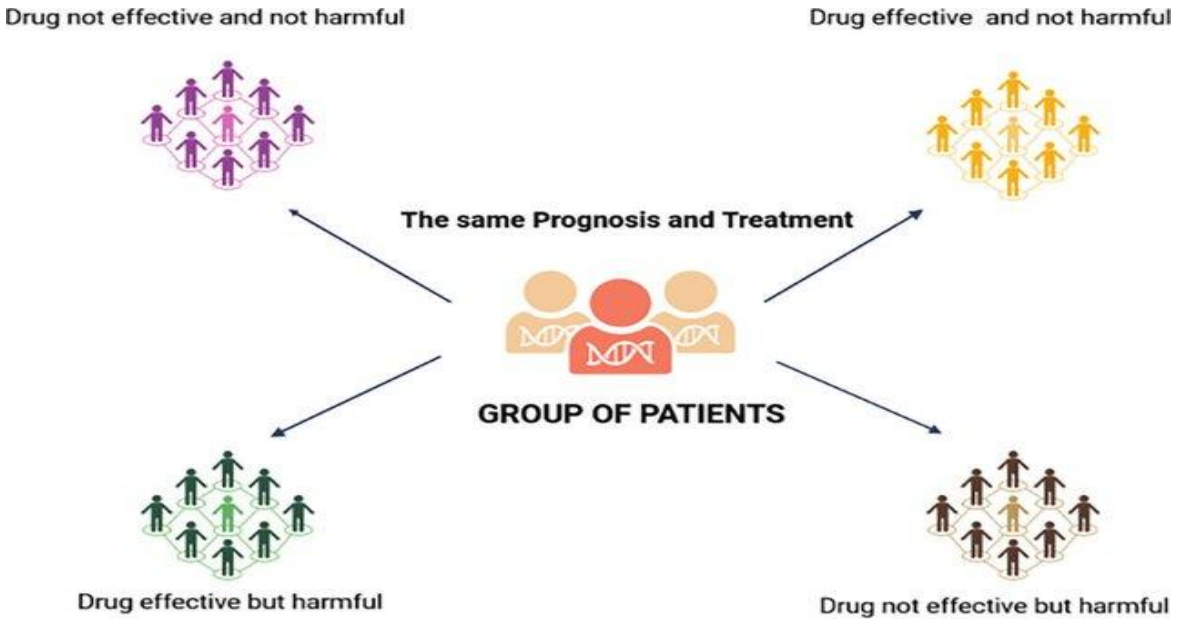

Fig. 2: Drug response variability between individuals (Freeman, 2023).

1.9 Genetic Polymorphisms

Gene polymorphisms are alterations in the sequence of DNA that happen naturally among people in a community. These alterations may involve modifications as small as one nucleotide or as big as a whole structure. They can change genes, regulatory areas, or non-coding DNA, as well as change attributes like how susceptible a person is to getting sick and how they respond to drugs, along with other biological traits. There are several kinds of genetic polymorphisms, such as single nucleotide polymorphisms (SNPs), insertion or deletion polymorphisms (indels), microsatellites (short tandem repeats, STRs), copy number variations (CNVs), and structural variants. Multiple approaches may be utilized to identify these polymorphisms, such as allele-specific PCR, restriction fragment length polymorphism, and the microarray technique, as well as whole genome sequencing (Ismail and Essawi, 2012). Gene polymorphisms could have happened by coincidence or been caused by external variables like viruses or radioactive substances. A genetic mutation is a change in DNA sequence that has been linked to disease in some people. External variables usually induce alterations in the DNA sequence, which are labeled "mutations" instead of "polymorphisms." Genome-wide association studies (GWAS)

have become extremely effective in recognizing variations in genes linked to complicated attributes and disorders (Pasaniuc and Price, 2017).

1.10 Single Nucleotide Polymorphisms

SNPs, or single-nucleotide polymorphisms, are the most predominant category of change in the genetic makeup of human beings. They are typically found in a minimum of 1% of humans. SNPs might be observed in coding regions (exons), non-coding areas (introns), or regulatory areas, including promoters and enhancers, as well as untranslated regions (UTRs). They are extremely valuable for different kinds of medical genetic research (Wang et al., 1998). A single DNA building component, termed a nucleotide, is what makes each SNP different. For instance, an SNP might change a particular part of DNA by replacing the nucleotide cytosine (C) with the nucleotide thymine (T).

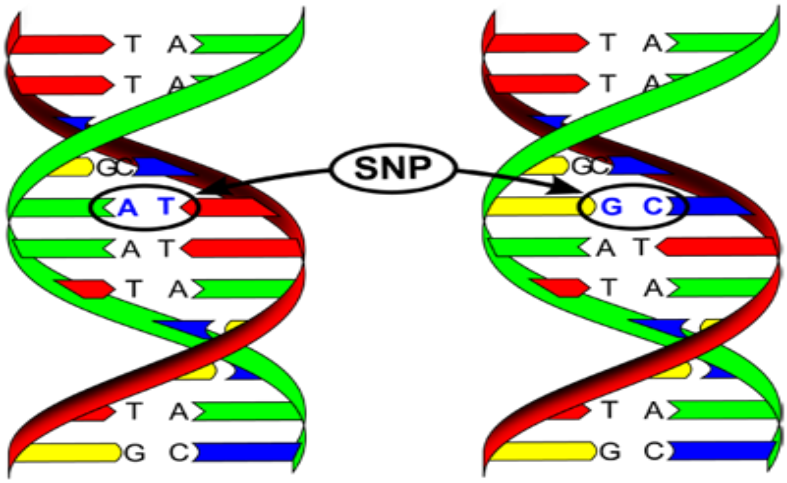

Fig. 3: Single Nucleotide Polymorphisms (Wang et al., 1998).

SNPs are common in an individual's DNA. Researchers may use them as biological indicators to identify genes linked to ailments. SNPs within a gene or in a regulatory region close to it may have a greater impact on disease by altering how the gene functions (Baird et al., 2008). When researchers uncover certain single nucleotide polymorphisms (SNPs) that are linked to a particular feature, they usually look at the genomic areas around those SNPs to identify the gene or genes that cause the phenotype (Grey et al., 2000). SNPs are the most common kind of variation in genes, making up 90% of all genetic differences in humans. It is thought that their density happens about every 500 to 1,000 bases in all human DNA sequences, which means that there are millions of SNPs in human beings as a whole. Researchers are particularly interested in SNPs found in coding sequences due to their increased potential to impact protein function (Hegele, 2002). Drug-metabolising enzymes are an excellent example of how genetic

variants can affect clinical outcomes. These enzymes affect the effectiveness as well as the safety of around 30% of all drugs. So, a treatment that works well for one person may not work for another. This diversity underscores the significance of personalised treatment, wherein the examination of an individual's SNP profile before therapy can assist in identifying the most suitable medication as well as the dose regimen (Eichelbaum et al., 2006).

1.11 Genetic Polymorphisms and AC-T Chemotherapy Outcomes in Breast Cancer

Individual differences in treatment efficacy as well as toxicity, resulting in varied clinical outcomes, are recognised problems in numerous therapeutic domains, including BC. This variability poses several challenges in clinical practice, as it can contribute to ineffective therapy and/or negative drug side effects. The availability of medications and their effectiveness can be impacted by several factors, including individual variations in pharmacokinetics as well as pharmacodynamics, gender, ethnicity, and eating habits. Genetic variations in carrier proteins and target molecules, as well as the enzymes that metabolize drugs, are still thought to be the most important factors among these variables. Changes in the genes that code for enzymes that metabolize medications and other foreign substances, as well as the impacts of these polymorphisms on how the body works, are crucial in determining how effectively therapy works. Single nucleotide polymorphisms (SNPs) in enzymes that metabolize drugs, including both phase I and phase II, along with ABC transporters (*ABCB1*, *ABCC1*, *ABCC2*, *ABCC3*, and *ABCG2*), are crucial in influencing chemotherapy effectiveness as well as adverse consequences (Islam et al., 2015; Haufroid, 2011). SNPs are little changes in genes, but they might have substantial impacts on how an individual appears, since chemotherapeutic drugs have a limited therapeutic index. Toxicity is predominantly determined by the individual's genotype, but the response to therapy is regulated by both the tumor and the individual's genetic traits (Candelaria et al., 2005). Phase I enzymes influence the activation or inactivation of AC-T therapies (anthracyclines, cyclophosphamide, and taxanes). Carbonyl reductase (CBR) enzymes break down doxorubicin into active metabolites, but cyclophosphamide needs CYP2C family enzymes to work (Afsar et al., 2010). *CYP3A4*, *CYP3A5*, and *CYP2C8* break down taxanes, and *CYP1B1* also breaks them down. *ABCB1* (P-glycoprotein) moves metabolites out of hepatocytes, which helps them leave the body through bile. Mutations in CYP genes alongside *ABCB1* have been correlated with the side effects associated with taxanes (Tulsyan et al., 2014). The activity of paclitaxel in the body is also influenced by certain transporters such as MRP2 (*ABCC2*) and OATP1B3 (*SLCO1B3*). As an efflux transporter, MRP2 contributes to hepatobiliary transport and, when overexpressed, can cause resistance to

paclitaxel (Hertz, 2013). OATP1B3 aids in the hepatic uptake of paclitaxel, and when absent or expressed at low levels, the systemic concentration of the medication can be high enough to induce some degree of resistance to chemotherapy (Smith et al., 2005; van de Steeg et al., 2011; Takano et al., 2009). The existence of such genetic variability among the genes that encode the enzymes for metabolism of drugs as well as those for the transport proteins, determines the effectiveness of AC-T chemotherapy, along with consequently justifying the assignment of such mutations for BC treatment personalization.

1.11.1 *SLCO1B3* Polymorphism and AC-T Chemotherapy Outcomes in Breast Cancer

The most important transporters for carrying medications are called solute carriers (SLCs); these transporters carry medications into the body and have the ultimate ability to determine the effectiveness (sensitivity and resistance) as well as the detrimental effects of chemotherapy (Tang et al., 2021). *SLCO1B3* means Solute Carrier Organic Anion Transporter Family Member 1B3 and is responsible for encoding the OATP1B3 transporter, which is a protein with the function of absorbing and transporting several endogenous compounds and anticancer drugs, including anthracyclines and taxanes (Lee et al., 2017; Smith et al., 2007). Likewise, OATP1B1 as well as OATP1B3 are fundamental for the uptake of docetaxel by the liver and the subsequent excretion of the surplus (Iusuf et al., 2015). OATP1B3 is excessively expressed in many human carcinomas, for example, breast, liver, ovary, colon, and pancreas. This protein might be a means for them to adjust and capture more steroids, particularly estrone-3-sulfate. Hence, OATPs are likely to accelerate the proliferation of cancer and also may be important for the development of anticancer agents (Schulte and Ho, 2019). There are greater chances of better therapeutic results to be gained with *SLCO1B3* upregulation due to more effective accumulation of chemotherapy agents like doxorubicin and paclitaxel at the tumor sites (Smith et al., 2007). In functional assays, it was shown that BC cell lines with downregulated *SLCO1B3* expression were able to grow, migrate, and invade more aggressively, while upregulation of *SLCO1B3* significantly attenuated these processes ($P < 0.05$). Results of these assays support *SLCO1B3* as the potential diagnostic tool and/or prognostic indicator for BC (Tang et al., 2021). *SLCO1B3* polymorphisms may be connected with variations in the pharmacodynamics as well as the toxicity of the drugs in a given population or ethnicity, which may explain the differences in the disposition and efficacy of doxorubicin and paclitaxel (Seithel et al., 2008).

The missense variant rs4149117 (334T>G; Ser112Ala), which has been thoroughly investigated, is on chromosome 12p12 and has been linked to differences in how drugs are transported between people. Functional analysis implies that this mutation may change how

transporters function and affect the pharmacokinetics as well as the efficiency of chemotherapy drugs in different ways (Smith et al., 2007). Smith *et al*. (2007) found that rs4149117 had no noteworthy impact on how paclitaxel was disposed of in Caucasian individuals. However, haplotype studies that included this variant revealed that it had a substantial effect on docetaxel pharmacokinetics, especially in Chinese individuals (Chew et al., 2012). Du *et al*. (2019) also found that rs4149117 was not significantly related to the prognosis of BC patients who underwent chemotherapy with the neoadjuvant TA regimen; however, its potential therapeutic significance cannot be dismissed. As of now, no particular investigations have explored the influence of rs4149117 on the pharmacokinetics of doxorubicin as well as cyclophosphamide. Consequently, while rs4149117 appears to be associated with modified transporter activity, its specific influence on the disposition of the drug and treatment-induced toxicities, as well as the clinical results of the prevalent AC-T regimen in BC remains ambiguous and necessitates additional research.

1.11.2 *ABCB1* Polymorphism and AC-T Chemotherapy Outcomes in Breast Cancer

Adenosine triphosphate (ATP)-binding cassette (ABC) subfamily B member 1 (*ABCB1*) is one of a vast group of major active transporters that exist in all forms of life. The multidrug resistance gene 1 (MDR1), or cluster of differentiation (243), is another name for this gene (Tulsyan et al., 2016). The *ABCB1* gene encodes P-glycoprotein (P-gp), a 170-kDa efflux transporter, and transports many different substances out of cells, including several chemotherapy drugs (Ueda et al., 1997; Gottesman et al., 1996). Phenomenon known as multi-drug resistance in cancer, as it reduces the intracellular concentration of chemotherapeutic agents, thereby limiting the effectiveness of therapy (Ambudkar et al., 1999). P-glycoprotein (P-gp) is extensively expressed in barrier as well as excretory tissues, where it modulates the absorption and distribution, along with the elimination of drugs (Schinkel et al., 1997; Fromm, 2000). According to Xiao *et al*. (2020), the human ABC superfamily has 48 functional transporters that are divided into seven subgroups (A-G). ABC transporters, especially P-gp, accelerate the medication's exit from cells, which acts as one of the primary reasons for drug resistance to BC. Multiple studies conducted over the last ten years have demonstrated how genetic variations in ABC transporter genes influence the efficacy as well as adverse consequences of medications. *ABCB1* has been the most studied of these genes. *ABCB1* genetic variants are widely acknowledged as prospective biomarkers for interindividual heterogeneity in pharmacokinetics, treatment results, and adverse drug events following typical AC-T therapy (anthracycline–cyclophosphamide followed by taxane) in BC (Xiao et al., 2020). Researchers

have found links between *ABCB1* gene variations and the metabolism, elimination, responsiveness, and toxic effects of paclitaxel as well as docetaxel, although the results have not always been consistent (Sissung et al., 2006; Bosch et al., 2006; Green et al., 2006; Marsh et al., 2006; Marzolini et al., 2004). The *ABCB1* gene contains a total of 990 variants that alter the amino acid sequence of the protein it encodes. But just three exonic along with one intronic variant were found to be linked with alterations in effectiveness as well as toxicity to BC treatment. The three exonic variants-rs2032582, rs1128503, and rs1045642-are found in a haplotype block that exceeds 40 kb in size and shows substantial linkage disequilibrium (D' > 0.7) in the Asian population, but the intronic variant rs3213619 has no linkage (Tang et al., 2002; Kim et al., 2001; Chen et al., 2014).

The rs2032582 (2677G>T/A; A893S) variant is a triallelic missense mutation at amino acid position 893 of MDR1 (P-gp). The reference allele G (MAF = 55%) codes for alanine, while the variant alleles T (MAF = 41.4%) and A (MAF = 3.6%) code for serine as well as threonine, accordingly. There is still conflicting evidence about how these variant alleles affect transporter function (Leschziner et al., 2007; Wolking et al., 2015). Numerous studies have indicated substantial correlations between rs2032582 and treatment effectiveness or toxic effects in the setting of BC. The variant alleles are linked to a lower risk of hematological (e.g., leukopenia) as well as nonhematological (e.g., fever, fatigue) adverse reactions for individuals treated with docetaxel (Tsai et al., 2009; Jabir et al., 2018; Tulsyan et al., 2014), and they are also linked to a lower risk of resistance to paclitaxel (Chang et al., 2009). In comparison, variant rs2032582 is linked with an increase in therapeutic efficacy as well as increased toxicity associated with anthracycline combinations, which denotes a drug-specific and substrate-specific influence of this polymorphism on P-gp mediated transport in the context of AC-T therapy (Chang et al., 2009; Ikeda et al., 2015; Wu et al., 2012).

The synonymous variant rs1045642 (3435C>T; I1145I) has been demonstrated to modify the function of MDR1 in vitro by altering cotranslational folding as well as substrate selectivity by the addition of an infrequent codon (Kimchi-Sarfaty et al., 2007). The T allele of the rs1045642 variant has been linked to higher toxicity (Jabir et al., 2018; Kim et al., 2012; Kus et al., 2016) and more favourable treatment results (Chang et al., 2009; Kim et al., 2015) in patients receiving taxane-based treatment for BC as part of AC-T chemotherapy (anthracycline–cyclophosphamide followed by taxane). On the contrary, the results for the anthracycline part of the regimen are still conflicting. Several studies have demonstrated that the variant allele is linked to improved (Wu et al., 2012; Lévy et al., 2013) and poorer (Cizmarikova et al., 2010; Ji

et al., 2012) responses as well as survival in the Chinese individuals. Like rs2032582, rs1045642 seems to have diverse impacts on pharmacokinetics, toxic effects, as well as clinical effectiveness depending on the substrate and population, which is similar to how it affects the AC-T therapy (Leschziner et al., 2007; Wolking et al., 2015).

The rs1128503 (1236C>T; G412G), a synonymous variant, is usually thought to be functionally neutral in in-vitro tests (Kimchi-Sarfaty et al., 2007). However, clinical investigations in BC have revealed that rs1128503 may have a substantial impact on how effectively treatments work. In Asian individuals who got AC-T chemotherapy (anthracycline–cyclophosphamide followed by taxane), the variant allele has been connected to higher systemic exposure (Cmax) to anthracyclines (Lal et al., 2008) and to better treatment response as well as adverse reactions to both anthracyclines (Chaturvedi et al., 2013) and taxanes (Priyadarshini et al., 2019; Eckhoff et al., 2015; Tanabe et al., 2017). Although rs1128503 appears neutral in vitro, it demonstrates substrate- and population-specific impacts on P-glycoprotein-driven drug disposition, highlighting its possible clinical significance in the pharmacogenomics of BC. These data suggest that rs1128503 may influence the effectiveness as well as toxic effects of drugs in the AC-T therapy, therefore impacting therapeutic results in BC individuals.

The 129T>C (rs3213619) promoter SNP is part of a haplotype that is linked to increased promoter function as well as elevated ABCB1 expression (Takane et al., 2004). Yamaguchi *et al*. (2006) accomplished an integrated haplotype study that included this genetic variant. They found a substantial association between this variant and paclitaxel clearance ($r = 0.673$; $p < 0.05$). This mutation has been documented in Caucasian (MAF: 0.03), Asian (MAF: 0.06–0.08), and African (MAF: 0.06) communities, despite its comparative rarity. Remarkably, until 2013, no research had assessed its correlation with clinical results for individuals undergoing paclitaxel treatment (Hertz, 2013). Consequently, Lévy *et al*. (2013) found no substantial relationship between the *ABCB1* 129T>C (rs3213619) variant and how well patients responded to doxorubicin-docetaxel neoadjuvant treatment in BC. Conversely, Abraham *et al*. (2014) revealed a substantial linkage between this variant and paclitaxel-related peripheral neuropathy in a European cohort. These results indicate that rs3213619 could influence paclitaxel-associated toxic effects; however, its impact on the therapeutic effectiveness of AC-T treatment is still unclear.

1.11.3 *ABCC2* Polymorphism and AC-T Chemotherapy Outcomes in Breast Cancer

The ATP-binding cassette (ABC) transporters are crucial for cancer cells' ability to resist several drugs. They work as pumps that facilitate efflux, transporting chemotherapy drugs out of cells as well as lowering their levels inside cells. Multidrug resistance-associated protein 2 (MRP2) is one of these. It is encoded by the *ABCC2* gene on chromosome 10q24 and spans about 69 kb with 32 exons (Han et al., 2011). Jedlitschky *et al*. (2006) show that *ABCC2* is located mainly on apical membranes of liver, pancreas (enterocytes), and renal tubular epithelial cells and is involved in the excretion of drugs or metabolites via biliary and renal pathways. Alterations of *ABCC2* gene expression or activity can greatly influence drug disposition and the potential toxicity as well as therapeutic efficacy of a drug in patients (Vlaming et al., 2011). Several polymorphisms of *ABCC2* have been linked with alterations of therapeutic drug efficacy or response of patients to chemotherapy. For instance, rs17222723 (3563T>A; V1188E) is associated with alteration of drug binding and/or drug influx (Pratt et al., 2015). The rs3740065 and rs8187710 polymorphisms are also linked with the therapeutic efficacy of patients with BC (Xiao et al., 2020). BC patients who were on the AC-T regimen and had a low BMI, either alone or with *ABCC2* (58626A>A), had a poorer response to chemotherapy as well as a shorter life span (Mistry et al., 2025). Additionally, *ABCC2* polymorphisms are implicated with gastrointestinal, hematologic, and taxane-associated sensory neuropathy adverse reactions in individuals undergoing AC-T or taxane therapy (Kim et al., 2012; Abraham et al., 2014). Since doxorubicin, cyclophosphamide, and paclitaxel are the key elements of the AC-T regimen and are *ABCC2* substrates, these genetic variants may explain why some people respond differently to treatment and experience more adverse reactions. However, not enough research has been done on the significance of *ABCC2* polymorphisms, specifically rs17222723, in BC risk, chemotherapy-related adverse consequences, and treatment results, particularly in South Asian populations.

1.11.4 *CYP2C8* Polymorphism and AC-T Chemotherapy Outcomes in Breast Cancer

Cytochrome P450 (CYP) enzymes are an extensive family of heme-containing monooxygenases that govern the production of sex hormones as well as the metabolism of medications. Mutations in CYP genes have been linked to BC risk, future-likelihood, and differences in how well chemotherapy works, which can lead to results ranging from less effective treatment to very undesirable adverse reactions (Attia et al., 2024). *CYP2C8* as well as *CYP2C9* are genes that change frequently, leading to over 14 and 34 distinct variants of themselves, respectively (Seredina et al., 2012). The key enzyme that breaks down paclitaxel is

CYP2C8. It also changes arachidonic acid into physiologically active epoxyeicosatrienoic acids (EETs) in the hepatocytes as well as kidneys (Rowbotham et al., 2010; Bergmann et al., 2011). Chemotherapy-induced peripheral neuropathy (CIPN) is a significant adverse consequence of chemotherapy, especially with taxane-based regimens. A meta-analysis encompassing 31 research studies with 4,179 patients indicated a CIPN prevalence rate of 48% (Seretny et al., 2014). Paclitaxel is closely related to CIPN. The differences between individuals may be partly due to genetic differences in the enzymes that metabolize drugs, particularly *CYP2C8* (Hertz, 2013). Polymorphic variants like *CYP2C8*3* (Arg139Lys and Lys399Arg substitutions) as well as *CYP2C8*2* have an immense effect on how paclitaxel is metabolized. The *3 allele is prevalent among Caucasians, and *2 among African-Americans, although both alleles are infrequent in Asian groups (Dai et al., 2001).

CYP2C8 is rather resistant to induction or suppression, although SNPs like *3 (rs11572080; 416G>A) decrease the rate of metabolism, which leads to increased drug levels in the body (Rowbotham et al., 2010; Bergmann et al., 2011). The rs11572080 allele, a missense variant, is linked to modified activity of enzymes, frequently resulting in enhanced paclitaxel metabolism. Clinical investigations have shown that individuals with the *CYP2C8*3* allele are at increased risk of paclitaxel-triggered neuropathy, particularly those who are homozygous for the allele (Gréen et al., 2009; Leskelä et al., 2011). Individuals with *3 may attain elevated clinical response rates; however, this is accompanied by a greater likelihood of grade ≥ 3 peripheral neuropathy (Hertz et al., 2013). These results show that *CYP2C8*3* has a role in the effectiveness as well as the toxic effects of paclitaxel. A lot of research has been done on *CYP2C8*3* in European and African communities, but not much is known about how prevalent it is or how it impacts people in South Asian communities, like Bangladesh.

1.12 Research Gap and Rationale of the Study

BC has become an emerging concern in South Asian nations (Begum et al., 2019). In Bangladesh and other low-or middle-income countries, limited resources, costs related to the provision of healthcare, and healthcare concerns result in not applying standard chemotherapy treatment protocols (Hossain, 2014). In addition, the absence of education, living in rural areas, and certain risk behaviors, such as smoking, have also contributed to the accessibility of treatment and to the effectiveness of treatment at less favorable levels in those areas (Mistry et al., 2024). Though the AC-T (doxorubicin, cyclophosphamide, and then a taxane) regimen is widely used globally (Vriens et al., 2013), little is known about its effectiveness and toxicity in BC patients in Bangladesh. This indicates that we must have evaluative studies conducted in

the area that assess how treatment is tolerated by patients and how accessible it is. Pharmacogenomic studies have explored the association between genetic polymorphisms and chemotherapy outcomes in BC patients worldwide, data from South Asian populations remain limited. In particular, Bangladeshi BC patients receiving the AC-T chemotherapy regimen have not been extensively investigated for genetic predictors of treatment response and toxicity. Variations in drug-transport and drug-metabolizing genes such as *SLCO1B3*, *ABCB1*, *ABCC2*, and *CYP2C8* may influence chemotherapy efficacy and adverse reactions, yet their roles have not been sufficiently evaluated in the Bangladeshi population (Islam et al., 2015; Haufroid, 2011; Smith et al., 2007). Identifying these pharmacogenetic markers could help predict individual differences in treatment response and toxicity, thereby supporting the development of precision medicine approaches. Therefore, the present study aimed to investigate the association between selected genetic polymorphisms and clinical outcomes of AC-T chemotherapy in Bangladeshi BC patients.

1. 13 Literature Review

BC is still one of the most prevalent carcinomas in women around the globe, and chemotherapy is still the most important way to treat it. Numerous treatment plans have been created and tested for their effectiveness in both early- and late-stage carcinoma during the past few decades. Anthracycline-containing regimens, especially those that include doxorubicin, have shown considerable improvements in survival and are commonly regarded as an essential aspect of treatment (Kaklamani & Gradishar, 2003). Later trials showed that combining taxanes like paclitaxel or docetaxel to treatment makes the situation more effective, especially for individuals who tend to be more vulnerable (Lyseng-Williamson & Fenton, 2005).

Cyclophosphamide is a cornerstone of combination therapy due to its activity as a DNA-alkylating agent and due to its side effects, which are important to consider in overall treatment (Pinto, 2009). The combination therapy of doxorubicin and cyclophosphamide and then taxanes (AC-T) is considered one of the most competent protocols for BC (Vriens et al., 2013). AC and AC-T protocols enhance disease-free survival (DFS) as well as overall survival (OS) metrics, focusing on the importance of these protocols in treatment (Gadisa et al., 2020). AC-T especially has been very effective in the neoadjuvant setting, leading to a noteworthy reduction in tumor incidence (Matikas et al., 2024).

Taxanes like paclitaxel or docetaxel are essential for treating BC. They were first used in the treatment of advanced disease, but their value is now acknowledged for early-stage BC and,

more specifically, for the lymph node-positive subtypes, for which taxanes have become the main ingredient of adjuvant therapy. Both drugs have demonstrated strong clinical action in metastatic situations, which has led to greater rates of response as well as survival without progression (Saloustros, 2008). Anthracycline alone and anthracycline–taxane regimens were compared in studies, and the latter had greater rates of responses as well as longer disease-free survival; however, the toxicity profiles were distinctive (Tia et al., 2015).

Even though AC as well as AC-T treatment plans could save lives, they come with many negative consequences. Research conducted in Ethiopia has indicated a significant occurrence of both haematological and non-haematological adverse effects. Patients receiving AC had a higher incidence of haematological problems along with raised alkaline phosphatase (ALP) levels, while individuals undergoing AC-T faced a greater likelihood of non-haematological toxic effects, such as arthralgia/myalgia, peripheral neuropathy, and gastritis (Gadisa et al., 2020). For instance, haematological toxicities were significantly elevated among individuals administered doxorubicin-cyclophosphamide than paclitaxel in the northwest region of Ethiopia (Tesfaye et al., 2013). These adverse consequences, along with mental and financial stress, can greatly lower the quality of life and make it challenging to stick to therapy (Friese et al., 2017; Groenvold, 2010).

Choulli *et al*. (2024) studied the side effects experienced by 122 Moroccan women who were diagnosed with localized BC and who were undergoing neoadjuvant and/or adjuvant treatments with anthracyclines and taxanes. Their study uncovered numerous complex and multi-dimensional side effects, including but not limited to systemic, gastrointestinal, dermatological, and neurological toxicities, which indicates the high level of difficulty in managing the side effects of these treatment regimens. These studies underscore the significance of enforcing the control of toxicities and the delivery of supportive care in under-resourced settings. These studies also allow the identification of treatment-related issues in populations with insufficient or no access to care.

Potential diagnostic, prognostic, and therapeutic decision-making genetic markers have gained recognition alongside enhancements in chemotherapy. Immunohistochemical studies have confirmed that *SLCO1B3* overexpression is associated with less BC recurrence. *SLCO1B3* is also associated with lower metastasis and lower mortality; that is why it is considered a prognostic predictive biomarker. The expression levels of *SLCO1B3* and their positive correlation with the DFS and OS in ER-positive BC have been recognized. This also suggests a possible relationship between estrogen and transporter activity. *SLCO1B3*'s potential role as

an anti-cancer agent is supported by in vitro studies demonstrating that it suppresses the proliferation, motility, and invasiveness of BC cells. However, there is still some dispute regarding the contributions of *SLCO1B3* in BC (Tang et al., 2021). Smith *et al*. (2007) explained that the missense variant rs4149117 (334T>G; Ser112Ala), which is positioned on chromosome 12p12 of the *SLCO1B3* gene, has altered functions of the transporters and the pharmacokinetics as well as the effects of certain chemotherapy drugs. Even though rs4149117 has been shown to alter transporter function, there is little to no understanding of how this variant contributes to drug pharmacokinetics, treatment toxicities, and outcomes in patients undergoing the standard AC-T treatment regimen in BC. This is a gap that needs to be explored.

OATP1B3 is encoded by *SLCO1B3* and is essential for the liver uptake of various chemotherapy agents, including doxorubicin, paclitaxel, and docetaxel. The protein modulates drug clearance, drug exposure, and the risk of toxicity from the drug (Lee et al. 2017, Smith et al. 2007, Iusuf et al. 2015). There is evidence that certain mutations in *SLCO1B3* can alter the transporter's function, which, in turn, may increase the risk of therapeutic failure, altered pharmacokinetics, increased drug-drug interactions, and toxicity (Anabtawi et al. 2022, Mbatchi et al. 2015). However, the clinical evidence so far is inconclusive. For example, de Graan et al. (2012), in their study of a Dutch cohort, found no substantial impact of *SLCO1B3* polymorphisms on docetaxel clearance and toxicity. In contrast, some researchers suggest that the presence of some polymorphisms in *SLCO1B3* could significantly influence the disposition of paclitaxel as well as docetaxel and, therefore, the efficacy of the therapy (Smith et al. 2007, Iusuf et al. 2015). It is remarkable that the impact of certain variants, like rs4149117, seems to depend on the substrate rather than being the same for all taxanes. This is because OATP1B3 exhibits distinct transport features for different substrates (Schwarz et al., 2011; Letschert et al., 2004). Additional extensive and ethnically varied studies are necessary to elucidate the therapeutic importance of *SLCO1B3* polymorphisms, especially for AC-T therapy results.

In the *ABCB1* gene, there are roughly 66 coding SNPs that code for P-glycoprotein (P-gp). These 66 SNPs contain 22 synonymous SNPs and 44 nonsynonymous SNPs (Wolf et al., 2011). Research has indicated that the variants can alter the levels of P-gp produced and the P-gp transport efficiency (Hoffmeyer et al., 2000; Ieiri et al., 2006). *ABCB1* gene variants can cause resistance to different medications and can lead to changes in the clinical outcome of BC, particularly in patients who are on AC-T chemotherapy. These mutations can alter the pharmacokinetics and pharmacodynamics of chemotherapy medications like doxorubicin and taxanes and can alter drug efficacy, drug toxicity, and the overall survival (OS) of the patients

(Clarke et al., 2005; Tulsyan et al., 2016). Furthermore, there are several studies that have documented significant associations of *ABCB1* gene variants with chemotherapy toxicities and clinical outcomes in BC patients undergoing AC-T, providing evidence of the clinical importance of these variants for the prediction of the clinical outcome as well as for the prediction of the chemotherapy response (Bray et al., 2010; Chaturvedi et al., 2013; Tulsyan et al., 2014).

There exists a triallelic variant 2677G>T/A (rs2032582) on the *ABCB1* gene that is positioned on the inside of P-glycoprotein, following the 10th transmembrane region (Tulsyan et al., 2016). Numerous studies have assessed the clinical significance of this variant in BC patients who underwent anthracycline-containing neoadjuvant therapy, but most found no significant association with therapeutic response. For example, Ji *et al*. (2012) found no relationship between this variant and therapy response among BC patients of the Chinese Han ethnicity, and this was similarly reported by Chaturvedi *et al*. (2013) among Indian females. Also, Wu *et al*. (2012) found no noteworthy relationship between the 2677G>T/A variant and the responsiveness to anthracycline-containing neoadjuvant therapy in the Chinese population. Tsai *et al*. (2009) stated that in Taiwanese women with BC, those with the 2677GG genotype had experienced fever and febrile neutropenia following docetaxel therapy. In contrast to the above studies, some studies found no substantial linkage of the 2677G>T/A variant and adverse effects of anthracycline (Bray et al., 2010; Ji et al., 2012; Yao et al., 2010). In addition, the association of this variant with overall survival (OS) is also not consistent in different populations. For example, based on the study of Wu *et al*. (2012), 2677G>T/A polymorphisms were linked with longer overall survival (OS) among HER2-negative BC patients in China after anthracycline-based therapies, but in the study of Bray *et al*. (2010), it was found that UK women who received the same therapy had shorter OS. The contradictory results suggest that ethnicity, treatment variation, and other personal characteristics might affect the clinical significance of the polymorphism of 2677G>T/A in BC, particularly in relation to AC-T chemotherapy.

The *ABCB1* 3435C>T (rs1045642) is a common synonymous variant shown to alter production and activity of P-gp (Hoffmeyer et al., 2000). This mutation has been related with the responsiveness of BC to chemotherapy. For those with a 3435C>T variant and locally advanced disease, there has been a lack of response to therapy (Kafka et al., 2003). Furthermore, the P-glycoprotein and *ABCB1* 3435C>T genotype expression was documented as independent predictors for therapeutic response as well as disease-free survival (Tsukamoto et al., 1997; Hoffmeyer et al., 2000; Kafka et al., 2003). The T allele in BC patients who received taxane-

containing protocols has been associated with increased treatment-triggered toxicities (Jabir et al., 2018; Kim et al., 2012; Kus et al., 2016) and positive clinical results (Chang et al., 2009; Kim et al., 2015). Nonetheless, the results regarding anthracycline-mediated chemotherapy are still not clear. Some studies have found that the variant allele is linked to better (Wu et al., 2012; Lévy et al., 2013) and worse (Cizmarikova et al., 2010; Ji et al., 2012) therapeutic responses as well as survival. Wu *et al*. (2012) found that Chinese BC females with the 3435C>T variant who underwent therapy with anthracycline-containing neoadjuvant treatment had a much better response to therapy. On the other hand, Chang *et al*. (2009) found that heterozygous individuals had diminished rates of illness control as well as were more likely to have a reduced overall survival compared to homozygous individuals. Tsai *et al*. (2009) observed that the CC genotype was linked to leukopenia in Taiwanese individuals who were being treated with anthracycline-containing therapies. Ji *et al*. (2012) revealed that there was no substantial connection between this particular mutation and adverse reactions related to anthracyclines in Chinese Han women. Overall, the findings imply that the *ABCB1* 3435C>T allele may differentially influence the efficiency or toxicity of chemotherapy agents, which might be useful as a predictive pharmacogenetic marker for BC patients undergoing the AC-T regimen.

ABCB1 1236C>T (rs1128503) is one of the other synonymous variants that has been connected to the risk as well as clinical outcomes of BC and may serve as a potential SNP for the sensitivity to anthracycline chemotherapy (Wu et al., 2012). Zhou *et al*.'s (2015) meta-analysis confirms the substantial relationship of the 1236C>T SNP in the dominant model and the response to chemotherapy among BC patients in Asia. Priyadarshini *et al*. (2019) also showed the noteworthy relationship of this SNP with the tumor response to neoadjuvant chemotherapy, which contains docetaxel, among patients with locally advanced BC in South India. 1236C>T has also been linked significantly with the response of North Indian patients to doxorubicin- and cyclophosphamide-based neoadjuvant chemotherapy, as demonstrated by Chaturvedi *et al*. (2013). On the contrary, Khan et al. (2007) and Alsaif et al. (2013) observed that individuals with the C1236T genotype showed a lower response to anthracyclines in South India and Saudi Arabia, respectively. Despite these discrepancies, the overall evidence suggests that *ABCB1* 1236C>T may have a significant role in determining the chemotherapy responses among BC patients in several Asian countries, especially India, China, and Saudi Arabia (Priyadarshini et al., 2019). Zhang *et al*. (2011), along with Ji *et al*. (2012), found no significant link between the 1236C>T variant and anthracycline-triggered toxicities in Chinese BC patients. In contrast, Chaturvedi *et al*. (2013) identified a strong correlation between this genetic variant and higher incidences of haematological and gastrointestinal damage in Indian women undergoing

anthracycline-containing therapy. In general, these outcomes indicate that the *ABCB1* 1236C>T variant may have different effects on treatment efficacy and the adverse consequences of chemotherapy in BC patients of different ethnicities. More research is needed on patients who are getting AC-T regimens.

The *ABCB1* 129T>C polymorphism is quite uncommon among the documented alleles, where the C allele on 129T>C has been verified to cause elevation in the expression of P-gp, which may result in the augmentation of drug transport activity (Takane et al., 2004). Lévy *et al*. (2013) studied the possible influence of the mentioned polymorphism on the clinical response to the neoadjuvant doxorubicin-docetaxel regimens, which constitute the main components of the AC-T regimen, among French BC patients and reported no statistically noteworthy associations. On the other hand, Abraham *et al*. (2014) analysed the same polymorphism in the European population and established robust evidence of the association between 129T>C and sensory neuropathy induced by paclitaxel. This suggested that this polymorphism may impact taxane-mediated toxicity rather than the efficacy of the treatment. These findings generally suggest the *ABCB1* 129T>C polymorphism may impact treatment results and toxicity, which may possibly reflect its pharmacogenetic value in BC patients, especially those on AC-T regimen combinations.

The *ABCC2* gene is responsible for producing MRP2, an efflux transporter that aids in the excretion of certain chemotherapeutic drugs from cells. *ABCC2* gene polymorphisms that have been attributed to the genetically based variation of rs17222723 (3563T>A; V1188E) (Pratt et al., 2015). Evidence suggests that the 3563T>A variant modifies the protein, altering the functioning of the efflux pump, which consequently affects the concentration of a chemotherapeutic agent in the body and influences the intended clinical outcome (Cui et al., 1999; Pratt et al., 2015). When BC patients who received AC-T chemotherapy were examined in relation to *ABCC2* polymorphisms, it was discovered that they responded poorly to the therapy and a subsequent low survival (Mistry et al., 2025). In addition to the low survival, the same polymorphisms have been correlated with a high incidence of toxicities such as gastrointestinal toxicities, and hematologic toxicities, especially taxane-triggered sensory neuropathy (Kim et al 2012; Abraham et al 2014). In summary, these all go to show that the polymorphisms of the *ABCC2* gene have a significant contribution to the survival and toxic effects associated with BC patients administered with the AC-T regimen.

The P450 (CYP) enzyme family is among the most important for the metabolic activities of many of the anticancer drugs, and the genetic differences among these enzymes influence the

efficacy as well as the toxic effects of the drugs. The polymorphisms of the genes that regulate the metabolic enzymes and the genes that code for the transport of drugs can cause great differences in the pharmacokinetics, and thus the outcomes in the patients are altered. The study by Seredina *et al*. (2012) examined the CYP gene variants in a cohort of 395 patients diagnosed with BC employing RFLP analysis, and this study examined polymorphisms of *CYP2B6*5*, *CYP2C8*2*, *CYP2C8*3*, *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*2*, and *CYP2C19*3*. Among these polymorphisms, there were poor responders to the neoadjuvant chemotherapy who were found. The odds ratio analysis revealed that there were probable unresponsive patients to the chemotherapy, and this indicates that the CYP polymorphism contributes to the variability in the treatment outcomes. These findings, however, are similar to the findings that discussed *CYP2C8* variants and the enhanced metabolism of paclitaxel and the *CYP2C19* variants that were discussed in the activation of the prodrugs like cyclophosphamide (Zhang et al., 2006; Van Schaik, 2005). Therefore, these findings need to be validated in larger and heterogeneous populations to approve the findings as well as to evaluate their therapeutic implications.

Paclitaxel therapy exhibits substantial interpatient variability in both efficacy and toxicity, much of which remains unexplained. Pharmacokinetic variability is a major contributor, with metabolism and transport processes critically influencing systemic drug exposure. Paclitaxel, a key component of the AC-T regimen, is primarily metabolized by *CYP2C8* to 6α-hydroxypaclitaxel. SNPs of this gene may lead to the over/under production of the enzyme or the over/under expression of the transporter, thereby affecting paclitaxel pharmacokinetics and altering the therapeutic effect and toxicity (Hertz, 2013; Marsh et al., 2007; Leskelä et al., 2011). The variant *CYP2C8*3* is linked with diminished paclitaxel clearance (Marsh et al., 2007) and has been found to pose a greater likelihood of developing neuropathy related to treatment (Gréen et al., 2009; Leskelä et al., 2011). However, results across populations remain inconsistent, partly due to variations in allele frequencies, research approaches, and clinical endpoints. Importantly, limited pharmacogenetic data are available for South Asian populations, including Bangladeshi BC patients, despite potential genetic and environmental factors that may influence treatment response. Investigating these determinants in understudied populations may provide valuable insights for optimizing AC-T chemotherapy regimens.

Genetic variability of *CYP2C8* affects the metabolism of paclitaxel and its clinical impacts. The *3 and *2 alleles are rare in Asians, while the former is more prevalent in Caucasians and the latter in African Americans (Dai et al., 2001). While *CYP2C8* is highly prone to being induction and inhibition resistant, some single nucleotide polymorphisms, such as *3 (rs11572080)

polymorphisms, do reduce metabolism of the enzyme, resulting in higher-than-normal systemic exposure for one or more coadministered drugs (Rowbotham et al., 2010; Bergmann et al., 2011). Missense variants (polymorphisms) like rs11572080 have been associated with altered enzyme activity that could change paclitaxel metabolism and/or alter clinical outcomes in patients on AC-T therapy. In the clinical arena, this polymorphism has been shown to have distinct effects on patients' responses to therapy. For example, Hertz *et al*. (2012) noted that carriers of *CYP2C8*3* polymorphisms had improved complete responses to neoadjuvant paclitaxel therapy compared to wild-type homozygotes. Seredina *et al*. (2012) pointed out that the heterozygous GA genotype might be related with an improved response to neoadjuvant chemotherapy than GG homozygotes, though this was a statistically insignificant difference. In comparison, Tulsyan *et al*. (2014) stated that patients who had the heterozygous GA genotype had a somewhat greater chance of being classified as non-responders than GG homozygotes, although this was also statistically insignificant. In addition to chemotherapy response, the *CYP2C8*3* allele was associated with toxicity from chemotherapy. Hertz *et al*. (2012) described that these patients were more likely to suffer higher-grade (≥3) adverse effects, which included neuropathy, neutropenia, myalgia, hypersensitivity, fatigue, gastrointestinal toxicity, and anaemia. All the studies cited suggest that these effects may result from the *CYP2C8*3* allele affecting the pharmacokinetics and pharmacodynamics of paclitaxel in the AC-T regimen.

Unfortunately, clinical evidence differs from one population to the next, indicating the need to study other populations, South Asians in particular. The present research was intended to examine the impact of gene polymorphisms on toxicity and clinical outcomes in BC patients undergoing the AC-T regimen in Bangladesh for the first time.

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Chapter-02
Observational Study

2.1 Introduction

Breast cancer (BC) endures as one of the most frequent as well as hazardous medical conditions in the entire globe. It was reported that nearly 2.3 million new instances occurred in the year 2022, corresponding to about 11.6% of total instances of cancer. Even though BC is less widespread in poorer countries in comparison to Western countries, it is expanding promptly. This highlights how crucial it is to recognise it quickly, boost the public's well-being, and develop the appropriate therapeutic actions. Also, the overall mortality frequency from BC is still substantially higher in developing countries than in advanced countries (Hossain, 2014). Women continue to be very terrified of BC, largely because of the anxiety as well as negative feelings that come with getting diagnosed or receiving therapy (Gadisa et al., 2021). Patients typically experience lumps in their breasts, pain in their breasts, discharge from their nipples, or retraction of their nipples when they are diagnosed. These symptoms tend to render it quite difficult for individuals to adhere to their therapy and can have a big impact on their overall well-being (Mistry et al., 2024). These difficulties often cause a lot of psychological discomfort and may make it more difficult to respond to therapy as well as control the illness. Apart from the biological nature of the ailment, several socio-demographic as well as clinical factors-for example, age, place of residence, educational attainment, employment status, and TNM stage-have been recognized as substantial indicators of variability in clinical responses as well as therapeutic outcomes among BC patients (Gadisa et al., 2021).

BC is now understood as a heterogeneous group of diseases, and treatment strategies vary according to tumor type, stage, and extent of dissemination (Iqbal, 2019). Common therapeutic approaches include hormone therapy, chemotherapy, radiation, and targeted therapy, administered either before or after surgery. Surgical interventions, particularly mastectomy and breast-conserving surgery, remain preferred treatment modalities (Deepa et al., 2020). Physicians frequently recommend chemotherapy with cytotoxic agents to stop tumor cells from growing and dividing (Islam et al., 2015). Chemotherapy regimens have changed considerably over the past few decades. They are crucial for lowering the chance of cancer coming back and raising the probability of survival. In developing nations, traditional chemotherapy drugs are still the best way to treat BC (Tesfaye et al., 2023). Currently, anthracycline- and taxane-based agents are often administered sequentially, and combination therapies targeting multiple pathways are generally more effective and less toxic than monotherapy (Mistry et al., 2024; Wang and Minden, 2022). In advanced BC, anthracycline along with cyclophosphamide (AC)

is often used as the first line of chemotherapy. Taxanes, such as paclitaxel as well as docetaxel, have also been demonstrated to be quite effective in both first- and second-line therapy (Biganzoli et al., 2002). Cyclophosphamide acts as a DNA-alkylating drug upon which the effectiveness as well as toxicity of the combination therapy relies (Pinto et al., 2009). The AC-T regimen, combining anthracyclines, cyclophosphamide, and taxanes, is considered among the most potent chemotherapy options for BC (Vriens et al., 2013). Among commonly used neoadjuvant chemotherapy (NACT) regimens for BC, AC-T has demonstrated the highest overall response rate (ORR) compared with regimens such as FEC (5-fluorouracil, epirubicin, cyclophosphamide) or TAC (docetaxel, doxorubicin, cyclophosphamide). This highlights the strong efficacy of AC-T and its substantial ability to reduce tumor burden in patients undergoing NACT. AC-T showed little advantage over the other regimens, but there was a substantial correlation between the response to therapy and disease-free survival (DFS) ($P < 0.0001$). This demonstrates exactly how important it is for boosting the well-being of patients (Matikas et al., 2024). Nevertheless, both AC and AC-T therapies cause a lot of stress on patients and their household members, both mentally and physically, as well as financially, which affects many aspects of clinical results (Friese et al., 2017; Groenvold, 2010).

Chemotherapy-related toxic exposure is a vital concern in the management of BC, since it can shorten the lifespan and extend treatment duration, as well as cause severe adverse reactions that may result in therapy discontinuation (Mustian et al., 2011). Standard combination chemotherapy has demonstrated improved overall survival (OS) and clinical efficacy, though it is associated with adverse events such as anemia, neutropenia, fever, diarrhea, constipation, alopecia, and arthralgia. These toxicities can affect patients' QoL, treatment adherence, and clinical outcomes (Mistry et al., 2024). The AC regimen is associated more frequently with hematological toxicities, whereas AC-T is linked to non-hematological adverse effects. Chemotherapy can also cause varying grades of hepatic as well as renal toxicities (Ellis et al., 2011; Gadisa et al., 2020). In Ethiopia, significant hematological and non-hematological toxicities were reported among women treated with AC (doxorubicin-cyclophosphamide) and AC-T (doxorubicin-cyclophosphamide then paclitaxel) regimens (Gadisa et al., 2020). Similarly, a study from Northwest Ethiopia found anemia, neutropenia, febrile neutropenia, and thrombocytopenia among women receiving AC-T (doxorubicin-cyclophosphamide then paclitaxel) therapy (Tesfaye et al., 2023).

In Bangladesh, various chemotherapy regimens are used for BC treatment; however, evidence regarding their effectiveness and safety is limited. The clinical presentation of the AC-T

(doxorubicin-cyclophosphamide followed by taxane) regimen, as well as the outcomes of frontline treatment for BC, are not well documented in Bangladesh. To address this gap, we performed the first observational study in the country to assess the clinical response as well as the level of toxicity of the AC-T therapy in BC patients.

2.2 Methods

2.2.1 Study Type

The current prospective observational study aimed to assess the toxicity profiles as well as clinical outcomes of doxorubicin, cyclophosphamide, and taxane-based (AC-T) chemotherapy in Bangladeshi women diagnosed with BC.

2.2.2 Calculation of Sample Size

To figure out the sample size, the following formula was employed.

$n = Z^2 P(1-P)/ d^2$	**$n = Z^2 P(1-P)/ d^2$**
Here, n = Sample size z = The level of confidence at 95% (standard value of 1.96) P = Prevalence (5-year prevalence: 42.4 per 100,000 women; then P = 0.0424) [1] d = Precision (If the precision is 5%, then d= 0.05) $n = (1.96)^2 \times 0.0424 \times (1-0.0424) / (0.05)^2$ = 62	Here, n = Sample size z = The level of confidence at 95% (standard value of 1.96) P = Recent incidence (incidence rate= 18%, then P = 0.18) [1] d = Precision (If the precision is 5%, then d= 0.05) $n = (1.96)^2 \times 0.18 \times (1-0.18) / (0.05)^2$ = 227

Here, [1] https://gco.iarc.who.int/media/globocan/factsheets/populations/50-bangladesh-fact-sheet.pdf

This study consisted of 249 patients with BC, which is more than the minimum number of participants required according to the sample size estimation equation.

2.2.3 Study Subjects and Inclusion/Exclusion Criteria

The present investigation encompassed 249 BC patients who received doxorubicin, cyclophosphamide, and taxane-based chemotherapy (AC-T; either paclitaxel or docetaxel) at the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh. This investigation started in October 2022 and ended in July 2025. As a major city as well as the

principal healthcare centre of Bangladesh, Dhaka draws patients from throughout the country (Fatima et al., 2016). Since NICRH is the principal cancer hospital in Bangladesh, we have found patients from all over the country. There are usually eight cycles in the AC-T chemotherapy treatment. The first four cycles use doxorubicin and cyclophosphamide, while the last four use a taxane drug, either paclitaxel or docetaxel. We included individuals who had undergone at least 5 cycles of chemotherapy in this study. Patients receiving either adjuvant or neoadjuvant chemotherapy were included, while individuals who underwent chemotherapy other than the AC-T regimen were excluded during this current investigation. The individuals who had insufficient medical records were excluded from this study. This study excluded patients younger than 18 and those who could not provide the necessary information.

2.2.4 Data Collection

Before commencing the research, everyone who participated submitted their written informed consent (**Fig. 4**).

রোগীর সম্মতি পত্র

আমি নিম্নে স্বাক্ষরিত, একজন গবেষণা ছাত্রকে তার গবেষণা কাজের জন্য আমাকে একজন রোগী হিসেবে বিবেচনা করার অনুমতি দিয়েছি। আমি বুঝতে পেরেছি যে, এই গবেষণা চলাকালীন যে কোন সময় নিজের মন পরবর্তন করতে পারি এবং এই গবেষণা হতে নিজেকে প্রত্যাহার করতে পারি।

চিকিৎসা অধ্যয়নের জন্য রোগীর সম্মতি- (উপযুক্ত স্থানে টিক চিহ্ন দাও)

১. আপনার কি গবেষণার ধরণ, চুড়ান্ত লক্ষ্য এবং পদ্ধতি সম্পর্কে পুরোপুরি ধারণা রয়েছে ?	হ্যাঁ	না
২. আপনি কি সচেতন যে, এরজন্য আপনাকে কোনপ্রকার শারীরিক, মানসিক এবং সামাজিক ঝুঁকির সম্মুখীন হতে হবে না ?	হ্যাঁ	না
৩. আপনার শরীরের কোন অঙ্গে আঘাতের কোন সম্ভাবনা থাকবে না; আপনি কি এই ব্যাপারে সচেতন?	হ্যাঁ	না
৪ এই পরীক্ষার ফলাফল সম্পর্কে আপনি কোন প্রকার ধারণা পেয়েছেন?	হ্যাঁ	না
৫ এই পরীক্ষাই অংশ নিতে কি ইচ্ছাকৃতভাবে সিদ্ধান্ত নিয়েছেন?	হ্যাঁ	না
৬ আপনি কি মনে করেন যে, এই পরীক্ষাটি আপনার মানবাধিকার লঙ্ঘন করেছে?	হ্যাঁ	না
৭ আপনি কি নিশ্চিত যে, আপনার সম্পর্কিত সকল তথ্য গোপন রাখা হবে?	হ্যাঁ	না
৮ এই পরীক্ষার জন্য কোন প্রকার পারিশ্রমিক দেয়া হবে না, আপনি কি এই সম্পকে সচেতন?	হ্যাঁ	না

উপরোল্লিখিত বিষয়গুলি পড়ার পরে আমি রোগী হিসেবে এই গবেষণায় অংশগ্রহনের ব্যাপারে সম্মতি প্রকাশ করছি।

রোগীর সাক্ষর ও তারিখ: ..... রোগীর নাম: .....

Fig. 4: Participant's consent form (written in the native (Bengali) language).

The aims, methodologies, and possible hazards of the investigation were thoroughly clarified to all participants. They were also told that they might quit the research at any time without any difficulties. To ensure confidentiality, all personal identifiers were removed and replaced with unique study codes, thereby anonymizing the data. A pre-structured questionnaire, based on similar research already published, was used to gather demographic and socioeconomic, as well as lifestyle, information from participants (Nguyen et al., 2022; Lee et al., 2017).

Pre-Structured Questionnaire

Research Title: Impact of gene polymorphism on toxicity and clinical outcome in breast cancer patients treated with doxorubicin, cyclophosphamide and taxane-based chemotherapy

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Section A: Patient's identification-

1. Patient's Name:
2. Mobile No:
3. Religion: ☐ Muslim ☐ Hindu ☐ Buddhist ☐ Christian
4. Region of the patient: ☐ Rural ☐ Urban ☐ S-urban
5. Sex: ☐ Male ☐ Female
6. BMI: Height (cm): Weight (kg):
7. Education level:

☐ No Education ☐ Primary ☐ SSC ☐ HSC ☐ Above HSC

8. Occupation:

☐ Employed ☐ Unemployed ☐ Housewife ☐ Student

9. Marital Status:

☐ Married ☐ Unmarried

10. Tobacco consumption history: ☐ Yes ☐ No
11. Dietary habit: ☐ Rich fibrous ☐ Moderately ☐ Poor fibrous

Section B: Clinicopathological Parameters-

I. Age-

☐ < 45 ☐ 45-55 ☐ > 55

II. Menstrual status-

☐ Regular menstruation (Premenopausal) ☐ Postmenopausal ☐ Perimenopausal

III. Patient's history-

a) When did you have your first child? ----------

b) How long do you have to wait before having another child? ----------

c) Breastfeeding duration ----------

d) Has she previously had treatment for BC? Yes ☐ No ☐

e) What was the age at which cancer first appeared? .....

f) Has she ever used contraceptives before? ☐ Yes ☐ No

- If yes, how long have you been taking contraceptives? ……

g) Has postmenopausal hormone therapy been used in the past? ☐ Yes ☐ No

h) Family History of breast cancer: ☐ Yes ☐ No

IV. **TNM Staging System (Clinical):**

T: size or direct extent of the primary tumor

- Tx: Tumor cannot be assessed
- Tis: Carcinoma in situ
- T_o: No evidence of tumor
- T1: Tumor is 2cm (3/4 of an inch) or less across
- T2: Tumor is more than 2 cm but not more than 5 cm (2 inches)
- T3: Tumor is more than 5 cm across
- T4: Tumor of any size growing into the chest wall or skin

N: degree of spread to regional lymph nodes

- Nx: Lymph nodes cannot be assessed
- N_o: No regional lymph nodes metastasis
- N1: Regional lymph node metastasis present; at some sites, tumor spread to closest or small number of regional lymph nodes
- N2: Tumor spread to an extent between N1 and N3 (N2 is not used at all sites)
- N3: Tumor spread to more distant or numerous regional lymph nodes (N3 is not used at all sites)

M: presence of distant metastasis

- M_o: No distant metastasis
- M1: Metastasis to distant organs (beyond regional lymph nodes)

V. Histology (Microscopic anatomy of breast cancer)-

☐ Ductal ☐ Lobular ☐ Mixed

VI. Tumor grade-

☐ Grade 1 ☐ Grade 2 ☐ Grade 3

VII. Hormone receptor status

Estrogen receptor (ER)-

☐ Negative ☐ Positive

Progesterone receptor (PR)-

☐ Negative ☐ Positive

Her-2/neu status-

☐ Negative ☐ Positive

Section C: Diagnosis of breast cancer-

How is breast cancer diagnosed?

☐ Biopsy ☐ FNAC ☐ Mammography ☐ Paraffin block method

☐ Mastectomy ☐ Lumpectomy ☐ Ultrasonography ☐ CT scan

☐ Chest X-ray ☐ Echocardiogram ☐ IHC analysis ☐ MRI

Section D: Treatment of breast cancer-

i. Are you treated by doxorubicin, cyclophosphamide and paclitaxel/docetaxel-based chemotherapy?

☐ Yes ☐ No

ii. Which types of chemotherapy is provided to you?

☐ Neoadjuvant chemotherapy (NACT)

☐ Adjuvant chemotherapy (ACT)

iii. How many cycles are taken in this combined chemotherapy? ……..

iv. After this kind of treatment, what kind of clinical response was observed?

☐ Positive (+) ve ☐ Negative (-) ve

v. What kinds of hormone therapy do patients take, if any? .....

vi. What kind of chemotherapy did the patient receive if they were HER2 (+)? .....

Section E: Toxicity-

Which type of toxicities are induced by AC-T-based chemotherapy?

I. Nausea-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

II. Vomiting-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

III. Diarrhoea-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

IV. Constipation-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

V. Fever

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

VI. Oral mucositis-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

VII. Dry mouth-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

VIII. Fatigue-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

IX. Dysgeusia-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

X. Skin hyperpigmentation-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

XI. Gastritis-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

XII. Peripheral neuropathy-

☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

XIII. Myalgia/arthralgia-

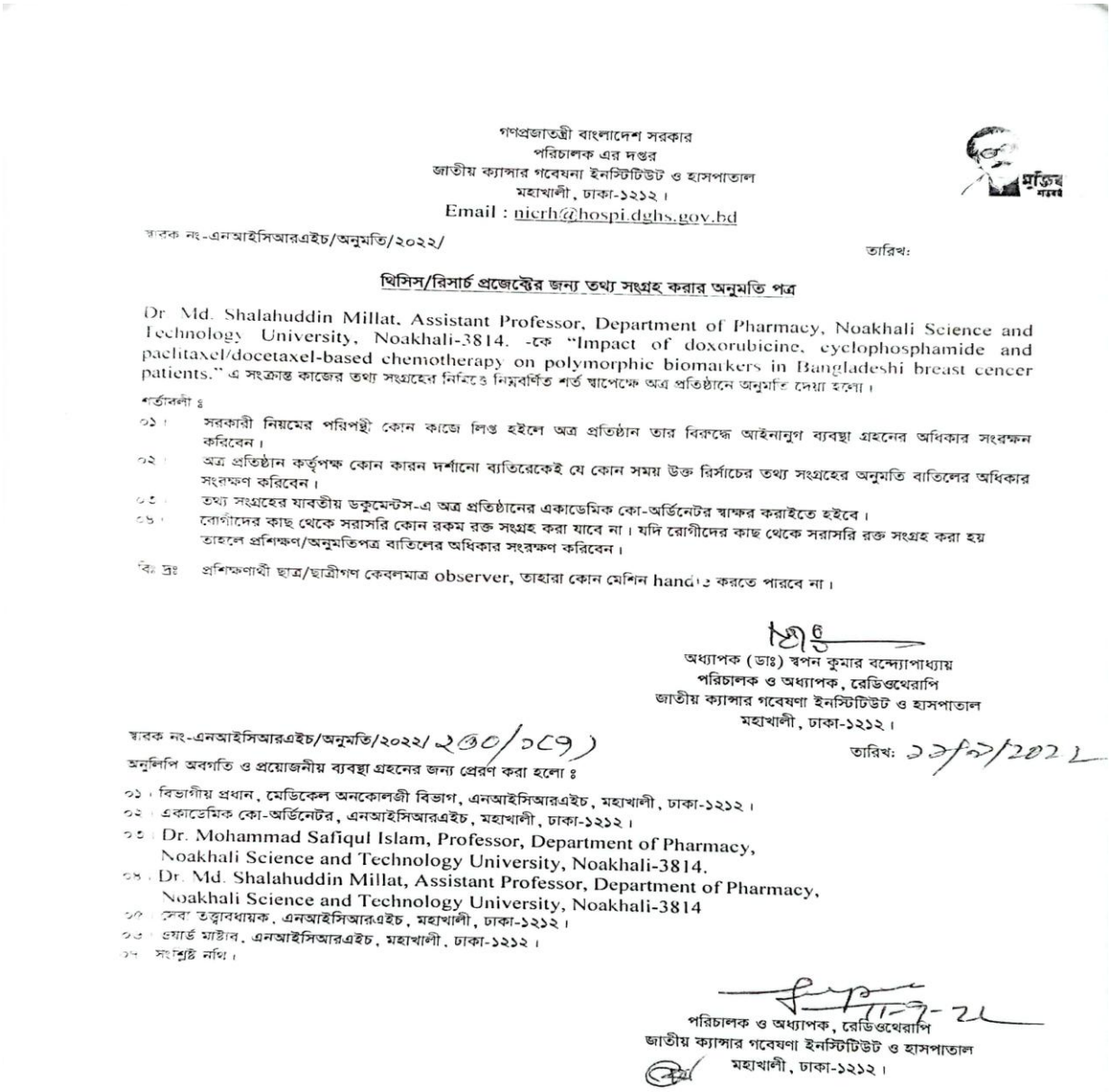
☐ Grade 0 ☐ Grade I ☐ Grade II ☐ Grade III ☐ Grade IV

Several clinicopathological features, such as height, weight, body mass index (BMI), previous health records, laboratory examinations including serum creatinine levels, liver function assessments, complete blood counts, etc., were recorded from patient medical files. Biopsy analyses detailing the tumor features, including tumor locations, growth, dimension, and grade,

as well as lymph node number, were obtained from the patient's medical records. In addition, hormone receptor conditions, chemotherapy types, chemotherapy regimens, chemotherapy cycles, and other related issues were also recorded. All phases of this investigation adhered to the Helsinki Principles as well as the fundamentals of effective clinical practice (World Medical Association, 2013).

2.2.5 Ethical Clearance

The National Institute of Cancer Research and Hospital's corresponding ethical panel gave permission for this research (NICRH/Permission/2022/230/1(7)) (**Fig. 5**).



গণপ্রজাতন্ত্রী বাংলাদেশ সরকার
পরিচালক এর দপ্তর
জাতীয় ক্যান্সার গবেষনা ইনস্টিটিউট ও হাসপাতাল
মহাখালী, ঢাকা-১২১২।
Email : nicrh@hospi.dghs.gov.bd

স্মারক নং-এনআইসিআরএইচ/অনুমতি/২০২২/ তারিখ:

থিসিস/রিসার্চ প্রজেক্টের জন্য তথ্য সংগ্রহ করার অনুমতি পত্র

Dr. Md. Shalahuddin Millat, Assistant Professor, Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814. -কে "Impact of doxorubicine, cyclophosphamide and paclitaxel/docetaxel-based chemotherapy on polymorphic biomarkers in Bangladeshi breast cancer patients." এ সংক্রান্ত কাজের তথ্য সংগ্রহের নিমিত্তে নিম্নবর্ণিত শর্ত স্বাপেক্ষে অত্র প্রতিষ্ঠানে অনুমতি দেয়া হলো।

শর্তাবলী ঃ

১। সরকারী নিয়মের পরিপন্থী কোন কাজে লিপ্ত হইলে অত্র প্রতিষ্ঠান তার বিরুদ্ধে আইনানুগ ব্যবস্থা গ্রহনের অধিকার সংরক্ষন করিবেন।

২। অত্র প্রতিষ্ঠান কর্তৃপক্ষ কোন কারন দর্শানো ব্যতিরেকেই যে কোন সময় উক্ত রির্সাচের তথ্য সংগ্রহের অনুমতি বাতিলের অধিকার সংরক্ষণ করিবেন।

৩। তথ্য সংগ্রহের যাবতীয় ডকুমেন্টস-এ অত্র প্রতিষ্ঠানের একাডেমিক কো-অর্ডিনেটর স্বাক্ষর করাইতে হইবে।

৪। রোগীদের কাছ থেকে সরাসরি কোন রকম রক্ত সংগ্রহ করা যাবে না। যদি রোগীদের কাছ থেকে সরাসরি রক্ত সংগ্রহ করা হয় তাহলে প্রশিক্ষণ/অনুমতিপত্র বাতিলের অধিকার সংরক্ষণ করিবেন।

বিঃ দ্রঃ প্রশিক্ষণার্থী ছাত্র/ছাত্রীগণ কেবলমাত্র observer, তাহারা কোন মেশিন hand'ও করতে পারবে না।

অধ্যাপক (ডাঃ) স্বপন কুমার বন্দ্যোপাধ্যায়
পরিচালক ও অধ্যাপক, রেডিওথেরাপি
জাতীয় ক্যান্সার গবেষণা ইনস্টিটিউট ও হাসপাতাল
মহাখালী, ঢাকা-১২১২।

স্মারক নং-এনআইসিআরএইচ/অনুমতি/২০২২/২৩০/১৫৭) তারিখ: ২৭/৭/২০২২

অনুলিপি অবগতি ও প্রয়োজনীয় ব্যবস্থা গ্রহনের জন্য প্রেরণ করা হলো ঃ

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৭। সংশ্লিষ্ট নথি।

পরিচালক ও অধ্যাপক, রেডিওথেরাপি
জাতীয় ক্যান্সার গবেষণা ইনস্টিটিউট ও হাসপাতাল
মহাখালী, ঢাকা-১২১২।

Fig. 5: Ethical permission letter (written in the native (Bengali) language) from the NICRH.

2.2.6 Criteria for Tumor Response Assessment

The Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) were used to assess the tumor responses to the AC-T regimen, comprising complete response, partial response, progressing disease, and stable disease (Eisenhauer et al., 2009). Tumor responses were assessed after two consecutive cycles, and the responses were verified by a follow-up assessment at four weeks interval (Chang et al., 2009). A documented approach was used for assessing tumour, node, metastasis (TNM) stages (Edge, 2010). All patients regularly had complete blood count and urine examinations before and after each chemotherapy cycle.

2.2.7 Criteria for Toxicity Assessment

All patients underwent routine complete blood count and urine analysis before and after each chemotherapy cycle, with the results documented in their clinical records. Hematological toxicities were recorded ninety days after each chemotherapy treatment. In addition, we also reviewed all medical recordings approximately for eighteen months after their inclusion to current investigation. Non-hematological toxicities were documented by combining the adverse events experienced by participants during their visits in two phases (1st follow-up: approximately 6 to 11 months; 2nd follow-up: approximately 12 to 18 months). In order to grade the observed toxicities, we merged self-identified adverse events with data from medical records (Nguyen et al., 2022). For patients who experienced repeated episodes of the same toxicity across multiple chemotherapy cycles, only the highest grade (i.e., the most severe event) was recorded and considered for analysis. The Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) has been implemented to grade toxicities caused by chemotherapy (Freites-Martinez et al., 2021).

2.2.8 Statistical Analysis

The statistics software IBM SPSS (Version 26.0; IBM Corporation, Armonk, NY, USA) was applied to collect data and perform statistical analysis. Chi-square (χ^2) tests were utilized to evaluate differences between categorical variables and to compare toxicities across the two chemotherapy regimens. The independent samples t-test was applied to identify differences in mean values (Khanom et al., 2024; Gadisa et al., 2020). The correlation between clinicopathological features and response status to the AC-T regimen was assessed using logistic regression analysis in MedCalc (version 19.0.7) (Bushra et al., 2020). Based on the p-values, we decided which one was statistically noteworthy. In statistics, $p < 0.05$ was the acceptable threshold for significance.

2.3 Results

2.3.1 Sociodemographic Information of Breast Cancer Patients

Table 2 shows the demographic information for those who participated in the investigation. The participants were between 26 and 71 years old, with an average age of 43.24 years. The average BMI of participants was 23.65 kg/m^2, where 5.62%, 57.83%, 31.33%, and 5.22% of participants were underweight, normal, overweight, and obese, respectively. A large percentage of patients (64.26%) were from remote regions, while 35.74% were from urban areas. Most participants (81.53%) were non-employed or housewives, and half of the total participants (51.81%) had no formal education. Out of the 249 women involved in the study, 247 were married, and only two were single. Again, 67.07% of patients received rich fibrous food in their daily life, while 23.29% had a history of tobacco use, compared to 76.71% who did not. Again, 34.54% of women did not receive any contraceptive pills, although 65.46% of women had used them previously.

Table 2: Sociodemographic information of BC patients.

Variables	**Patients (N= 249)**	**%**
Age (years)		
<45	125	50.20
45-60	103	41.37
>60	21	8.43
Mean age ± SD (range)	43.24 ± 9.17 (26-71)	-
BMI (kg/m^2)		
Underweight (<18.5)	14	5.62
Normal (18.5-24.9)	144	57.83
Overweight (25.0-29.9)	78	31.33
Obese (≥30)	13	5.22
Mean BMI ± SD (range)	23.65 ± 3.60 (16.0-37.3)	-
Region		
Rural	160	64.26
Urban	89	35.74
Education		
No education	129	51.81
Primary-secondary	99	39.76
Higher secondary or above	21	8.43
Occupation		
Non-employed	203	81.53
Employed	46	18.47

Marital status		
Married	247	99.20
Unmarried	02	0.80
Dietary habit		
Rich fibrous	167	67.07
Moderate fibrous	54	21.69
Poor fibrous	28	11.24
Tobacco consumption history		
Yes	58	23.29
No	191	76.71
Previous history of contraceptives		
Yes	163	65.46
No	86	34.54

2.3.2 Clinicopathological Features of Breast Cancer Patients

The clinicopathological profiles of 249 BC patients are thoroughly evaluated in **Table 3**. Among them, the primary tumour site was almost evenly distributed between the right (52.21%) and left (47.79%) breasts. Menstrual history revealed that 38.95% of patients had regular menstruation (premenopausal), 34.54% were postmenopausal, and 26.51% had irregular menstruation (perimenopausal) due to various causes. Breastfeeding for ≥2 years was reported by 88.35% of patients, while 11.65% had either no or shorter than two years of breastfeeding history. An ancestral background of BC was present in 12.05% of cases. The onset of cancer symptoms occurred at or before the age of 45 in 60.64% of patients, with the remaining 39.36% reporting onset after 45 years of age. Biopsy was the most employed diagnostic technique (84.74%), followed by FNAC (58.23%), paraffin block method (48.19%), mammography (27.31%), and surgical methods, including mastectomy (32.53%) and lumpectomy (10.04%). Additional diagnostic tools included ultrasonography (65.06%), chest X-rays (38.55%), echocardiography (27.31%), CT scan (16.87%), MRI (2.41%), and IHS analysis (1.20%). In terms of histology, invasive carcinoma of the ducts was the most common form (53.82%), then infiltrating ductal carcinoma (45.78%) and duct cell carcinoma (26.51%). Additionally, less prevalent forms included metastatic ductal, mucinous, mixed, medullary, invasive lobular, and tubular carcinomas.

It was found that 47.79% of the tumours were grade II, 35.74% were grade III, as well as 16.47% were grade I. Most patients were diagnosed at stage II (46.98%) or stage III (34.54%), with a smaller proportion at stage I (10.44%), stage 0 (4.02%), and stage IV (4.02%). Lymphovascular invasion was present in 17.67% of the cases. Regarding hormone receptor status, HER2-negative was the most prevalent, observed in 58.63% of patients. The majority of

patients (87.53%) received a combination regimen of doxorubicin, cyclophosphamide, and paclitaxel, while the remaining 12.45% were treated with doxorubicin, cyclophosphamide, and docetaxel. Adjuvant chemotherapy was more commonly administered (70.28%) than neoadjuvant chemotherapy (29.72%). The majority of patients (83.53%) underwent 5-8 cycles of chemotherapy, while the remaining 16.47% received more than 8 cycles.

Table 3: Clinical along with pathological characteristics of BC patients.

Variables	**No. of patients (N= 249)**	**%**
Primary tumour site		
Right breast	130	52.21
Left breast	119	47.79
Menstruation status		
Premenopausal	97	38.95
Perimenopausal	66	26.51
Postmenopausal	86	34.54
Breastfeeding period (years)		
No or < 2	29	11.65
≥ 2	220	88.35
Family history of BC		
Yes	30	12.05
No	219	87.95
Cancer showed its first symptom (age)		
≤45	151	60.64
>45	98	39.36
Diagnosis technique		
Biopsy	211	84.74
FNAC	145	58.23
Mammography	68	27.31
Paraffin block method	120	48.19
Mastectomy	81	32.53
Lumpectomy	25	10.04
Ultrasonography	162	65.06
CT scan	42	16.87
Chest X-ray	96	38.55
Echocardiogram	68	27.31
IHC analysis	03	1.20
MRI	06	2.41
Histology		
Ductal carcinoma	66	26.51
Invasive ductal carcinoma	134	53.82
Infiltrating ductal carcinoma	114	45.78
Metastatic ductal carcinoma	53	21.29
Mucinous carcinoma	04	1.61
Mixed (ductal and mucinous) carcinoma	03	1.20

Medullary carcinoma	03	1.20
Invasive lobular	05	2.00
Tubular carcinoma	02	0.80
Tumor grade		
Grade I	41	16.47
Grade II	119	47.79
Grade III	89	35.74
Cancer stage		
Stage 0	10	4.02
Stage I	26	10.44
Stage II	117	46.98
Stage III	86	34.54
Stage IV	10	4.02
Lymphovascular invasion		
Present	44	17.67
Absent	119	47.79
No data	86	34.54
Hormone receptor status		
ER (-)	127	51.00
ER (+)	122	49.00
PR (-)	132	53.01
PR (+)	117	46.99
HER-2 (-)	146	58.63
HER-2 (+)	79	31.73
HER-2 (equivocal)	25	10.04
Triple (-)	63	25.30
Chemotherapy regimen		
Doxorubicin-cyclophosphamide- paclitaxel	218	87.55
Doxorubicin-cyclophosphamide- docetaxel	31	12.45
Types of chemotherapy		
Neoadjuvant chemotherapy (NACT)	74	29.72
Adjuvant chemotherapy (ACT)	175	70.28
Chemotherapy cycle		
5-8 cycle	208	83.53
> 8 cycle	41	16.47

2.3.3 Clinical Response of Breast Cancer Patients to AC-T Chemotherapy

Table 4 illustrates how 249 BC patients responded to the AC-T (doxorubicin, cyclophosphamide, and taxane-based) chemotherapy regimens in a clinical setting. Among the 218 patients who received the doxorubicin-cyclophosphamide-paclitaxel regimen, 117 patients (53.67%) were classified as responders, exhibiting either a complete response (44.50%) or a partial response (9.17%). The remaining 101 patients (46.33%) exhibited no substantial tumor regression, classified as non-responsive patients with stable disease (10.09%) or progressing disease (36.24%). In contrast, 31 patients received the doxorubicin-cyclophosphamide-

docetaxel regimen. Out of these, 18 patients (35.48% with complete response and 22.58% with partial response), accounting for 58.06%, responded favorably to the treatment. In contrast, 13 patients (12.90% with stable disease and 29.03% with progressive disease), or 41.93%, were non-responsive. Although both regimens demonstrated notable response rates, a marginally higher proportion of responders was observed in the docetaxel-treated cohort, suggesting potentially greater efficacy. However, because of the reduced sample size in that subgroup, this finding should be considered cautiously.

Table 4: Clinical responses of BC patients to AC-T chemotherapy regimen (RECIST v.1.1).

Variables	**Total patients (N= 249)**	**%**
Doxorubicin-cyclophosphamide- paclitaxel (N=218)		
Complete Response (CR)	97/218	44.50
Partial Response (PR)	20/218	9.17
Responder (CR+PR)	117/218	53.67
Stable Disease (SD)	22/218	10.09
Progressive Disease (PD)	79/218	36.24
Non-responder (SD+PD)	101/218	46.33
Doxorubicin-cyclophosphamide- docetaxel (N=31)		
Complete Response (CR)	11/31	35.48
Partial Response (PR)	7/31	22.58
Responder (CR+PR)	18/31	58.06
Stable Disease (SD)	4/31	12.90
Progressive Disease (PD)	9/31	29.03
Non-responder (SD+PD)	13/31	41.93

2.3.4 Correlation Between Clinicopathological Features and Response Status of AC-T Regimen

The relationship between clinical response and various demographic and clinicopathological features was evaluated among patients undergoing treatment for breast cancer with the AC-T chemotherapy regimen, as presented in **Table 5**. In the AC-paclitaxel regimen, underweight patients (BMI < 18.5) had a considerably lower likelihood of responding to chemotherapy than individuals with a normal BMI (OR = 0.24; 95% CI = 0.06-0.93; p = 0.039). In terms of histological subtype, individuals with infiltrating ductal carcinoma (OR = 1.96; 95% CI = 1.01-3.78; p = 0.045) and metastatic ductal carcinoma (OR = 2.38; 95% CI = 1.08-5.27; p = 0.031) had substantially greater odds of treatment response than those with ductal carcinoma. Patients with triple-negative hormone receptor type had a markedly reduced responsiveness to therapy

compared to those with HER-2 negative type (OR = 0.43; 95% CI = 0.23-0.84; p = 0.012). Additional variables, including age, tobacco consumption history, contraceptives, tumor site, menstruation, breastfeeding period (years), family history of BC, previous BC treatment, tumor grade, cancer stage, types of chemotherapy, and chemotherapy cycle, did not demonstrate statistically significant associations with chemotherapy response. Conversely, no statistically noteworthy linkages were identified between any demographic or clinicopathological features and clinical response in patients treated with the AC-docetaxel regimen.

2.3.5 Toxicity Status of the AC-T Regimen in Bangladeshi Women with Breast Cancer

Table 6 depicts the toxicity pattern of the AC-T regimen among BC patients from Bangladesh. Despite all study participants receiving antiemetic medication, nausea and vomiting remained the most common gastrointestinal (GI) toxicities, affecting 190 patients (76.31%) and 150 patients (60.24%), respectively. Again, 34.94% and 44.18% of participants experienced diarrhea and constipation, respectively; however, the AC-docetaxel regimen showed no cases of higher-grade (grade 4) diarrhea or constipation. The majority of the participants (72.29%) experienced fever, while in comparison, 35.74% patients had gastritis, and 53.41% patients had mouth sores. Approximately 55.82%, 53.01%, 59.44%, 78.71%, and 41.37% of participants experienced edema, dry mouth, fatigue, dysgeusia, and skin hyperpigmentation, respectively. Myalgia/arthralgia was reported in a higher proportion of cases (77.51%) compared to peripheral neuropathy (61.04%). Anemia was the most prevalent haematological toxicity related with the AC-T regimen, affecting 61.45% of patients. However, our study did not observe any instances of grade 4 anemia. Among various organ toxicities, hepatotoxicity was the most common (30.92%), followed by bone toxicity (20.48%), lung toxicity (15.66%), nephrotoxicity (6.43%), and cardiotoxicity (5.62%).

Significant differences in toxicity grade between the AC-paclitaxel and AC-docetaxel regimens were observed for several non-haematological toxicities, including vomiting ($p = 0.034$), edema ($p = 0.026$), mouth sores ($p = 0.008$), fatigue ($p < 0.001$), dysgeusia ($p = 0.034$), and myalgia ($p = 0.017$). Thrombocytosis and cardiotoxicity were observed exclusively in patients receiving the AC-paclitaxel regimen, with no events reported in the AC-docetaxel group. As there were no incidents in one group, statistical testing couldn't be done. There were no substantial variations in toxicity grades between the two regimens for any other toxicities ($p > 0.05$).

2.3.6 Death Outcomes of Breast Cancer Patients Receiving AC-T Regimen

Table 7 presents a brief overview of BC patients who passed away between October 2022 and July 2025 following treatment with the AC-T regimen. Among the patients, 27 (10.84%) died within five years of their cancer diagnosis; of these, 15 (55.56%) were diagnosed at stage III and 5 (18.52%) at stage IV. In contrast, 18 patients (7.23%) died more than five years after their diagnosis, of whom 8 (44.44%) were in stage III, while none were in stage IV. A total of 45 (18.07%) patients died who received AC-paclitaxel or AC-docetaxel chemotherapy regimen. As of the last follow-up in July 2025, 204 patients (81.93%) were still alive.

Table 5: Correlation between clinicopathological features and response status of AC-T regimen among Bangladeshi BC patients.

Characteristics		Doxorubicin-cyclophosphamide- paclitaxel (N=218)				Doxorubicin-cyclophosphamide- docetaxel (N=31)			
		Responder (N=117)	Non-responder (N=101)	OR (95% CI)	p value	Responder (N=18)	Non-responder (N=13)	OR (95% CI)	p value
Age (years)	< 45	64	46	1 (reference)	-	11	4	1 (reference)	-
	45-60	46	44	0.75 (0.43-1.31)	0.317	5	8	0.22 (0.05-1.12)	0.069
	> 60	7	11	0.46 (0.16-1.26)	0.133	2	1	0.73 (0.05-10.39)	0.814
BMI (kg/m2)	Normal (18.5-24.9)	72	52	1 (reference)		14	6	1 (reference)	
	Underweight (<18.5)	3	9	0.24 (0.06-0.93)	**0.039**	0	2	0.08 (0.00-2.14)	0.136
	Overweight (25.0-29.9)	39	33	0.85 (0.47-1.53)	0.595	2	4	0.21 (0.03-1.50)	0.121
	Obese (≥30)	3	7	0.31 (0.07-1.25)	0.100	2	1	0.86 (0.06-11.35)	0.906
Tobacco consumption history	No	94	71	1 (reference)	-	15	11	1 (reference)	-
	Yes	23	30	0.57 (0.31-1.08)	0.086	3	2	1.10 (0.15-7.73)	0.923
Contraceptives	No	40	36	1 (reference)	-	6	4	1 (reference)	-
	Yes	77	65	1.06 (0.61-1.86)	0.822	12	9	0.89 (0.19-4.11)	0.880
Tumor Site	Right breast	59	53	1 (reference)	-	12	6	1 (reference)	-
	Left breast	58	48	1.08 (0.64-1.84)	0.762	6	7	0.43 (0.09-1.85)	0.257
Menstruation	Premenopausal	48	37	1 (reference)	-	7	5	1 (reference)	-
	Perimenopausal	32	24	1.03 (0.52-2.03)	0.937	6	4	1.07 (0.19-5.91)	0.936
	Postmenopausal	37	40	0.71 (0.38-1.32)	0.284	5	4	0.89 (0.16-5.11)	0.898
Breastfeeding period	No or < 2 years	17	8	1 (reference)	-	3	1	1 (reference)	-
	≥ 2 years	100	93	0.50 (0.21-1.22)	0.132	15	12	0.42 (0.04-4.53)	0.472
Family history of BC	No	99	94	1 (reference)	-	16	10	1 (reference)	-
	Yes	18	7	2.44 (0.98-6.11)	0.056	2	3	0.42 (0.06-2.94)	0.380

Previous BC treatment	No	97	87	1 (reference)	-	17	9	1 (reference)	-
	Yes	20	14	1.28 (0.61-2.69)	0.512	1	4	0.13 (0.01-1.36)	0.089
Histology	Ductal carcinoma	23	38	1 (reference)	-	3	2	1 (reference)	-
	Invasive ductal carcinoma	61	55	1.83 (0.97-3.45)	0.060	11	7	1.05 (0.14-7.93)	0.964
	Infiltrating ductal carcinoma	51	43	1.96 (1.01-3.78)	**0.045**	13	7	1.24 (0.17-9.25)	0.835
	Metastatic ductal carcinoma	26	18	2.38 (1.08-5.27)	**0.031**	4	5	0.53 (0.05-4.91)	0.579
	Mucinous carcinoma	2	0	8.19 (0.38-178.14)	0.180	2	0	3.57 (0.11-111.71)	0.468
	Mixed carcinoma	0	2	0.33 (0.02-7.12)	0.477	1	0	2.14 (0.06-77.54)	0.677
	Medullary carcinoma	2	0	8.19 (0.38-178.14)	0.180	1	0	2.14 (0.06-77.54)	0.677
	Tubular carcinoma	1	0	4.91 (0.19-125.68)	0.335	0	1	0.23 (0.00-8.61)	0.433
	Invasive lobular	2	3	1.10 (0.17-7.09)	0.919	0	0	0.71 (0.01-49.71)	0.876
Tumor grade	Grade I	21	16	1 (reference)	-	3	1	1 (reference)	-
	Grade II	55	49	0.86 (0.40-1.82)	0.685	8	7	0.38 (0.03-4.54)	0.445
	Grade III	41	36	0.87 (0.39-1.91)	0.724	7	5	0.47 (0.04-4.90)	0.556
Cancer stage	Stage 0	6	2	1 (reference)	-	2	0	1 (reference)	-
	Stage I	15	6	0.83 (0.13-5.35)	0.847	3	2	0.28 (0.00-8.75)	0.468
	Stage II	59	42	0.47 (0.09-2.43)	0.367	9	7	0.25 (0.01-6.11)	0.398
	Stage III	34	44	0.26 (0.05-1.35)	0.109	4	4	0.20 (0.00-5.45)	0.339
	Stage IV	3	7	0.14 (0.12-1.16)	0.068	0	0	0.20 (0.00-28.47)	0.524
Hormone receptor status	ER (-)	46	59	1 (reference)	-	8	14	1 (reference)	-
	ER (+)	57	44	1.66 (0.96-2.88)	0.070	12	9	2.33 (0.68-7.94)	0.175

	PR (-)	49	63	1 (reference)	-	7	13	1 (reference)	-
	PR (+)	56	46	1.57 (0.91-2.68)	0.103	9	6	2.79 (0.70-11.10)	0.146
	HER-2 (-)	71	54	1 (reference)	-	14	7	1 (reference)	-
	HER-2 (+)	33	39	0.64 (0.36-1.15)	0.138	4	3	0.67 (0.12-3.83)	0.649
	HER-2 (equivocal)	12	9	1.01 (0.40-2.58)	0.976	1	3	0.16 (0.01-1.90)	0.14
	Triple (-)	20	35	0.43 (0.23-0.84)	**0.012**	5	3	0.83 (0.15-4.54)	0.833
Chemotherapy types	Neoadjuvant	29	35	1 (reference)	-	4	6	1 (reference)	-
	Adjuvant	88	66	1.61 (0.90-2.89)	0.111	14	7	3.00 (0.63-14.23)	0.166
Chemotherapy cycle	5-8 cycle	103	82	1 (reference)	-	14	9	1 (reference)	-
	> 8 cycle	14	19	0.58 (0.28-1.24)	0.162	4	4	0.64 (0.13-3.24)	0.592

Table 6: Toxicity profile of AC-T chemotherapy regimen among Bangladeshi BC patients.

Toxicity	**Doxorubicin-cyclophosphamide- paclitaxel (N=218) (%)**				**Doxorubicin-cyclophosphamide- docetaxel (N=31) (%)**				**Overall (N=249) (%)**	**Toxicity difference between two regimens: p-value**
	Grade				**Grade**					
	I	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**		
Nausea	83 (38.07)	63 (28.90)	10 (4.58)	5 (2.29)	15 (48.39)	10 (32.26)	2 (6.45)	2 (6.45)	190 (76.31)	0.776
Vomiting	50 (22.94)	45 (20.64)	30 (13.76)	3 (1.38)	2 (6.45)	13 (41.94)	7 (22.58)	0 (0)	150 (60.24)	**0.034**
Diarrhoea	19 (8.72)	28 (12.84)	20 (9.17)	10 (4.59)	5 (16.13)	1 (3.23)	4 (12.90)	0 (0)	87 (34.94)	0.125
Constipation	43 (19.72)	37 (16.97)	12 (5.50)	1 (0.46)	5 (16.13)	7 (22.58)	5 (16.13)	0 (0)	110 (44.18)	0.296
Fever	76 (34.86)	28 (12.84)	38 (17.43)	15 (6.88)	12 (38.71)	8 (25.81)	3 (9.67)	0 (0)	180 (72.29)	0.099
Edema	56 (25.69)	30 (13.76)	28 (12.84)	8 (3.67)	5 (16.13)	10 (32.26)	1 (3.23)	1 (3.23)	139 (55.82)	**0.026**
Dry mouth	51 (23.39)	50 (22.94)	9 (14.86)	2 (0.92)	7 (22.58)	9 (29.03)	4 (12.90)	0 (0)	132 (53.01)	0.354

Mouth sores	65 (29.82)	32 (14.68)	9 (4.13)	4 (1.83)	5 (16.13)	12 (38.71)	5 (16.13)	1 (3.23)	133 (53.41)	**0.008**
Fatigue	48 (22.02)	51 (23.39)	25 (11.47)	7 (3.21)	2 (6.45)	4 (12.90)	4 (12.90)	7 (22.58)	148 (59.44)	**<0.001**
Dysgeusia	43 (19.72)	71 (32.57)	38 (17.43)	17 (7.80)	7 (22.58)	5 (16.13)	8 (25.81)	7 (22.58)	196 (78.71)	**0.034**
Skin hyperpigmentation	43 (19.72)	36 (16.51)	10 (4.59)	5 (2.29)	2 (6.45)	3 (9.68)	2 (6.45)	2 (6.45)	103 (41.37)	0.126
Gastritis	40 (18.35)	26 (11.93)	9 (4.13)	3 (1.38)	4 (12.90)	3 (9.68)	3 (9.68)	1 (3.23)	89 (35.74)	0.275
Peripheral neuropathy	58 (26.61)	46 (21.10)	25 (11.47)	7 (3.21)	4 (12.90)	8 (25.81)	2 (6.45)	2 (6.45)	152 (61.04)	0.292
Myalgia/arthralgia	37 (16.97)	66 (30.28)	48 (22.01)	20 (9.17)	7 (22.58)	3 (9.68)	5 (16.13)	7 (22.58)	193 (77.51)	**0.017**
Anaemia	98 (44.95)	31 (14.22)	9 (4.13)	0 (0)	8 (25.81)	5 (16.13)	2 (6.45)	0 (0)	153 (61.45)	0.530
Leukopenia	17 (7.80)	10 (4.59)	7 (3.21)	0 (0)	2 (6.45)	2 (6.45)	3 (9.68)	0 (0)	41 (16.47)	0.630
Leukocytosis	25 (11.47)	12 (5.50)	2 (0.92)	0 (0)	8 (25.81)	3 (9.68)	0 (0)	0 (0)	50 (20.08)	0.870
Neutropenia	18 (8.26)	22 (10.09)	8 (3.67)	2 (0.92)	2 (6.45)	2 (6.45)	2 (6.45)	0 (0)	56 (22.49)	0.730
Febrile neutropenia	7 (3.21)	17 (7.80)	10 (4.59)	0 (0)	2 (6.45)	1 (3.23)	1 (3.23)	0 (0)	38 (15.26)	0.620
Neutrophilia	17 (7.80)	2 (0.92)	0 (0)	0 (0)	3 (9.68)	0 (0)	0 (0)	0 (0)	22 (8.84)	0.560
Lymphocytosis	25 (11.47)	10 (4.59)	10 (4.59)	0 (0)	2 (6.45)	0 (0)	0 (0)	0 (0)	47 (18.88)	0.670
Lymphocytopenia	10 (4.59)	5 (2.29)	0 (0)	0 (0)	3 (9.68)	2 (6.45)	0 (0)	0 (0)	20 (8.03)	0.790
Thrombocytopenia	7 (3.21)	0 (0)	0 (0)	2 (0.92)	2 (6.45)	2 (6.45)	0 (0)	0 (0)	13 (5.22)	0.128
Thrombocytosis	3 (1.38)	7 (3.21)	5 (2.29)	2 (0.92)	0 (0)	0 (0)	0 (0)	0 (0)	17 (6.82)	-
Hepatotoxicity	31 (14.22)	12 (5.50)	10 (4.59)	15 (6.88)	2 (6.45)	3 (9.68)	2 (6.45)	2 (6.45)	77 (30.92)	0.509
Nephrotoxicity	7 (3.21)	3 (1.38)	4 (1.83)	0 (0)	2 (6.45)	0 (0)	0 (0)	0 (0)	16 (6.43)	0.410
Cardiotoxicity	10 (4.59)	2 (0.92)	2 (0.92)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	14 (5.62)	-
Pulmonary toxicity	10 (4.59)	7 (3.21)	12 (5.50)	3 (1.38)	2 (6.45)	2 (6.45)	0 (0)	3 (9.68)	39 (15.66)	0.069
Bone toxicity	5 (2.29)	10 (4.59)	8 (3.67)	20 (9.17)	2 (6.45)	4 (12.90)	1 (3.23)	1 (3.23)	51 (20.48)	0.199

Here, $p < 0.05$= statistically significant; Nail abnormalities along with total alopecia were developed by all patients of both regimens.

Table 7: Overall mortality rate of Bangladeshi BC patients receiving the AC-T regimen from October 2022 to July 2025.

Time after diagnosis	No. of death (N) (%)				Overall death (N=249) (%)	Cancer stage (%)			
	Doxorubicin-cyclophosphamide- paclitaxel		Doxorubicin-cyclophosphamide- docetaxel						
	Responder (N=117)	Non-responder (N=101)	Responder (N=18)	Non-responder (N=13)		I	II	III	IV
≤ 5 years	3 (2.56)	21 (20.79)	0 (0)	3 (23.08)	27 (10.84)	2 (7.41)	5 (18.52)	15 (55.56)	5 (18.52)
> 5 years	2 (1.71)	13 (12.87)	0 (0)	3 (23.08)	18 (7.23)	4 (22.22)	6 (33.33)	8 (44.44)	0 (0)

2.4 Discussion

The present investigation offers noteworthy perspectives on the sociodemographic characteristics, clinicopathological characteristics, therapeutic outcomes, and toxicological profiles of BC patients in Bangladesh undergoing treatment by doxorubicin, cyclophosphamide, and taxane-based (AC-T) chemotherapy. The findings are especially significant considering Bangladesh is a low-resource country with limited access to advanced treatment modalities, early detection, comprehensive supportive care and patients' misunderstanding regarding therapeutic applications.

In this study, 50.20% of participants were under 45, 41.37% were between 45 and 60, and just 8.43% were above 60. This means that a large number of BC cases happened at a younger age than usual. In Western populations, most cases are found after the age of 50. Other studies from South Asian countries have shown the same results, which suggest that inheritance, surroundings, as well as habits may all have an integral role in the earlier manifestation of BC in this region (Hossain et al., 2014). A substantial number of our patients (51.81%) lacked formal education as well as lived in remote regions (64.26%). This result is in line with what has been claimed in India, where 69.4% of BC patients had low levels of literacy along with 70.20% were from remote regions (Mistry et al., 2024). Only a small number of patients (23.29%) said they had used tobacco before, which is in line with previous research that found that only a tiny number of breast cancer patients engaged in consuming alcohol or cigarettes, or other tobacco-associated habits (Mistry et al., 2024).

Presence of a tumour in the breast site is a contentious parameter, with previous studies indicating a greater vulnerability to cancer in the left breast (Amer, 2014). Our study indicates that the occurrence is very similar for both the left and right breasts. In this research, 38.95% of the participants were premenopausal, while 87.95% had no ancestral occurrence of BC. These results agree with the research carried out by Mistry *et al*. (2024) in India, which revealed that 42.10% of BC women were premenopausal and 86.0% lacked family records of this cancer. The similarity in the research findings suggests that reproductive conditions, as well as ancestral history, may show similar trends in women with BC within this neighbourhood community. The method most frequently used to identify BC was through a biopsy. It was the best way to do a conclusive histological evaluation of both visible and invisible tumours in the breast (Tseng, 2023). The study's emphasis on biopsy underscores its vital function in determining proper treatment options involving surgical strategy, chemotherapy, or targeted therapies,

ensuring that patients get accurate and personalized care. Most of the individuals with BC had either grade 2 or grade 3 tumours, which have been linked to more rapid tumour growth as well as a greater likelihood of metastasis (Feng et al., 2018). This data implies that it's important to work harder to find tumours earlier in time. A substantial amount (60.64%) of participants first displayed cancer-related symptoms by the age of 45, suggesting an earlier initiation of the disease in comparison to the developed world (DeSantis et al., 2015). The high proportion of HER-2-negative tumours in research groups also indicates that BC often has this receptor status, which helps doctors decide how to treat the disease, such as with taxane-based chemotherapy (Garutti et al., 2023; Montero et al., 2012; Verrill et al., 2020). Our study revealed that the majority of the participants (70.28%) received adjuvant chemotherapy. This figure is comparable with the findings that Mistry *et al.* (2024) discovered, which showed that 73.6% of the recipients had adjuvant chemotherapy. This resemblance illustrates the prevalent nature of adjuvant chemotherapy in BC management as well as how important it is for better disease management and outcomes for patients.

In this study, AC-paclitaxel was the best treatment plan since a large number of patients had a full to partial response. On the other hand, the AC-docetaxel regimen had similar rates of partial, stable, as well as disease progressing responses, even though it had fewer complete responses. The clinical evidence showed that the AC-paclitaxel regimen seems to be better at achieving full responses; however, many patients still fail to respond to both therapies (AC-paclitaxel and AC-docetaxel). This demonstrates how important it is to add individualized chemotherapy or make treatment plans more effective to obtain better results, especially for individuals with disease-resistant histories (Masoud and Pagès, 2017).

Adverse events caused by chemotherapy were common among the participants. This was the primary cause for discontinuing the drug, prolonging treatment, changing it, or extending it; all of these instances could make patients' lives more difficult (Gadisa et al., 2020). To optimize chemotherapy outcomes, it is essential to understand the challenges encountered by BC patients. The present research found that the AC-docetaxel and AC-paclitaxel regimens had quite different toxicity profiles. This shows how important it is to adjust treatment plans to each person's tolerance. The toxicity analysis indicated that gastrointestinal (GI) toxicities were common among the participants. This finding is in line with what is known about the side effects of taxane and doxorubicin, which don't often lead to life-threatening circumstances when treated properly (Akbarali et al., 2022). The hematological toxicities found may have been caused by anthracyclines (Al-mahayri, 2020), while the simultaneous use of taxanes did not

make these toxicities worse or better. This result is comparable with a former study by Rašić *et al*. (2019), which indicated that the intensity of hematological toxicities diminished while taxanes were co-administered with anthracyclines. The most common organ toxicities caused by treatment in both the AC-paclitaxel and AC-docetaxel regimens were liver toxicity, bone toxicity, and lung toxicity. This suggests that these treatment methods may have a higher likelihood of harming such organs. This could mean that they are more likely to be harmed by toxic substances that affect several organs without providing an equivalent benefit to therapy (Gadisa et al., 2020; Lee et al., 2017). Earlier studies also found that patients with BC who received paclitaxel and radiation therapy at the same time (Taghian et al., 2001) or docetaxel, another taxane medication (Kim et al., 2012), had damage to their lungs.

Within five years of their BC diagnosis, 10.84% of the patients died, and after that period, 7.23% more died. Overall, 18.07% of individuals who got either the AC-paclitaxel or AC-docetaxel chemotherapy regimen died, but 81.93% remained alive at the time of last follow-up. These findings agree with the study by Vriens et al. (2017), which reported 5-year survival rates of 76% for patients receiving AC-docetaxel and 84% for those receiving AC-paclitaxel. In conclusion, our findings underscore the essential need for rapid identification and accurate implementation of the AC-T chemotherapy regimen, along with meticulous management of treatment-related toxicities, to improve outcomes for BC patients.

2.5 Limitations

The present investigation has a number of limitations. The efficacy of these two regimens concerning overall survival (OS) as well as disease-free survival (DFS), along with the potential for delayed toxicities that may arise long after the completion of chemotherapy, was not revealed. Participants' self-reporting of non-haematological toxicities at two distinct phases may have affected their impression of dangers due to memory prejudice. Furthermore, this investigation did not explicitly consider various modes of treatment, particularly surgical ones, which could have influenced the reported clinical responses. To evaluate the prognostic as well as therapeutic implications of this research, more precise environmental exposure data and larger numbers of samples are necessary.

2.6 Conclusion

In conclusion, this extensive study elucidates the intricate clinicopathological features, therapeutic findings, as well as toxicity profiles of BC patients in Bangladesh, emphasizing the difficulties arising from inadequate medical facilities along with the necessity for individualized treatment strategies. The effectiveness of doxorubicin, cyclophosphamide, and taxane-based chemotherapy has been established, with most patients achieving positive outcomes; however, considerable variation in toxicity and therapeutic response underscores the need for personalized care. To improve early diagnosis, survival rates, and overall patient outcomes in communities with limited resources, it is important to address differences in education, access to healthcare in rural areas, and early detection. It is also important to reduce the adverse effects of chemotherapy by providing better supportive care and personalized treatment plans.

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Chapter-03
Pharmacogenomics Study

3.1 Introduction

In South Asian countries, including Bangladesh, breast cancer (BC) has become an increasing concern and it is getting worse because people lack knowledge about it (Alam et al., 2012; Begum et al., 2019). Finding the factors that trigger women at risk for BC is an important part of screening them for the illness early on; that, in turn, provides them a better chance of responding well to treatment. There are substantially greater incidences of BC in industrialized nations because of factors including early menstruation, delayed childbirth, childlessness, being overweight, alcohol consumption, lack of exercise, or not breastfeeding (Alam et al., 2024). One of the most significant things to think about when figuring out how likely someone is to get cancer is the genetic distribution of the proteins that turn on or off their activity in humans (Nabil et al., 2024). Mutations in genes are responsible for about 8-10% of BC cases. These alterations can make the disease more aggressive (Klassen et al., 2022). The *BRCA1* as well as *BRCA2* genes are the most substantial components of this genetic system. Mutations in such genes greatly raise the risk of BC (Lee, 2020). Genome-wide association studies (GWAS) have acknowledged several genes as well as single-nucleotide polymorphisms (SNPs) that contribute to the disease's development and progression (Aziz et al., 2022). These genetic variables show that most inherited BC cases follow an autosomal dominant inheritance pattern, and that there is a complex interaction between high-penetrance genes and many moderate-to-low penetrance genes across distinct populations (Harbeck et al., 2019; Taylor et al., 2018). The link between SNPs in different genes and the likelihood of BC clearly shows that they represent the main triggers for the disease (Shabnaz et al., 2016).

Chemotherapy regimens that contain anthracyclines are widely used. Cyclophosphamide acts as a DNA-alkylating agent that affects the effectiveness as well as safety of the combination chemotherapy (Pinto, 2009). Paclitaxel as well as docetaxel are cytotoxic taxanes that work against microtubules and are useful in treating BC patients (Lyseng-Williamson and Fenton, 2005; Perez, 1998). Doxorubicin-cyclophosphamide (AC) and Doxorubicin-cyclophosphamide-paclitaxel (AC-T) are typical chemotherapy treatments for BC employed all over the world (Tesfaye et al., 2023). Although AC and AC-T chemotherapy are essential for BC treatment, they are frequently associated with significant toxicities. Standard doses can produce highly variable adverse effects among patients, partly due to genetic and racial differences (Gadisa et al., 2020).

Genetic variations in drug transporters as well as enzymes that break down drugs, can cause differences in how patients respond to chemotherapy and how toxic it is. The hepatic uptake transporter OATP1B3, encoded by *SLCO1B3*, regulates the disposition of several anticancer agents, including doxorubicin, paclitaxel, and docetaxel. Transporter deficit decreases hepatic uptake and increases systemic exposure (Lee et al., 2017; Smith et al., 2007; Iusuf et al., 2015). In cancers like BC, OATP1B3 is often overexpressed, which may help drugs accumulate within the tumour (Schulte & Ho, 2019). Higher levels of *SLCO1B3* have been related to decreased development as well as progression of malignant cells, which underscores its possibility as a predictive indicator (Tang et al., 2021). But prior research has demonstrated different things regarding its impact on BC (Muto et al., 2007; Kindla et al., 2011). The protein *SLCO1B3* appears on the cell's outer layer and within the cytoplasm of both healthy and malignant tissues in the breast. 80% of BC tissue specimens reveal positive expression, while 24% of these specimens exhibit high positivity (Tang et al., 2021). The rs4149117 missense mutation has been shown to change the function of the transporter, and both paclitaxel and docetaxel have been demonstrated as potential substrates of OATP1B3. However, clinical trials have not shown any significant relationship between rs4149117 and the way paclitaxel works in the body (Smith et al., 2007). Preclinical models showed how important it is since OATP1B3 knockout animals had higher levels of paclitaxel along with lower levels of hepatic absorption (van de Steeg et al., 2011), and suppression of OATP1B3 expression under laboratory conditions makes cells resistant to paclitaxel (Takano et al., 2009). Even if these results are true, clinical trials have always failed to prove that OATP1B3 polymorphisms have any effect on how paclitaxel works (Bergmann et al., 2011; Smith et al., 2007) or how well it works (Leskelä et al., 2011; Bergmann et al., 2012; Bergmann & Gréen, 2011). The therapeutic significance of *SLCO1B3* rs4149117 in influencing drug transport, chemotherapy-related adverse effects, and therapeutic responses in women with BC undergoing the AC-T regimen remains ambiguous.

The ATP-binding cassette (ABC) superfamily is essential for transporting and eliminating medicines and xenobiotics from the body. The *ABCB1* gene, also referred to as MDR1, encodes P-glycoprotein (P-gp), a protein located in the cell membrane that functions as an energy-driven efflux pump for various chemotherapy agents commonly used to treat metastatic BC (Clarke et al., 2005). P-gp is found in large amounts in the intestinal tract, liver, kidney, and blood-brain barrier. Its polarised positioning in the plasma membrane makes it easier for medications and other foreign substances to pass in one direction (Xiao et al., 2020). The *ABCB1* gene has more than 50 single-nucleotide polymorphisms (SNPs) as well as some insertion or deletion variants. A number of these affect how P-gp works as well as how much it is expressed (Salama et al.,

2006; Fromm, 2002). SNPs in *ABCB1* may alter P-gp function, allowing antineoplastic drugs to exit cancer cells or the body. Such changes could lower plasma drug levels and make treatments less effective. ABCB1 SNPs are significantly linked to variations in treatment effectiveness and survival in BC individuals undergoing anthracycline- and taxane-based therapies, including the AC-T regimen (Chang et al., 2009).

2677G>T/A (exon 21, rs2032582) is one of the most researched variants. It is a triallelic SNP where the wild-type guanine (G) is replaced with either adenine (A) or thymine (T). This variant is found within the P-glycoprotein (P-gp) subsequent to transmembrane region 10, resulting in an alteration of the amino acids located at codon 893 from alanine to serine or threonine (Ala893Ser/Thr; ABCB1∗10) (Tulsyan et al., 2016). The 2677G>T/A polymorphism has been linked to the efficacy of paclitaxel monotherapy in patients (Tanabe et al., 2001) and to the resistance of metastatic BC to both paclitaxel and anthracyclines (Chang et al., 2009). In Taiwanese patients who received anthracycline-containing regimens, it was also linked to a higher risk of fever as well as febrile neutropenia (Tsai et al., 2009). However, in North American patients who received identical treatment, there was no link between it and haematological or gastrointestinal toxicities (Yao et al., 2014). This variant has also been linked to swift tumor growth along with a lower overall survival rate in patients receiving doxorubicin and cyclophosphamide (Bray et al., 2010).

The 3435C>T (exon 26, rs1045642) SNP serves as a synonymous variant that possesses no effect on the encoded amino acid isoleucine. Its prevalence of alleles has considerable ethnic heterogeneity, highlighting distinctions across Asian, African, and Caucasian groups (Balram et al., 2003; Tang et al., 2002). Within Asia, the distribution in Chinese and Malay populations resembles that of Caucasians, whereas Indian populations show distinct patterns, with Thai-Indians having a variant allele frequency of approximately 33% (Sensorn et al., 2013). The 3435C>T has been shown to influence P-gp expression and function via altered mRNA stability or translational efficiency (Hoffmeyer et al., 2000), with functional studies reporting both increased and decreased protein levels (Illmer et al., 2002; Kim et al., 2001; Nakamura et al., 2002; Brinkmann et al., 2001; Siegsmund et al., 2002; Wang et al., 2005; Hitzl et al., 2001). Clinically, 3435C>T has been implicated in drug detoxification and outcomes among Chinese Han patients receiving anthracycline-based neoadjuvant chemotherapy (Ji et al., 2012), and has been connected with variability in response to anthracycline- and taxane-based chemotherapy in locally advanced BC (Kafka et al., 2003). It was also correlated with overall outcomes and clinical toxicity in patients receiving docetaxel (Tran et al., 2006), as well as longer overall

survival in individuals receiving docetaxel and doxorubicin neoadjuvant chemotherapy (Kim et al., 2015).

Another synonymous SNP, 1236C>T (exon 12, rs1128503), is located within transmembrane domain 6 (TM6), critical for substrate binding. It does not alter the amino acid located at position 412 (Gly), but it may alter how proteins fold as well as how stable they are in their shape, especially when it occurs with 3435C>T (Kimchi-Sarfaty et al., 2007; Hung et al., 2008). No significant link was seen between the 1236C>T variant and the extent to which Indian patients responded to anthracycline-based neoadjuvant chemotherapy, though hematological toxicity was significantly correlated (Chaturvedi et al., 2013). Conversely, Wu *et al*. (2012) observed a significant association with chemotherapy response in Chinese patients. This variant has also been linked to altered docetaxel clearance (Bosch et al., 2006; Green et al., 2006).

The 129T>C (rs3213619) variant has been noticed within the proximal promoter region (exon 1b) of the MDR1 (*ABCB1*) gene, which is a low-frequency polymorphism (Horinouchi et al., 2002). The frequency of the genotype of the 129T>C variant was comparable between Japanese and Caucasian populations (Hoffmeyer et al., 2000). In 101 BC individuals who underwent neoadjuvant chemotherapy (NACT) with doxorubicin and docetaxel, there appeared a lack of substantial correlation between the *ABCB1* 129T>C variant and how well the treatment worked (Lévy et al., 2013). Conversely, more extensive replication research with 1,303 European patients revealed a strong correlation between rs3213619 variant and taxane-triggered sensory nerve damage (Abraham et al., 2014). Collectively, the *ABCB1* 2677G>T/A, 3435C>T, 1236C>T, and 129T>C genetic variants may function as possible indicators of treatment efficacy, toxic effects, and overall outcomes among individuals receiving AC-T–based chemotherapy.

ABCC2 is present in many tumor types, and excessive levels of it have been related to the emergence of resistance to different therapies (Han et al., 2011). *ABCC2* has a significant impact on the pharmacokinetics as well as the therapeutic effectiveness of different chemotherapy drugs, such as paclitaxel and docetaxel, by controlling how drugs are disposed of and eliminated (Vlaming et al., 2011; Xiao et al., 2020). A functional mutation in this gene, 3563T>A (V1188E; rs17222723), has been identified to alter the activity of the transporter, which may explain why people respond differently to drugs (Cui et al., 1999; Pratt et al., 2015). Moreover, BC treatment outcomes have been linked with a number of additional *ABCC2* variants, such as rs3740065 and rs8187710 (Xiao et al., 2020). The genotype of *ABCC2* (58626AA) is linked to a decreased

response to chemotherapy as well as a lower likelihood of survival in BC individuals who received the AC-T regimen (Mistry et al., 2025). Overall, the findings suggest that *ABCC2* genetic variants, particularly rs17222723, significantly impact responses to AC-T chemotherapy and the associated toxicities. More research is needed in South Asian communities.

The cytochrome P450 (CYP) superfamily of enzymes is crucial for phase I xenobiotic metabolic processes. They help to oxidize an extensive number of substances that come from inside and outside the body, and they also help to metabolize about 90% of the medications that physicians prescribe (Seredina et al., 2012). *CYP2C8* is one of these enzymes that is very crucial for metabolizing chemotherapy drugs like cyclophosphamide and paclitaxel. The AC-T regimen (doxorubicin and cyclophosphamide, then paclitaxel) includes these drugs as key elements (Zhang et al., 2006; van Schaik, 2005). The *CYP2C8*3* variant, which is common in Caucasian people, causes mutations that are non-synonymous and can lower the activity of enzymes (Robert et al., 2005). Functional investigations have indicated that this polymorphic allele is linked to lower activity of paclitaxel 6α-hydroxylase in cell lines from humans as well as liver microsomes, respectively (Marsh et al., 2007). SNPs found in the *CYP2C8* gene, such as the *3 (rs11572080 R139K) variant, reduced the efficacy of paclitaxel in its metabolism, resulting in elevated drug storage in the body (Rowbotham et al., 2010; Bergmann et al., 2011). The *CYP2C8* rs11572080 missense variant appears to be associated with a modified function of the enzyme, occasionally resulting in enhanced paclitaxel breakdown (Gréen et al., 2009). Clinical investigations revealed that patients carrying the *CYP2C8*3* allele, particularly homozygotes, demonstrated an increased susceptibility to paclitaxel-induced neuropathy (Leskelä et al., 2011), but certain data suggest that these individuals may exhibit a more favorable response to paclitaxel therapy (Hertz et al., 2013). The overall data indicate that *CYP2C8*3* variants may affect drug metabolism and adverse effects, along with overall therapeutic outcomes in breast carcinoma patients undergoing the AC-T regimen.

The study aimed to determine how the genetic variants *SLCO1B3* (rs4149117), *ABCB1* (rs1128503, rs2032582, rs3213619, rs1045642), *ABCC2* (rs17222723), and *CYP2C8* (rs11572080) affect toxicity as well as clinical outcomes in Bangladeshi BC patients receiving the AC-T regimen. This would help to move personalized medicine forward in the treatment of BC in Bangladesh.

3.2 Methods

3.2.1 Type of Study

In the current prospective study, pharmacogenomic techniques were used to investigate how gene polymorphisms influenced the toxicity and clinical outcomes of Bangladeshi women with BC who underwent doxorubicin, cyclophosphamide, and taxane-based (AC-T) chemotherapy.

3.2.2 Sample Size Calculation

Sample sizes for case-control studies were calculated using a priori estimation with G*Power. *SLCO1B3* rs4149117 was chosen as the model SNP because of its proven function as a drug transporter. Based on pilot estimates and previously reported allele frequencies in Asian populations, we assumed a dominant genetic model, with an expected genotype difference of approximately 19% in cases and 31% in controls. Using a two-sided alpha of 0.05, 80 percent power, and equal allocation, the required sample size was 204 participants per group (408 in total). Our study included 249 patients with BC in the case group and 251 healthy individuals in the control group (total of 500), exceeding the required sample size. For the cohort analysis, the primary endpoint chosen for planning was grade III to IV paclitaxel-induced peripheral neuropathy. Published data indicate that severe neuropathy occurs in about 20 percent of paclitaxel-treated patients (Seretny et al., 2014; Andreas et al., 2012). To justify an effect size that would be clinically meaningful, we considered prior reports showing higher toxicity among carriers of reduced-function *CYP2C8* variants and assumed an increase to 38 percent in carriers of the *CYP2C8* rs11572080 variant. Using a two-sided alpha of 0.05 and 80 percent power, the required cohort size was approximately 198 patients. These a priori estimates guided the recruitment targets for both components of the study. Our AC-paclitaxel cohort included 218 patients, exceeding the priori target.

3.2.3 Protocol for Genotyping Assessment

The genotyping analysis was done at the Laboratory of Molecular Biology and Pharmacogenomics, Department of Pharmacy, Noakhali Science and Technology University, Bangladesh. **Fig. 6** shows an outline of the whole genotyping approach.

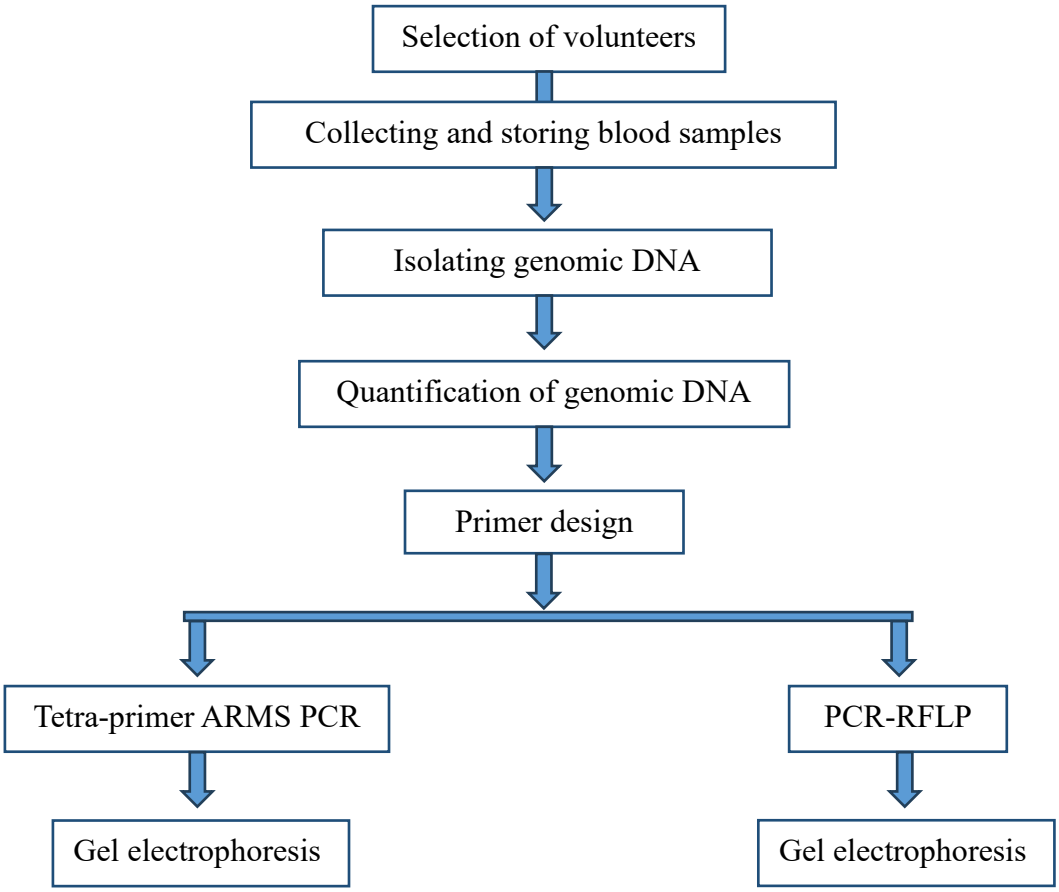


Fig. 6: Brief flow chart of the genotyping method.

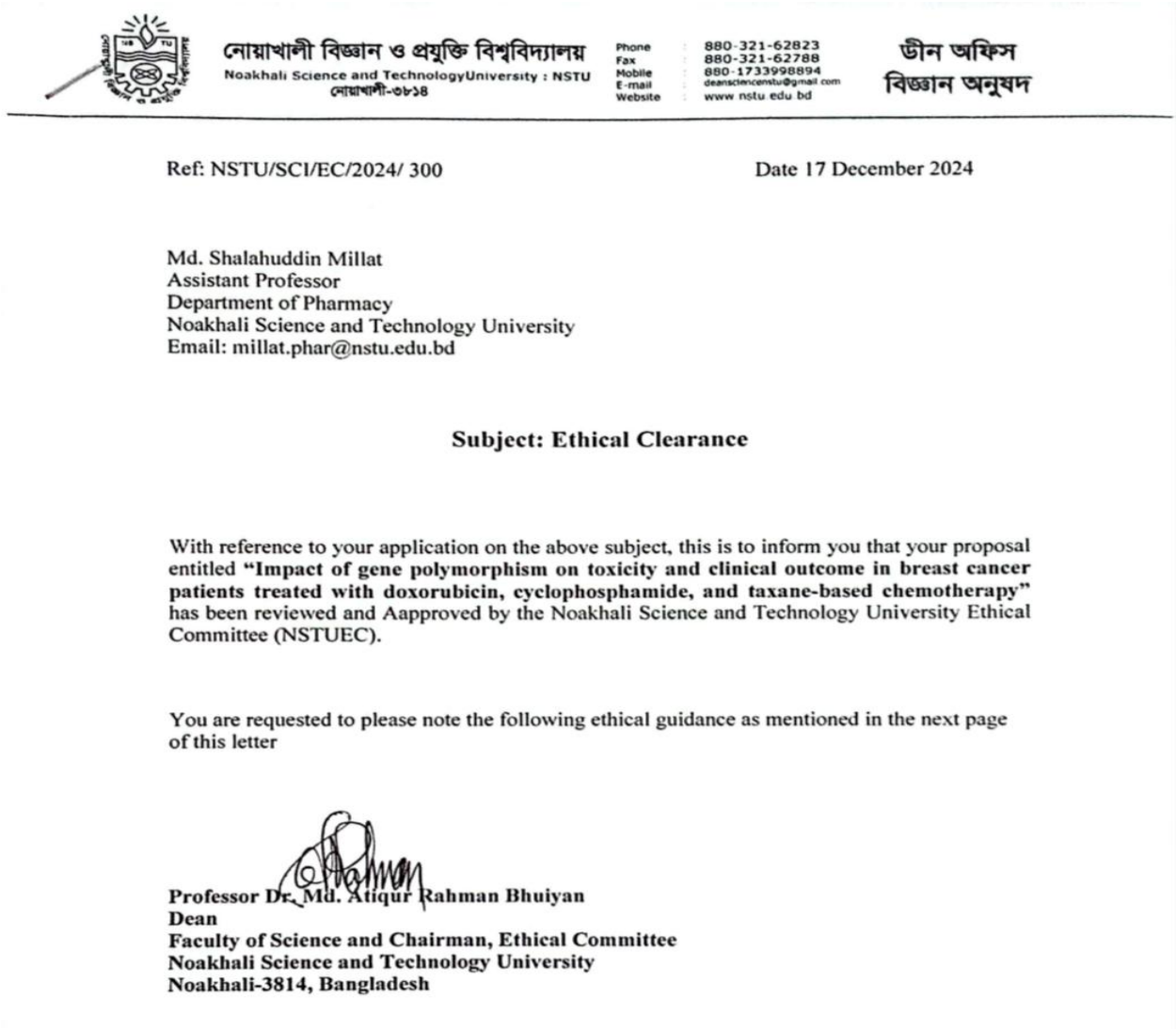
3.2.3.1 Volunteers' Selection

This pharmacogenomics study was performed on those 249 women with BC who participated in the previous observational study. In addition, 251 healthy women with no history of chronic diseases were enrolled as controls. The control volunteers were chosen from various regions of Bangladesh and were identical in age and BMI to the BC patients. The socioeconomic backgrounds of the controls as well as the cases were very similar. Most of the people in both groups came from families with low incomes. This study did not include pregnant women, younger than 18, or incapable of giving the required information in the control group. All phases of this investigation adhered to the Helsinki Principles as well as the fundamentals of effective clinical practice (World Medical Association, 2013).

3.2.3.2 Ethical Clearance

The ethics board of Noakhali Science and Technology University provided ethical consent to conduct this pharmacogenomics research (Ref: NSTU/SCI/EC/2024/300) (**Fig. 7**). We carried out this pharmacogenomics study in the Laboratory of Pharmacogenomics and Molecular

Biology, Department of Pharmacy, Noakhali Science and Technology University, Noakhali-3814, Bangladesh.



নোয়াখালী বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়
Noakhali Science and TechnologyUniversity : NSTU
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ডীন অফিস
বিজ্ঞান অনুষদ

Ref: NSTU/SCI/EC/2024/ 300 Date 17 December 2024

Md. Shalahuddin Millat
Assistant Professor
Department of Pharmacy
Noakhali Science and Technology University
Email: millat.phar@nstu.edu.bd

Subject: Ethical Clearance

With reference to your application on the above subject, this is to inform you that your proposal entitled **"Impact of gene polymorphism on toxicity and clinical outcome in breast cancer patients treated with doxorubicin, cyclophosphamide, and taxane-based chemotherapy"** has been reviewed and Aapproved by the Noakhali Science and Technology University Ethical Committee (NSTUEC).

You are requested to please note the following ethical guidance as mentioned in the next page of this letter

Professor Dr. Md. Atiqur Rahman Bhuiyan
Dean
Faculty of Science and Chairman, Ethical Committee
Noakhali Science and Technology University
Noakhali-3814, Bangladesh

Fig. 7: Ethical approval from the ethics board of Noakhali Science and Technology University.

3.2.3.3 Collection and Storage of Blood Sample

Every participant of both groups had almost 3 mL of blood drawn using a sterile plastic syringe. The blood was then put into tubes holding EDTA to keep it from clotting and refrigerated at −80°C until the DNA could be extracted.

3.2.3.4 Isolation of Genomic DNA

Genomic DNA was extracted from whole blood samples using the FavorPrep™ Blood Genomic DNA Extraction Mini Kit following the manufacturer's protocol with slight modifications. The procedure was carried out as follows:

1. Approximately 200 µL of whole blood was transferred into a sterile microcentrifuge tube.

2. 20 µL of Proteinase K and 200 µL of FABG buffer were added to the sample, and the mixture was thoroughly vortexed to ensure proper mixing. (Proteinase K was added directly to the sample and not to the FABG buffer alone.)

3. The mixture was incubated at 60 °C for 15 minutes to facilitate cell lysis and protein digestion. During incubation, the sample was briefly vortexed every 3-5 minutes.

4. The tube was briefly centrifuged for 30 seconds to remove any liquid droplets from the tube lid.

5. 200 µL of absolute ethanol (100%) was added, and the sample was mixed thoroughly by vortexing for approximately 10 seconds.

6. The tube was centrifuged again for 30 seconds to collect any remaining droplets.

7. The lysate was then transferred to an FABG mini column placed in a collection tube, ensuring that any precipitate was also transferred. The column was centrifuged at 6000 × g for 1 minute, and the filtrate was discarded. The column was subsequently placed in a new collection tube.

8. 400 µL of W1 buffer was added to the column, followed by centrifugation at maximum speed for 30 seconds. The flow-through was discarded. (Ethanol had been added to the W1 buffer prior to its use as instructed by the manufacturer.)

9. Subsequently, 750 µL of wash buffer was added to the column and centrifuged at maximum speed for 30 seconds, after which the flow-through was discarded. (Ethanol had also been added to the wash buffer before use.)

10. The column was then centrifuged at maximum speed for an additional 3 minutes to ensure complete drying of the membrane.

11. The FABG mini column was transferred into a clean elution tube.

12. 60 µL of pre-warmed elution buffer was carefully applied to the center of the membrane and allowed to stand for 3 minutes.

13. The column was centrifuged at maximum speed for 1 minute to elute the purified genomic DNA.

14. The extracted DNA samples were finally stored at 4 °C for short-term use or at −20 °C for long-term preservation.

3.2.3.5 Quantification of Genomic DNA

We used a Berthold Colibri microvolume spectrophotometer (Model LB 915) to determine the concentration of the isolated DNA. Two microliters (µL) of DNA were put on the spectrophotometer for each measurement, and the concentration was recorded in micrograms per milliliter (µg/mL). The absorbance ratio at 260 nm and 280 nm was used to determine how pure the DNA was. For pure DNA, a proportion of approximately 1.8 was preferable. If the proportion is very different, it could mean that RNA, proteins, or other things have gotten into the DNA.

3.2.3.6 Primer Design

For tetra-primer ARMS-PCR primer design, the web-based tool Primer1 (http://primer1.soton.ac.uk/primer1.html) was utilized, and for PCR-RFLP primer sequence design, Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was utilized. There are some guidelines for primer design:

- **Primer Length**: PCR primers ought to possess a length of 15 to 30 nucleotides.
- **GC Content**: An optimal GC content of 40-60% is recommended, with an even distribution of G and C bases throughout the primer sequence.
- **3'-End Stability**: To minimize the chance of nonspecific binding, avoid positioning in excess of three consecutive G or C bases at the 3'-end.
- **Template Complementarity**: To prevent off-target amplification, make sure that the primers and the template DNA don't complement each other in ways that you don't want them to.
- **Melting Temperature (Tm)**: The melting temperatures of the forward and reverse primers should be similar, ideally within 5 °C of each other.

Considering all the key factors for primer design, specific primers were developed for the study. For the SLCO1B3 rs4149117 variant, a tetra-primer ARMS-PCR approach was used, involving four primers such as forward inner, reverse inner, forward outer, and reverse outer. For the remaining SNPs examined in the study, a conventional two-primer design was utilized, comprising a forward primer and a reverse primer. **Table 8** shows the primer sequences along with GC content for all the SNPs that were examined in this study.

Table 8: Primer sequences along with GC content for all studied SNPs.

Variant	Primer Sequence (5'-3')	% GC
rs4149117 (SLCO1B3)	FI: TCCTTATGGGAACTGGAAGTATTTTGATAG	37.0
	RI: ACTATCCCATGAAGAAATGTGGTAAGGA	39.0
	FO: TTGGGGCATTCAGTTCTACTAGATACAA	39.0
	RO: TCAAAAGGTAACTGCCCACTTACAAGTA	39.0
rs1128503 (ABCB1)	FP: CACTCTGCACCTTCAGGTTC	55.0
	RP: TCTATTGAATGAAGAGTTTCTG	32.0
rs2032582 (ABCB1)	FP: TTTAGTTTGACTCACCTTCCC	43.0
	RP: TATCCTTCATCTATGGTTGGC	43.0
rs3213619 (ABCB1)	FP: TTTGCGTGCCCCTACCTC	50.0
	RP: TCAGCATTCAGTCAATCCGG	61.0
rs1045642 (ABCB1)	FP: ACATTAGGCAGTGACTCGATGAAGGCA	48.0
	RP: TGCTGGTCCTGAAGTTGATCTGTGAAC	48.0
rs17222723 (ABCC2)	FP: GTAAGCTGTGCCCATCAAGG	55.0
	RP: CCTCCCACCGCTAATATCAA	50.0
rs11572080 (CYP2C8)	FP: CAGGATGCGCAATGAAGA	45.0
	RP: AGGCAATTCCCCAATATCTC	50.0

3.2.3.7 Tetra-Primer ARMS-PCR

The Tetra-Primer ARMS-PCR approach was used to amplify the *SLCO1B3* rs4149117 variant. Gradient PCR was conducted within a temperature range of 54°C to 65°C, utilizing different amounts of primers as well as magnesium chloride to enhance the amplification settings. For this SNP, an annealing temperature of 60°C was chosen following the melting temperatures of the primers as well as the amplification effectiveness. Premix Taq™ Master Mix and specially designed primers were used to create the PCR reaction mixture for the Tetra-Primer ARMS-PCR approach. Each PCR tube contained 10 µL of the working mixture, and then 1 µL of genomic DNA was incorporated, raising the total reaction volume to 11 µL. **Table 9** presents the composition of the working mix for the Tetra-Primer ARMS PCR. After the tetra-primer ARMS-PCR was completed, the products that were amplified were placed on a 2% agarose gel that was stained with ethidium bromide (EtBr) and then seen under ultraviolet (UV) illumination. Three separate bands were seen at 154 bp, 206 bp, and 302 bp. This data shows that the target alleles were effectively and precisely amplified. **Table 10** shows the conditions

used to amplify rs4149117, along with the lengths of the resultant PCR products for each genotype under this SNP.

Table 9: Composition of the working mix for the Tetra-Primer ARMS-PCR approach.

Name of the reagent	Quantity for 16 (sixteen) samples (µl)
Premix Taq ™ Master Mix	80
Forward Inner (FI)	3.0
Reverse Inner (RI)	3.0
Forward Outer (FO)	2.5
Reverse Outer (RO)	2.5
Nuclease Free Water	69
Total	160
PCR Run	10 µl working mix + 1 µl DNA (per sample)

Table 10: PCR conditions and the lengths of the resulting PCR products for the rs4149117 allele.

SNP	PCR conditions	No. of PCR cycles	Polymorphism	Size of PCR products (bp)
rs4149117 (*SLCO1B3*)	95°C for 5 minutes 95°C for 30 seconds 60°C for 45 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35 cycles	G > T	NH: GG: 154, 302 HE: GT: 154, 206, 302 MH: TT: 206, 302

Here, NH: Normal Homozygote; HE: Heterozygote; MH: Mutant Homozygote

3.2.3.8 PCR-RFLP

PCR-based RFLP analysis was employed to genotype the following alleles: rs1128503 (*ABCB1*), rs2032582 (*ABCB1*), rs3213619 (*ABCB1*), rs1045642 (*ABCB1*), rs17222723 (*ABCC2*), and rs11572080 (*CYP2C8*). We made a PCR reaction mixture by combining the previously designed primers with Premix Taq™ Master Mix. The final volume of each reaction was 21 µL, which was made up of 20 µL of the working mix as well as 1 µL of genomic DNA. The composition of the working mix for PCR is summarized in **Table 11**. After PCR amplification, 10 µL of each PCR product was put on the gel to evaluate the size of the products as well as to verify that the target genomic area was amplified correctly. We applied 1.5% (w/v)

agarose gel electrophoresis to see the PCR amplification products. For RFLP analysis, 5 µL of each PCR product was combined with 5 µL of the RFLP working solution and incubated for the recommended time under appropriate conditions. **Table 12** summarizes the RFLP working mix. A 2% (w/v) agarose gel that was stained with ethidium bromide (EtBr) was used to see the RFLP patterns of the studied SNPs after they had been digested. A DNA ladder with 100 base pairs was employed to correctly measure the sizes of the restriction fragments to confirm that the genotyping was accurate. Detailed information on PCR conditions, amplicon sizes, restriction enzymes used, digestion conditions, and the expected fragment sizes for each genotype of the studied SNPs is provided in **Table 13**.

Table 11: Composition of the working mix for PCR.

Name of the reagent	**Quantity for 16 (sixteen) samples (µl)**
Premix Taq™ Master Mix	160
Forward Primer (FP)	3.5
Reverse Primer (RP)	3.5
Nuclease Free Water	153
Total	320
PCR Run	20µl working mix + 1µl DNA (per sample)

Table 12: Composition of the working mix for RFLP.

Name of the reagent	**Quantity for 16 (sixteen) samples (µl)**
Restriction Enzyme	3
CutSmart® Buffer	8
Nuclease Free Water	69
Total	80
Digestion	5 µl PCR products + 5 µl RFLP mix (per sample)

Table 13: PCR conditions, amplicon sizes, restriction enzymes, digestion conditions, and expected fragment sizes for each genotype of the studied SNPs.

SNP	PCR conditions	No. of PCR cycle	Size of PCR products (bp)	Restriction enzyme (RE)	Digestion condition	Polymorphism	Digested fragments (bp)
rs1128503	95°C for 5 minutes 95°C for 30 seconds 56°C for 30 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	174	HaeIII	Incubation at 37°C for 6 hours	A > G	AA: 174 AG: 23, 151, 174 GG: 23, 151
rs2032582	95°C for 5 minutes 95°C for 30 seconds 54°C for 45 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	155	BsrI	Incubation at 37°C for overnight	A > T	AA: 155 AT: 20, 135, 155 TT: 20, 135
rs3213619	95°C for 5 minutes 95°C for 30 seconds 56°C for 30 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	273	MspA1I	Incubation at 37°C for overnight	A > G	AA: 103, 170 AG: 32, 103, 138, 170 GG: 32, 103, 138
rs1045642	95°C for 5 minutes 95°C for 30 seconds						

	63°C for 30 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	248	MboI	Incubation at 37°C for overnight	A > G	AA: 20, 228 AG: 20, 56, 172, 192 GG: 20, 56, 172
rs17222723	95°C for 5 minutes 95°C for 30 seconds 55°C for 30 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	383	BseRI	Incubation at 37°C for overnight	T > A	TT: 383 TA: 113, 270, 383 AA: 113, 270
rs11572080	95°C for 5 minutes 95°C for 30 seconds 51°C for 30 seconds 72°C for 30 seconds 72°C for 10 minutes 4°C for ∞	35	467	BseRI	Incubation at 37°C for overnight	C > T	CC: 46, 113, 308 CT: 46, 113, 308, 354 TT: 113, 354

3.2.3.9 Agarose Gel Electrophoresis

1X TAE buffer was used to prepare and run all the agarose gels, which were prepared by diluting a 10X stock solution.

The stages followed in the procedure are:

I. To make a 1% (w/v) agarose gel, put 1 gram of dried agarose in a conical flask with 100 ml of 1X TAE buffer. The mixture was then placed in the microwave and boiled until it was clear.
II. The resultant mixture was cooled to nearly 60°C, and then 5 µl of ethidium bromide (EtBr) solution was transferred to the mixture from the EtBr stock solution (10 mg/ml) to make it easier to recognize DNA under UV light.
III. Then, the semi-liquid gel was transferred onto a gel molding tray as well as given time to cool and harden. A comb was put within the tray to create wells in the gel so that samples could be loaded.
IV. After the gels had solidified, the samples, along with the molecular ladder, were put into the wells of the gel. The gel was then inserted into an electrophoresis device filled with 1X TAE buffer solution.
V. After the gel was placed in the chamber, the lid was carefully positioned on the device, and the electric cables were connected, as well as the power supply, which was set to 140 volts for about 45 minutes. The electric field pushes the DNA into the positive (red) electrode from the negative (black) electrode throughout the run.
VI. The gel was displayed on a transilluminator with UV light after the run was over so that the DNA could be visible. A picture was captured and stored for further analysis.

3.2.3.10 Statistical Analysis

The statistics program IBM SPSS (version 26.0; IBM Corporation, Armonk, NY, USA) was utilized to gather data and run statistical tests. Chi-square (χ^2) tests were employed to examine deviations from Hardy-Weinberg equilibrium (HWE) (Khanom et al., 2024). Odds ratios (ORs) along with 95% confidence intervals (CIs) were computed employing MedCalc (version 19.0.7) to assess the relationships between genetic polymorphisms and BC susceptibility, their correlation with treatment response, and their link to chemotherapy-triggered toxicities (Khanom et al., 2024; Islam et al., 2015; Bushra et al., 2020). The acceptable level for statistical significance was $p < 0.05$.

3.3 Materials

Table 14: A summary of the instruments utilized during this investigation.

Sl. No.	Name	Source	Country of origin
1.	SimpliAmp Thermal Cycler (PCR)	Applied Biosystems	USA
2.	SimpliAmp Thermal Cycler (PCR)	Applied Biosystems	Taiwan
3.	Colibri microvolume spectrophotometer (Model LB 915)	Berthold Technologies	Germany
4.	Laminar Air Flow Cabinet	Esco Technologies, Inc.	USA
5.	Micro Centrifuge	Hermle Labortechnik GmbH	Germany
6.	Mini Centrifuge	Hawach Scientific	China
7.	Binder GmbH	Asia Pacific	Germany
8.	Noka Microwave Oven	Electro Mart Ltd.	Bangladesh
9.	multiSUB Midi, Midi Horizontal Electrophoresis System	Cleaver Scientific	UK
10.	Gel Documentation Machine	Cleaver Scientific	UK
11.	Vortex Mixer	Digisystem	Taiwan
12.	Illuminator (UV)	Alfa Innotech	UK
13.	Electronic Balance	Ohaus	USA
14.	Water Purification System	Lab Tech	UK
15.	Freeze (−80 ºC)	Wise Cryo	UK
16.	Freeze (−16 to -32 ºC)	Siemens	USA
17.	Refrigerator (2 to 8 ºC)	Walton	Bangladesh

Table 15: Summary of supportive accessories employed in this research.

Sl. No.	Name	Source	Country of origin
1.	Micropipettes (100-1000; 20-200; 10-100; 0.5-10 µl)	Precolor	Germany
2.	Microtips (10, 200, 1000) µl	Forlabo	Russia
3.	PCR Tube	Forlabo	Russia
4.	Eppendorf Tube	Forlabo	Russia
5.	Disposable Syringe (3 ml)	JMI Healthcare	Bangladesh
6.	One-time bandages	JMI Healthcare	Bangladesh
7.	Alcohol pad	JMI Healthcare	Bangladesh
8.	Measuring cylinder (50,100 & 1000 ml)	Bio Basic	Canada
9.	Conical flask	Bio Basic	Canada
11.	DNA tube	Forlabo	Russia
13.	EDTA Tube	InterVacTechnology	Estonia

Table 16: Reagents and Chemicals.

SI. No	Name of items	Quantity
1.	FavorPrep ™ DNA Extraction Mini Kit	500 unit (5 Boxes)
2.	100% Ethanol	3 Lit.
3.	Isopropanol	500 ml
4.	Premix Taq TM Master Mix	6 packs
5.	Tris	500 gm
6.	EDTA	250 gm
7.	Agarose	500 gm
8.	Ethidium Bromide (EtBr)	5 ml
9.	100 BP DNA Ladder	1.5 ml
10.	Water for injection	50 Lit.

Table 17: Other accessories.

SI. No	Name of items	Quantity
1	Marker	3
2	Vinyl Gloves	100pcs×5
3	Mask	2 packs
4	Hexisol	500ml×2
5	Tissue Paper	5 packs
6	Aluminum foil	4 rolls

3.4 Results

3.4.1 Demographic Information about Cases and Controls

The baseline demographic information about the cases as well as controls is summed up in **Table 18**. The average age of the patients was 43.24 ± 9.17 years, while that of the control was 41.75 ± 9.79 years. Most of the individuals in both groups were under 45 years of age. In terms of BMI, the mean BMI among patients was 23.65 ± 3.60 kg/m^2, which was higher than that of controls (22.69±3.32 kg/m^2). A higher percentage of patients were overweight than controls (31.33% vs. 23.91%). Most of the patients and controls were normal in their BMI (57.83% of patients and 65.74% of controls). Regarding residence, a higher proportion of controls resided in rural areas (70.52%) compared to patients (64.26%). Most participants in both groups were married (99.20% of patients, 97.21% of controls). Dietary analysis revealed that 67.07% of patients and 72.11% of controls had a rich fibrous diet, while poor fibrous intake was comparable in both groups (11.24% in patients vs. 13.15% in controls). Tobacco use was reported in 23.29% of patients and 28.69% of controls. The history of contraceptive use was more prevalent among controls (72.11%) compared to patients (65.46%). No substantial differences ($p > 0.05$) were observed between patients and controls in terms of age category, mean age, BMI category, region, occupation, marital status, dietary habits, tobacco consumption history, or prior contraceptive use. But there were substantial differences in mean BMI and educational level between the two distinct groups ($p < 0.05$).

Table 18: Basic demographic details of the patients as well as controls.

Variables	Patients (N= 249) (%)	Controls (N=251) (%)	p value
Age (years)			
<45	125 (50.20)	144 (57.37)	
45-60	103 (41.37)	91 (36.26)	0.253
>60	21 (8.43)	16 (6.37)	
Mean age ± SD (range)	43.24 ± 9.17 (26-71)	41.75 ± 9.79 (25-69)	0.080
BMI (kg/m^2)			
Underweight (<18.5)	14 (5.62)	10 (3.98)	
Normal (18.5-24.9)	144 (57.83)	165 (65.74)	0.192
Overweight (25.0-29.9)	78 (31.33)	60 (23.91)	
Obese (≥30)	13 (5.22)	16 (6.37)	
Mean BMI ± SD (range)	23.65 ± 3.60 (16.0-37.3)	22.69 ± 3.32 (17.5-35.9)	**0.002**
Region			
Rural	160 (64.26)	177 (70.52)	
Urban	89 (35.74)	74 (29.48)	0.135
Education			
No education	129 (51.81)	97 (38.64)	
Primary-secondary	99 (39.76)	115 (45.82)	**0.003**
Higher secondary or above	21 (8.43)	39 (15.54)	
Occupation			
Non-employed	203 (81.53)	191 (76.10)	0.169
Employed	46 (18.47)	60 (23.90)	
Marital status			
Married	247 (99.20)	244 (97.21)	
Unmarried	02 (0.80)	7 (2.79)	0.095
Dietary habit			
Rich fibrous	167 (67.07)	181 (72.11)	
Moderate fibrous	54 (21.69)	37 (14.74)	0.126
Poor fibrous	28 (11.24)	33 (13.15)	
Tobacco consumption history			
Yes	58 (23.29)	72 (28.69)	
No	191 (76.71)	179 (71.31)	0.169
Previous history of contraceptives			
Yes	163 (65.46)	181 (72.11)	
No	86 (34.54)	70 (27.89)	0.108

3.4.2 Genotyping of *SLCO1B3* rs4149117

The tetra-primer ARMS-PCR approach was used to recognize the *SLCO1B3* rs4149117 polymorphism as well as to explore its association with BC among Bangladeshi women.

3.4.2.1 Linkage between *SLCO1B3* rs4149117 Polymorphism and Breast Cancer Susceptibility

Fig. 8 shows the digestion patterns for the GG, GT, and TT genotypes. **Table 19** shows that the genotypic distribution of the *SLCO1B3* rs4149117 polymorphism in the patient and control groups followed Hardy-Weinberg equilibrium (HWE). As observed for the rs4149117 polymorphism, the frequency of the GG normal homozygote was greater in cases compared to controls (80.27% vs. 69.32%). In contrast, the GT heterozygote genotype was less common in cases as compared to controls (17.27% vs. 25.90%). Interestingly, the TT mutant homozygote was found to be more prevalent in controls than in cases (4.78% vs. 2.01%), indicating a possible protective association. In the allelic model (T vs. G: p = 0.001; OR = 0.55; 95% CI = 0.38-0.80), codominant model 1 (GT vs. GG: p = 0.012; OR = 0.57; 95% CI = 0.37-0.88), dominant model (GT+TT vs. GG: p = 0.003; OR = 0.54; 95% CI = 0.36-0.82), and over-dominant model (GT vs. GG+TT: p = 0.019; OR = 0.60; 95% CI = 0.39-0.92), the associations were statistically noteworthy, as all p-values were below 0.05. Notably, in all genetic models, the odds ratios (ORs) were below 1.00, suggesting a possible protective impact. These findings suggest that the *SLCO1B3* rs4149117 polymorphism is substantially associated with a reduced risk of BC in the studied population. Conversely, the associations observed in the codominant model 2 (TT vs. GG: p = 0.060; OR = 0.36; 95% CI = 0.12-1.04) and the recessive model (TT vs. GG+GT: p = 0.097; OR = 0.40; 95% CI = 0.14-1.17) were statistically non-significant, as the p-values exceeded the threshold of 0.05.

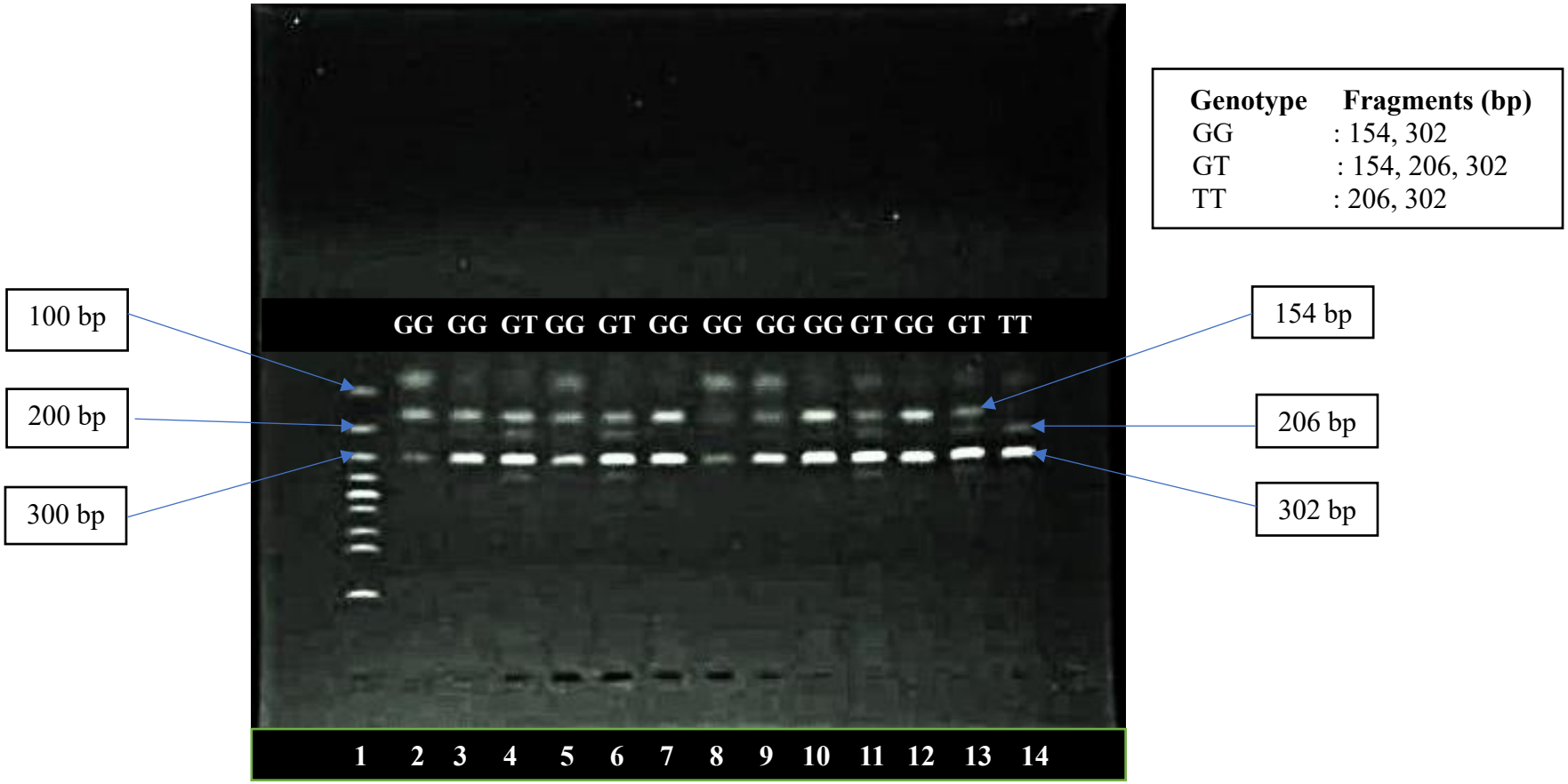


Fig. 8: Tetra Primer ARMS-PCR products for *SLCO1B3* rs4149117 after 1.5% agarose gel electrophoresis. Lane-1: 100 bp DNA ladder; Lanes- 2, 3, 5, 7, 8, 9, 10, 12: GG; Lanes-4, 6, 11, 13: GT; Lane- 14: TT.

Table 19: Genotypic association of *SLCO1B3* rs4149117 variant with BC susceptibility.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
GG	201 (80.72)			174 (69.32)			1	
Codominant model 1 (GT vs. GG)	43 (17.27)	2.11	0.146	65 (25.90)	3.16	0.075	0.57 (0.37-0.88)	**0.012**
Codominant model 2 (TT vs. GG)	5 (2.01)			12 (4.78)			0.36 (0.12-1.04)	0.060
Dominant model								
GG	201 (80.72)			174 (69.32)			1	
GT+TT	48 (19.28)			77 (30.68)			0.54 (0.36-0.82)	**0.003**
Recessive model								
GG+GT	244 (97.99)			239 (95.22)			1	
TT	5 (2.01)			12 (4.78)			0.40 (0.14-1.17)	0.097
Over-dominant model								
GG+TT	206 (82.73)			186 (74.10)			1	
GT	43 (17.27)			65 (25.90)			0.60 (0.39-0.92)	**0.019**
Allele								
G	445 (89.36)			413 (82.27)			1	
T	53 (10.64)			89 (17.73)			0.55 (0.38-0.80)	**0.001**

3.4.2.2 Association between *SLCO1B3* rs4149117 Polymorphism and AC-T Chemotherapy Response

The relationship between *SLCO1B3* rs4149117 genotypes and clinical response to AC-T therapy was assessed in 249 BC patients, categorized into AC-paclitaxel and AC-docetaxel treatment subgroups, as illustrated in **Table 20**. In patients undergoing the AC-paclitaxel regimen, those with the GT+TT genotype showed a diminished response compared with those with the GG genotype (GT+TT vs. GG: OR = 0.84; 95% CI: 0.41-1.71; p = 0.629). But the relationship was not statistically noteworthy.

Conversely, among the smaller group receiving the AC-docetaxel regimen, individuals having the GT+TT genotype unveiled a diminished response compared to those with the GG genotype (GT+TT vs. GG: OR = 0.33; 95% CI: 0.07-1.47; p = 0.147). However, the relationship was not statistically noteworthy.

Overall, the *SLCO1B3* rs4149117 variant did not demonstrate a substantial linkage with clinical response to either AC-paclitaxel or AC-docetaxel regimens.

3.4.2.3 Association between *SLCO1B3* rs4149117 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The association between *SLCO1B3* rs4149117 genotypes and the incidence of toxicities induced by AC-T chemotherapy regimen was evaluated among BC patients, as presented in **Table 21**. Among patients undergoing the AC-paclitaxel regimen, the GT+TT genotype had a significant linkage with a greater risk of grade III-IV toxicities than the GG genotype. These included nausea (OR = 3.84; 95% CI: 1.28-11.59; p = 0.016), diarrhoea (OR = 3.12; 95% CI: 1.31-7.40; p = 0.010), constipation (OR= 3.50; 95% CI= 1.08-11.43; p= 0.037), fever (OR = 2.71; 95% CI: 1.27-5.75; p = 0.009), edema (OR= 2.40; 95% CI= 1.03-5.57; p= 0.041), fatigue (OR= 2.80; 95% CI= 1.19-6.58; p= 0.018), dysgeusia (OR = 3.39; 95% CI: 1.61-7.15; p = 0.001), and hepatotoxicity (OR= 2.77; 95% CI= 1.09-7.04; p= 0.031). The other toxicities associated with the AC-paclitaxel regimen did not demonstrate a statistically noteworthy correlation among the genotypes.

Conversely, in the smaller group treated with AC-docetaxel, no toxicity showed significant links to genotype.

Table 20: Impact of *SLCO1B3* rs4149117 polymorphisms on the response of AC-T chemotherapy regimen.

Variant	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
***SLCO1B3* rs4149117**	Non-carrier GG (182)	99	83	1 (reference)		GG (19)	13	6	1	
	Carrier GT+TT (36)	18	18	0.84 (0.41-1.71)	0.629	GT+TT (12)	5	7	0.33 (0.07-1.47)	0.147

Table 21: Impact of *SLCO1B3* rs4149117 polymorphisms on the toxicities triggered by AC-T chemotherapy regimen.

Toxicities		Doxorubicin-cyclophosphamide- paclitaxel (N=218)		Doxorubicin-cyclophosphamide- docetaxel (N=31)	
		Non-carrier GG (182)	**Carrier GT + TT (36)**	**Non-carrier GG (19)**	**Carrier GT + TT (12)**
Nausea	Grade III + IV	9	6	2	2
	Grade ≤ II	173	30	17	10
	Odd ratio (95% CI)	Reference	3.84 (1.28-11.59)	Reference	1.70 (0.21-14.02)
	p-value		**0.016**		0.622
Vomiting	Grade III + IV	24	9	5	2
	Grade ≤ II	158	27	14	10
	Odd ratio (95% CI)	Reference	2.19 (0.92-5.23)	Reference	0.56 (0.09-3.49)
	p-value		0.076		0.534
Diarrhoea	Grade III + IV	20	10	3	1
	Grade ≤ II	162	26	16	11
	Odd ratio (95% CI)	Reference	3.12 (1.31-7.40)	Reference	0.48 (0.04-5.29)
	p-value		**0.010**		0.552

Constipation	Grade III + IV	8	5	4	1
	Grade ≤ II	174	31	15	11
	Odd ratio (95% CI)	Reference	3.50 (1.08-11.43)	Reference	0.34 (0.03-3.49)
	p-value		**0.037**		0.364
Fever	Grade III + IV	38	15	2	1
	Grade ≤ II	144	21	17	11
	Odd ratio (95% CI)	Reference	2.71 (1.27-5.75)	Reference	0.77 (0.06-9.58)
	p-value		**0.009**		0.840
Edema	Grade III + IV	26	10	2	0
	Grade ≤ II	156	26	17	12
	Odd ratio (95% CI)	Reference	2.40 (1.03-5.57)	Reference	-
	p-value		**0.041**		-
Dry mouth	Grade III + IV	9	2	2	2
	Grade ≤ II	173	34	17	10
	Odd ratio (95% CI)	Reference	1.13 (0.23-5.47)	Reference	1.70 (0.21-14.02)
	p-value		0.878		0.622
Mouth sores	Grade III + IV	9	4	4	2
	Grade ≤ II	173	32	15	10
	Odd ratio (95% CI)	Reference	2.40 (0.70-8.28)	Reference	0.75 (0.11-4.90)
	p-value		0.164		0.763
Fatigue	Grade III + IV	22	10	7	4
	Grade ≤ II	160	26	12	8
	Odd ratio (95% CI)	Reference	2.80 (1.19-6.58)	Reference	0.86 (0.19-3.92)
	p-value		**0.018**		0.842
Dysgeusia	Grade III + IV	38	17	9	6
	Grade ≤ II	144	19	10	6
	Odd ratio (95% CI)	Reference	3.39 (1.61-7.15)	Reference	1.11 (0.26-4.72)
	p-value		**0.001**		0.886
Skin hyperpigmentation	Grade III + IV	10	5	4	0
	Grade ≤ II	172	31	15	12
	Odd ratio (95% CI)	Reference	2.77 (0.89-8.67)	Reference	-

	p-value		0.079		-
Gastritis	Grade III + IV	9	3	3	1
	Grade ≤ II	173	33	16	11
	Odd ratio (95% CI)	Reference	1.75 (0.45-6.80)	Reference	1.09 (0.10-11.57)
	p-value		0.420		0.942
Peripheral neuropathy	Grade III + IV	23	9	2	2
	Grade ≤ II	159	27	17	10
	Odd ratio (95% CI)	Reference	2.30 (0.96-5.51)	Reference	1.70 (0.21-14.02)
	p-value		0.060		0.622
Myalgia/arthralgia	Grade III + IV	52	16	8	4
	Grade ≤ II	130	20	11	8
	Odd ratio (95% CI)	Reference	2.00 (0.96-4.16)	Reference	0.69 (0.15-3.10)
	p-value		0.063		0.626
Anaemia	Grade III + IV	8	1	2	0
	Grade ≤ II	174	35	17	12
	Odd ratio (95% CI)	Reference	0.62 (0.08-5.13)	Reference	-
	p-value		0.658		-
Leukopenia	Grade III + IV	7	0	2	1
	Grade I + II	175	36	17	11
	Odd ratio (95% CI)	Reference	-	Reference	0.77 (0.06-9.58)
	p-value		-		0.840
Leukocytosis	Grade III + IV	2	0	0	0
	Grade I + II	180	36	19	12
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	7	3	2	0
	Grade I + II	175	33	17	12
	Odd ratio (95% CI)	Reference	2.27 (0.56-9.24)	Reference	-
	p-value		0.251)		-

Febrile neutropenia	Grade III + IV	7	3	1	0
	Grade I + II	175	33	18	12
	Odd ratio (95% CI)	Reference	2.27 (0.56-9.24)	Reference	-
	p-value		0.251)		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	182	36	19	12
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	9	1	0	0
	Grade I + II	173	35	19	12
	Odd ratio (95% CI)	Reference	0.55 (0.07-4.47)	Reference	-
	p-value		0.575		-
Lymphocytopenia	Grade III + IV	0	0	0	0
	Grade I + II	182	36	19	12
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	180	36	19	12
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	5	2	0	0
	Grade I + II	177	34	19	12
	Odd ratio (95% CI)	Reference	2.08 (0.39-11.18)	Reference	-
	p-value		0.392		-
Hepatotoxicity	Grade III + IV	17	8	3	1
	Grade I + II	165	28	16	11
	Odd ratio (95% CI)	Reference	2.77 (1.09-7.04)	Reference	0.48 (0.04-5.29)
	p-value		**0.031**		0.552
Nephrotoxicity	Grade III + IV	3	1	0	0
	Grade I + II	179	35	19	12
	Odd ratio (95% CI)	Reference	1.70 (0.17-16.87)	Reference	-

	p-value		0.648		-
Cardiotoxicity	Grade III + IV	2	0	0	0
	Grade I + II	180	36	19	12
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	10	5	2	1
	Grade I + II	172	31	17	11
	Odd ratio (95% CI)	Reference	2.77 (0.89-8.67)	Reference	0.77 (0.06-9.58)
	p-value		0.079		0.840
Bone toxicity	Grade III + IV	20	8	1	1
	Grade I + II	162	28	18	11
	Odd ratio (95% CI)	Reference	2.31 (0.93-5.77)	Reference	1.64 (0.09-28.90)
	p-value		0.071		0.736

When a zero cell was present, the OR was not estimable

3.4.3 Genotyping of *ABCB1* rs1128503

The PCR-RFLP method was employed to identify the *ABCB1* rs1128503 polymorphism as well as to determine whether it was linked to BC in Bangladeshi women.

3.4.3.1 PCR of ABCB1 rs1128503

PCR product of rs1128503

Forward Primer

Polymorphism of interest
R= A > G

CACTCTGCACCTTCAGGTTCAGRCCCTTCAAGATCTACCAGGACGAGTGAGAAAAAA
ACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGACTGTTCACTAGGTTTAAAT
ATACATGCACTTTTTTATAATCTCTACAAGAAAACATCAGAAACTCTTCATTCAATAGA
= 174 bp

Reverse Primer

Forward Primer (FP): 5'-CACTCTGCACCTTCAGGTTC-3'

Reverse Primer (RP): 5'-TCTATTGAATGAAGAGTTTCTG-3

After PCR amplification, the PCR product of the rs1128503 allele of the *ABCB1* gene was visualized on a 1.5% (w/v) agarose gel that had been stained with ethidium bromide (**Fig 9**).

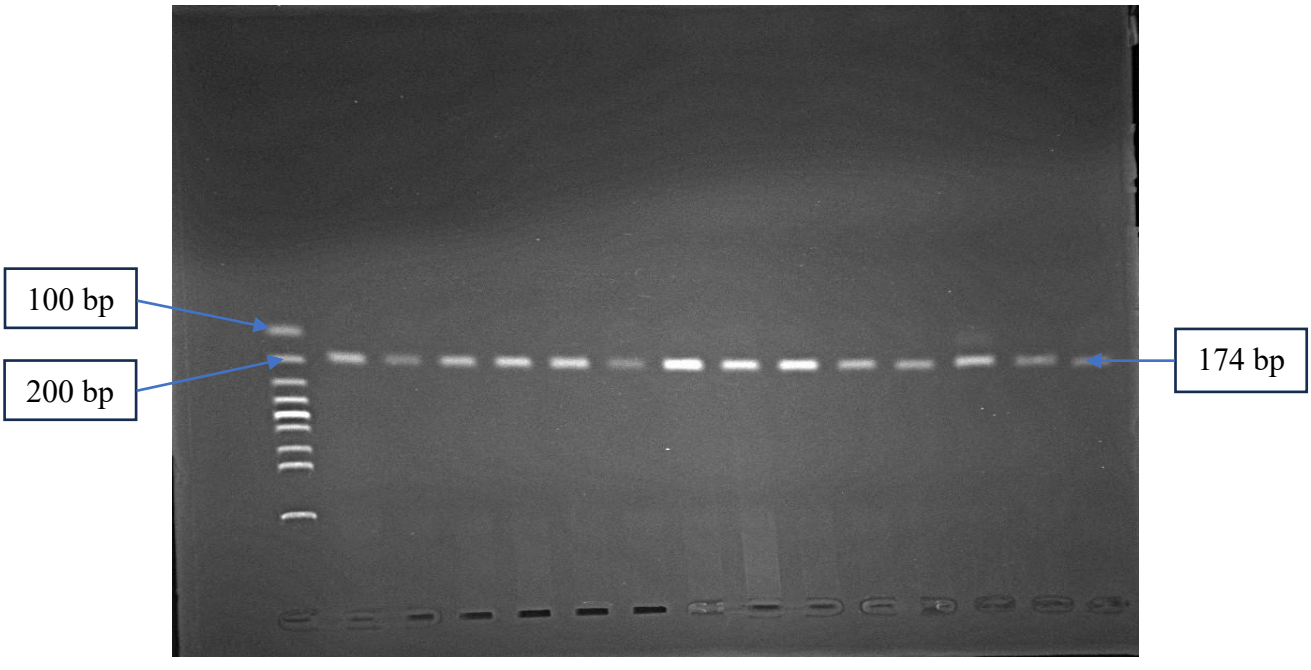


Fig. 9: PCR product of the *ABCB1* rs1128503 allele following electrophoresis on a 1.5% agarose gel.

3.4.3.2 RFLP Fragmentation Pattern of *ABCB1* rs1128503

Table 22: Restriction enzyme along with its digestion sites for *ABCB1* rs1128503.

Restriction Enzyme	Sites of Digestion
HaeIII	Cutting Site 5'... G G C C ... 3' 3'... C C G G ... 5

When R= A in both copies of the gene, there is no cutting site and we will get one fragment.

CACTCTGCACCTTCAGGTTCAGRCCCTTCAAGATCTACCAGGACGAGTG
AGAAAAAAACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGAC
TGTTCACTAGGTTTAAATATACATGCACTTTTTTATAATCTCTACAAGAAA
ACATCAGAAACTCTTCATTCAATAGA

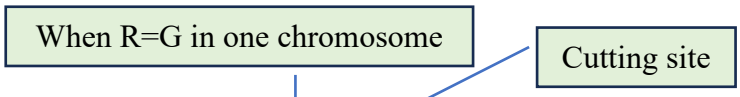
When R= A in both chromosomes, there is no cutting site

Restriction Enzyme (HaeIII)

CACTCTGCACCTTCAGGTTCAGACCCTTCAAGATCTACCAGGACGAGTG
AGAAAAAAACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGAC
TGTTCACTAGGTTTAAATATACATGCACTTTTTTATAATCTCTACAAGAAA
ACATCAGAAACTCTTCATTCAATAGA = 174 bp (Fragment 1)

Wild: AA: 174 bp

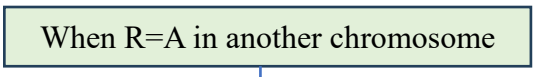
When R=A in one copy of the gene and R=G in another copy of the gene, there is one cutting site and we will get 3 fragments.



CACTCTGCACCTTCAGGTTCAGGCCCTTCAAGATCTACCAGGACGAGTGAGAA
AAAAACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGACTGTTCACTA
GGTTTAAATATACATGCACTTTTTTATAATCTCTACAAGAAAACATCAGAAACT
CTTCATTCAATAGA

CACTCTGCACCTTCAGGTTCAGG = 23 bp (Fragment 1)

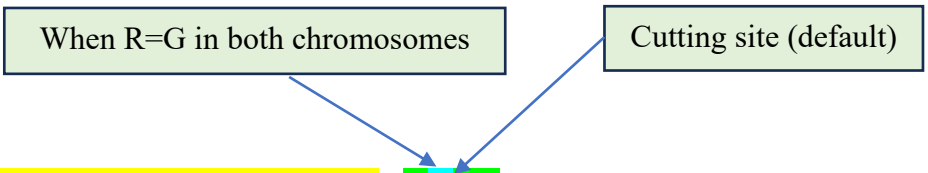
CCCTTCAAGATCTACCAGGACGAGTGAGAAAAAAACTTCAAGGCAATTCACA
GACACAGGATATAGGAACTGACTGTTCACTAGGTTTAAATATACATGCACTTTT
TTATAATCTCTACAAGAAAACATCAGAAACTCTTCATTCAATAGA = 151 bp
(Fragment 2)



CACTCTGCACCTTCAGGTTCAGACCCTTCAAGATCTACCAGGACGAGTGAGAA
AAAAACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGACTGTTCACTA
GGTTTAAATATACATGCACTTTTTTATAATCTCTACAAGAAAACATCAGAAACT
CTTCATTCAATAGA = 174 bp (Uncut Fragment; Fragment 3)

Hetero: AG: 23, 151, 174 bp

When R=G in both copy of the gene, then we will get 2 fragments.



CACTCTGCACCTTCAGGTTCAGGCCCTTCAAGATCTACCAGGACGAGTGAGAA
AAAAACTTCAAGGCAATTCACAGACACAGGATATAGGAACTGACTGTTCACTA
GGTTTAAATATACATGCACTTTTTTATAATCTCTACAAGAAAACATCAGAAACT
CTTCATTCAATAGA

CACTCTGCACCTTCAGGTTCAGG = 23 bp (Fragment 1)

CCCTTCAAGATCTACCAGGACGAGTGAGAAAAAAACTTCAAGGCAATTCACA
GACACAGGATATAGGAACTGACTGTTCACTAGGTTTAAATATACATGCACTTTT
TATAATCTCTACAAGAAAACATCAGAAACTCTTCATTCAATAGA = 151 bp
(Fragment 2)

Mutant: GG: 23, 151 bp

Table 23: Nucleotide changes, fragment sizes, and genotypes for the rs1128503 allele of the *ABCB1* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=A in both chromosomes (AA)	174	AA
When R=A in one chromosome and R=G in another chromosome (AG)	23, 151, 174	AG
When R=G in both chromosome (GG)	23, 151	GG

3.4.3.3 Linkage between *ABCB1* rs1128503 Polymorphism and Breast Cancer Susceptibility

The resulting digestion fragments for the AA, AG, and GG genotypes are shown in **Fig. 10**. The genotypic distribution of the *ABCB1* rs1128503 polymorphism in both case and control categories followed the Hardy–Weinberg equilibrium (HWE) (**Table 24).** The codominant model 2, recessive model, and allelic model indicated a significantly higher risk of BC susceptibility among Bangladeshi women (codominant model 2: GG vs. AA, OR = 1.91, 95% CI = 1.14-3.20, p = 0.014; recessive model: GG vs. AA+AG, OR = 1.64, 95% CI = 1.05-2.56, p = 0.030; allelic model: G vs. A, OR = 1.36, 95% CI = 1.06-1.74, p = 0.016). Similarly, the codominant and dominant models suggested a higher risk of BC progression (codominant model 1: AG vs. AA, OR = 1.27, 95% CI = 0.85-1.92, p = 0.241; dominant model: AG+GG vs. AA, OR = 1.43, 95% CI = 0.97-2.10, p = 0.071), whereas the over dominant model indicated a reduced risk (over dominant model: AG vs. AA+GG, OR = 0.98, 95% CI = 0.69-1.40, p = 0.929); though, none of these outcomes were statistically noteworthy. In conclusion, Bangladeshi women have a significantly higher risk of BC when they carry the rs1128503 allele of the *ABCB1* gene.

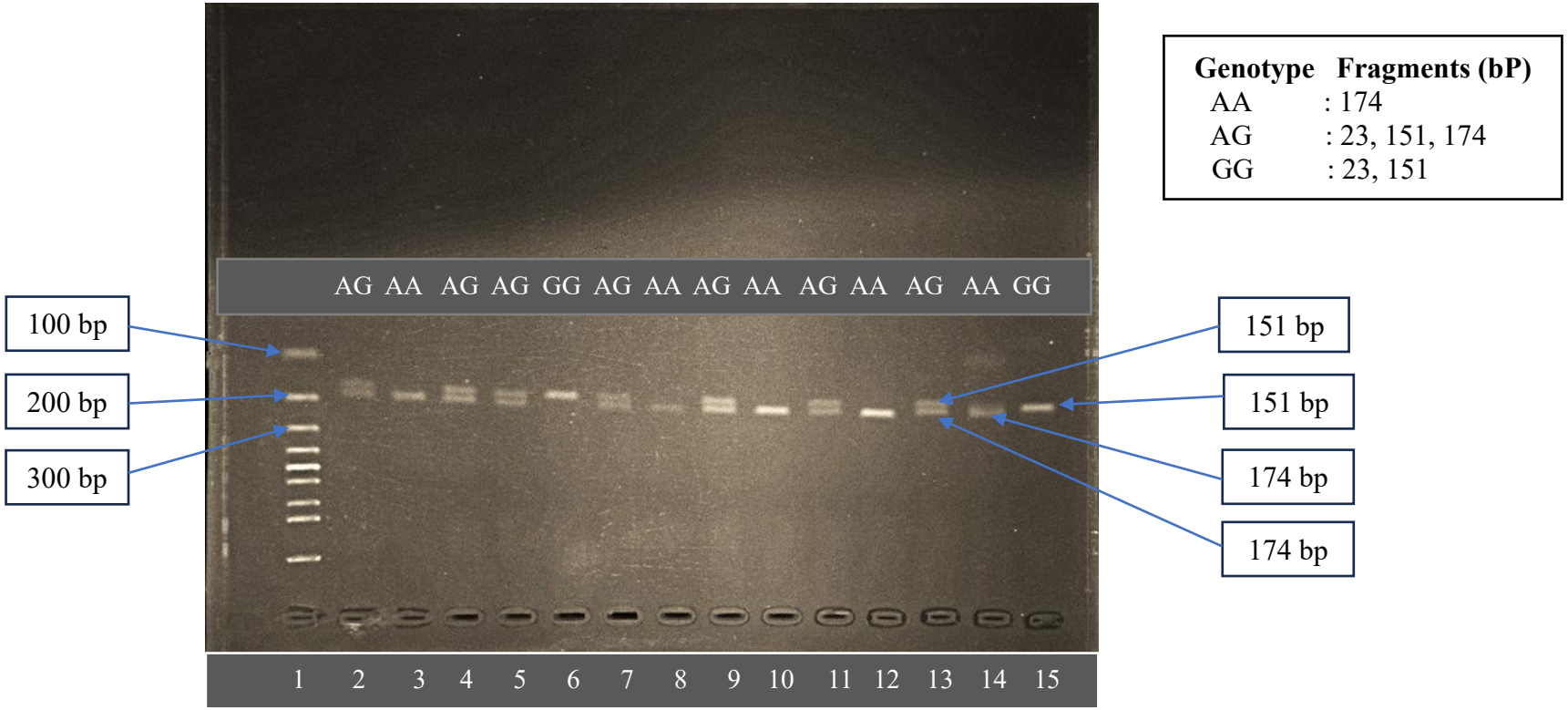


Fig. 10: Restriction Endonuclease (HaeIII) digestion fragments of rs1128503 allele after 2.0% agarose gel electrophoresis. Lane-1: 100 bp DNA ladder; Lanes-3, 8, 10, 12, 14: AA; Lanes-2, 4, 5, 7, 9, 11, 13: AG; Lanes-6, 15: GG.

Table 24: Genotypic association of *ABCB1* rs1128503 variant with BC risk.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
AA	64 (25.70)			83 (33.07)			1	
Codominant model 1 (AG vs. AA)	126 (50.60)	0.04	0.844	128 (50.99)	0.65	0.421	1.27 (0.85-1.92)	0.241
Codominant model 2 (GG vs. AA)	59 (23.70)			40 (15.94)			1.91 (1.14-3.20)	**0.014**
Dominant model								
AA	64 (25.70)			83 (33.07)			1	
AG+GG	185 (74.30)			168 (66.93)			1.43 (0.97-2.10)	0.071
Recessive model								
AA+AG	190 (76.30)			211 (84.06)			1	
GG	59 (23.70)			40 (15.94)			1.64 (1.05-2.56)	**0.030**
Over-dominant model								
AA+GG	123 (49.40)			123 (49.01)			1	
AG	126 (50.60)			128 (50.99)			0.98 (0.69-1.40)	0.929
Allele								
A	254 (51.00)			294 (58.57)			1	
G	244 (49.00)			208 (41.43)			1.36 (1.06-1.74)	**0.016**

3.4.3.4 Association between *ABCB1* rs1128503 Polymorphism and AC-T Chemotherapy Response

The linkage between *ABCB1* rs1128503 genotypes and clinical response to AC-T chemotherapy was evaluated in 249 BC patients, stratified into AC-paclitaxel and AC-docetaxel treatment subgroups, as presented in **Table 25**. Among patients receiving the AC-paclitaxel regimen, those with the AG+GG genotype had substantially lower clinical response rates than those with the AA genotype (AG+GG vs. AA: OR = 0.53, 95% CI = 0.28-0.99, p = 0.049).

In contrast, among the smaller subset of patients treated with the AC-docetaxel regimen, patients carrying the AG+GG genotype showed lower clinical responses; however, this association didn't reach statistical significance (AG+GG vs. AA: OR = 0.47, 95% CI = 0.07-2.93, p = 0.421).

Overall, the *ABCB1* rs1128503 polymorphism showed a borderline significant association with reduced clinical response to the AC-paclitaxel regimen. but not to the AC-docetaxel regimen.

3.4.3.5 Association between *ABCB1* rs1128503 Polymorphism and AC-T Chemotherapy-Induced Toxicity

Table 26 illustrates the correlation between *ABCB1* rs1128503 genotypes and the incidence of toxicities associated with AC-T therapy in BC patients. Among patients treated with the AC-paclitaxel regimen, the AG+GG genotype exhibited a significantly greater likelihood of various higher-grade toxicities than those who had the AA genotype. These included vomiting (OR = 4.12, 95% CI = 1.20-14.08, p = 0.023), fever (OR = 2.86, 95% CI = 1.20-6.76, p = 0.016), hepatotoxicity (OR = 4.58, 95% CI = 1.04-20.10, p = 0.043), and bone toxicity (OR = 5.29, 95% CI = 1.21-23.08, p = 0.026). Other toxicities under the AC-paclitaxel regimen did not show statistically significant associations across genotypes.

Conversely, in the smaller cohort treated with AC-docetaxel, no toxicities demonstrated significant correlations with genotypes.

Table 25: Impact of the *ABCB1* rs1128503 variant on the responsiveness of the AC-T chemotherapy regimen.

Variant	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs1128503	Non-carrier AA (57)	37	20	1 (reference)	-	AA (7)	5	2	1 (reference)	-
	Carrier AG+GG (161)	80	81	0.53 (0.28-0.99)	**0.049**	AG+GG (24)	13	11	0.47 (0.07-2.93)	0.421

Table 26: Impact of *ABCB1* rs1128503 polymorphisms on the toxicities induced by AC-T chemotherapy regimen.

Toxicities		Doxorubicin-cyclophosphamide- paclitaxel (N=218)		Doxorubicin-cyclophosphamide- docetaxel (N=31)	
		Non-carrier AA (57)	Carrier AG + GG (161)	Non-carrier AA (7)	Carrier AG + GG (24)
Nausea	Grade III + IV	3	12	1	3
	Grade ≤ II	54	149	6	21
	Odd ratio (95% CI)	Reference	1.45 (0.39-5.33)	Reference	0.86 (0.07-9.81)
	p-value		0.576		0.901
Vomiting	Grade III + IV	3	30	2	5
	Grade ≤ II	54	131	5	19
	Odd ratio (95% CI)	Reference	4.12 (1.20-14.08)	Reference	0.66 (0.09-4.45)
	p-value		**0.023**		0.667
Diarrhoea	Grade III + IV	5	25	0	4
	Grade ≤ II	52	136	7	20
	Odd ratio (95% CI)	Reference	1.91 (0.69-5.25)	Reference	-
	p-value		0.209		-

Constipation	Grade III + IV	4	9	1	4
	Grade ≤ II	53	152	6	20
	Odd ratio (95% CI)	Reference	0.78 (0.23-2.65)	Reference	1.20 (0.11-12.88)
	p-value		0.696		0.880
Fever	Grade III + IV	7	46	1	2
	Grade ≤ II	50	115	6	22
	Odd ratio (95% CI)	Reference	2.86 (1.20-6.76)	Reference	0.54 (0.04-7.08)
	p-value		**0.016**		0.643
Edema	Grade III + IV	9	27	0	2
	Grade ≤ II	48	134	7	22
	Odd ratio (95% CI)	Reference	1.07 (0.47-2.44)	Reference	-
	p-value		0.864		-
Dry mouth	Grade III + IV	2	9	2	2
	Grade ≤ II	55	152	5	22
	Odd ratio (95% CI)	Reference	1.63 (0.34-7.77)	Reference	0.22 (0.02-2.02)
	p-value		0.540		0.184
Mouth sores	Grade III + IV	4	9	1	5
	Grade ≤ II	53	152	6	19
	Odd ratio (95% CI)	Reference	0.78 (0.23-2.65)	Reference	1.58 (0.15-16.31)
	p-value		0.696		0.701
Fatigue	Grade III + IV	8	24	3	8
	Grade ≤ II	49	137	4	16
	Odd ratio (95% CI)	Reference	1.07 (0.45-2.54)	Reference	0.67 (0.12-3.72)
	p-value		0.873		0.644
Dysgeusia	Grade III + IV	11	44	4	11
	Grade ≤ II	46	117	3	13
	Odd ratio (95% CI)	Reference	1.57 (0.74-3.30)	Reference	0.63 (0.11-3.46)
	p-value		0.232		0.599

Skin hyperpigmentation	Grade III + IV	2	13	0	4
	Grade ≤ II	55	148	7	20
	Odd ratio (95% CI)	Reference	2.42 (0.52-11.05)	Reference	-
	p-value		0.255		-
Gastritis	Grade III + IV	2	10	1	3
	Grade ≤ II	55	151	6	21
	Odd ratio (95% CI)	Reference	1.82 (0.38-8.57)	Reference	0.86 (0.07-9.81)
	p-value		0.448		0.901
Peripheral neuropathy	Grade III + IV	6	26	1	3
	Grade ≤ II	51	135	6	21
	Odd ratio (95% CI)	Reference	1.63 (0.63-4.20)	Reference	0.86 (0.07-9.81)
	p-value		0.306		0.901
Myalgia/arthralgia	Grade III + IV	13	55	3	9
	Grade ≤ II	44	106	4	15
	Odd ratio (95% CI)	Reference	1.75 (0.87-3.53)	Reference	0.80 (0.14-4.42)
	p-value		0.114		0.798
Anaemia	Grade III + IV	1	8	1	1
	Grade ≤ II	56	153	6	23
	Odd ratio (95% CI)	Reference	2.93 (0.36-23.94)	Reference	0.26 (0.01-4.81)
	p-value		0.316		0.366
Leukopenia	Grade III + IV	2	5	1	2
	Grade I + II	55	156	6	22
	Odd ratio (95% CI)	Reference	0.88 (0.16-4.67)	Reference	0.54 (0.04-7.08)
	p-value		0.882		0.643
Leukocytosis	Grade III + IV	0	2	0	0
	Grade I + II	57	159	7	24
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	3	7	0	2
	Grade I + II	54	154	7	22
	Odd ratio (95% CI)	Reference	0.82 (0.20-3.27)	Reference	-

	p-value		0.776		-
Febrile neutropenia	Grade III + IV	3	7	0	1
	Grade I + II	54	154	7	23
	Odd ratio (95% CI)	Reference	0.82 (0.20-3.27)	Reference	-
	p-value		0.776		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	57	161	7	24
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	2	8	0	0
	Grade I + II	55	153	7	24
	Odd ratio (95% CI)	Reference	1.44 (0.29-6.98)	Reference	-
	p-value		0.652		-
Lymphocytopenia	Grade III + IV	0	0	0	0
	Grade I + II	57	161	7	24
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	0	2	0	0
	Grade I + II	57	159	7	24
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	2	5	0	0
	Grade I + II	55	156	7	24
	Odd ratio (95% CI)	Reference	0.88 (0.16-4.67)	Reference	-
	p-value		0.882		-
Hepatotoxicity	Grade III + IV	2	23	0	4
	Grade I + II	55	138	7	20
	Odd ratio (95% CI)	Reference	4.58 (1.04-20.10)	Reference	-
	p-value		**0.043**		-

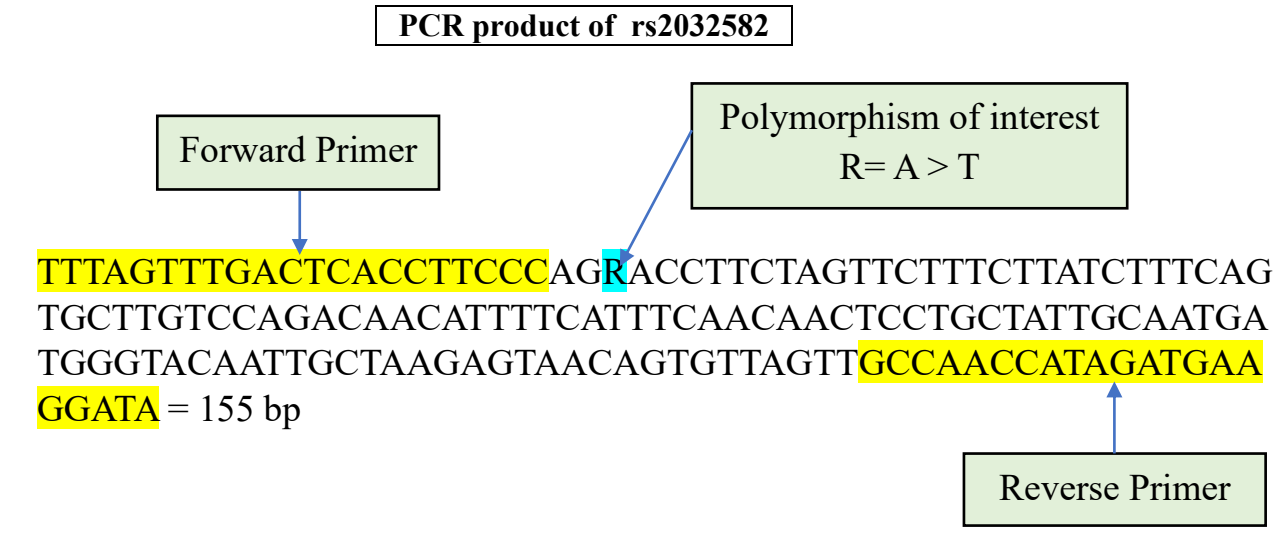
Nephrotoxicity	Grade III + IV	1	3	0	0
	Grade I + II	56	158	7	24
	Odd ratio (95% CI)	Reference	1.06 (0.10-10.43)	Reference	-
	p-value		0.958)		-
Cardiotoxicity	Grade III + IV	0	2	0	0
	Grade I + II	57	159	7	24
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lung toxicity	Grade III + IV	3	12	0	3
	Grade I + II	54	149	7	21
	Odd ratio (95% CI)	Reference	1.45 (0.39-5.33)	Reference	-
	p-value		0.576		-
Bone toxicity	Grade III + IV	2	26	0	2
	Grade I + II	55	135	7	22
	Odd ratio (95% CI)	Reference	5.29 (1.21-23.08)	Reference	-
	p-value		**0.026**		-

When a zero cell was present, the OR was not estimable.

3.4.4 Genotyping of *ABCB1* rs2032582

The PCR-RFLP approach was employed to identify the *ABCB1* rs2032582 polymorphism along with its linkage with BC in Bangladeshi women.

3.4.4.1 PCR of *ABCB1* rs2032582



Forward Primer (FP): 5'-TTTAGTTTGACTCACCTTCCC-3'
Reverse Primer (RP): 5'-TATCCTTCATCTATGGTTGGC-3'

After PCR amplification, the PCR product of the rs2032582 allele of the *ABCB1* gene was visualized on a 1.5 % (w/v) agarose gel that was stained with ethidium bromide (**Fig. 11**).

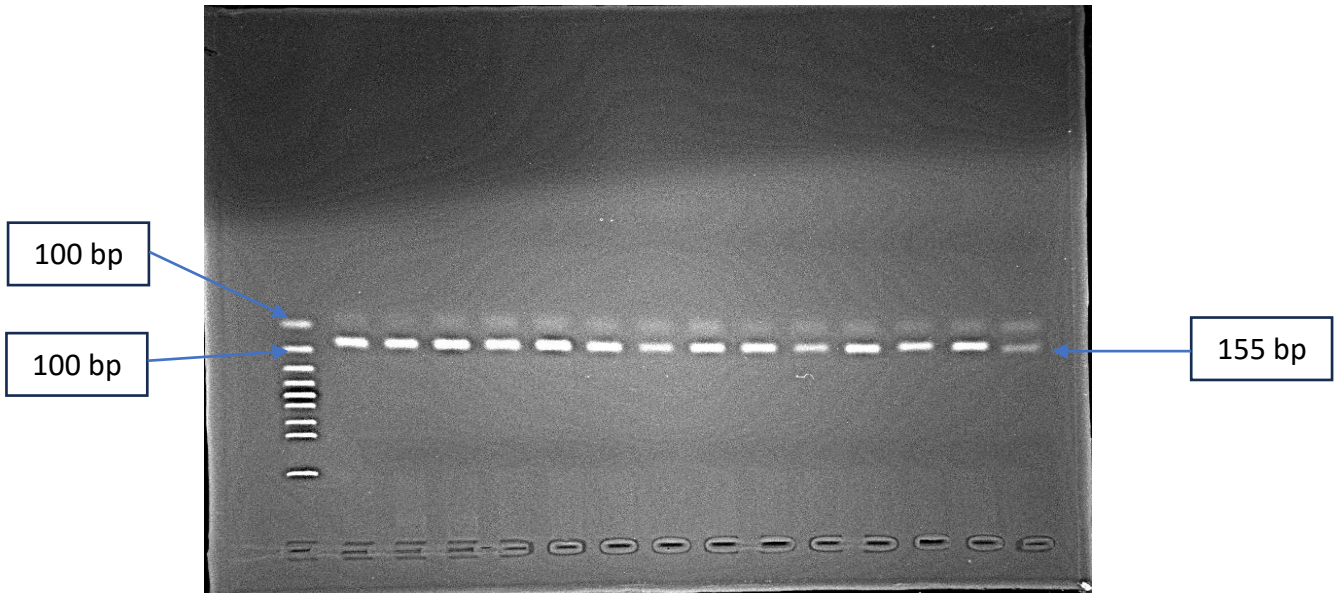


Fig. 11: Amplification band of rs2032582 (*ABCB1*) allele after 1.5% agarose gel electrophoresis.

3.4.4.2 RFLP Fragmentation Pattern of *ABCB1* rs2032582

Table 27: Restriction enzyme along with its digestion sites for *ABCB1* rs2032582.

Restriction Enzyme	Sites of Digestion
BsrI	5'...A C T G G N▼... 3' 3'...T G A C▲C N ... 5' Cutting Site

When R= A in both copies of the gene, there is no cutting site and we will get one fragment.

When R= A in both chromosomes, there is no cutting site

↓

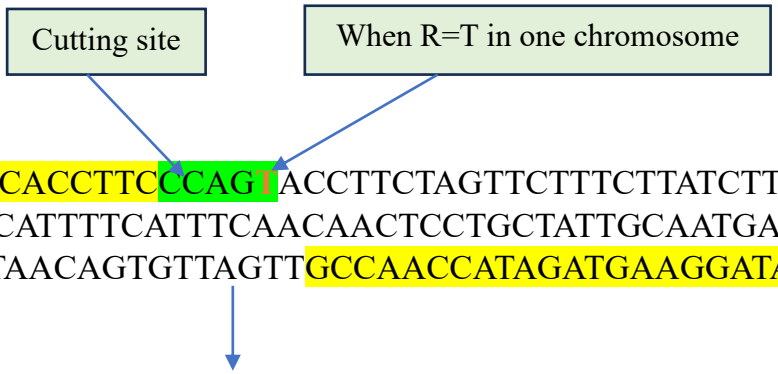
TTTAGTTTGACTCACCTTCCCAGAACCTTCTAGTTCTTTCTTATCTTTCAG
TGCTTGTCCAGACAACATTTTCATTTCAACAACTCCTGCTATTGCAATGA
TGGGTACAATTGCTAAGAGTAACAGTGTTAGTTGCCAACCATAGATGAA
GGATA

↓ Restriction enzyme (BsrI)

TTTAGTTTGACTCACCTTCCCAGAACCTTCTAGTTCTTTCTTATCTTTCAG
TGCTTGTCCAGACAACATTTTCATTTCAACAACTCCTGCTATTGCAATGA
TGGGTACAATTGCTAAGAGTAACAGTGTTAGTTGCCAACCATAGATGAA
GGATA = 155 bp (Fragment 1)

Wild: AA: 155 bp

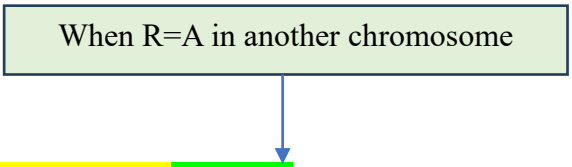
When R=A in one copy of the gene and R=T in another copy of the gene, there is one cutting site and we will get 3 fragments.



TTTAGTTTGACTCACCTTCCCAGTACCTTCTAGTTCTTTCTTATCTTTCAGTGCT
TGTCCAGACAACATTTTCATTTCAACAACTCCTGCTATTGCAATGATGGGTACA
ATTGCTAAGAGTAACAGTGTTAGTTGCCAACCATAGATGAAGGATA

TTTAGTTTGACTCACCTTCC = 20 bp (Fragment 1)

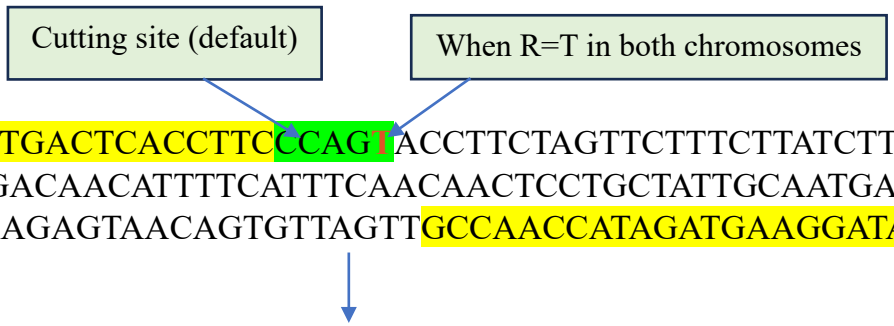
CAGTACCTTCTAGTTCTTTCTTATCTTTCAGTGCTTGTCCAGACAACATTTTCAT
TTCAACAACTCCTGCTATTGCAATGATGGGTACAATTGCTAAGAGTAACAGTGT
TAGTTGCCAACCATAGATGAAGGATA = 135 bp (Fragment 2)



TTTAGTTTGACTCACCTTCCCAGAACCTTCTAGTTCTTTCTTATCTTTCAGTGCT
TGTCCAGACAACATTTTCATTTCAACAACTCCTGCTATTGCAATGATGGGTACA
ATTGCTAAGAGTAACAGTGTTAGTTGCCAACCATAGATGAAGGATA = 155 bp
(Uncut Fragment, Fragment 3)

Hetero: AT: 20, 135, 155 bp

When R=T in both copies of the gene, then we will get 2 fragments.



TTTAGTTTGACTCACCTTCCCAGTACCTTCTAGTTCTTTCTTATCTTTCAGTGCT
TGTCCAGACAACATTTTCATTTCAACAACTCCTGCTATTGCAATGATGGGTACA
ATTGCTAAGAGTAACAGTGTTAGTTGCCAACCATAGATGAAGGATA

TTTAGTTTGACTCACCTTCC = 20 bp (Fragment 1)

CAGTACCTTCTAGTTCTTTCTTATCTTTCAGTGCTTGTCCAGACAACATTTTCAT
TTCAACAACTCCTGCTATTGCAATGATGGGTACAATTGCTAAGAGTAACAGTGT
TAGTTGCCAACCATAGATGAAGGATA = 135 bp (Fragment 2)

Mutant: TT: 20, 135 bp

Table 28: Nucleotide changes, fragment sizes, and genotypes for the rs2032582 allele of the *ABCB1* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=A in both chromosomes (AA)	155	AA
When R=A in one chromosome and R=T in another chromosome (AT)	20, 135, 155	AT
When R=T in both chromosome (TT)	20, 135	TT

3.4.4.3 Linkage between *ABCB1* rs2032582 Polymorphism and Breast Cancer Susceptibility

The digestion fragment patterns for the AA, AT, and TT genotypes are shown in **Fig. 12**. **Table 29** shows that the genotype pattern of the *ABCB1* rs2032582 polymorphism in both case and control populations followed Hardy-Weinberg equilibrium (HWE). All genetic models indicated a reduced risk of BC; however, none reached statistical significance (codominant 1: AT vs. AA: OR = 0.79, 95% CI = 0.51-1.24, p = 0.318; codominant 2: TT vs. AA: OR = 0.82, 95% CI = 0.27-2.49, p = 0.730; dominant: AT+TT vs. AA: OR = 0.80, 95% CI = 0.52-1.22, p = 0.302; recessive: TT vs. AA+AT: OR = 0.86, 95% CI = 0.28-2.59, p = 0.790; over dominant: AT vs. AA+TT: OR = 0.80, 95% CI = 0.51-1.25, p = 0.330; allelic: T vs. A: OR = 0.82, 95% CI = 0.56-1.20, p = 0.312). Overall, these findings indicate that the *ABCB1* rs2032582 polymorphism is not significantly linked with BC risk in Bangladeshi women.

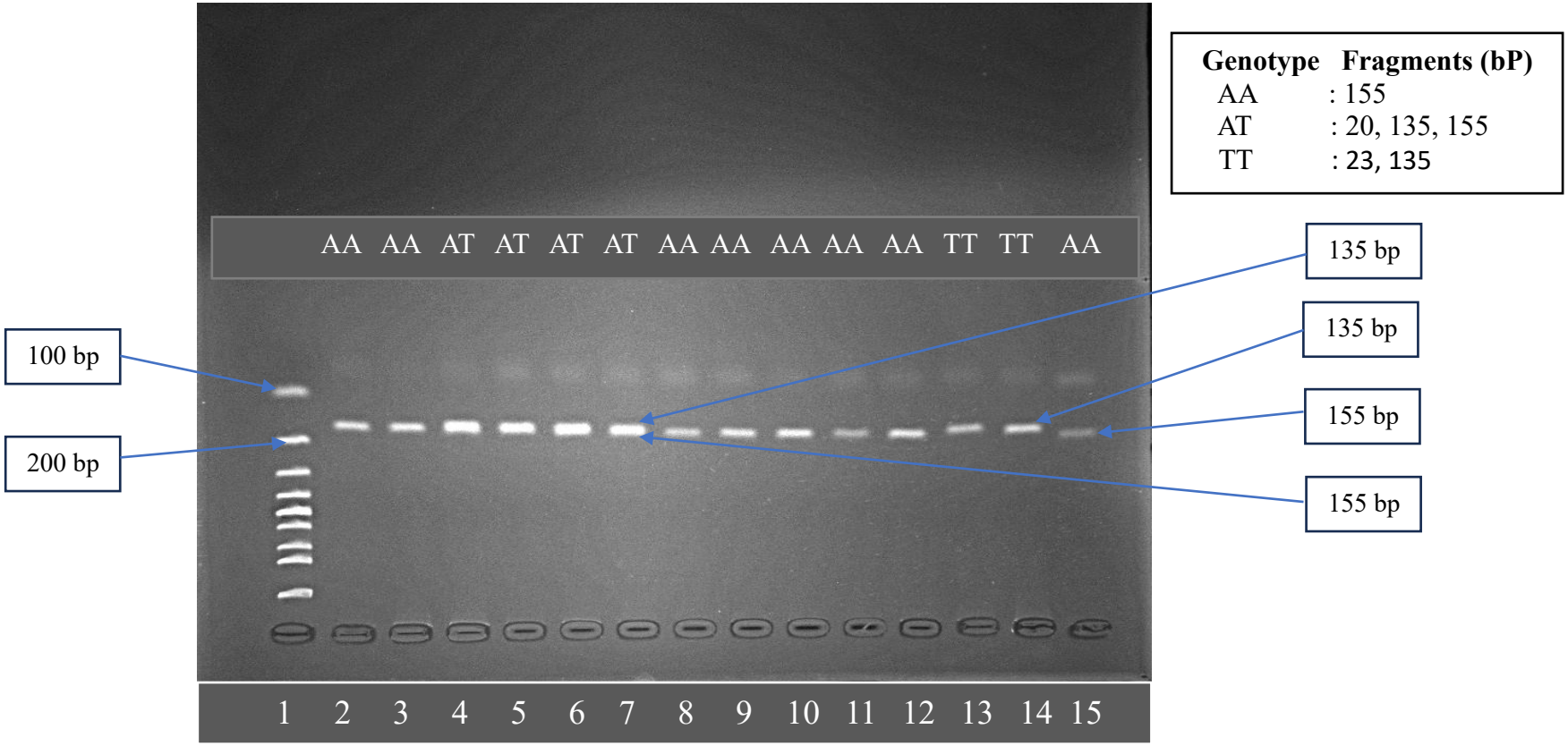


Fig. 12: Restriction Endonuclease (BsrI) digestion fragments of rs2032582 allele after 2.0% agarose gel electrophoresis. Lane-1: 100 bp DNA ladder. Lane-1: 100 bp DNA ladder; Lanes-2, 3, 8, 9, 10, 11, 12, 15: AA; Lanes-4, 5, 6, 7: AT; Lanes-13, 14: TT.

Table 29: Genotypic association of *ABCB1* (rs2032582) variant with BC susceptibility.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% CI)	*p*-value
AA	199 (79.92)			191 (76.09)			1	
Codominant model 1 (AT vs. AA)	44 (17.67)	3.27	0.07	53 (21.12)	1.90	0.167	0.79 (0.51-1.24)	0.318
Codominant model 2 (TT vs. AA)	6 (2.41)			7 (2.79)			0.82 (0.27-2.49)	0.730
Dominant model								
AA	199 (79.92)			191 (76.09)			1	
AT+TT	50 (20.08)			60 (23.91)			0.80 (0.52-1.22)	0.302
Recessive model								
AA+AT	243 (97.59)			244 (97.21)			1	
TT	6 (2.41)			7 (2.79)			0.86 (0.28-2.59)	0.790
Over-dominant model								
AA+TT	205 (82.33)			198 (78.88)			1	
AT	44 (17.67)			53 (21.12)			0.80 (0.51-1.25)	0.330
Allele								
A	442 (88.76)			435 (86.65)			1	
T	56 (11.24)			67 (13.35)			0.82 (0.56-1.20)	0.312

3.4.4.4 Association between *ABCB1* rs2032582 Polymorphism and AC-T Chemotherapy Response

The relationship between *ABCB1* rs2032582 genotypes and clinical responses to AC-T regimen was assessed in 249 BC patients, categorized into AC-paclitaxel and AC-docetaxel subgroups (**Table 30**). In the AC-paclitaxel subgroup, patients carrying the AT+TT genotype exhibited higher clinical response rates than the patients who carried the AA genotype (AT+TT vs. AA: OR = 1.41, 95% CI = 0.72-2.79, p = 0.319), although this correlation was statistically not significant.

Similarly, in the smaller AC-docetaxel subgroup, the patients with AT+TT genotype indicated higher clinical response rates compared with the AA genotype, but without statistical significance (AT+TT vs. AA: OR = 2.12, 95% CI = 0.34-13.13, p = 0.421).

Overall, *ABCB1* rs2032582 genotypes were not significantly linked with clinical response to either AC-paclitaxel or AC-docetaxel chemotherapy regimens.

3.4.4.5 Association between *ABCB1* rs2032582 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The association between *ABCB1* rs2032582 genotypes and the occurrence of toxicities induced by the AC-T chemotherapy was evaluated in women with BC (**Table 31**). In the AC-paclitaxel subgroup, the patients with the AT+TT genotype demonstrated significant linkage with a greater likelihood of several higher-grade toxicities in comparison with the AA genotype, including diarrhoea (OR = 2.82, 95% CI = 1.22-6.50, p = 0.014), fever (OR = 2.21, 95% CI = 1.08-4.53, p = 0.030), fatigue (OR = 2.52, 95% CI = 1.10-5.74, p = 0.027), dysgeusia (OR = 2.07, 95% CI = 1.01-4.22, p = 0.046), myalgia (OR = 2.04, 95% CI = 1.02-4.04, p = 0.042), and hepatotoxicity (OR= 2.63; 95% CI= 1.07-6.45; p= 0.034). Other toxicities in this treatment group did not show statistically significant associations with genotype.

Conversely, in the smaller AC-docetaxel subgroup, no toxicities were significantly associated with genotypes.

Table 30: Impact of *ABCB1* (rs2032582) polymorphisms on the response of AC-T chemotherapy regimen.

Variant	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs2032582	Non-carrier AA (175)	91	84	1 (reference)	-	AA (24)	13	11	1 (reference)	-
	Carrier AT+TT (43)	26	17	1.41 (0.72-2.79)	0.319	AT+TT (7)	5	2	2.12 (0.34-13.13)	0.421

Table 31: Impact of *ABCB1* (rs2032582) polymorphisms on the toxicities induced by AC-T chemotherapy regimen.

Toxicities		**Doxorubicin-cyclophosphamide- paclitaxel (N=218)**		**Doxorubicin-cyclophosphamide- docetaxel (N=31)**	
		Non-carrier AA (175)	**Carrier AT + TT (43)**	**Non-carrier AA (24)**	**Carrier AT + TT (7)**
Nausea	Grade III + IV	11	4	3	1
	Grade ≤ II	164	39	21	6
	Odd ratio (95% CI)	Reference	1.53 (0.46-5.06)	Reference	1.17 (0.10-13.36)
	p-value		0.486		0.901
Vomiting	Grade III + IV	23	10	5	2
	Grade ≤ II	152	33	19	5
	Odd ratio (95% CI)	Reference	2.00 (0.87-4.60)	Reference	1.52 (0.22-10.29)
	p-value		0.102		0.667
Diarrhoea	Grade III + IV	19	11	3	1
	Grade ≤ II	156	32	21	6
	Odd ratio (95% CI)	Reference	2.82 (1.22-6.50)	Reference	1.17 (0.10-13.36)
	p-value		**0.014**		0.901

Constipation	Grade III + IV	10	3	3	2
	Grade ≤ II	165	40	21	5
	Odd ratio (95% CI)	Reference	1.24 (0.33-4.71)	Reference	2.80 (0.36-21.48)
	p-value		0.754		0.322
Fever	Grade III + IV	37	16	2	1
	Grade ≤ II	138	27	22	6
	Odd ratio (95% CI)	Reference	2.21 (1.08-4.53)	Reference	1.83 (0.14-23.82)
	p-value		**0.030**		0.643
Edema	Grade III + IV	25	11	2	0
	Grade ≤ II	150	32	22	7
	Odd ratio (95% CI)	Reference	2.06 (0.92-4.61)	Reference	-
	p-value		0.078		-
Dry mouth	Grade III + IV	8	3	3	1
	Grade ≤ II	167	40	21	6
	Odd ratio (95% CI)	Reference	1.56 (0.39-6.16)	Reference	1.17 (0.10-13.36)
	p-value		0.521		0.901
Mouth sores	Grade III + IV	11	2	4	2
	Grade ≤ II	164	41	20	5
	Odd ratio (95% CI)	Reference	0.73 (0.16-3.41)	Reference	2.00 (0.28-14.20)
	p-value		0.686		0.488
Fatigue	Grade III + IV	21	11	7	4
	Grade ≤ II	154	32	17	3
	Odd ratio (95% CI)	Reference	2.52 (1.10-5.74)	Reference	3.24 (0.57-18.38)
	p-value		**0.027**		0.184
Dysgeusia	Grade III + IV	39	16	11	4
	Grade ≤ II	136	27	13	3
	Odd ratio (95% CI)	Reference	2.07 (1.01-4.22)	Reference	1.57 (0.28-8.61)
	p-value		**0.046**		0.599
Skin hyperpigmentation	Grade III + IV	12	3	3	1
	Grade ≤ II	163	40	21	6
	Odd ratio (95% CI)	Reference	1.02 (0.27-3.78)	Reference	1.17 (0.10-13.36)

	p-value		0.977		0.901
Gastritis	Grade III + IV	10	2	3	1
	Grade ≤ II	165	41	21	6
	Odd ratio (95% CI)	Reference	0.80 (0.17-3.82)	Reference	1.17 (0.10-13.36)
	p-value		0.784		0.901
Peripheral neuropathy	Grade III + IV	23	9	3	1
	Grade ≤ II	152	34	21	6
	Odd ratio (95% CI)	Reference	1.75 (0.74-4.11)	Reference	1.17 (0.10-13.36)
	p-value		0.200		0.901
Myalgia/arthralgia	Grade III + IV	49	19	9	3
	Grade ≤ II	126	24	15	4
	Odd ratio (95% CI)	Reference	2.04 (1.02-4.04)	Reference	1.25 (0.22-6.91)
	p-value		**0.042**		0.798
Anaemia	Grade III + IV	8	1	2	0
	Grade ≤ II	167	42	22	7
	Odd ratio (95% CI)	Reference	0.49 (0.06-4.08)	Reference	-
	p-value		0.515		-
Leukopenia	Grade III + IV	6	1	2	1
	Grade I + II	169	42	22	6
	Odd ratio (95% CI)	Reference	0.67 (0.07-5.72)	Reference	1.83 (0.14-23.82)
	p-value		0.714		0.643
Leukocytosis	Grade III + IV	2	0	0	0
	Grade I + II	173	43	24	7
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	8	2	2	0
	Grade I + II	167	41	22	7
	Odd ratio (95% CI)	Reference	1.02 (0.21-4.98)	Reference	-
	p-value		0.982		-

Febrile neutropenia	Grade III + IV	8	2	1	0
	Grade I + II	167	41	23	7
	Odd ratio (95% CI)	Reference	1.02 (0.21-4.98)	Reference	-
	p-value		0.982		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	175	43	24	7
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	9	1	0	0
	Grade I + II	166	42	24	7
	Odd ratio (95% CI)	Reference	0.44 (0.05-3.56)	Reference	-
	p-value		0.441		-
Lymphocytopenia	Grade III + IV	0	0	0	0
	Grade I + II	175	43	24	7
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	173	43	24	7
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	5	2	0	0
	Grade I + II	170	41	24	7
	Odd ratio (95% CI)	Reference	1.66 (0.31-8.85)	Reference	-
	p-value		0.553		-
Hepatotoxicity	Grade III + IV	16	9	3	1
	Grade I + II	159	34	21	6
	Odd ratio (95% CI)	Reference	2.63 (1.07-6.45)	Reference	1.17 (0.10-13.36)
	p-value		**0.034**		0.901
Nephrotoxicity	Grade III + IV	3	1	0	0
	Grade I + II	172	42	24	7
	Odd ratio (95% CI)	Reference	1.37 (0.14-13.46)	Reference	-

	p-value		0.789		-
Cardiotoxicity	Grade III + IV	2	0	0	0
	Grade I + II	173	43	24	7
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	10	5	2	1
	Grade I + II	165	38	22	6
	Odd ratio (95% CI)	Reference	2.17 (0.70-6.72)	Reference	1.83 (0.14-23.82)
	p-value		0.178		0.643
Bone toxicity	Grade III + IV	21	7	2	0
	Grade I + II	154	36	22	7
	Odd ratio (95% CI)	Reference	1.43 (0.56-3.61)	Reference	-
	p-value		0.454		-

When a zero cell was present, the OR was not estimable.

3.4.5 Genotyping of *ABCB1* rs3213619

The polymorphism of the *ABCB1* rs3213619 variant and its association with BC in Bangladeshi women was investigated using the PCR-RFLP approach.

3.4.5.1 PCR of *ABCB1* rs3213619

PCR product of rs3213619

Forward Primer

Polymorphism of interest
R= A > G

TTTGCGTGCCCCTACCTCGCGCTCCTTGGAACGGCCACCAAGACGTGAAATTT
TGGAAGAAGATACTCCGACTTTAGTGGAAAGACCTAAAGGAAACGAACAGCG
GCCTCTGCTTCTTTGAGCTTGGAAGAGCCGCTRCTCGAATGAGCTCAGGCTTC
CTGTGGCAAAGAGAGCGAAGCGGCTGTGCTCAGCCCACGCCCCGGCGCTGTT
CCTGCCCAGCCAATCAGCCTCACCACAGATGACTGCTCCCGGCCCGGATTGAC
TGAATGCTGA = 273 bp

Reverse Primer

Forward Primer (FP): 5'-TTTGCGTGCCCCTACCTC-3'
Reverse Primer (RP): 5'-TCAGCATTCAGTCAATCCGG-3'

After PCR amplification, the PCR product of the rs2032582 allele of the *ABCB1* gene was visualized on a 1.5 % (w/v) agarose gel that was stained with ethidium bromide (**Fig. 13**).

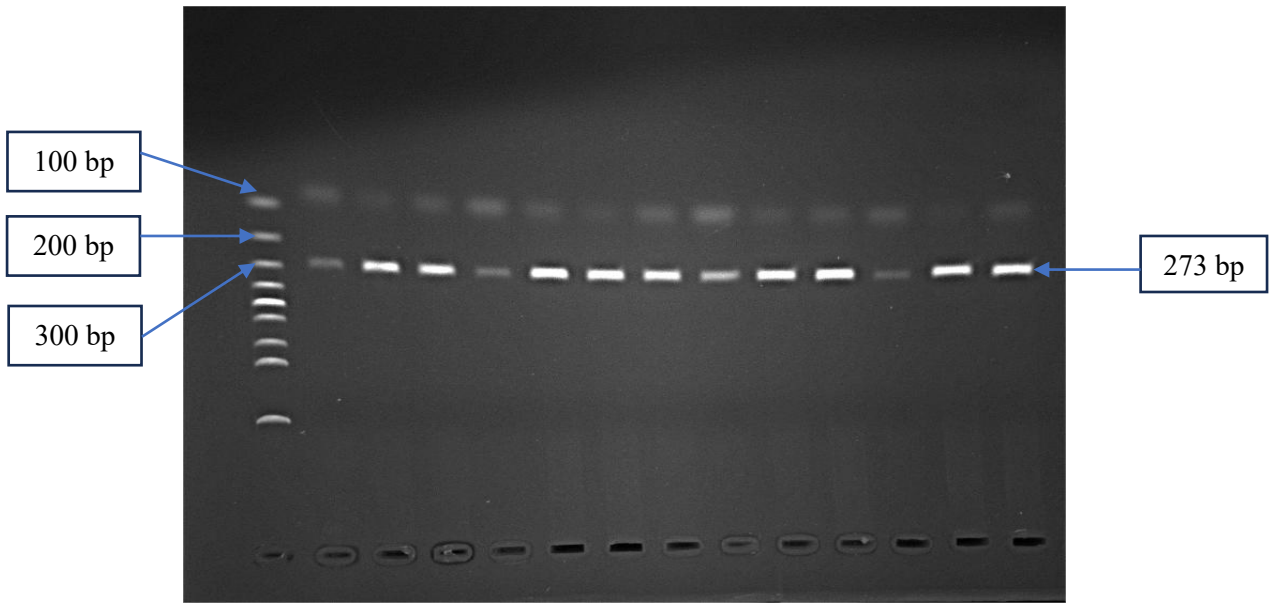


Fig. 13: Amplification band of rs3213619 (*ABCB1*) allele after 1.5% agarose gel electrophoresis.

3.4.5.2 RFLP Fragmentation Pattern of *ABCB1* rs3213619

Table 32: Restriction enzyme along with its digestion sites for *ABCB1* rs3213619.

Restriction Enzyme	Sites of Digestion
MspA1I	Cutting Site 5'... C M G ▼ C K G ... 3' 3'... G K C ▲ G M C ... 5' Cutting Site

Here, M= A or C; K= G or T

When R= A in both copies of the gene, there is one cutting site and we will get 2 fragments.

Cutting site

When R= A in both chromosomes

TTTGCGTGCCCCTACCTCGCGCTCCTTGGAACGGCCACCAAGACGTGAA
ATTTTGGAAGAAGATACTCCGACTTTAGTGGAAAGACCTAAAGGAAACG
AACAGCGGCCTCTGCTTCTTTGAGCTTGGAAGAGCCGCTACTCGAATGA
GCTCAGGCTTCCTGTGGCAAAGAGAGCGAAGCGGCTGTGCTCAGCCCA
CGCCCCGGCGCTGTTCCTGCCCAGCCAATCAGCCTCACCACAGATGACT
GCTCCCGGCCCGGATTGACTGAATGCTGA

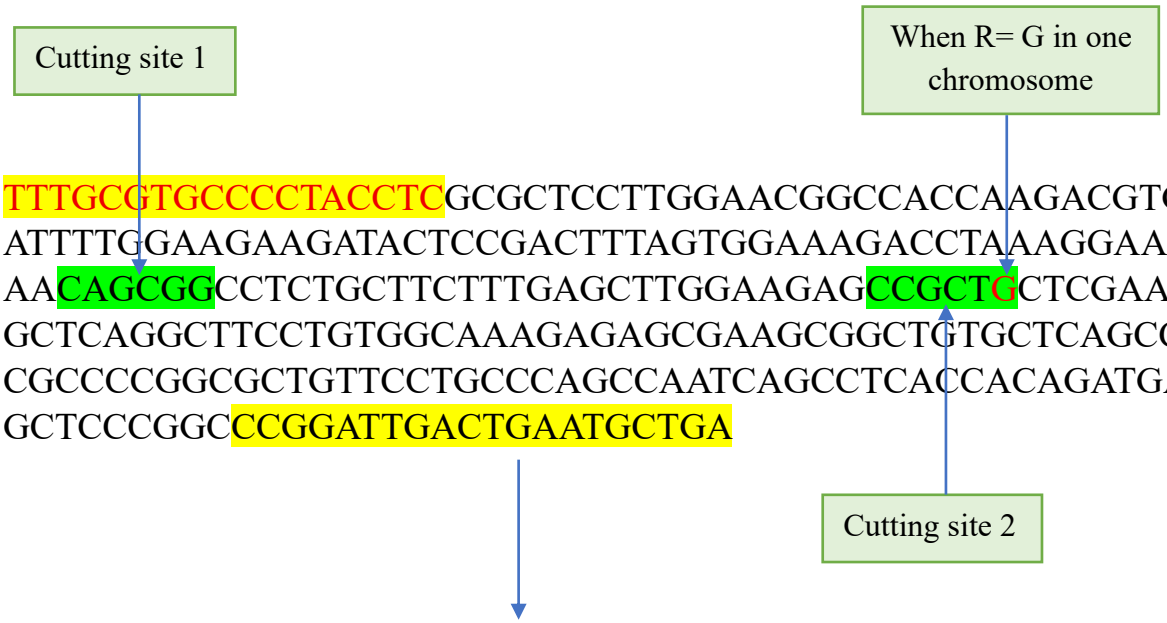
MspA1I

TTTGCGTGCCCCTACCTCGCGCTCCTTGGAACGGCCACCAAGACGTGAA
ATTTTGGAAGAAGATACTCCGACTTTAGTGGAAAGACCTAAAGGAAACG
AACAG = 103 bp

CGGCCTCTGCTTCTTTGAGCTTGGAAGAGCCGCTACTCGAATGAGCTCA
GGCTTCCTGTGGCAAAGAGAGCGAAGCGGCTGTGCTCAGCCCACGCCC
CGGCGCTGTTCCTGCCCAGCCAATCAGCCTCACCACAGATGACTGCTCC
CGGCCCGGATTGACTGAATGCTGA = 170 bp

Wild: AA: 103, 170 bp

When R=A in one copy of the gene and R=G in another copy of the gene, there are two cutting site and we will get 4 fragments.



TTTGCGTGCCCCTACCTCGCGCTCCTTGGAACGGCCACCAAGACGTGAA
ATTTTGGAAGAAGATACTCCGACTTTAGTGGAAAGACCTAAAGGAAACG
AACAG = 103 bp

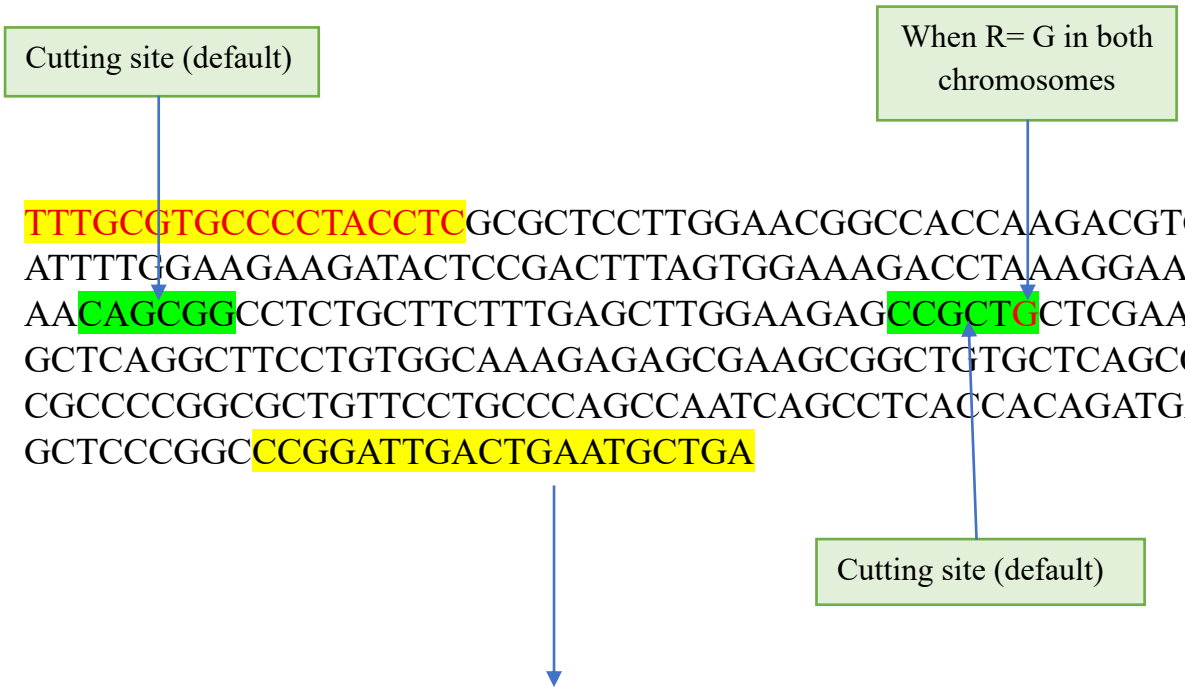
CGGCCTCTGCTTCTTTGAGCTTGGAAGAGCCG = 32 bp

CTGCTCGAATGAGCTCAGGCTTCCTGTGGCAAAGAGAGCGAAGCGGCT
GTGCTCAGCCCACGCCCCGGCGCTGTTCCTGCCCAGCCAATCAGCCTCA
CCACAGATGACTGCTCCCGGCCCGGATTGACTGAATGCTGA = 138 bp

CGGCCTCTGCTTCTTTGAGCTTGGAAGAGCCGCTGCTCGAATGAGCTCA
GGCTTCCTGTGGCAAAGAGAGCGAAGCGGCTGTGCTCAGCCCACGCCC
CGGCGCTGTTCCTGCCCAGCCAATCAGCCTCACCACAGATGACTGCTCC
CGGCCCGGATTGACTGAATGCTGA = 170 bp

Hetero: AG: 32, 103, 138, 170 bp

When R= G in both copies of the gene, we will get 3 fragments.



TTTGCGTGCCCCTACCTCGCGCTCCTTGGAACGGCCACCAAGACGTGAA
ATTTTGGAAGAAGATACTCCGACTTTAGTGGAAAGACCTAAAGGAAACG
AACAG = 103 bp

CGGCCTCTGCTTCTTTGAGCTTGGAAGAGCCG = 32 bp

CTGCTCGAATGAGCTCAGGCTTCCTGTGGCAAAGAGAGCGAAGCGGCT
GTGCTCAGCCCACGCCCCGGCGCTGTTCCTGCCCAGCCAATCAGCCTCA
CCACAGATGACTGCTCCCGGCCCGGATTGACTGAATGCTGA = 138 bp

Mutant: GG: 32, 103, 138 bp

Table 33: Nucleotide changes, fragment sizes, and genotypes for the rs3213619 allele of the *ABCB1* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=A in both chromosomes (AA)	103, 170	AA
When R=A in one chromosome and R=G in another chromosome (AG)	32, 103, 138, 170	AG
When R=G in both chromosome (GG)	32, 103, 138	GG

3.4.5.3 Linkage between *ABCB1* rs3213619 Polymorphism and Breast Cancer Susceptibility

The digestion fragment patterns for the AA, AG, and GG genotypes are shown in **Fig. 14**. As summarized in **Table 34**, the genotype pattern of the *ABCB1* rs3213619 polymorphism in both case and control categories was in line with Hardy-Weinberg equilibrium (HWE). All the genetic models showed that Bangladeshi women had a higher chance of getting BC; however, none reached statistical significance (codominant 1: AG vs. AA: OR = 1.31, 95% CI = 0.77-2.23, p = 0.320; codominant 2: GG vs. AA: OR = 1.40, 95% CI = 0.31-6.32, p = 0.664; dominant: AG+GG vs. AA: OR = 1.32, 95% CI = 0.79-2.19, p = 0.286; recessive: GG vs. AA+AG: OR = 1.35, 95% CI = 0.30-6.09, p = 0.696; over-dominant: AG vs. AA+GG: OR = 1.30, 95% CI = 0.77-2.22, p = 0.329; allelic: G vs. A: OR = 1.30, 95% CI = 0.81-2.08, p = 0.270). Overall, these results indicate that the *ABCB1* rs3213619 variant is not substantially linked with BC risk in Bangladeshi women.

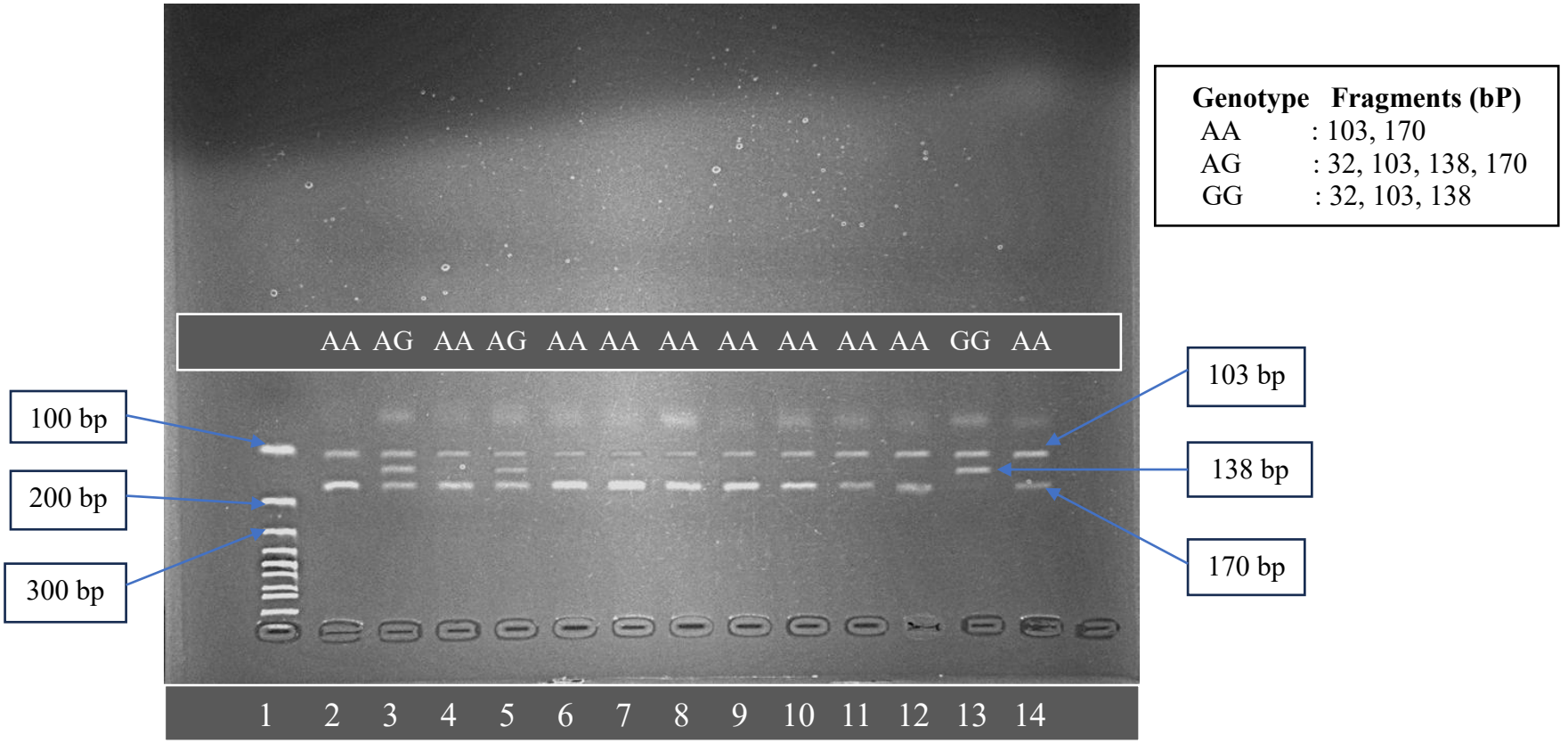


Fig. 14: Restriction Endonuclease (MspA1I) digestion fragments of rs3213619 allele after 2.0 % agarose gel. Lane-1: 100 bp DNA ladder; Lanes-2, 4, 6, 7, 8, 9, 10, 11, 12, 14: AA; Lanes-3, 5: AG; Lane: 13: GG.

Table 34: Genotypic association of *ABCB1* rs3213619 variant with BC risk.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
AA	210 (84.34)			220 (87.65)			1	
Codominant model 1 (AG vs. AA)	35 (14.05)	2.96	0.08	28 (11.16)	3.41	0.06	1.31 (0.77-2.23)	0.320
Codominant model 2 (GG vs. AA)	4 (1.61)			3 (1.19)			1.40 (0.31-6.32)	0.664
Dominant model								
AA	210 (84.34)			220 (87.65)			1	
AG+GG	39 (15.66)			31 (12.35)			1.32 (0.79-2.19)	0.286
Recessive model								
AA+AG	245 (98.39)			248 (98.81)			1	
GG	4 (1.61)			3 (1.19)			1.35 (0.30-6.09)	0.696
Over-dominant model								
AA+GG	214 (85.95)			223 (88.84)			1	
AG	35 (14.05)			28 (11.16)			1.30 (0.77-2.22)	0.329
Allele								
A	455 (91.37)			468 (93.23)			1	
G	43 (8.63)			34 (6.77)			1.30 (0.81-2.08)	0.270

3.4.5.4 Association between *ABCB1* rs3213619 Polymorphism and AC-T Chemotherapy Response

Table 35 illustrates the connection between *ABCB1* rs3213619 genotypes and clinical responses to AC-T regimen among 249 BC patients, divided into AC-paclitaxel and AC-docetaxel subgroups. In the AC-paclitaxel subgroup, patients having the AG+GG genotype showed higher clinical response rates than the patients having the AA genotype (AG+GG vs. AA: OR = 1.23, 95% CI = 0.57-2.66, p = 0.596). However, the correlation did not appear statistically noteworthy.

In contrast, in the smaller AC-docetaxel subgroup, the AG+GG genotype indicated lower clinical response rates compared with the AA genotype; this correlation also did not appear to be statistically noteworthy (AG+GG vs. AA: OR = 0.64, 95% CI = 0.13-3.25, p = 0.592).

Overall, *ABCB1* rs3213619 genotypes had no significant association with clinical responses to either AC-paclitaxel or AC-docetaxel chemotherapy regimens.

3.4.5.5 Association between *ABCB1* rs3213619 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The association between *ABCB1* rs3213619 genotypes and the incidence of toxicities induced by the AC-T chemotherapy was evaluated in BC women (**Table 36**). In the AC-paclitaxel subgroup, the carrier patients having the AG+GG genotype were significantly related to a greater risk of several higher-grade toxicities than those with the AA genotype, including diarrhea (OR= 2.61; 95% CI= 1.04-6.54; p= 0.040), fever (OR= 2.25; 95% CI= 1.01-5.01; p= 0.047), mouth sores (OR = 4.30, 95% CI = 1.31-14.15, p = 0.016), dysgeusia (OR = 2.93, 95% CI = 1.33-6.44, p = 0.007), and peripheral neuropathy (OR = 2.91, 95% CI = 1.20-7.10, p = 0.018). There were no statistically noteworthy links between genotypes and other toxicities in this subgroup.

Conversely, in the smaller AC-docetaxel subgroup, no toxicities were significantly associated with genotypes.

Table 35: Impact of *ABCB1* rs3213619 polymorphisms on the response of AC-T chemotherapy regimen.

Variants	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs3213619	Non-carrier AA (187)	99	88	1 (reference)	-	AA (23)	14	9	1 (reference)	-
	Carrier AG+GG (31)	18	13	1.23 (0.57-2.66)	0.596	AG+GG (8)	4	4	0.64 (0.13-3.25)	0.592

Table 36: Impact of *ABCB1* rs3213619 polymorphisms on the toxicities induced by AC-T chemotherapy regimen.

Toxicities		**Doxorubicin-cyclophosphamide- paclitaxel (N=218)**		**Doxorubicin-cyclophosphamide- docetaxel (N=31)**	
		Non-carrier AA (187)	**Carrier AG + GG (31)**	**Non-carrier AA (23)**	**Carrier AG + GG (8)**
Nausea	Grade III + IV	11	4	3	1
	Grade ≤ II	176	27	20	7
	Odd ratio (95% CI)	Reference	2.37 (0.70-7.98)	Reference	0.95 (0.08-10.72)
	p-value		0.163		0.968
Vomiting	Grade III + IV	27	6	5	2
	Grade ≤ II	160	25	18	6
	Odd ratio (95% CI)	Reference	1.42 (0.53-3.78)	Reference	1.20 (0.18-7.88)
	p-value		0.481		0.849
Diarrhoea	Grade III + IV	22	8	2	2
	Grade ≤ II	165	23	21	6
	Odd ratio (95% CI)	Reference	2.61 (1.04-6.54)	Reference	3.50 (0.40-30.34)
	p-value		**0.040**		0.255

Constipation	Grade III + IV	10	3	3	2
	Grade ≤ II	177	28	20	6
	Odd ratio (95% CI)	Reference	1.90 (0.49-7.32)	Reference	2.22 (0.30-16.56)
	p-value		0.353		0.435
Fever	Grade III + IV	41	12	2	1
	Grade ≤ II	146	19	21	7
	Odd ratio (95% CI)	Reference	2.25 (1.01-5.01)	Reference	1.50 (0.12-19.18)
	p-value		**0.047**		0.755
Edema	Grade III + IV	29	7	1	1
	Grade ≤ II	158	24	22	7
	Odd ratio (95% CI)	Reference	1.59 (0.63-4.03)	Reference	3.14 (0.17-57.08)
	p-value		0.329		0.438
Dry mouth	Grade III + IV	10	1	2	2
	Grade ≤ II	177	30	21	6
	Odd ratio (95% CI)	Reference	0.59 (0.07-4.77)	Reference	3.50 (0.40-30.34)
	p-value		0.621		0.255
Mouth sores	Grade III + IV	8	5	5	1
	Grade ≤ II	179	26	18	7
	Odd ratio (95% CI)	Reference	4.30 (1.31-14.15)	Reference	0.51 (0.05-5.22)
	p-value		**0.016**		0.573
Fatigue	Grade III + IV	26	6	9	2
	Grade ≤ II	161	25	14	6
	Odd ratio (95% CI)	Reference	1.48 (0.55-3.97)	Reference	0.52 (0.08-3.15)
	p-value		0.429		0.476
Dysgeusia	Grade III + IV	41	14	11	4
	Grade ≤ II	146	17	12	4
	Odd ratio (95% CI)	Reference	2.93 (1.33-6.44)	Reference	1.09 (0.22-5.45)
	p-value		**0.007**		0.915
Skin hyperpigmentation	Grade III + IV	12	3	3	1
	Grade ≤ II	175	28	20	7
	Odd ratio (95% CI)	Reference	1.56 (0.41-5.88)	Reference	0.95 (0.08-10.72)

	p-value		0.509		0.968
Gastritis	Grade III + IV	10	2	3	1
	Grade ≤ II	177	29	20	7
	Odd ratio (95% CI)	Reference	1.22 (0.25-5.85)	Reference	0.95 (0.08-10.72)
	p-value		0.803		0.968
Peripheral neuropathy	Grade III + IV	23	9	3	1
	Grade ≤ II	164	22	20	7
	Odd ratio (95% CI)	Reference	2.91 (1.20-7.10)	Reference	0.95 (0.08-10.72)
	p-value		**0.018**		0.968
Myalgia/arthralgia	Grade III + IV	55	13	9	3
	Grade ≤ II	132	18	14	5
	Odd ratio (95% CI)	Reference	1.73 (0.79-3.78)	Reference	0.93 (0.18-4.90)
	p-value		0.166		0.935
Anaemia	Grade III + IV	8	1	2	0
	Grade ≤ II	179	30	21	8
	Odd ratio (95% CI)	Reference	0.74 (0.09-6.18)	Reference	-
	p-value		0.785		-
Leukopenia	Grade III + IV	7	0	2	1
	Grade I + II	180	31	21	7
	Odd ratio (95% CI)	Reference	-	Reference	1.50 (0.12-19.18)
	p-value		-		0.755
Leukocytosis	Grade III + IV	2	0	0	0
	Grade I + II	185	31	23	8
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	9	1	2	0
	Grade I + II	178	30	21	8
	Odd ratio (95% CI)	Reference	0.66 (0.08-5.39)	Reference	-
	p-value		0.697		-

Febrile neutropenia	Grade III + IV	9	1	1	0
	Grade I + II	178	30	22	8
	Odd ratio (95% CI)	Reference	0.66 (0.08-5.39)	Reference	-
	p-value		0.697		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	187	31	23	8
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	8	2	0	0
	Grade I + II	179	29	23	8
	Odd ratio (95% CI)	Reference	1.54 (0.31-7.63)	Reference	-
	p-value		0.594		-
Lymphocytopenia	Grade III + IV	0	0	0	0
	Grade I + II	187	31	23	8
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	185	31	23	8
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	6	1	0	0
	Grade I + II	181	30	23	8
	Odd ratio (95% CI)	Reference	1.01 (0.12-8.65)	Reference	-
	p-value		0.996		-
Hepatotoxicity	Grade III + IV	20	5	3	1
	Grade I + II	167	26	20	7
	Odd ratio (95% CI)	Reference	1.61 (0.55-4.65)	Reference	0.95 (0.08-10.73)
	p-value		0.382		0.968
Nephrotoxicity	Grade III + IV	3	1	0	0
	Grade I + II	184	30	23	8
	Odd ratio (95% CI)	Reference	2.04 (0.21-20.31)	Reference	-

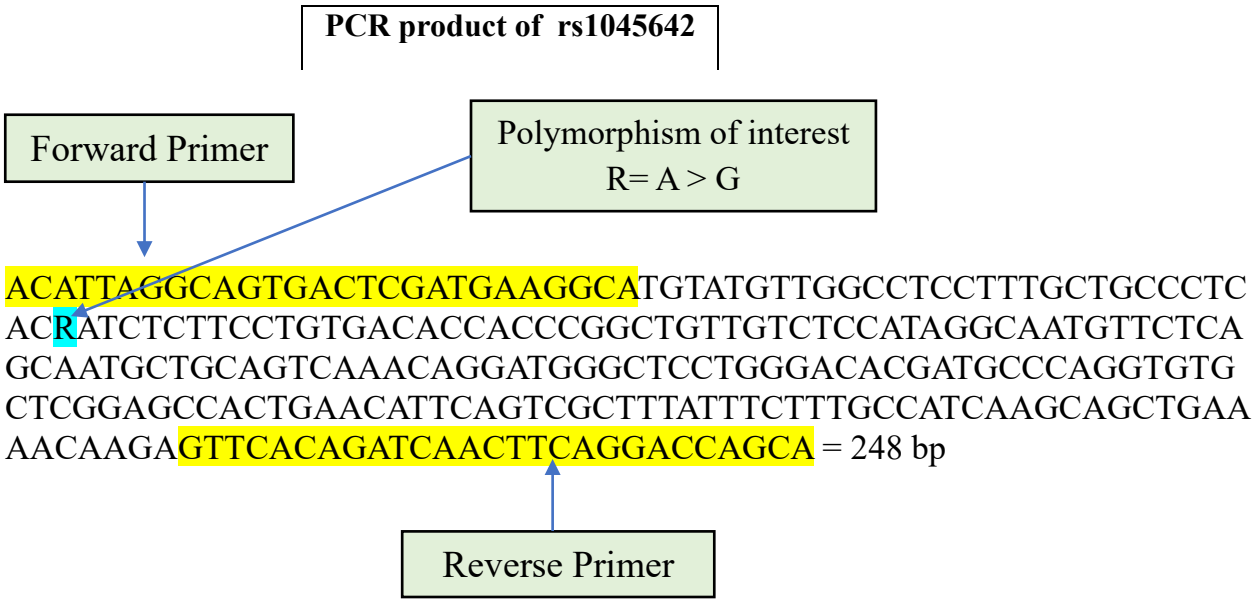
	p-value		0.541		-
Cardiotoxicity	Grade III + IV	2	0	0	0
	Grade I + II	185	31	23	8
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	11	4	2	1
	Grade I + II	176	27	21	7
	Odd ratio (95% CI)	Reference	2.37 (0.70-7.98)	Reference	1.50 (0.12-19.18)
	p-value		0.163		0.755
Bone toxicity	Grade III + IV	22	6	1	1
	Grade I + II	165	25	22	7
	Odd ratio (95% CI)	Reference	1.80 (0.66-4.87)	Reference	3.14 (0.17-57.08)
	p-value		0.247		0.438

When a zero cell was present, the OR was not estimable.

3.4.6 Genotyping of *ABCB1* rs1045642

The polymorphism of the *ABCB1* rs1045642 variant and its linkage with BC in Bangladeshi women was demonstrated using the PCR-RFLP approach.

3.4.6.1 PCR of *ABCB1* rs1045642



Forward Primer (FP): 5'-ACATTAGGCAGTGACTCGATGAAGGCA-3'
Reverse Primer (RP): 5'-TGCTGGTCCTGAAGTTGATCTGTGAAC-3'

After PCR amplification, the PCR product of the rs1045642 allele of the *ABCB1* gene was visualized on a 1.5 % (w/v) agarose gel which was stained with ethidium bromide (**Fig. 15**).

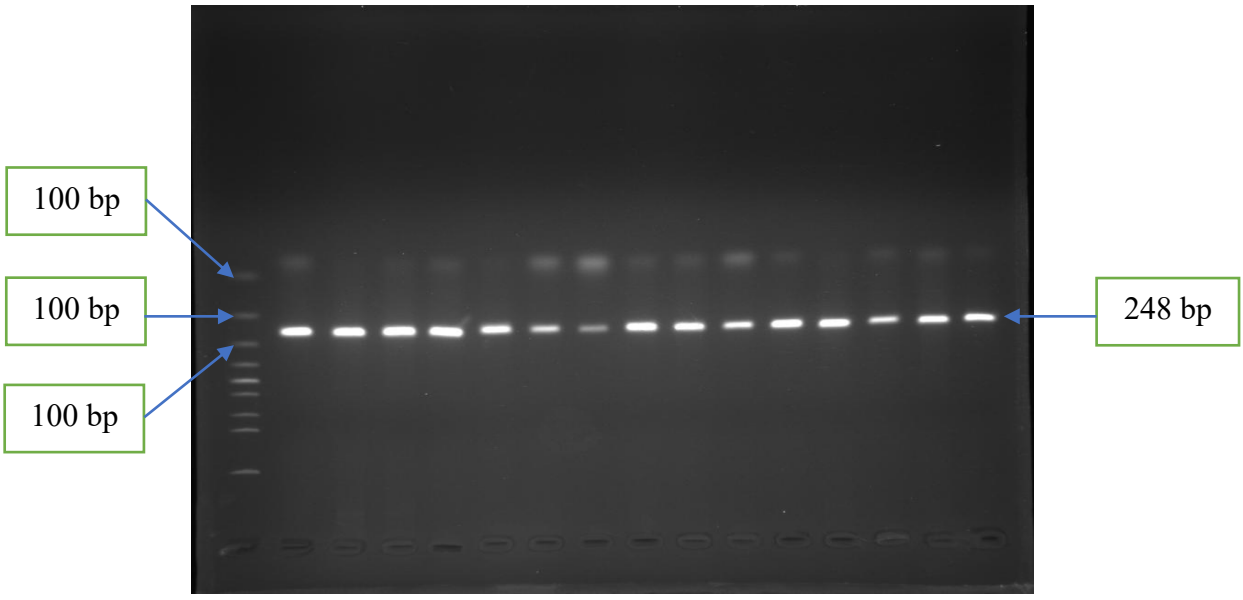


Fig. 15: Amplification band of rs1045642 (*ABCB1*) allele after 1.5% agarose gel electrophoresis.

3.4.6.2 RFLP Fragmentation Pattern of *ABCB1* rs1045642

Table 37: Restriction enzyme along with its digestion sites for *ABCB1* rs1045642.

Restriction Enzyme	Sites of Digestion
MboI	Cutting Site 5'... G A T C ... 3' 3'... C T A G ... 5'

When R= A in both copies of the gene, there are one cutting site and we will get 2 fragments.

When R= A in both chromosomes

ACATTAGGCAGTGACTCGATGAAGGCATGTATGTTGGCCTCCTTTGCTGCCCTC
ACAATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAATGTTCTCA
GCAATGCTGCAGTCAAACAGGATGGGCTCCTGGGACACGATGCCCAGGTGTG
CTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGCCATCAAGCAGCTGAA

AACAAGAGTTCACAGATCAACTTCAGGACCAGCA

Cutting site

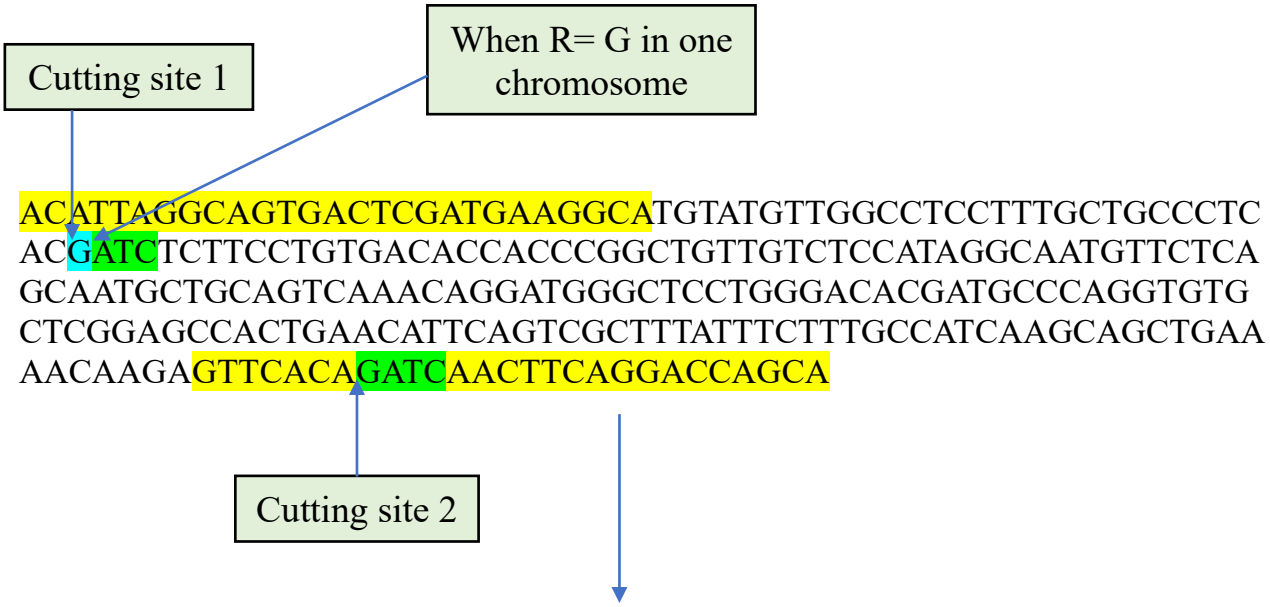
MboI

ACATTAGGCAGTGACTCGATGAAGGCATGTATGTTGGCCTCCTTTGCTGC
CCTCACAATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAA
TGTTCTCAGCAATGCTGCAGTCAAACAGGATGGGCTCCTGGGACACGAT
GCCCAGGTGTGCTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGC
CATCAAGCAGCTGAAAACAAGAGTTCACA = 228 bp (Fragment 1)

GATCAACTTCAGGACCAGCA = 20 bp (fragment 2)

Wild: AA: 20, 228 bp

When R=A in one copy of the gene and R=G in another copy of the gene, there is two cutting sites and we will get 4 fragments.



ACATTAGGCAGTGACTCGATGAAGGCATGTATGTTGGCCTCCTTTGCTGC
CCTCAC = 56 bp (Fragment 1)

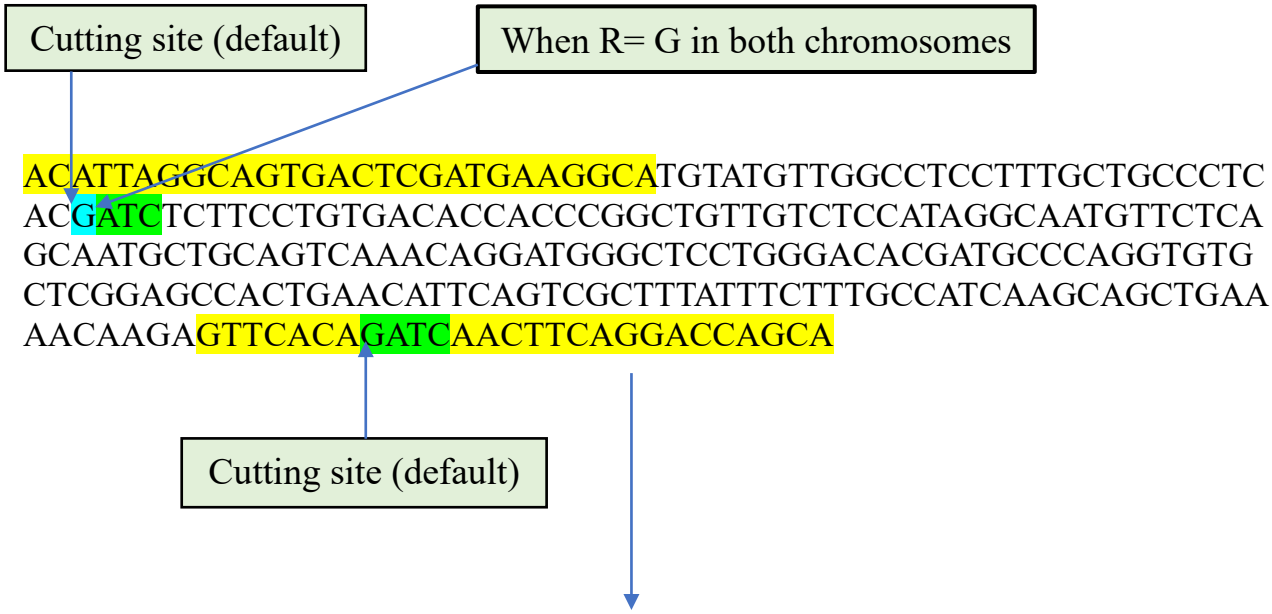
GATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAATGTTCT
CAGCAATGCTGCAGTCAAACAGGATGGGCTCCTGGGACACGATGCCCA
GGTGTGCTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGCCATCA
AGCAGCTGAAAACAAGAGTTCACA = 172 bp (Fragment 2)

GATCAACTTCAGGACCAGCA = 20 bp (Fragment 3)

GATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAATGTTCT
CAGCAATGCTGCAGTCAAACAGGATGGGCTCCTGGGACACGATGCCCA
GGTGTGCTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGCCATCA
AGCAGCTGAAAACAAGAGTTCACAGATCAACTTCAGGACCAGCA = 192
bp (Fragment 4)

Hetero: AG: 20, 56, 172, 192

When R= G in both copies of the gene, we will get 3 fragments.



ACATTAGGCAGTGACTCGATGAAGGCATGTATGTTGGCCTCCTTTGCTGC
CCTCAC = 56 bp (Fragment 1)

GATCTCTTCCTGTGACACCACCCGGCTGTTGTCTCCATAGGCAATGTTCT
CAGCAATGCTGCAGTCAAACAGGATGGGCTCCTGGGACACGATGCCCA
GGTGTGCTCGGAGCCACTGAACATTCAGTCGCTTTATTTCTTTGCCATCA
AGCAGCTGAAAACAAGAGTTCACA = 172 bp (Fragment 2)

GATCAACTTCAGGACCAGCA = 20 bp (Fragment 3)

Mutant: GG: 20, 56, 172 bp

Table 38: Nucleotide changes, fragment sizes, and genotypes for the rs1045642 allele of the *ABCB1* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=A in both chromosomes (AA)	20, 228	AA
When R=A in one chromosome and R=G in another chromosome (AG)	20, 56, 172, 192	AG
When R=G in both chromosome (GG)	20, 56, 172	GG

3.4.6.3 Linkage between *ABCB1* rs1045642 Polymorphism and Breast Cancer Susceptibility

The digestion patterns for the AA, AG, and GG genotypes are shown in **Fig. 16**. As shown in **Table 39**, the genotypic pattern of the *ABCB1* rs1045642 polymorphism in both case and control populations was in line with Hardy-Weinberg equilibrium (HWE). In Bangladeshi women, the codominant model 2 and the dominant model, as well as the allelic model, indicated a substantially higher risk of BC (codominant model 2: GG vs. AA, OR = 1.98, 95% CI = 1.09-3.60, p = 0.024; dominant model: AG + GG vs. AA: OR = 1.45, 95% CI = 1.01-2.09, p = 0.042; allelic model: G vs. A: OR = 1.36, 95% CI = 1.05-1.76, p = 0.019). The codominant model 1, recessive model, and over dominant model also suggested a greater risk (codominant model 1: AG vs. AA: OR = 1.35, 95% CI = 0.93-1.97, p = 0.116; recessive model: GG vs. AA + AG: OR = 1.68, 95% CI = 0.96-2.92, p = 0.068; over dominant model: AG vs. AA + GG: OR = 1.15, 95% CI = 0.81-1.64, p = 0.421), although these results were not statistically noteworthy. Overall, Bangladeshi women with the rs1045642 *ABCB1* allele are significantly more likely to develop BC.

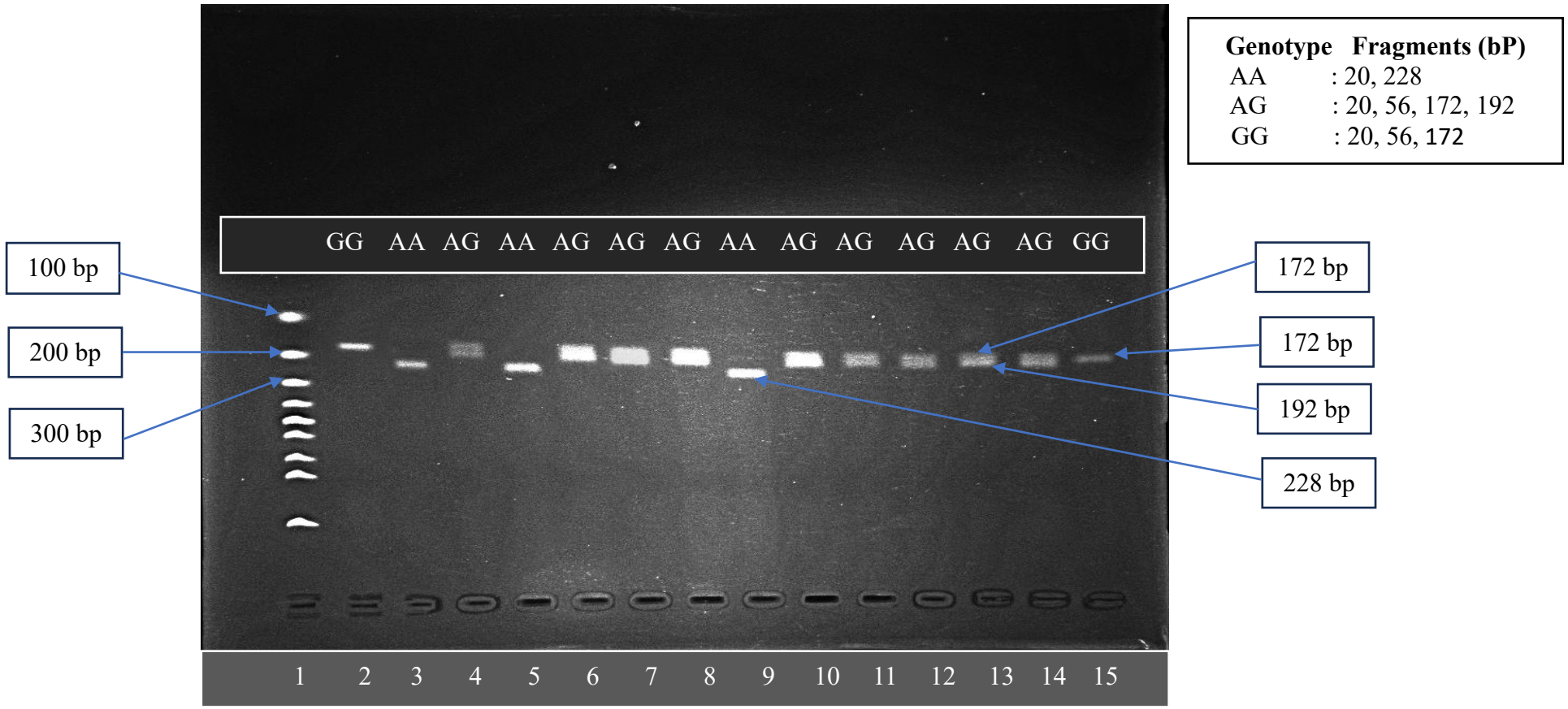


Fig. 16: Restriction Endonuclease (MboI) digestion fragments of rs1045642 allele after 2.0 % agarose gel. Lane-1: 100 bp DNA ladder; Lanes-3, 5, 9: AA; Lanes-4, 6, 7, 8, 10, 11, 12, 13, 14: AG; Lanes-2, 15: GG.

Table 39: Genotypic association of *ABCB1* rs1045642 variant with BC susceptibility.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
AA	86 (34.54)			109 (43.43)			1	
Codominant model 1 (AG vs. AA)	127 (51.00)	0.98	0.320	119 (47.41)	1.39	0.239	1.35 (0.93-1.97)	0.116
Codominant model 2 (GG vs. AA)	36 (14.46)			23 (9.16)			1.98 (1.09-3.60)	**0.024**
Dominant model								
AA	86 (34.54)			109 (43.43)			1	
AG+GG	163 (65.46)			142 (56.57)			1.45 (1.01-2.09)	**0.042**
Recessive model								
AA+AG	213 (85.54)			228 (90.84)			1	
GG	36 (14.46)			23 (9.16)			1.68 (0.96-2.92)	0.068
Over-dominant model								
AA+GG	122 (49.00)			132 (52.59)			1	
AG	127 (51.00)			119 (47.41)			1.15 (0.81-1.64)	0.421
Allele								
A	299 (60.04)			337 (67.13)			1	
G	199 (39.96)			165 (32.87)			1.36 (1.05-1.76)	**0.019**

3.4.6.4 Association between *ABCB1* rs1045642 Polymorphism and AC-T Chemotherapy Response

The linkage between *ABCB1* rs1045642 genotypes and clinical responses to AC-T chemotherapy was assessed in 249 BC patients, stratified into AC-paclitaxel and AC-docetaxel treatment subgroups (**Table 40**). In the AC-paclitaxel subgroup, carrier patients with the AG+GG genotype exhibited a significantly lower likelihood of clinical responses in comparison with the AA genotype (AG+GG vs. AA: OR = 0.55, 95% CI = 0.31-0.98, p = 0.040).

Similarly, in the smaller AC-docetaxel subgroup, the patients having the AG+GG genotype also indicated lower clinical responses; but this association didn't reach statistical significance (AG+GG vs. AA: OR = 0.23, 95% CI = 0.04-1.33, p = 0.101).

Overall, the *ABCB1* rs1045642 polymorphism was substantially linked with reduced clinical response to the AC-paclitaxel regimen, but not to AC-docetaxel.

3.4.6.5 Association between *ABCB1* rs1045642 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The relationship between *ABCB1* rs1045642 genotypes and the incidence of toxicities induced by the AC-T chemotherapy was evaluated among BC patients, as shown in **Table 41**. Among patients treated with the AC-paclitaxel regimen, the AG+GG genotype unveiled significant linkage with a greater risk of several higher-grade toxicities in contrast to the AA genotype. These included vomiting (OR= 2.74, 95% CI= 1.08-6.96, p=0.034), fever (OR = 3.34, 95% CI= 1.53-7.30, p=0.002), dysgeusia (OR = 2.32, 95% CI= 1.13-4.72, p=0.020), peripheral neuropathy (OR= 2.61, 95% CI= 1.03-6.67, p= 0.044), myalgia (OR= 1.95, 95% CI= 1.02-3.68, p= 0.041), and hepatotoxicity (OR = 3.12, 95% CI= 1.03-9.46, p=0.044). Other toxicities under the AC-paclitaxel regimen did not show statistically noteworthy correlations across genotypes.

Conversely, in the smaller cohort treated with AC-docetaxel, no toxicities demonstrated significant correlations with genotypes.

Table 40: Impact of *ABCB1* rs1045642 polymorphisms on the response of AC-T chemotherapy regimen.

Variants	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs1045642	Non-carrier AA (76)	48	28	1 (reference)	-	AA (10)	8	2	1 (reference)	-
	Carrier AG+GG (142)	69	73	0.55 (0.31-0.98)	**0.040**	AG+GG (21)	10	11	0.23 (0.04-1.33)	0.101

Table 41: Impact of *ABCB1* rs1045642 polymorphisms on the toxicities induced by AC-T chemotherapy regimen.

Toxicities		Doxorubicin-cyclophosphamide-paclitaxel (N=218)		Doxorubicin-cyclophosphamide-docetaxel (N=31)	
		Non-carrier AA (76)	**Carrier AG + GG (142)**	**Non-carrier AA (10)**	**Carrier AG + GG (21)**
Nausea	Grade III + IV	4	11	1	3
	Grade ≤ II	72	131	9	18
	Odd ratio (95% CI)	Reference	1.51 (0.46-4.92)	Reference	1.50 (0.13-16.54)
	p-value		0.492		0.740
Vomiting	Grade III + IV	6	27	1	6
	Grade ≤ II	70	115	9	15
	Odd ratio (95% CI)	Reference	2.74 (1.08-6.96)	Reference	3.60 (0.37-34.93)
	p-value		**0.034**		0.269
Diarrhoea	Grade III + IV	6	24	1	3
	Grade ≤ II	70	118	9	18
	Odd ratio (95% CI)	Reference	2.37 (0.92-6.08)	Reference	1.50 (0.13-16.54)
	p-value		0.072		0.740

Constipation	Grade III + IV	2	10	1	4
	Grade ≤ II	74	132	9	17
	Odd ratio (95% CI)	Reference	2.80 (0.60-13.13)	Reference	2.12 (0.20-21.88)
	p-value		0.190		0.528
Fever	Grade III + IV	9	44	1	2
	Grade ≤ II	67	98	9	19
	Odd ratio (95% CI)	Reference	3.34 (1.53-7.30)	Reference	0.95 (0.07-11.87)
	p-value		**0.002**		0.966
Edema	Grade III + IV	12	24	1	1
	Grade ≤ II	64	118	9	20
	Odd ratio (95% CI)	Reference	1.08 (0.51-2.31)	Reference	0.45 (0.02-8.02)
	p-value		0.833		0.587
Dry mouth	Grade III + IV	2	9	2	2
	Grade ≤ II	74	133	8	19
	Odd ratio (95% CI)	Reference	2.50 (0.53-11.90)	Reference	0.42 (0.05-3.53)
	p-value		0.248		0.425
Mouth sores	Grade III + IV	3	10	1	5
	Grade ≤ II	73	132	9	16
	Odd ratio (95% CI)	Reference	1.84 (0.49-6.91)	Reference	2.81 (0.28-27.97)
	p-value		0.364		0.377
Fatigue	Grade III + IV	7	25	3	8
	Grade ≤ II	69	117	7	13
	Odd ratio (95% CI)	Reference	2.10 (0.86-5.12)	Reference	1.43 (0.28-7.21)
	p-value		0.100		0.660
Dysgeusia	Grade III + IV	12	43	4	11
	Grade ≤ II	64	99	6	10
	Odd ratio (95% CI)	Reference	2.32 (1.13-4.72)	Reference	1.65 (0.36-7.60)
	p-value		**0.020**		0.520
Skin hyperpigmentation	Grade III + IV	3	12	2	2
	Grade ≤ II	73	130	8	19
	Odd ratio (95% CI)	Reference	2.24 (0.61-8.21)	Reference	0.42 (0.05-3.53)

	p-value		0.221		0.425
Gastritis	Grade III + IV	2	10	1	3
	Grade ≤ II	74	132	9	18
	Odd ratio (95% CI)	Reference	2.80 (0.60-13.13)	Reference	1.50 (0.13-16.54)
	p-value		0.190		0.740
Peripheral neuropathy	Grade III + IV	6	26	1	3
	Grade ≤ II	70	116	9	18
	Odd ratio (95% CI)	Reference	2.61 (1.03-6.67)	Reference	1.50 (0.13-16.54)
	p-value		**0.044**		0.740
Myalgia/arthralgia	Grade III + IV	17	51	4	8
	Grade ≤ II	59	91	6	13
	Odd ratio (95% CI)	Reference	1.95 (1.02-3.68)	Reference	0.92 (0.20-4.31)
	p-value		**0.041**		0.918
Anaemia	Grade III + IV	2	7	0	2
	Grade ≤ II	74	135	10	19
	Odd ratio (95% CI)	Reference	1.92 (0.39-9.47)	Reference	-
	p-value		0.423		-
Leukopenia	Grade III + IV	0	7	0	3
	Grade I + II	76	130	10	18
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Leukocytosis	Grade III + IV	0	2	0	0
	Grade I + II	76	140	10	21
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	2	8	1	1
	Grade I + II	74	134	9	20
	Odd ratio (95% CI)	Reference	2.21 (0.46-10.67)	Reference	0.45 (0.02-8.02)
	p-value		0.324		0.587

Febrile neutropenia	Grade III + IV	2	8	0	1
	Grade I + II	74	134	10	20
	Odd ratio (95% CI)	Reference	2.21 (0.46-10.67)	Reference	-
	p-value		0.324		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	76	142	10	21
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	2	8	0	0
	Grade I + II	74	134	10	21
	Odd ratio (95% CI)	Reference	2.21 (0.46-10.67)	Reference	-
	p-value		0.324		-
Lymphocytopenia	Grade III + IV	0	0	0	0
	Grade I + II	76	142	10	21
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	0	2	0	0
	Grade I + II	76	140	10	21
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	2	5	0	0
	Grade I + II	74	137	10	21
	Odd ratio (95% CI)	Reference	1.35 (0.26-7.13)	Reference	-
	p-value		0.723		-
Hepatotoxicity	Grade III + IV	4	21	0	4
	Grade I + II	72	121	10	17
	Odd ratio (95% CI)	Reference	3.12 (1.03-9.46)	Reference	-
	p-value		**0.044**		-

Nephrotoxicity	Grade III + IV	1	3	0	0
	Grade I + II	75	139	10	21
	Odd ratio (95% CI)	Reference	1.62 (0.16-15.83)	Reference	-
	p-value		0.678		-
Cardiotoxicity	Grade III + IV	0	2	0	0
	Grade I + II	76	140	10	21
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	3	12	1	2
	Grade I + II	73	130	9	19
	Odd ratio (95% CI)	Reference	2.15 (0.61-8.21)	Reference	0.95 (0.07-11.87)
	p-value		0.221		0.966
Bone toxicity	Grade III + IV	5	23	1	1
	Grade I + II	71	119	9	20
	Odd ratio (95% CI)	Reference	2.74 (0.99-7.54)	Reference	0.45 (0.02-8.02)
	p-value		0.050		0.587

When a zero cell was present, the OR was not estimable.

3.4.7 Genotyping of *ABCC2* rs17222723

The polymorphism of *ABCC2* rs17222723 and its relationship with BC in Bangladeshi women was detected using the PCR-RFLP technique.

3.4.7.1 PCR of *ABCC2* rs17222723

PCR product of rs17222723

Forward Primer

Polymorphism of interest
R= T > A

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGGAAGA
TGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGTCCAGATGTT
TTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCACCAGGTCCCCAATC
TACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTATCCGTGCCTTTGAGCACC
AGCAGCGATTTCTGAAACACAATGAGGRGAGGATTGACACCAACCAGAAATGTGTCT
TTTCCTGGATCACCTCCAACAGGTGAGGCTTCCCCTGGGTATTTACCCATGTGTGTAC
TTTGGGGTTCTATATGTTTGATATTAGCGGTGGGAGG = 383 bp

Reverse Primer

Forward Primer (FP): 5'-GTAAGCTGTGCCCATCAAGG-3'
Reverse Primer (RP): 5'-CCTCCCACCGCTAATATCAA -3'

After PCR run, the PCR product of the rs17222723 allele of the *ABCC2* gene was visualized on a 1.5 % (w/v) agarose gel that was stained with ethidium bromide (**Fig. 17**).

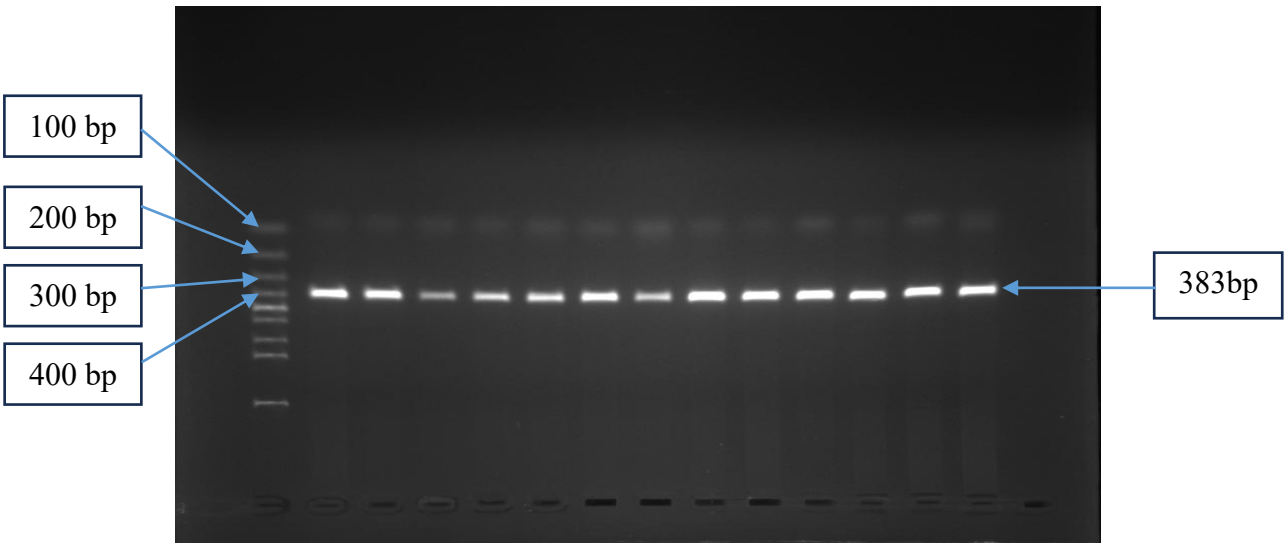


Fig. 17: Visualization of PCR product of rs17222723 (*ABCC2*) allele after 1.5% agarose gel electrophoresis.

3.4.7.2 RFLP Fragmentation Pattern of *ABCC2* rs17222723

Table 42: Restriction enzyme along with its digestion sites for *ABCC2* rs17222723.

Restriction Enzyme	Sites of Digestion
BseRI	Cutting Site 5'... G A G G A G $(N)_{10}$ ... 3' 3'... C T C C T C $(N)_8$ ... 5'

Here, N= A or T or G or C

When R= T in both copies of the gene, there is no cutting site and we will get 1 fragment.

When R= T in both chromosomes

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGTGAGGATTG
ACACCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTTC
CCCTGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGCG
GTGGGAGG

BseRI

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGTGAGGATTG
ACACCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTTC
CCCTGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGCG
GTGGGAGG = 383 bp (Uncut Fragment, Fragment 1)

Wild: TT: 383 bp

When R=T in one copy of the gene and R=A in another copy of the gene, there is one cutting site and we will get 3 fragments.

When R= A in one chromosome

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGAGAGGATT
GACACCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTT
CCCCTGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGC
GGTGGGAGG

cutting site

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGAGAGGATT
GACA = 270 bp (Fragment 1)

CCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTTCCCC
TGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGCGGTG
GGAGG = 113 bp (Fragment 2)

When R= T in another chromosome

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGTGAGGATTG
ACACCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTTC
CCCTGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGCG
GTGGGAGG = 383 bp (Uncut Fragment, Fragment 3)

Hetero: TA: 113, 270, 383 bp

When R= A in both copies of the gene, we will get 2 fragments.

GTAAGCTGTGCCCATCAAGGGGAAGAATGCTGGGTTGTGGCCAGAAAGGAGG
AAGATGGTGGATGCCTCATGACTGCGGGACTGGCTGATTCTTTACTTTTTGTGT
CCAGATGTTTTATGTGTCTACCTCCCGCCAGCTGAGGCGTCTGGACTCTGTCAC
CAGGTCCCCAATCTACTCTCACTTCAGCGAGACCGTATCAGGTTTGCCAGTTAT
CCGTGCCTTTGAGCACCAGCAGCGATTTCTGAAACACAATGAGGAGAGGATT
GACA = 270 bp (Fragment 1)

CCAACCAGAAATGTGTCTTTTCCTGGATCACCTCCAACAGGTGAGGCTTCCCC
TGGGTATTTACCCATGTGTGTACTTTGGGGTTCTATATGTTTGATATTAGCGGTG
GGAGG = 113 bp (Fragment 2)

Mutant: AA: 113, 270 bp

Table 43: Nucleotide changes, fragment sizes, and genotypes for the rs17222723 allele of the *ABCC2* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=T in both chromosomes (TT)	383	TT
When R=T in one chromosome and R=A in another chromosome (TA)	113, 270, 383	TA
When R=A in both chromosome (AA)	113, 270	AA

3.4.7.3 Linkage between *ABCC2* rs17222723 Polymorphism and Breast Cancer Susceptibility

The digestion fragment patterns for the TT, TA, and AA genotypes are shown in **Fig. 18**. The genotype pattern of the *ABCC2* rs17222723 polymorphism across both the patient and control categories was in line with Hardy-Weinberg equilibrium (HWE) (**Table 44**). All the genetic models showed an elevated risk of BC among Bangladeshi women; however, none reached statistical significance (codominant 1: TA vs. TT: OR = 1.44, 95% CI= 0.86-2.41, p= 0.169; codominant 2: AA vs. TT: OR = 1.78, 95% CI= 0.42-7.55, p= 0.433; dominant: TA+AA vs. TT: OR = 1.47, 95% CI= 0.90-2.41, p= 0.126; recessive: AA vs. TT+TA: OR = 1.69, 95% CI= 0.40-7.16, p= 0.473; over dominant: TA vs. TT+AA: OR = 1.42, 95% CI= 0.85-2.38, p=0.181; allelic: A vs. T: OR = 1.46, 95% CI= 0.93-2.29, p= 0.103). Overall, these outcomes indicate that the *ABCC2* rs17222723 polymorphism is not substantially associated with BC susceptibility in Bangladeshi women.

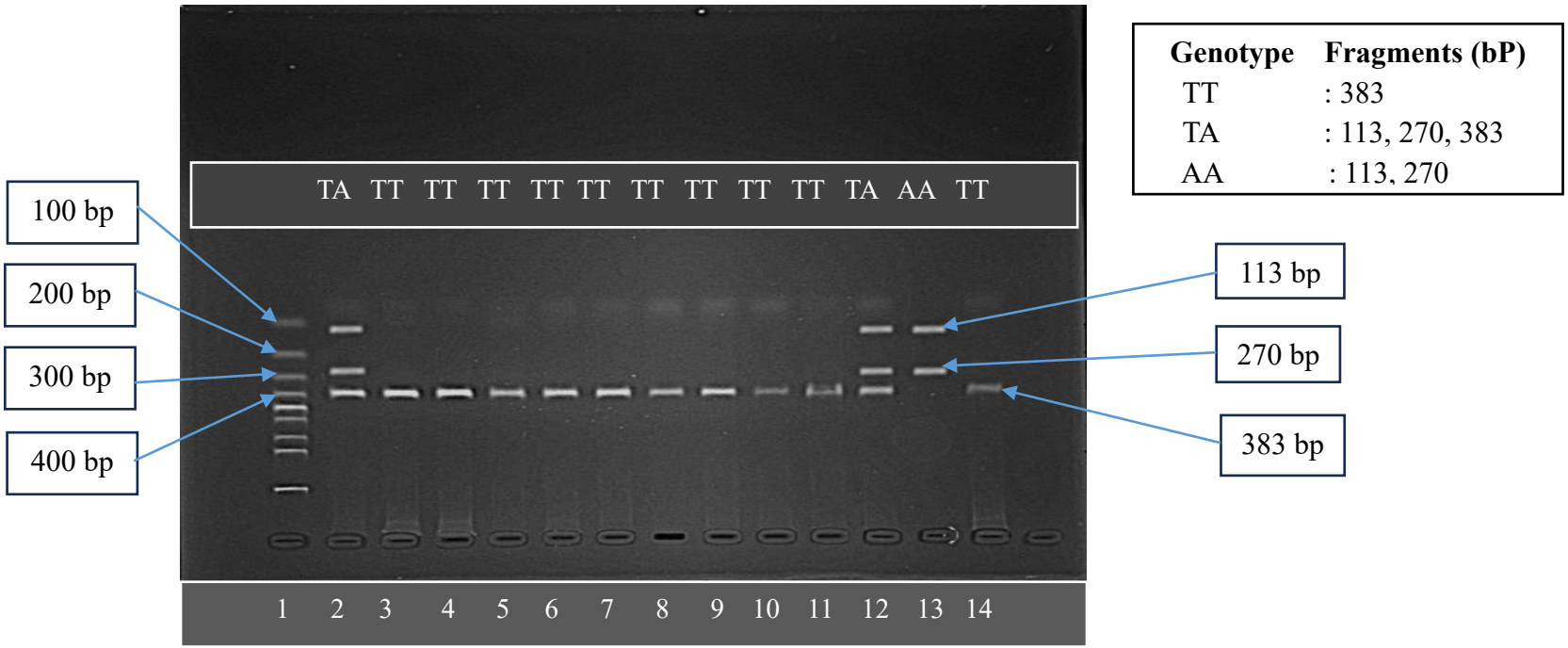


Fig. 18: Restriction Endonuclease (BseRI) digestion fragments of rs17222723 allele after 2.0 % agarose gel. Lane-1: 100 bp DNA ladder; Lanes-3, 4, 5, 6, 7, 8, 9, 10, 11, 14: TT; Lanes- 2, 12: TA; Lane-13: AA.

Table 44: Genotypic association of *ABCC2* rs17222723 variant with BC risk.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
TT	205 (82.33)			219 (87.25)			1	
Codominant model 1 (TA vs. TT)	39 (15.66)	3.42	0.064	29 (11.55)	3.00	0.083	1.44 (0.86-2.41)	0.169
Codominant model 2 (AA vs. TT)	5 (2.01)			3 (1.20)			1.78 (0.42-7.55)	0.433
Dominant model								
TT	205 (82.33)			219 (87.25)			1	
TA+AA	44 (17.67)			32 (12.75)			1.47 (0.90-2.41)	0.126
Recessive model								
TT+TA	244 (97.99)			248 (98.80)			1	
AA	5 (2.01)			3 (1.20)			1.69 (0.40-7.16)	0.473
Over-dominant model								
TT+AA	210 (84.34)			222 (88.45)			1	
TA	39 (15.66)			29 (11.55)			1.42 (0.85-2.38)	0.181
Allele								
T	449 (90.16)			467 (93.03)			1	
A	49 (9.84)			35 (6.97)			1.46 (0.93-2.29)	0.103

3.4.7.4 Association between *ABCC2* rs17222723 Polymorphism and AC-T Chemotherapy Response

The association between *ABCC2* rs17222723 genotypes and clinical response to AC-T chemotherapy was assessed in 249 BC patients, stratified into AC-paclitaxel and AC-docetaxel subgroups (**Table 45**). In the AC-paclitaxel subgroup, patients carrying the combined TA+AA genotype ere associated with slightly lower response rates than those with the TT genotype (TA+AA vs. TT: OR = 0.98, 95% CI = 0.45-2.13, p = 0.968). Though this response was statistically not significant.

In the smaller AC-docetaxel cohort, the TA+AA genotype was also associated with slightly lower clinical response rates than the TT genotype (TA+AA vs. TT: OR = 0.93, 95% CI = 0.22-3.91, p = 0.924). This linkage was also not statistically significant.

Overall, no substantial associations were found between *ABCC2* rs17222723 genotypes and clinical responses to either AC-paclitaxel or AC-docetaxel regimens.

3.4.7.5 Association between *ABCC2* rs17222723 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The association between *ABCC2* rs17222723 genotypes and the incidence of AC-T chemotherapy–induced toxicities was evaluated in BC patients (**Table 46**). In the AC-paclitaxel subgroup, the TA+AA genotype exhibited substantial association with a greater risk of several higher-grade toxicities in contrast to the TT genotype, including nausea (OR= 3.56; 95% CI= 1.12-11.27; p= 0.030), vomiting (OR = 2.93, 95% CI = 1.20-7.14, p = 0.018), diarrhea (OR = 2.74, 95% CI = 1.09-6.91, p = 0.032), fever (OR = 2.39, 95% CI = 1.07-5.36, p = 0.034), dysgeusia (OR = 2.25, 95% CI = 1.00-5.03, p = 0.048), and hepatotoxicity (OR = 2.87, 95% CI = 1.08-7.63, p = 0.033). Other toxicities caused by the AC-paclitaxel regimen were not statistically significant regarding genotypes.

In the smaller AC-docetaxel subgroup, there was no significant association between *ABCC2* rs17222723 genotypes and chemotherapy-related toxicities.

Table 45: Impact of *ABCC2* rs17222723 polymorphisms on the response of AC-T chemotherapy regimen.

Variants	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs17222723	Non-carrier TT (188)	101	87	1 (reference)	-	TT (17)	10	7	1 (reference)	-
	Carrier TA+AA (30)	16	14	0.98 (0.45-2.13)	0.968	TA+AA (14)	8	6	0.93 (0.22-3.91)	0.924

When a zero cell was present, the OR was not estimable.

Table 46: Impact of *ABCC2* rs17222723 polymorphisms on the toxicities triggered by the AC-T chemotherapy regimen.

Toxicities		Doxorubicin-cyclophosphamide-paclitaxel (N=218)		Doxorubicin-cyclophosphamide-docetaxel (N=31)	
		Non-carrier TT (188)	**Carrier TA + AA (30)**	**Non-carrier TT (17)**	**Carrier TA + AA (14)**
Nausea	Grade III + IV	10	5	2	2
	Grade ≤ II	178	25	15	12
	Odd ratio (95% CI)	Reference	3.56 (1.12-11.27)	Reference	1.25 (0.15-10.23)
	p-value		**0.030**		0.835
Vomiting	Grade III + IV	24	9	4	3
	Grade ≤ II	164	21	13	11
	Odd ratio (95% CI)	Reference	2.93 (1.20-7.14)	Reference	0.89 (0.16-4.85)
	p-value		**0.018**		0.889
Diarrhoea	Grade III + IV	22	8	2	2
	Grade ≤ II	166	22	15	12
	Odd ratio (95% CI)	Reference	2.74 (1.09-6.91)	Reference	1.25 (0.15-10.23)
	p-value		**0.032**		0.835

Constipation	Grade III + IV	10	3	3	2
	Grade ≤ II	178	27	14	12
	Odd ratio (95% CI)	Reference	1.98 (0.51-7.65)	Reference	0.78 (0.11-5.46)
	p-value		0.322		0.800
Fever	Grade III + IV	41	12	2	1
	Grade ≤ II	147	18	15	13
	Odd ratio (95% CI)	Reference	2.39 (1.07-5.36)	Reference	0.58 (0.05-7.12)
	p-value		**0.034**		0.667
Edema	Grade III + IV	29	7	1	1
	Grade ≤ II	159	23	16	13
	Odd ratio (95% CI)	Reference	1.67 (0.66-4.25)	Reference	1.23 (0.07-21.64)
	p-value		0.282		0.887
Dry mouth	Grade III + IV	10	1	3	1
	Grade ≤ II	178	29	14	13
	Odd ratio (95% CI)	Reference	0.61 (0.07-4.98)	Reference	0.36 (0.03-3.90)
	p-value		0.647		0.400
Mouth sores	Grade III + IV	11	2	3	3
	Grade ≤ II	177	28	14	11
	Odd ratio (95% CI)	Reference	1.15 (0.24-5.46)	Reference	1.27 (0.21-7.58)
	p-value		0.861		0.791
Fatigue	Grade III + IV	24	8	6	5
	Grade ≤ II	164	22	11	9
	Odd ratio (95% CI)	Reference	2.48 (0.99-6.21)	Reference	1.02 (0.23-4.46)
	p-value		0.051		0.980
Dysgeusia	Grade III + IV	43	12	9	6
	Grade ≤ II	145	18	8	8
	Odd ratio (95% CI)	Reference	2.25 (1.00-5.03)	Reference	0.67 (0.16-2.77)
	p-value		**0.048**		0.576
Skin hyperpigmentation	Grade III + IV	12	3	2	2
	Grade ≤ II	176	27	15	12
	Odd ratio (95% CI)	Reference	1.63 (0.43-6.15)	Reference	1.25 (0.15-10.23)

	p-value		0.471		0.835
Gastritis	Grade III + IV	11	1	2	2
	Grade ≤ II	177	29	15	12
	Odd ratio (95% CI)	Reference	0.55 (0.06-4.46)	Reference	1.25 (0.15-10.23)
	p-value		0.579		0.835
Peripheral neuropathy	Grade III + IV	24	8	2	2
	Grade ≤ II	164	22	15	12
	Odd ratio (95% CI)	Reference	2.48 (0.99-6.21)	Reference	1.25 (0.15-10.23)
	p-value		0.051		0.835
Myalgia/arthralgia	Grade III + IV	54	14	7	5
	Grade ≤ II	134	16	10	9
	Odd ratio (95% CI)	Reference	2.17 (0.99-4.75)	Reference	0.79 (0.18-3.41)
	p-value		0.052		0.756
Anaemia	Grade III + IV	8	1	1	1
	Grade ≤ II	180	29	16	13
	Odd ratio (95% CI)	Reference	0.78 (0.09-6.44)	Reference	1.23 (0.07-21.64)
	p-value		0.814		0.887
Leukopenia	Grade III + IV	6	1	2	1
	Grade I + II	182	29	15	13
	Odd ratio (95% CI)	Reference	1.05 (0.12-9.01)	Reference	0.58 (0.05-7.12)
	p-value		0.967		0.667
Leukocytosis	Grade III + IV	2	0	0	0
	Grade I + II	186	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	9	1	1	1
	Grade I + II	179	29	16	13
	Odd ratio (95% CI)	Reference	0.69 (0.08-5.62)	Reference	1.23 (0.07-21.64)
	p-value		0.725		0.887

Febrile neutropenia	Grade III + IV	9	1	1	0
	Grade I + II	179	29	16	14
	Odd ratio (95% CI)	Reference	0.69 (0.08-5.62)	Reference	-
	p-value		0.725		-
Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	188	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	8	2	0	0
	Grade I + II	180	28	17	14
	Odd ratio (95% CI)	Reference	1.61 (0.32-7.80)	Reference	-
	p-value		0.561		-
Lymphocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	186	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	186	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	6	1	0	0
	Grade I + II	182	29	17	14
	Odd ratio (95% CI)	Reference	1.05 (0.12-9.01)	Reference	-
	p-value		0.967		-
Hepatotoxicity	Grade III + IV	18	7	3	1
	Grade I + II	170	23	14	13
	Odd ratio (95% CI)	Reference	2.87 (1.08-7.63)	Reference	0.36 (0.03-3.90)
	p-value		**0.033**		0.400

Nephrotoxicity	Grade III + IV	4	0	0	0
	Grade I + II	184	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Cardiotoxicity	Grade III + IV	2	0	0	0
	Grade I + II	186	30	17	14
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	12	3	2	1
	Grade I + II	176	27	15	13
	Odd ratio (95% CI)	Reference	1.63 (0.43-6.15)	Reference	0.58 (0.05-7.12)
	p-value		0.471		0.669
Bone toxicity	Grade III + IV	21	7	1	1
	Grade I + II	167	23	16	13
	Odd ratio (95% CI)	Reference	2.42 (0.93-6.32)	Reference	1.23 (0.07-21.64)
	p-value		0.071		0.887

When a zero cell was present, the OR was not estimable.

3.4.8 Genotyping of *CYP2C8* rs11572080

The PCR-RFLP approach was employed to detect the relationship of the *CYP2C8* rs11572080 polymorphism with BC susceptibility in Bangladeshi women.

3.4.8.1 PCR of *CYP2C8* rs11572080

PCR product of rs11572080

Forward Primer

Polymorphism of interest
R= C > T

CAGGATGCGCAATGAAGACCTGGCCACCCCTGAAATGTTTCCAAGGACGTCA
CTAGTGAAGACAGGTAACTGTTAAGGTCAATGACGCAGAGTAGAGTCACCCA
CCCTTGGTTTTTCTCAACTCCTCCACAAGGCAGTGAGCTTCCTCTTGAACACG
GTCCTCAATGCTCRTCTTCCCCATCCCAAAATTCCGCAAGGTTGTGAGGGAGA
AACGCCGGATCTCCTTCCATCTCTTTCCATTGCTGGAAATGATTCCTAATAAAA
AAAGGGGCAGAAACTGGGAGAATTCACAGCCAAGGAAGAAAGTGCTGCAAC
ACTTGGCAGCCATGCAGATAGGCTAAGCTCTGCTGAGAAGCTTTTTAGGGCTC
TGTTTTCCATCCCCCTCACCCCAGTTACCAAAGCTGACACAGAAATATGTGCA
CCTACCAAGTCCTTTAGTAATTCTTTGAGATATTGGGGAATTGCCT = 467 bp

Reverse Primer

Forward Primer (FP): 5'-CAGGATGCGCAATGAAGA-3'
Reverse Primer (RP): 5'-AGGCAATTCCCCAATATCTC-3'

After PCR amplification, the PCR product of the rs11572080 allele of the *CYP2C8* gene was visualized on a 1.5 % (w/v) agarose gel that was stained with ethidium bromide (**Fig. 19**).

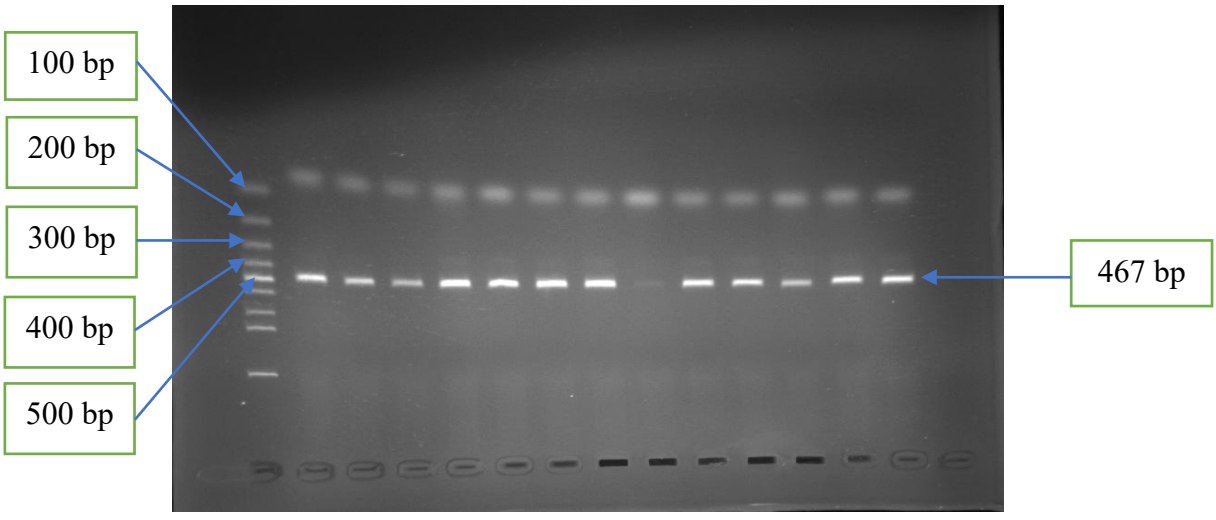


Fig. 19: Visualization of PCR product of *CYP2C8* rs11572080 allele after 1.5% agarose gel electrophoresis.

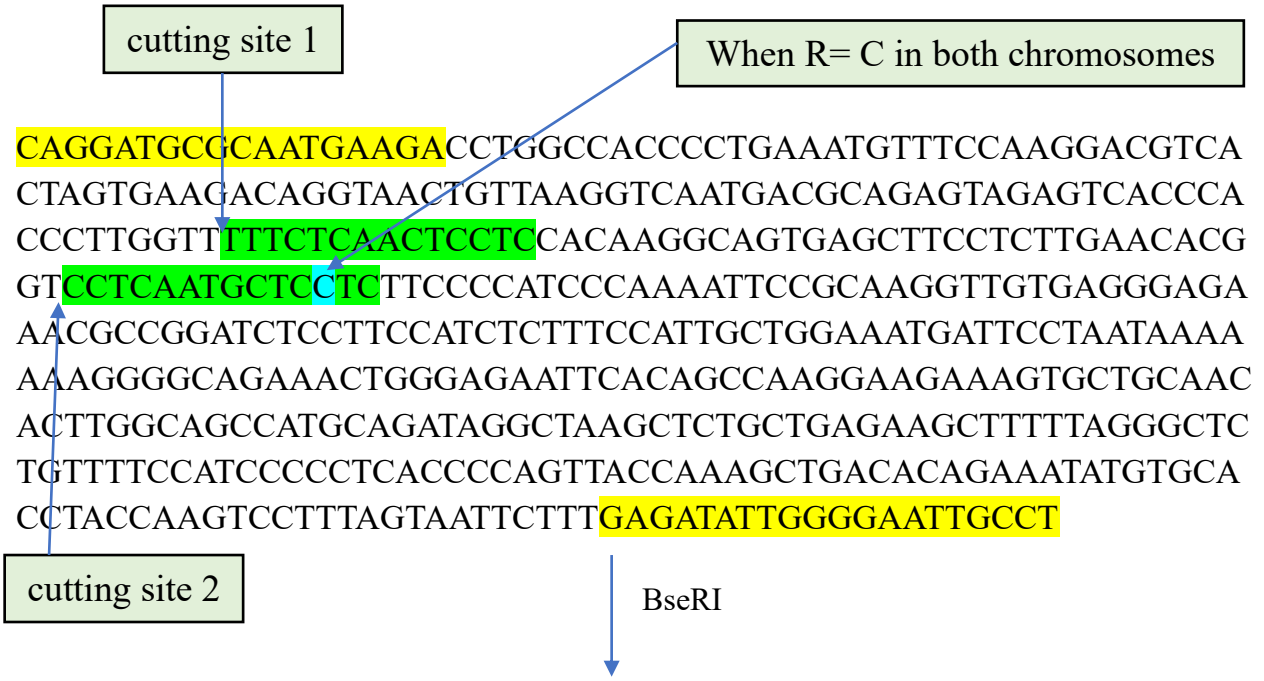
3.4.8.2 RFLP Fragmentation Pattern of *CYP2C8* rs11572080

Table 47: Restriction enzyme along with its digestion sites for *CYP2C8* rs11572080.

Restriction Enzyme	Sites of Digestion
BseRI	5'... G A G G A G $(N)_{10}$ ▼ ... 3' 3'... C T C C T C $(N)_8$ ▲ ... 5' Cutting Site

Here, N= A or T or G or C

When R= C in both copies of the gene, there are two cutting sites and we will get 3 fragments.



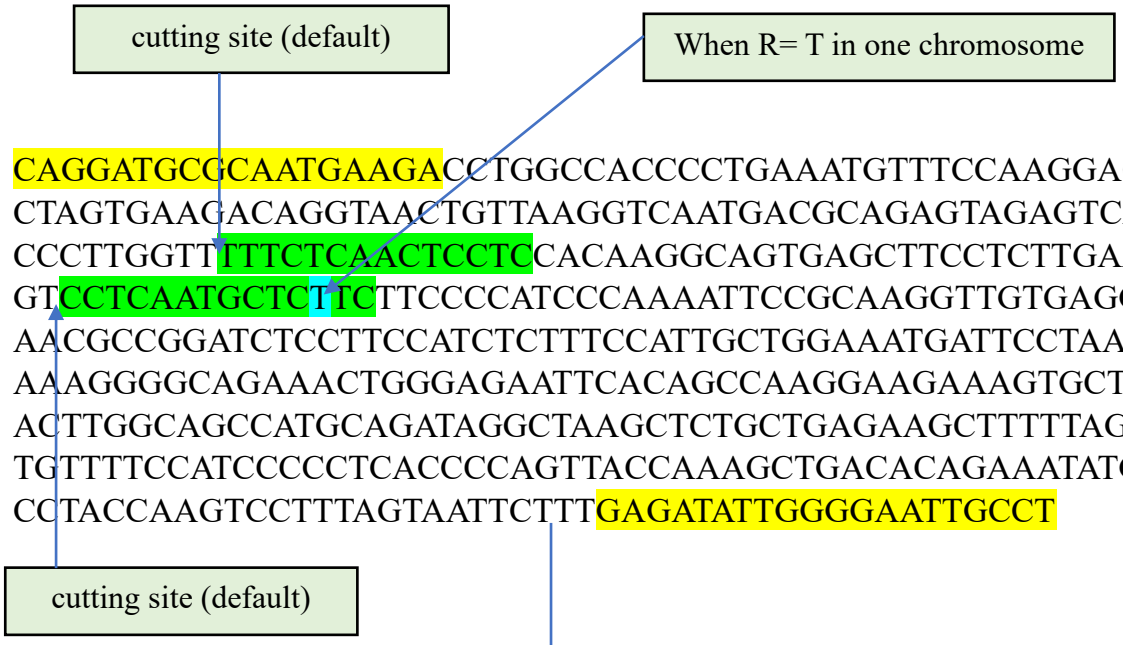
CAGGATGCGCAATGAAGACCTGGCCACCCCTGAAATGTTTCCAAGGACGTCACTA
GTGAAGACAGGTAACTGTTAAGGTCAATGACGCAGAGTAGAGTCACCCACCCTT
GGTT = 113 bp

TTTCTCAACTCCTCCACAAGGCAGTGAGCTTCCTCTTGAACACGGT = 46 bp

CCTCAATGCTCCTCTTCCCCATCCCAAAATTCCGCAAGGTTGTGAGGGAGAAACG
CCGGATCTCCTTCCATCTCTTTCCATTGCTGGAAATGATTCCTAATAAAAAAAGGG
GCAGAAACTGGGAGAATTCACAGCCAAGGAAGAAAGTGCTGCAACACTTGGCA
GCCATGCAGATAGGCTAAGCTCTGCTGAGAAGCTTTTTAGGGCTCTGTTTTCCATC
CCCCTCACCCCAGTTACCAAAGCTGACACAGAAATATGTGCACCTACCAAGTCCT
TTAGTAATTCTTTGAGATATTGGGGAATTGCCT = 308 bp

Wild: CC: 46, 113, 308 bp

When R=C in one copy of the gene and R=T in another copy of the gene, we will get 4 fragments.



CAGGATGCGCAATGAAGACCTGGCCACCCCTGAAATGTTTCCAAGGACGTCACTA
GTGAAGACAGGTAACTGTTAAGGTCAATGACGCAGAGTAGAGTCACCCACCCTT
GGTT = 113 bp

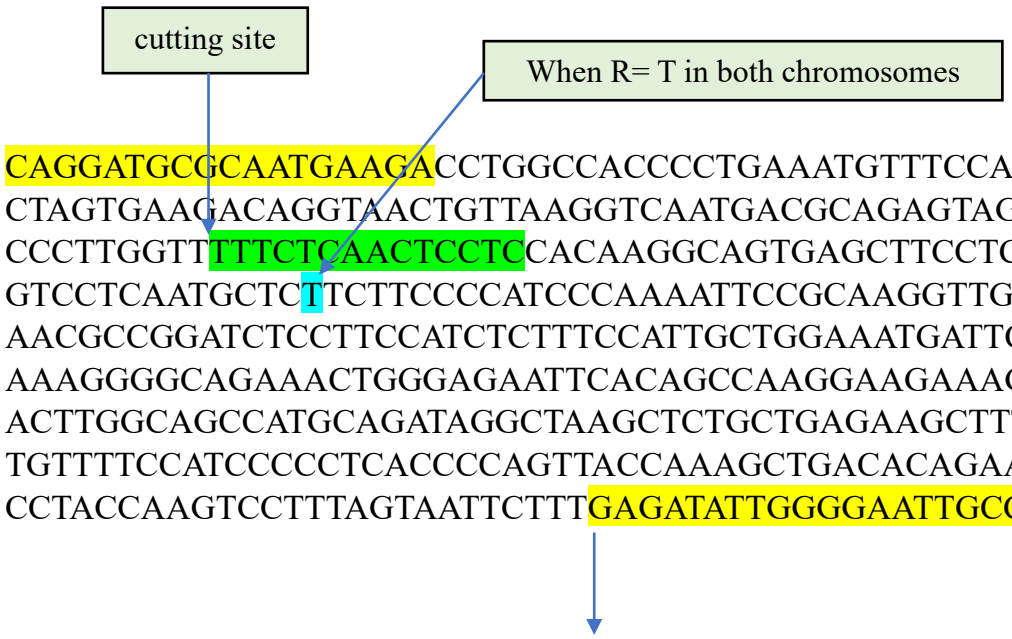
TTTCTCAACTCCTCCACAAGGCAGTGAGCTTCCTCTTGAACACGGT = 46 bp

CCTCAATGCTCTTCTTCCCCATCCCAAAATTCCGCAAGGTTGTGAGGGAGAAACG
CCGGATCTCCTTCCATCTCTTTCCATTGCTGGAAATGATTCCTAATAAAAAAAGGG
GCAGAAACTGGGAGAATTCACAGCCAAGGAAGAAAGTGCTGCAACACTTGGCA
GCCATGCAGATAGGCTAAGCTCTGCTGAGAAGCTTTTTAGGGCTCTGTTTTCCATC
CCCCTCACCCCAGTTACCAAAGCTGACACAGAAATATGTGCACCTACCAAGTCCT
TTAGTAATTCTTTGAGATATTGGGGAATTGCCT = 308 bp

TTTCTCAACTCCTCCACAAGGCAGTGAGCTTCCTCTTGAACACGGTCCTCAATGC
TCTTCTTCCCCATCCCAAAATTCCGCAAGGTTGTGAGGGAGAAACGCCGGATCTC
CTTCCATCTCTTTCCATTGCTGGAAATGATTCCTAATAAAAAAAGGGGCAGAAACT
GGGAGAATTCACAGCCAAGGAAGAAAGTGCTGCAACACTTGGCAGCCATGCAGA
TAGGCTAAGCTCTGCTGAGAAGCTTTTTAGGGCTCTGTTTTCCATCCCCCTCACCC
CAGTTACCAAAGCTGACACAGAAATATGTGCACCTACCAAGTCCTTTAGTAATTCT
TTGAGATATTGGGGAATTGCCT = 354 bp

Hetero: CT: 46, 113, 308, 354 bp

When R= T in both copies of the gene, there is one cutting site and we will get 2 fragments.



CAGGATGCGCAATGAAGACCTGGCCACCCCTGAAATGTTTCCAAGGACGTCA
CTAGTGAAGACAGGTAACTGTTAAGGTCAATGACGCAGAGTAGAGTCACCCA
CCCTTGGTT = 113 bp

TTTCTCAACTCCTCCACAAGGCAGTGAGCTTCCTCTTGAACACGGTCCTCAAT
GCTCTTCTTCCCCATCCCAAAATTCCGCAAGGTTGTGAGGGAGAAACGCCGGA
TCTCCTTCCATCTCTTTCCATTGCTGGAAATGATTCCTAATAAAAAAAGGGGCA
GAAACTGGGAGAATTCACAGCCAAGGAAGAAAGTGCTGCAACACTTGGCAG
CCATGCAGATAGGCTAAGCTCTGCTGAGAAGCTTTTTAGGGCTCTGTTTTCCAT
CCCCCTCACCCCAGTTACCAAAGCTGACACAGAAATATGTGCACCTACCAAGT
CCTTTAGTAATTCTTTGAGATATTGGGGAATTGCCT = 354 bp

Mutant: TT: 113, 354 bp

Table 48: Nucleotide changes, fragment sizes, and genotypes for the rs11572080 allele of the *CYP2C8* gene.

Nucleotide Changes	Fragments (bp)	Genotype
When R=C in both chromosomes (CC)	46, 113, 308	CC
When R=C in one chromosome and R=T in another chromosome (CT)	46, 113, 308, 354	CT
When R=T in both chromosome (TT)	113, 354	TT

3.4.8.3 Linkage between *CYP2C8* rs11572080 Polymorphism and Breast Cancer Susceptibility

The digestion fragment patterns for the CC, CT, and TT genotypes are presented in **Fig. 20**. As summarized in **Table 49**, the genotyping pattern of the *CYP2C8* rs11572080 polymorphism across the patient and control categories was in line with Hardy-Weinberg equilibrium (HWE). All genetic models indicated an elevated risk of BC among Bangladeshi women; however, none reached statistical significance (codominant 1: CT vs. CC: OR = 1.26, 95% CI= 0.74-2.16, p= 0.387; codominant 2: TT vs. CC: OR = 1.39, 95% CI= 0.31-6.28, p= 0.668; dominant: CT+TT vs. CC: OR = 1.28, 95% CI= 0.77-2.13, p= 0.346; recessive: TT vs. CC+CT: OR = 1.35, 95% CI= 0.30-6.09, p= 0.696; over dominant: CT vs. CC+TT: OR = 1.26, 95% CI= 0.74-2.15, p= 0.397; allelic: T vs. C: OR = 1.27, 95% CI= 0.79-2.03, p= 0.322). Overall, these results indicate that the *CYP2C8* rs11572080 polymorphism is not substantially associated with BC risk in Bangladeshi women.

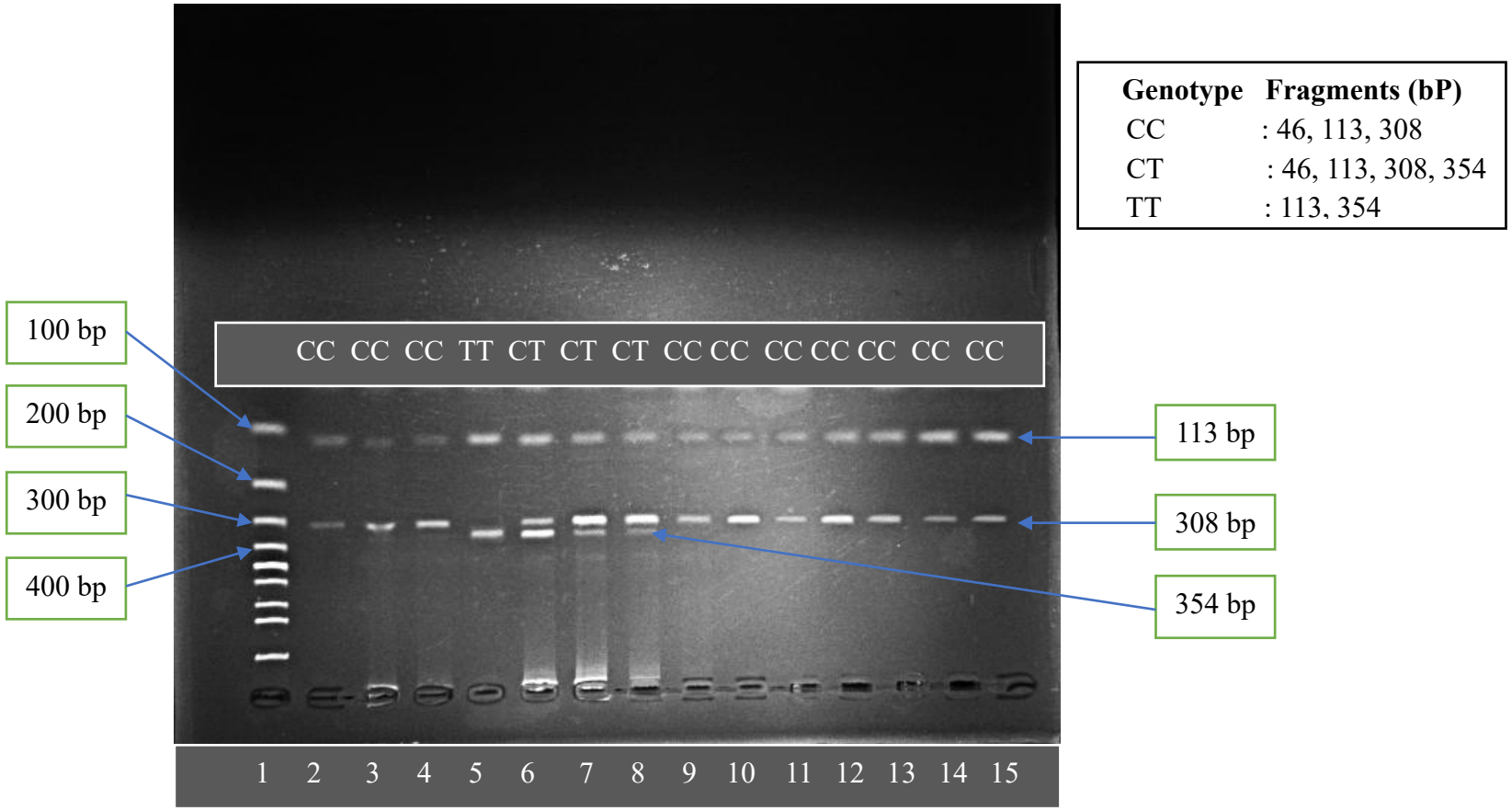


Fig. 20: Restriction Endonuclease (BseRI) digestion fragments of rs11572080 allele after 2.0 % agarose gel. Lane-1: 100 bp DNA ladder. Lanes-2, 3, 4, 9, 10, 11, 12, 13, 14, 15: CC; Lanes- 6, 7, 8: CT; Lane- 5: TT.

Table 49: Genotypic association of *CYP2C8* rs11572080 variant with BC risk.

Genetic Models	Cases (%)	HWE		Controls (%)	HWE		Crude Analysis	
	N = 249	χ^2	p-value	N = 251	χ^2	p-value	OR (95% Cl)	*p*-value
CC	211 (84.74)			220 (87.65)			1	
Codominant model 1 (CT vs. CC)	34 (13.65)	3.34	0.067	28 (11.16)	3.41	0.064	1.26 (0.74-2.16)	0.387
Codominant model 2 (TT vs. CC)	4 (1.61)			3 (1.19)			1.39 (0.31-6.28)	0.668
Dominant model								
CC	211 (84.74)			22 (87.65)			1	
CT+TT	38 (15.26)			31 (12.35)			1.28 (0.77-2.13)	0.346
Recessive model								
CC+CT	245 (98.39)			248 (98.81)			1	
TT	4 (1.61)			3 (1.19)			1.35 (0.30-6.09)	0.696
Over-dominant model								
CC+TT	215 (86.35)			223 (88.84)			1	
CT	34 (13.65)			28 (11.16)			1.26 (0.74-2.15)	0.397
Allele								
C	456 (91.57)			468 (93.23)			1	
T	42 (8.43)			34 (6.77)			1.27 (0.79-2.03)	0.322

3.4.8.4 Association between *CYP2C8* rs11572080 Polymorphism and AC-T Chemotherapy Response

The linkage between *CYP2C8* rs11572080 genotypes and clinical responses to AC-T chemotherapy was evaluated in 249 BC patients, categorized into AC-paclitaxel and AC-docetaxel subgroups (**Table 50**). In the AC-paclitaxel subgroup, carrier patients with the CT+TT genotype showed a higher response rate than non-carrier patients with the CC genotype (CT+TT vs. CC: OR = 2.25, 95% CI = 0.94-5.39, p = 0.068). However, this response was statistically not significant.

Conversely, in the AC-docetaxel subgroup, carrier patients possessing the CT+TT genotype exhibited reduced response rates compared to non-carrier patients possessing the CC genotype; however, this correlation did not achieve statistical significance (CT+TT vs. CC: OR = 0.80, 95% CI = 0.18-3.54, p = 0.768).

Overall, no significant correlation between the *CYP2C8* rs11572080 polymorphisms and AC-T chemotherapy response was observed among Bangladeshi BC patients.

3.4.8.5 Association between *CYP2C8* rs11572080 Polymorphism and AC-T Chemotherapy-Induced Toxicity

The association between *CYP2C8* rs11572080 genotypes and the incidence of AC-T chemotherapy–induced toxicities was evaluated in BC patients (**Table 51**). In the AC-paclitaxel subgroup, the carrier patients having the CT+TT genotype exhibited substantial linkage with a greater risk of several higher-grade toxicities compared to the CC genotype, including vomiting (OR = 2.80, 95% CI = 1.11-7.06, p = 0.029), fever (OR = 2.44, 95% CI = 1.05-5.65, p = 0.037), peripheral neuropathy (OR = 2.93, 95% CI = 1.16-7.43, p = 0.023), myalgia (OR = 2.29, 95% CI = 1.01-5.20, p = 0.046), hepatotoxicity (OR = 3.36, 95% CI = 1.25-9.04, p = 0.016), pulmonary toxicity (OR = 4.11, 95% CI = 1.29-13.14, p = 0.017), and bone toxicity (OR = 2.83, 95% CI = 1.07-7.50, p = 0.035). Other toxicities associated with the AC-paclitaxel regimen were not statistically significant with regard to the *CYP2C8* rs11572080 polymorphism.

In the smaller AC-docetaxel cohort, none of the toxicities were significantly linked to *CYP2C8* rs11572080 polymorphisms.

Table 50: Impact of *CYP2C8* rs11572080 polymorphisms on the response of AC-T chemotherapy regimen.

Variants	Doxorubicin-cyclophosphamide- paclitaxel (N=218)					Doxorubicin-cyclophosphamide- docetaxel (N=31)				
	Genotype	Responder (N=117)	Non-responder (N=101)	Odds ratio (95 % Cl)	p value	Genotype	Responder (N=18)	Non-responder (N=13)	Odds ratio (95 % Cl)	p value
rs11572080	Non-carrier group CC (191)	98	93	1 (reference)	-	CC (20)	12	8	1 (reference)	-
	Carrier group CT+TT (27)	19	8	2.25 (0.94-5.39)	0.068	CT+TT (11)	6	5	0.80 (0.18-3.54)	0.768

Table 51: Impact of *CYP2C8* rs11572080 polymorphisms on the toxicities induced by AC-T chemotherapy regimen.

Toxicities		Doxorubicin-cyclophosphamide-paclitaxel (N=218)		Doxorubicin-cyclophosphamide-docetaxel (N=31)	
		Non-carrier CC (191)	**Carrier CT + TT (27)**	**Non-carrier CC (20)**	**Carrier CT + TT (11)**
Nausea	Grade III + IV	12	3	2	2
	Grade ≤ II	179	24	18	9
	Odd ratio (95% CI)	Reference	1.86 (0.49-7.09)	Reference	2.00 (0.24-16.61)
	p-value		0.360		0.521
Vomiting	Grade III + IV	25	8	4	3
	Grade ≤ II	166	19	16	8
	Odd ratio (95% CI)	Reference	2.80 (1.11-7.06)	Reference	1.50 (0.27-8.38)
	p-value		**0.029**		0.644
Diarrhoea	Grade III + IV	23	7	2	2
	Grade ≤ II	168	20	18	9
	Odd ratio (95% CI)	Reference	2.56 (0.97-6.71)	Reference	2.00 (0.24-16.61)
	p-value		0.056		0.521

Constipation	Grade III + IV	10	3	3	2
	Grade ≤ II	181	24	17	9
	Odd ratio (95% CI)	Reference	2.26 (0.58-8.80)	Reference	1.26 (0.18-8.97)
	p-value		0.238		0.818
Fever	Grade III + IV	42	11	2	1
	Grade ≤ II	149	16	18	10
	Odd ratio (95% CI)	Reference	2.44 (1.05-5.65)	Reference	0.90 (0.07-11.21)
	p-value		**0.037**		0.934
Edema	Grade III + IV	30	6	1	1
	Grade ≤ II	161	21	19	10
	Odd ratio (95% CI)	Reference	1.53 (0.57-4.12)	Reference	1.90 (0.11-33.70)
	p-value		0.396		0.661
Dry mouth	Grade III + IV	9	2	2	2
	Grade ≤ II	182	25	18	9
	Odd ratio (95% CI)	Reference	1.62 (0.33-7.92)	Reference	2.00 (0.24-16.61)
	p-value		0.552		0.521
Mouth sores	Grade III + IV	11	2	4	2
	Grade ≤ II	180	25	16	9
	Odd ratio (95% CI)	Reference	1.31 (0.27-6.25)	Reference	0.89 (0.14-5.85)
	p-value		0.735		0.902
Fatigue	Grade III + IV	25	7	7	4
	Grade ≤ II	166	20	13	7
	Odd ratio (95% CI)	Reference	2.32 (0.89-6.06)	Reference	1.06 (0.23-4.92)
	p-value		0.084		0.939
Dysgeusia	Grade III + IV	44	11	10	5
	Grade ≤ II	147	16	10	6
	Odd ratio (95% CI)	Reference	2.30 (0.99-5.31)	Reference	0.83 (0.19-3.64)
	p-value		0.051		0.808
Skin hyperpigmentation	Grade III + IV	13	2	2	2
	Grade ≤ II	178	25	18	9
	Odd ratio (95% CI)	Reference	1.10 (0.23-5.14)	Reference	2.00 (0.24-16.61)
	p-value		0.908		0.521

Gastritis	Grade III + IV	10	2	1	3
	Grade ≤ II	181	25	19	8
	Odd ratio (95% CI)	Reference	1.45 (0.30-6.99)	Reference	7.13 (0.64-79.27)
	p-value		0.645		0.110
Peripheral neuropathy	Grade III + IV	24	8	2	2
	Grade ≤ II	167	19	18	9
	Odd ratio (95% CI)	Reference	2.93 (1.16-7.43)	Reference	2.00 (0.24-16.61)
	p-value		**0.023**		0.521
Myalgia/arthralgia	Grade III + IV	55	13	7	5
	Grade ≤ II	136	14	13	6
	Odd ratio (95% CI)	Reference	2.29 (1.01-5.20)	Reference	1.55 (0.35-6.94)
	p-value		**0.046**		0.568
Anaemia	Grade III + IV	7	2	2	0
	Grade ≤ II	184	25	18	11
	Odd ratio (95% CI)	Reference	2.10 (0.41-10.69)	Reference	-
	p-value		0.370		-
Leukopenia	Grade III + IV	6	1	2	1
	Grade I + II	185	26	18	10
	Odd ratio (95% CI)	Reference	1.19 (0.14-10.25)	Reference	0.90 (0.07-11.21)
	p-value		0.876		0.934
Leukocytosis	Grade III + IV	2	0	0	0
	Grade I + II	189	27	20	11
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Neutropenia	Grade III + IV	8	2	2	0
	Grade I + II	183	25	18	11
	Odd ratio (95% CI)	Reference	1.83 (0.37-9.11)	Reference	-
	p-value		0.460		-
Febrile neutropenia	Grade III + IV	8	2	1	0
	Grade I + II	183	25	19	11
	Odd ratio (95% CI)	Reference	1.83 (0.37-9.11)	Reference	-
	p-value		0.460		-

Neutrophilia	Grade III + IV	0	0	0	0
	Grade I + II	191	27	20	11
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Lymphocytosis	Grade III + IV	8	2	0	0
	Grade I + II	183	25	20	11
	Odd ratio (95% CI)	Reference	1.83 (0.37-9.11)	Reference	-
	p-value		0.460		-
Lymphocytopenia	Grade III + IV	1	1	0	0
	Grade I + II	190	26	20	11
	Odd ratio (95% CI)	Reference	7.31 (0.44-120.41)	Reference	-
	p-value		0.164		-
Thrombocytopenia	Grade III + IV	2	0	0	0
	Grade I + II	189	27	20	11
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Thrombocytosis	Grade III + IV	5	2	0	0
	Grade I + II	186	25	20	11
	Odd ratio (95% CI)	Reference	2.98 (0.55-16.16)	Reference	-
	p-value		0.206		-
Hepatotoxicity	Grade III + IV	18	7	2	2
	Grade I + II	173	20	18	9
	Odd ratio (95% CI)	Reference	3.36 (1.25-9.04)	Reference	2.00 (0.24-16.61)
	p-value		**0.016**		0.521
Nephrotoxicity	Grade III + IV	3	1	0	0
	Grade I + II	188	26	20	11
	Odd ratio (95% CI)	Reference	2.41 (0.24-24.04)	Reference	-
	p-value		0.453		-

Cardiotoxicity	Grade III + IV	2	0	0	0
	Grade I + II	189	27	20	11
	Odd ratio (95% CI)	Reference	-	Reference	-
	p-value		-		-
Pulmonary toxicity	Grade III + IV	10	5	2	1
	Grade I + II	181	22	18	10
	Odd ratio (95% CI)	Reference	4.11 (1.29-13.14)	Reference	0.90 (0.07-11.21)
	p-value		**0.017**		0.934
Bone toxicity	Grade III + IV	21	7	1	1
	Grade I + II	170	20	19	10
	Odd ratio (95% CI)	Reference	2.83 (1.07-7.50)	Reference	1.90 (0.11-33.70)
	p-value		**0.035**		0.661

When a zero cell was present, the OR was not estimable.

3.5 Discussion

The current study assessed the impact of multiple pharmacogenetic polymorphisms—*SLCO1B3* (rs4149117), *ABCB1* (rs1128503, rs2032582, rs3213619, rs1045642), *ABCC2* (rs17222723), and *CYP2C8* (rs11572080)-on treatment response and chemotherapy-induced toxicity in Bangladeshi women with BC undergoing the AC-T treatment regimen. These genes were chosen for their known roles in drug transport and metabolic processes, which may influence therapeutic efficacy and toxicity.

SLCO1B3 encodes a 702-amino acid transmembrane protein that acts as a liver-specific uptake transporter (König et al., 2000; Abe et al., 2001). Former research has shown that aberrant expression and functional alterations of *SLCO1B3* occur in different malignancies, yet the mechanisms regulating its expression in tumor tissues are still not well elucidated (Sun et al., 2014; Hamada et al., 2008; Justenhoven et al., 2011). Importantly, *SLCO1B3* gene expression is absent in healthy breast tissue (Muto et al., 2007), suggesting that its activation in breast cancer may be tumor-specific rather than physiological. In the current investigation, we revealed a substantial link between the rs4149117 polymorphism of *SLCO1B3* and a lower probability of BC in Bangladeshi women. Specifically, the co-dominant 1 (OR=0.57, 95% CI=0.37-0.88, p=0.012), dominant (OR=0.54, 95% CI=0.36-0.82, p=0.003), over-dominant (OR=0.60, 95% CI=0.39-0.92, p=0.019), and allelic (OR=0.55, 95% CI=0.38-0.80, p=0.001) models demonstrated significant protective effects. The co-dominant 2 and recessive models also suggested a reduced risk (OR<1.0), but these relationships were not statistically noteworthy (p>0.05). Taken together, these findings indicate that rs4149117 may exert a protective influence against BC. Our results are identical with those of Tang *et al.* (2021), who indicated that *SLCO1B3* expression decreased the progression, movement, and spreading of BC cells in in-vitro settings, suggesting a potential tumor-suppressive role. Tang *et al*. (2021) further demonstrated that the *SLCO1B3* gene is abundantly expressed at the cell membrane and in the cytoplasm of breast carcinoma tissues, but not in normal breast tissues (Muto et al., 2007). This differential expression pattern corroborates the concept that *SLCO1B3* may serve as a context-dependent factor, potentially operating as a tumor suppressor in BC. The current investigation found no substantial relationships (p>0.05) between rs4149117 genotypes and chemotherapy responses in patients undergoing either AC-paclitaxel or AC-docetaxel regimens. This finding implies that, although rs4149117 may have a protective effect on breast cancer susceptibility, the variant may not influence treatment outcomes in patients undergoing taxane-based chemotherapy. Our findings corroborate those of Schwarz *et al*. (2011), who similarly

indicated no substantial impact of rs4149117 on paclitaxel pharmacokinetics in Caucasian populations. In the AC-paclitaxel cohort, patients carrying the GT+TT genotype of rs4149117 had a substantially greater likelihood ($p<0.05$; OR>1.0) of developing higher grade (≥3) toxicities, including nausea, diarrhea, constipation, fever, edema, fatigue, dysgeusia, and hepatotoxicity, pointing to a potential role of rs4149117 in paclitaxel-triggered adverse events. Conversely, no substantial correlation was observed between *SLCO1B3* rs4149117 genotypes and higher-grade toxicities in patients treated with the AC-docetaxel regimen. This observation was in line with the report by de Graan *et al*. (2012), who found no significant influence of *SLCO1B3* polymorphisms on docetaxel clearance or toxicity in a Dutch population. Taken together, our findings reveal a protective role of rs4149117 in BC susceptibility and a potential influence on paclitaxel-induced toxicity, but not on treatment response or docetaxel-triggered adverse reactions.

In our Bangladeshi cohort, the genotype distribution of *ABCB1* (2677G>T/A; rs2032582) among BC patients was 79.92% for the wild-type homozygote (AA), 17.67% for heterozygotes (AT), and 2.41% for mutant homozygotes (TT), indicating a high prevalence of the wild-type allele. The major allele (A) was present in 88.76% of patients, and the minor allele (T) was observed in 11.24% of patients. Similarly, Wu *et al*. (2012) reported in a Chinese cohort that 31.3% were normal homozygotes (GG), 48.3% heterozygotes (GT or GA), and 20.4% mutant homozygotes (TT or AA), with major (G) and minor (T or A) allele frequencies of 55.5% and 44.5%, respectively. Analysis of all genetic models of the rs2032582 variant in our study suggested a lower risk for BC (OR<1.0); however, all of these linkages were statistically not noteworthy ($p>0.05$). Therefore, the rs2032582 variant does not appear to impact BC susceptibility in the Bangladeshi women significantly. Wu *et al*. (2012), conversely, revealed that the TT genotype or T allele of 2677G>T/A had significant linkage to a greater risk of BC in Chinese women ($p<0.05$; OR=1.827), suggesting potential population-specific genetic differences. In terms of treatment response, carrier patients (AT+TT) in our cohort had greater responses to the AC-T regimen compared to non-carrier (AA) patients; nevertheless, none achieved statistical significance ($p>0.05$). Consistent with this, Chaturvedi *et al*. (2013) reported no substantial linkage between the *ABCB1* 2677G>T/A variant and the response to anthracycline-based neoadjuvant treatment in Indian women with BC. Similarly, Chang *et al*. (2009) demonstrated that *ABCB1* 2677G>T/A genotypes as well as haplotypes had no relationship with the total rate of response or survival rate in BC patients undergoing anthracycline- and paclitaxel-based chemotherapy. Patients having the AT+TT genotype had a substantially greater incidence of many high-grade (≥3) toxicities after AC-paclitaxel treatment

than those with the AA genotype. These toxicities included diarrhoea, fever, fatigue, dysgeusia, myalgia, and hepatotoxicity. Similarly, Tsai *et al*. (2009) reported a substantial relationship of the GG genotype with fever or febrile neutropenia in Taiwanese BC patients, while Sissung *et al*. (2006) observed a substantial linkage between the *ABCB1* 2677G>T/A variant and paclitaxel-triggered peripheral neuropathy and neutropenia in German individuals. In contrast, Chang *et al*. (2009) and Rizzo *et al*. (2010) observed no substantial relationship between the *ABCB1* 2677G>T/A variant and taxane-triggered hematological, non-hematological, or neurological toxicities. Overall, the *ABCB1* 2677G>T/A polymorphism in Bangladeshi BC patients is significantly associated with AC-T-related toxicities, while it does not appear to markedly influence cancer risk or chemotherapy response, highlighting the importance of population-specific pharmacogenetic studies to optimize AC-T treatment strategies.

In our study, the *ABCB1* 3435C>T (rs1045642) variant showed a genotype distribution of 34.54% AA (normal homozygote), 51.00% AG (heterozygote), and 14.46% GG (mutant homozygote) among BC patients. The corresponding allele frequencies were 60.04% for A (major allele) and 39.96% for G (minor allele). The heterozygous genotype (AG) was the most common in our cohort. Wu *et al*. (2012) found a similar pattern in a Chinese population, with CC, CT, and TT genotypes at 33.10%, 48.20%, and 18.70%, respectively. The allele frequencies were 57.20% (C) and 42.80% (T). The close similarity in genotype and allele distributions suggests that the rs1045642 variant may follow a comparable inheritance pattern across Asian populations. Additionally, in our research, the rs1045642 variant was substantially correlated with an elevated risk of BC across different genetic models, including co-dominant 2, dominant, and allelic models, underscoring its possible role in disease susceptibility in Bangladesh. In line with our findings, Wu *et al*. (2012) also identified a substantial correlation between this variant and BC possibility in Chinese Han women, therefore reinforcing the polymorphism's broader significance across Asian groups. Regarding chemotherapy response, the dominant model for rs1045642 showed a substantially lower response to the AC-paclitaxel regimen (AG+GG vs. AA: OR = 0.55, 95% CI = 0.31-0.98, p = 0.040). Priyadarshini *et al*. (2019) similarly found that the co-dominant 1 model was correlated with diminished responses (OR < 1.0) to taxane-containing chemotherapy in Indian women with BC, but this correlation did not appear statistically noteworthy (p > 0.05). Furthermore, Wu *et al*. (2012), along with Ji *et al*. (2012), demonstrated that the co-dominant 2 (TT) of the 3435C>T polymorphism was correlated with disease-free survival (DFS), progression-free survival (PFS), as well as overall survival (OS) in anthracycline-treated BC individuals in China. Collectively, these findings suggest that the rs1045642 variant may influence chemotherapy efficacy, although the magnitude and

significance of its effect appear to vary across populations. Patients with the AG+GG genotype had markedly elevated incidences of various high-grade (≥3) toxicities during the AC-paclitaxel regimen, in contrast to those with the AA genotype. These toxicities included vomiting, fever, dysgeusia, peripheral neuropathy, myalgia, and hepatotoxicity. No substantial toxicities were observed in the AC-docetaxel regimen. Earlier research confirmed the impact of the 3435C>T variant in chemotherapy-related toxicity. Tran *et al*. (2006) and Sissung *et al*. (2006) demonstrated a significant correlation between rs1045642 polymorphisms and taxane-induced neutropenia in BC patients. In contrast, several studies-including Bray *et al*. (2010), Cizmarikova *et al*. (2010), Zhang *et al*. (2011), Ji *et al*. (2012), Chaturvedi *et al*. (2013), and Yao *et al*. (2014)-found no substantial relation between 3435C>T genotypes and anthracycline-containing chemotherapy toxicity. Collectively, these findings indicate that the influence of rs1045642 on chemotherapy-related toxicity is specific to genotype and treatment regimen, highlighting the necessity of individualized surveillance for BC patients undergoing AC-T therapy.

In the present study, the *ABCB1* 1236C>T (rs1128503) variant exhibited a genotype distribution of 25.70% wild-type homozygotes (AA), 50.60% heterozygotes (AG), and 23.70% mutant homozygotes (GG) among breast cancer patients. The corresponding major (A) and minor (G) allele frequencies were 51.00% and 49.00%, indicating a relatively balanced allele distribution within the studied population. Wu *et al*. (2012) demonstrated a distinct distribution among Chinese women with BC, noting CC, CT, and TT genotypes at frequencies of 11.70%, 48.20%, and 40.10%, respectively, alongside C and T allele rates of 35.80% and 64.20%. These findings indicate that although the heterozygous genotype is prevalent across populations, the mutant T allele appears to be more prevalent in the Chinese cohort than in the Bangladeshi population. All genetic models of the *ABCB1* rs1128503 variant were correlated with an elevated risk of BC (OR > 1.0), including the co-dominant-2, recessive, and allelic models demonstrating statistically noteworthy relationships ($p < 0.05$). These outcomes underscore the possible significance of this mutation as a marker for risk within the Bangladeshi community. Regarding chemotherapy response, dominant model of the *ABCB1* rs1128503 variant was substantially linked with lower responses to the AC-paclitaxel regimen (AG+GG vs. AA: OR = 0.53, 95% CI = 0.28-0.99, $p = 0.049$). This dominant model also demonstrated reduced responses to the AC-docetaxel regimen, however this correlation lacked statistical significance ($p > 0.05$). These findings imply that the *ABCB1* 1236C>T variant may affect therapeutic responses to the AC-paclitaxel regimen in Bangladeshi BC individuals. Similarly, Wu *et al*. (2012) observed lower response rates across all genetic models in Chinese patients undergoing anthracycline-

containing neoadjuvant chemotherapy, although none of these associations reached statistical significance. Carrier patients (AG+GG) were more likely to have higher-grade (≥ 3) adverse effects from AC-paclitaxel chemotherapy than non-carrier patients (AA). These side effects included vomiting, fever, hepatotoxicity, and bone toxicity (OR > 1.0; $p < 0.05$). Conversely, multiple prior investigations indicated no significant correlation between the *ABCB1* 1236C>T genotypes and anthracycline-triggered toxicities in breast carcinoma patients (Bray et al., 2010; Tsai et al., 2009; Yao et al., 2014; Cizmarikova et al., 2010), suggesting that the impact of this variant on toxicity may vary between populations or treatment regimens. Overall, these findings indicate that the *ABCB1* 1236C>T variant not only contributes to BC susceptibility but may also influence both the therapeutic response and toxicity profile of AC-T regimens in the Bangladeshi population.

In the current research, the allele frequency of the *ABCB1* 129T>C (rs3213619) variant among Bangladeshi women with BC was 91.37% for the major allele (A) and 8.63% for the minor allele (G). Lévy *et al*. (2013) similarly demonstrated that the allele frequency of the *ABCB1* 129T>C variant among women with BC in France was 93.07% for the major allele T and 6.93% for the minor allele C. All genetic models, including co-dominant 1 (AG vs. AA), co-dominant 2 (GG vs. AA), dominant (AG+GG vs. AA), recessive (GG vs. AA+AG), over-dominant (AG vs. AA+GG), and allelic model (G vs. A), indicated a marginally elevated risk (OR>1.0) for BC development in Bangladeshi women. However, these correlations were not statistically noteworthy ($p>0.05$). Furthermore, no published data currently exists that elucidates the correlation between the *ABCB1* 129T>C polymorphisms and BC susceptibility. Patients with the AG+GG genotype demonstrated a slightly elevated response (OR=1.23) to the AC-paclitaxel regimen and a diminished response (OR=0.64) to the AC-docetaxel regimen when compared to the AA genotype. However, none of these correlations attained statistical significance ($p>0.05$). Lévy *et al*. (2013) similarly showed no significant correlation between *ABCB1* T-129C genotypes and clinical responses to docetaxel-based neoadjuvant treatment in individuals with BC. Carrier patients with the AG+GG genotype experienced substantially higher toxicities (grade≥3) than patients with the AA genotype during the AC-paclitaxel regimen, including diarrhea, fever, mouth sores, dysgeusia, and peripheral neuropathy (OR > 1.0; $p < 0.05$). Abraham *et al*. (2014) also demonstrated a substantial association between *ABCB1* T-129C genotypes and taxane-induced sensory neuropathy in European BC patients. Overall, the 129T>C rs3213619 polymorphism may significantly influence AC-T induced toxicity among Bangladeshi women with BC.

The current study reveals that among BC patients, the genotypic pattern for *ABCC2* rs17222723 involves 82.33% wild-type homozygotes (TT), 15.66% heterozygotes (TA), and 2.01% mutant homozygotes (AA). This indicates a predominance of the normal-type allele and a relatively small frequency of the mutant allele. Similarly, Sensorn *et al*. (2016) reported a comparable pattern for *ABCC2-24C>T* among Thai BC patients, with 76.90% normal homozygotes (CC), 23.10% heterozygotes (CT), and no mutant homozygotes (TT). The low frequency of homozygous mutants in both populations may promote interindividual variability in *ABCC2*-mediated drug transport and chemotherapy response. In the current analysis, all genetic models suggested a higher risk for BC susceptibility (OR > 1.0); however, none reached statistical significance ($p > 0.05$). Consistent with these observations, Sensorn *et al*. (2016) reported overexpression of *ABCC2* in tamoxifen-resistant BC cells, further supporting its role in treatment resistance. Regarding chemotherapy response, carrier patients (TA+AA) had lower response rates to both the AC-paclitaxel and AC-docetaxel regimens compared with non-carrier patients (TT); however, no statistically significant correlations were observed ($p > 0.05$). In the same way, Mistry *et al*. (2025) discovered that BC patients who underwent the AC-T regimen and had the 58626AA genotype of the *ABCC2* gene did not respond well to chemotherapy and did not live long. With respect to chemotherapy-induced toxicities, patients carrying the TA+AA genotype experienced significantly higher incidences of multiple adverse effects (grade ≥3), including nausea, vomiting, diarrhea, fever, dysgeusia, and hepatotoxicity, during AC-paclitaxel treatment (OR>1.0; $p < 0.05$), pointing to a potential role of this genotype in increasing toxicity. Conversely, Abraham *et al*. (2014) indicated that the heterozygotes (TA) of rs17222723 was correlated with a diminished risk of taxane-triggered sensory neuropathy (OR = 0.63; 95% CI = 0.42-0.93; $p = 0.02$), suggesting that the functional implications of *ABCC2* variants may differ based on the particular toxicity and treatment setting. Overall, *ABCC2* rs17222723 may influence variability in AC-T response and toxicity, though treatment response associations were not statistically significant.

The mutant allele of *CYP2C8*3* is mostly present in Caucasians, occurring at a frequency of about 13%, and is infrequently observed in Asian groups (Seredina et al., 2012). In our Bangladeshi cohort, the genotype distribution of *CYP2C8*3* among BC patients was 84.74% normal homozygotes (CC), 13.65% heterozygotes (CT), and 1.61% mutant homozygotes (TT). This closely aligns with previous reports: Marsh *et al*. (2007) observed 85.71% CC, 13.09% CT, and 1.19% TT, while Seredina *et al*. (2012) reported 83.85%, 15.64%, and 0.51%, respectively. The major and minor allele frequencies in our study were 91.57% and 8.43%, consistent with Seredina *et al*. (2012), who reported 91.67% and 8.33%. The concordance of

these frequencies suggests that the allele distribution of *CYP2C8*3* in the Bangladeshi population is similar to formerly studied populations, supporting the potential generalizability of pharmacogenetic findings regarding this variant. Each genetic model of rs11572080 in our research indicated a tendency towards heightened BC susceptibility (OR>1.0), but none achieved statistical significance (p>0.05). Conversely, Attia *et al.* (2024) revealed that the heterozygous CT genotype (OR=2.199, 95% CI=1.071-4.513, p=0.023) and the minor T allele (OR=2.491, 95% CI=1.204-5.154, p=0.01) were substantially correlated with a greater likelihood of breast carcinoma in women who have not yet reached menopause. The lack of significance in our cohort may reflect population-specific differences or sample size limitations. Compared with non-carrier (CC) patients, carrier patients (CT+TT) had higher response rates to the AC-paclitaxel regimen (OR > 1.0), but lower response rates following the AC-docetaxel regimen. A similar trend was observed by Seredina *et al.* (2012), where the heterozygous GA genotype was associated with higher neoadjuvant chemotherapy response compared to homozygous GG, although the correlation didn't appear statistically noteworthy (OR=3.08, 95% CI=0.61-13.53, p=0.09). These consequences imply that heterozygosity at the *CYP2C8*3* variant may affect the response to chemotherapy. In addition, patients having the CT+TT genotype were correlated with a substantially elevated risk (OR>1.0; p<0.05) of multiple high-grade (≥3) chemotherapy-induced toxicities in the AC-paclitaxel cohort. These adverse events included vomiting, fever, peripheral neuropathy, myalgia, hepatotoxicity, pulmonary toxicity, and bone toxicity. These observations are in line with Hertz *et al.* (2013), who revealed that the genotypes of *CYP2C8*3* variant significantly elevated the incidence of paclitaxel-triggered neuropathy among European, African-American, and mixed-race breast cancer patients. Additionally, multiple studies have recognized *CYP2C8* rs11572080 as one of the most frequently identified variants linked to taxane-related sensory neuropathy (TRSN) (Marsh et al., 2007; Gréen et al., 2009; Leskelä et al., 2011). The association, however, is still controversial. For instance, Abraham *et al*. (2014) found an insignificant association between *CYP2C8*3* and TRSN risk (OR = 1.22; 95% CI = 0.93-1.59; p = 0.14), suggesting that variations in demographics, study design, and sample size may account for these inconsistencies. Taken together, the CT genotype of *CYP2C8* rs11572080 may increase susceptibility to paclitaxel-related toxicities in the AC-T regimen. Larger trials with people from different ethnic backgrounds are required to confirm this relationship and determine how it could be employed in individualized chemotherapy.

3.6 Limitations

This study exclusively examined the *SLCO1B3* rs4149117, *ABCB1* rs1128503, *ABCB1* rs1045642, *ABCB1* rs2032582, *ABCC2* rs17222723, and *CYP2C8* rs11572080 variants, skipping the assessment of other variants, haplotypes, or associated transporter genes that could also affect chemotherapy response and toxicity. Drug levels in plasma and transporter activity were not assessed, limiting the comprehension of the direct relationship between genotype and drug exposure or adverse consequences. Reporting bias may affect adverse events, especially subjective complaints like nausea, vomiting, fatigue, dysgeusia, or peripheral neuropathy. Moreover, the results were derived from a single, ethnically homogeneous population in Bangladesh, thus limiting their applicability to other communities. Despite these limitations, the study underscores the possibility of *SLCO1B3* rs4149117, *ABCB1* rs1128503, *ABCB1* rs1045642, *ABCB*1 rs2032582, *ABCC*2 rs17222723, or *CYP2C8* rs11572080 genotyping to predict chemotherapy-triggered toxicity along with individualised therapies.

3.7 Conclusion

This research provides strong evidence that the *SLCO1B3* rs4149117 polymorphisms confer a protective effect against BC risk, whereas *ABCB1* rs1128503 and rs1045642 are associated with heightened risk. There were no substantial correlations between the *ABCB1* rs2032582, ABCB1 rs3213619, *ABCC2* rs17222723, or *CYP2C8* rs11572080 genetic variants and the likelihood of developing BC. Patients with carrier genotypes (heterozygotes and mutant homozygotes) for *ABCB1* rs1128503 and *ABCB1* rs1045642 exhibited significantly poorer responses to AC-paclitaxel therapy. Other studied SNPs showed no significant associations with treatment response to either AC-paclitaxel or AC-docetaxel. Additionally, carrier patients of the studied genetic variants experienced a broad spectrum of toxicities, including nausea, vomiting, diarrhea, constipation, fever, edema, mouth sores, fatigue, dysgeusia, myalgia, peripheral neuropathy, hepatotoxicity, pulmonary toxicity, and bone toxicity. The above findings suggest that the genotyping of *SLCO1B3* rs4149117, *ABCB1* rs1128503, *ABCB1* rs1045642, *ABCB1* rs2032582, *ABCC2* rs17222723, or *CYP2C8* rs11572080 may facilitate personalised chemotherapy and mitigate treatment-related toxicity. To utilize genotype-based dosing in typical oncology practice, it needs to be tested in larger, more diverse groups and in planned clinical studies, particularly in countries with low or middle incomes like Bangladesh.

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Chapter-04
Discussion

4.1 Discussion

This research explained the sociodemographic and clinicopathological presentation of breast cancer (BC) patients in Bangladesh as well as their response to treatment and toxicities induced by a chemotherapy regimen comprising doxorubicin-cyclophosphamide and taxane (AC-T). This study also highlighted how gene polymorphisms affect clinical responses and toxicities in BC women receiving AC-T treatment.

The mean age of our studied patients was 43 years, and over half (50.20%) of them were younger than 45 years. This also reveals that BC is being diagnosed in comparatively younger ages in Bangladesh compared to Western cultures, where diagnosis typically occurs after the age of 50. The same has been suggested by other reports in South Asia, indicating that lifestyle factors, environmental exposures, and genetic predisposition could be responsible for breast cancer being diagnosed at younger ages (Hossain et al., 2014). A vast majority of our studied patients, approximately 64.26%, were from rural backgrounds. Such a rural majority is a sign of possible deficiencies in the provision of effective, timely cancer care since facilities and the resource base are typically limited in rural areas compared to urban settings. Parallel gaps have been found in the United States, too, with rural residence being linked with inequality in cancer incidence and mortality (Zahnd et al., 2018). Education-wise and job-wise, the majority of our patients (81. 53%) were housewives and more than half (51. 81%) were illiterate. This deficit can result in the ignorance of their illness and treatment, which can impact their following the prescription of the doctor and outcomes of health. Sen *et al*. (2023) and Chowdhury *et al*. (2023) have also found the same barriers among Bangladeshi women, highlighting how the educational and work status can impact cancer management. A mere 0.80% of our patients were unmarried, while an immense 99.20% were married, in line with Mistry *et al*. (2024) in an Indian cohort, where most were also married. The vast majority of married women may be indicative of cultural patterns in South Asia, where marriage in early life is widespread. Most important was that 76. 71% of the patients were non-users of tobacco, which may have reduced chemotherapy side effects. It has previously been postulated in literature, such as by Bergman *et al*. (2022), that smoking can be a promoter of some toxicities and an inhibitor of cancer treatment efficacy. It seems then that, for the majority of these patients, the use of tobacco is not a concern in terms of side effects experienced.

The clinicopathological pattern among the patients showed that tumors were predominantly found on the right breast (52.21%), and the majority of the participants (87.95%) had no family

history of BC, as previously documented in Bangladeshi BC patients (Chowdhury et al., 2023). During the current study, 65.46% of the subjects reported a history of contraceptive use and 38.95% were premenopausal. The result is critical because prolonged exposure to exogenous hormones through oral contraceptives has been suggested to influence BC risk, especially in women with premenopausal status (Hossain et al., 2014). There are many factors that contribute to the complexity of the linkage between contraceptive use and BC; some of them include age at contraceptive use, the length of time spent using them, and the form of contraceptive was used. The high prevalence observed in our cohort highlights the importance of analysing reproductive health patterns to determine the risk of BC among Bangladeshi women. In the current research, the most frequent approach used for BC detection was biopsy, followed by fine needle aspiration cytology (FNAC), mammography, lumpectomy, magnetic resonance imaging (MRI), and immunohistochemistry (IHC). Biopsy is the most sensitive method to obtain the true histological picture of palpable as well as nonpalpable tumors of the breast. It provides specific information regarding the grade, category, and other pathological features of the tumour that are relevant to clinical management (Tseng, 2023). Most participants (87.95%) reported no family history of BC, suggesting that environmental and lifestyle factors (Al Ajmi et al., 2020), rather than genetic factors, may play a substantial role in the aetiology of BC in this study population. Most of our participants (60.64%) indicated symptom onset before 45, indicating earlier disease development than among Western populations (DeSantis et al., 2015). Chowdhury *et al*. (2023) also documented an increasing average age at diagnosis of BC in Bangladeshi women to 51.1 years, suggesting the possibility of heterogeneity in disease presentation from the country, perhaps arising due to genetic, environmental, and lifestyle determinants. In this study, 88.35% of the participants had the history of proper breastfeeding, in line with the findings of Mistry *et al*. (2024), where 81.8% of mothers also shared the same history of breastfeeding. Histologically, the most prevalent form of BC was invasive ductal carcinoma (53.82%), as has been seen globally, where it is accountable for the vast majority of cases of BC (Thennavan et al., 2021). Most of our patients had grade-2 (47.79%) or grade-3 (35.74%) tumors, while stage-2 carcinoma accounted for 46.98% of all cases. These findings show that most patients were diagnosed at advanced to moderate stages and would therefore have needed more intensive treatment protocols. This aligns with previous work on several varied populations that exhibited the clinical complication related to managing advanced-stage BC (Biswas et al., 2017; Antonini et al., 2023; Kaplan, 2013). In our study, the most common endocrine receptor profile was that of HER-2 negative, which was found in 58.63% of the subjects. This pattern is similar to what is happening with BC globally. HER-2 positive cancers

are usually much more aggressive and require the use of more expensive targeted therapies, for example, trastuzumab (Kurosumi, 2009). The elevated levels of HER-2-negative tumors within our investigative group highlight a common receptor distribution in BC that can be of great value in planning treatment strategies, including taxane-based chemotherapy (Garutti et al., 2023; Montero et al., 2012; Verrill et al., 2020). Mistry *et al*. (2024) revealed that 73.60% of patients got adjuvant treatment, while our study found that 70.28% of patients underwent adjuvant chemotherapy. The high percentage of patients who had adjuvant chemotherapy demonstrates contemporary oncology's emphasis on efforts to minimize the probability of cancer's recurrence after surgical interventions, especially in patients with more advanced stages of the illness or in instances of tumors that are of moderate to high grade.

In this investigation, participants underwent different chemotherapy protocols, with the most prevalent being AC-paclitaxel, where 87.55% of patients received this treatment. 53.67% of patients of this group had a positive clinical response, while 46.33% of patients had a negative response. On the other hand, 12.45% of patients received an AC-docetaxel regimen. 58.06% of patients in this group had a positive clinical response, while 41.93% had a negative response. The result suggests both taxane-based regimens had a comparable overall benefit in this study. Response diversity for different therapies underscores the necessity of implementing individualised treatments in a more tailored fashion for each individual, as evidenced in BC (Masoud, 2017; Aleskandarany et al., 2018). The differing responses to the AC-Paclitaxel regimen between underweight and normal-weight individuals, particularly the finding that underweight individuals in the cohort had the least response, suggest that the cohort's nutritional status may influence response to different chemotherapy regimens (Gupta et al., 2022). Metastatic ductal carcinoma as well as infiltrating ductal carcinoma ultimately demonstrated stronger responses to the very same regimen, which appears to be higher in comparison to the rest of the other ductal carcinoma subtypes. The results obtained underscore the significance of body mass, other patient-related factors, and tumor characteristics regarding treatment outcomes and the need for personalized treatment approaches in BC (Gota et al., 2021; Rakha et al., 2010). Furthermore, patients whose tumor was triple negative for hormones exhibited significantly lower responses to the AC-paclitaxel regimen than patients with HER2-negative tumors. The observation correlates with other studies that reported triple-negative BC (TNBC) to be one of the more aggressive cases, which also lacks appropriate targeted treatment and also has lower responses to standard therapies (Bianchini et al., 2016; Foulkes et al., 2010).

Adverse events caused by chemotherapy were the leading cause of the withdrawal, delay, alteration, or extension of treatment in this study. Due to the negative influence on patients' quality of life, these interruptions are of great clinical importance (Gadisa et al., 2020). To improve therapeutic efficacy, it is vital to understand the issues faced by patients undergoing chemotherapy for BC. Adverse events were mostly of the gastrointestinal (GIT) type. Patients on either the AC-paclitaxel or AC-docetaxel treatment plans experienced a moderate to severe level of nausea (76.31%) and vomiting (60.24%), which suggests the inadequacy of the routine antiemetic regimen. While ondansetron, a blocker of the 5-HT3 receptor, is commonly prescribed, Gadisa *et al*. (2020) have shown that almost 88% of patients still experience chemotherapy-induced nausea and vomiting (CINV). According to Smith (2012), an alternative, the neurokinin-1 receptor antagonist aprepitant, is more likely to be successful and has the added benefit of reducing anxiety and sleep issues, as reported by Lee *et al*. (2017). Diarrhea (34.94%) and constipation (44.18%) were particularly noted in patients on the AC-paclitaxel regimen, which is consistent with Nyrop *et al*. (2019), who noted that 35% of patients and 47% of patients had significant diarrhea and constipation. In addition, 35.74% gastritis was noted, and only a few of these were of high severity (grade 3 or 4). The literature supports that gastritis is more prevalent among patients on AC-paclitaxel in comparison to AC-docetaxel. Gadisa *et al*. (2020) also reported patients on AC-paclitaxel to have gastritis more frequently. The gastrointestinal (GIT) toxicities are severe enough to cause interruption and discontinuation of the patient's treatment. Some of the toxicities can be lessened by actions taken in supportive care or by simple changes to the infusion chemotherapy sequence, e.g., docetaxel and cyclophosphamide, as noted by Hanaoka *et al*. (2013). Fever and oral mucositis were also part of the observations and were the cause of concern in many patients due to severe fever that was possibly due to the unrestrained activation of the COX-2 and prostaglandin pathways (Mehran et al., 2021). Participants receiving AC-T regimen also developed mouth sores (Gadisa et al., 2020). The frequency of myalgia, edema, and peripheral neuropathy was greater in those receiving AC-paclitaxel therapy, mirroring previous findings (Foster et al., 2010; Waterhouse et al., 2026). Myalgia/arthralgia and peripheral neuropathy can be attributed to taxanes (Gadisa et al., 2020), who highlighted that, in contrast to the AC therapy, the patients who received the AC-T therapy had far greater rates of peripheral neuropathy, and the incidence of severe myalgia or arthralgia was substantial. Numerous previous studies have also reported the same type of differences between AC and AC-paclitaxel therapies, where the myalgia and arthralgia alongside the peripheral neuropathy were significantly enhanced in scenarios where AC was administered with the addition of paclitaxel (Hershman et al., 2016; Kim et al., 2011; Fernandes

et al., 2015). Fatigue and dysgeusia were common and observed in many of our patients, with fatigue occurring in approximately 59.44% of cases and dysgeusia in about 78.71% of cases, in alignment with Gadisa *et al*. (2020), where 97.33% and 96.00% of patients receiving the AC-T regimen also reported fatigue and dysgeusia, respectively. In our study, participants also reported having dry mouth (xerostomia) with a prevalence of 53.01%. Xerostomia can lead to a range of functional impairments, such as difficulties in eating, speaking, and swallowing, and can also exacerbate the risks of dental caries and oral candidiasis, as well as worsening one's nutritional and psychological well-being (Monreal et al., 2022). All patients were presented with total hair loss along with onycholysis with or without discoloration. Onycholysis is presumably due to the anti-mitotic reactions of taxanes on dividing cells of the nail matrix (Alessandrini et al., 2019; Alshari et al., 2020). The neurogenic pathways induced by anthracyclines may also have caused nail alterations (Hinds & Thomas, 2008). Also, 41.37% of the patients developed skin hyperpigmentation of different degrees, supporting the studies on pigmentary changes due to chemotherapy (Ferreira et al., 2019).

Patients undergoing the AC-T treatment in the present study had significant hematological adverse events that included anemia (61.45%), neutropenia (22.49%), febrile neutropenia (15.26%), and thrombocytopenia (5.22%). In the investigation by Tesfaye *et al*. (2023), the rates of anemia (44.1%) were lower; however, neutropenia (61.8%) and thrombocytopenia (16.4%) were higher than in this study, and the rates of febrile neutropenia (10%) were comparable. The mentioned differences are likely attributable to the differences in the study populations and treatment of the study, the ethnic backgrounds, and the presence of supportive care. Vriens *et al*. (2013) documented grade ≥3 haematological toxicities, including anemia (1.0%), neutropenia (20.0%), febrile neutropenia (23.0%), and thrombocytopenia (5.0%). In this study, the following were reported as the frequency of grade ≥3 events: anemia (4.42%), neutropenia (4.82%), febrile neutropenia (4.42%), and thrombocytopenia (0.80%). These results corresponded relatively to the aforementioned study. Out of organ-specific toxicities, hepatotoxicity was of the highest prevalence, as it concerned 30.92% of the population. As documented by Moezian *et al*. (2022), doxorubicin-induced hepatotoxicity is usually highlighted as a prominent issue in BC management. Bone toxicity (20.48%) perhaps signifies the presence or development of pre-existing conditions such as osteoporosis or osteopenia (this is during/after treatment or during survivorship) (Choksi et al. 2013). The AC-T chemotherapeutic regimen has been reported to cause nephrotoxicity in 6.43% of patients, findings supported by Santos *et al*. (2020), who reported that nephrotoxicity can reduce the therapeutic effectiveness of anticancer drugs. The findings of cardiotoxicity in 5.62% of

patients also support the findings of Perez *et al*. (2008) concerning doxorubicin-related cardiotoxicity, especially at cumulative doses of > 300 mg/m2. In our cohort, 15.66% of patients had pulmonary toxicity. Similarly, Bielopolski *et al*. (2017) reported respiratory distress in individuals with BC who had received the AC-T regimen. To improve the benefits of therapy, it is also necessary to comprehend the complications of BC patients undergoing chemotherapy. Based on this research, 10.84% of patients passed away within 5 years of receiving a cancer diagnosis, while 7.23% passed away thereafter, indicating an overall mortality of 18.07% of patients subjected to an AC-T treatment. As per the last follow-up, 81.93% of patients were successful. These survival outcomes are somewhat similar to the results of Vriens *et al*. (2017), where the 5-year overall survival rate was in the range of 76% for AC-T and 84% for TAC, indicating the overall survival benefits with the taxane-based AC regimen.

Bushra *et al*. (2020) explain how pharmacogenetics predicts therapeutic outcomes based on interindividual genetic variation, which is extremely relevant in Bangladesh due to having little to no population-specific data. As far we know, this study is the first to examine the effects of the polymorphisms of *SLCO1B3* (rs4149117), *ABCB1* (rs1128503, rs2032582, rs3213619, rs1045642), *ABCC2* (rs17222723), and *CYP2C8*3* (rs11572080) on toxicity and clinical outcomes in Bangladeshi women with BC receiving the AC-T regimen. Lee *et al*. (2017), Smith *et al*. (2007), and Iusuf *et al*. (2015) explained that OATP1B3, the protein that *SLCO1B3* encodes, functions to facilitate the hepatic uptake of doxorubicin, paclitaxel, and docetaxel, which consequently impacts the drugs' clearance, systemic exposure, and toxicity risk. The presence of certain functional polymorphisms in OATP1B3 may determine how effective a given treatment is, how certain drugs may interact with each other, as well as increase the odds of negative side effects (Anabtawi et al. 2022), which may ultimately determine the pharmacokinetics and pharmacodynamics with regard to the treatment (Mbatchi et al. 2015). In our case, all co-dominant, dominant, over-dominant, and allelic models of rs4149117 showed a statistically significant association with a reduced risk of BC, suggesting a protective effect in the Bangladeshi population ($p < 0.05$). These findings correspond to those of Tang *et al*. (2021), who in vitro showed that *SLCO1B3* had the ability to stop the development, movement, and spreading of BC cells and therefore may have a tumor-suppressing function. However, this is contrary to Justenhoven *et al*. (2011), who showed no substantial linkage with any of the *SLCO1B3* variants and BC, which reflects the debate surrounding the role of *SLCO1B3* in carcinogenesis and BC. In regards to toxicity outcomes, it was evident that there was an increased risk of gastrointestinal, systemic, and organ-specific toxicities, which included, but were not limited to, nausea, diarrhea, constipation, fever, edema, fatigue, dysgeusia, and

hepatotoxicity, for patients with the GT+TT genotype, as there were higher odds ratios of 2.40 to 3.84 for patients on AC-paclitaxel. Therefore, it was demonstrated that the presence of the T allele had a higher predisposition of patients to more severe and harmful side effects due to chemotherapy when undergoing the AC-paclitaxel regimen. Conversely, none of the patients who underwent the AC-docetaxel treatment showed any substantial relationship between the *SLCO1B3* rs4149117 genotype and the higher-grade toxicity levels. These findings correspond to what de Graan *et al*. (2012) observed; there is no effect of *SLCO1B3* polymorphisms (without considering rs4149117) on docetaxel clearance and toxicity within the Dutch population. Also, there were no considerable rs4149117 polymorphism and chemo response relationships in the AC-paclitaxel and AC-docetaxel subgroups. In comparison to the findings from Smith *et al*. (2007) and Iusuf *et al*. (2015), it is suggested that there could be an impact of some OATP1B3 polymorphisms on the disposition of docetaxel/paclitaxel and, thus, the effectiveness of these drugs. However, there was no influence of rs4149117 on the pharmacokinetics of paclitaxel in Caucasians, indicating that the impact of this polymorphism on the pharmacogenetics of paclitaxel may be irrelevant in clinical practice in some populations. The impact of *SLCO1B3* rs4149117 may also be substrate-predictable rather than consistent across all taxanes. This reflects the substrate-specific transport mechanisms of OATP1B3 (Schwarz et al., 2011; Letschert et al., 2004). For instance, some studies showed that rs4149117 influences the uptake of certain substrates (e.g., mycophenolic acid and cholecystokinin) and has little or no effect on the uptake of other substrates (Schwarz et al., 2011; Picard et al., 2010). The implication is that the polymorphism does not change the primary response to the tumor despite having an ability to change the level of exposure to the drug and an ability to change the risk of toxicity through some other pathways. Even though information is still very inadequate, particularly concerning *SLCO1B3* rs4149117 in BC, our results do show consistency in broader evidence of pharmacogenomics.

In this study, among BC patients, most of the genotype distribution for the *ABCB1* (2677G>T/A; rs2032582) was the wild-type homozygote (AA) at 79.92%; the remaining patient distribution was heterozygote (AT) at 17.67%, while the least was the mutant homozygote (TT) at 2.41%. The wild-type homozygote also contributed the most to the allele frequencies, with 88.76% for the major allele (A) and 11.24% for the minor allele (T). There was, therefore, a predominance of the wild-type allele in this cohort from Bangladesh. On the other hand, Rizzo *et al*. (2010) recorded a more even distribution for an Italian population; 27% normal homozygotes, 46% heterozygotes, and 27% mutant homozygotes, which also resulted in populations with equal major and minor allele frequencies (50% each). For every genetic

model analyzed-co-dominant, dominant, recessive, over-dominant, and allelic-all the odds ratios were less than 1, which indicates that the *ABCB1* rs2032582 variant is negatively associated with the risk of BC. However, these odds ratios were all non-significant ($p>0.05$), indicating that this polymorphism likely does not decrease the risk of developing BC in the Bangladeshi population. In contrast, Wu *et al*. (2012) found that in all models of *ABCB1* G2677T/A, there was a positive association with BC in Chinese women ($OR>1.0$; $p<0.05$), indicating that there are likely population-specific effects. With regard to response to chemotherapy, the present investigation found that the dominant (AT+TT vs. AA) model predicted higher response rates to both AC-paclitaxel and AC-docetaxel regimens; however, no relations appeared statistically noteworthy ($p>0.05$). Hence, the *ABCB1* 2677G>T/A variant is likely not materially affecting the response to AC-T chemotherapy among Bangladeshi BC patients. In the same way, Wu *et al*. (2012) and Ji *et al*. (2012) did not report substantial correlations between this variant and response to anthracycline-based neoadjuvant chemotherapy among Chinese Han BC patients. Conversely, Gréen *et al*.'s (2006) findings showed that BC patients who had the T or A allele at the 2677 position were more inclined to possess a favorable response to paclitaxel treatment. Furthermore, Wu *et al*. (2012) revealed no substantial relationship between the G2677T/A variants and BC patients' progression-free survival (PFS) and overall survival (OS) who received anthracycline-based chemotherapy. Relating to toxicity, carrier patients having AT+TT genotype were at a much greater risk for a variety of high-grade (≥3) toxicities, like diarrhea, fever, fatigue, dysgeusia, myalgia, and hepatotoxicity, after AC-paclitaxel. Comparable to Ikeda *et al*. (2015), who found that the *ABCB1* 2677G>T/A polymorphism had a meaningful risk of grade ≥3 leukopenia for BC patients receiving doxorubicin and cyclophosphamide. Yet another series of publications published discrepancies. Tsai *et al*. (2009), Jabir *et al*. (2018) and Tulsyan *et al*. (2014) identified the polymorphism as substantially lowering the risk of fever, febrile neutropenia, fatigue and leukopenia of patients under taxane-based chemotherapy. In contrast to the above authors, Chaturvedi *et al*. (2013) documented no association of this polymorphism/variant and any of the hematological as well as non-hematological toxicities triggered by anthracycline chemotherapy in the Indian females with BC. Bray *et al*. (2010) and Yao *et al*. (2014) similarly reported no association between the 2677G>T/A variant and chemotherapy-induced toxicities in patients receiving doxorubicin-cyclophosphamide or anthracycline regimens. It is evident that the *ABCB1* 2677G>T/A variant in BC and its correlation with response/toxicity to chemotherapy are population dependent. As a result, pharmacogenetic investigations should be

conducted in the Bangladeshi population with an emphasis on ethnicity in an attempt to optimize AC-T chemotherapy.

In our research, *ABCB1* 3435C>T (rs1045642), among BC patients, had a genotype distribution of 34.54% AA (wild-type), 51.00% AG (heterozygous), and 14.46% GG (mutant homozygous), with allele frequencies of 60.04% for A (major allele) and 39.96% for G (minor allele). These results have some resemblance to those of Priyadarshini *et al*. (2019) for Indian women with locally advanced BC, with CC, CT, and TT genotype frequencies of 25.6%, 51.1%, and 23.3%, respectively, with allele frequencies of 51.2% (C) and 48.8% (T). Closely similar heterozygote frequencies likely indicate some common ancestry among these groups in South Asia. The *ABCB1* 3435C>T variant has been found to be linked with a greater risk of developing BC in all of the models of the study. This was especially pronounced in the case of the co-dominant model 2, the dominant model, and the allelic model, which all indicated a significant risk (OR > 1.0, $p < 0.05$) and hence a significant relationship with the risk of BC in the Bangladeshi case study. Likewise, Wu *et al*. (2012) observed the existence of a substantial relationship in all of the genetic models in the risk of BC in Chinese Han females, especially in the case of co-dominant model 2 (OR = 1.386, 95% CI = 1.091-1.761, p = 0.008) and the allelic model (OR = 1.281, 95% CI = 1.021-1.285, p = 0.020). On the whole, it has been proposed that the *ABCB1* 3435C>T variant affects the risk of BC and advanced-stage BC based on the cumulative research that has been done. It was observed that the rs1045642 polymorphism might have negative effects on the efficacy of treatment among Bangladeshi patients with BC undergoing AC-paclitaxel chemotherapy. Patients expressing the dominant model (AG+GG vs. AA) had significantly lower response rates (dominant model; OR = 0.55, 95% CI = 0.31-0.98, p = 0.040). In the AC-docetaxel regimen, these patients had reduced clinical response as well, but the linkage was not statistically noteworthy ($p>0.05$). In line with these findings are those of other researchers on the polymorphism, with varying results. Ji *et al*. (2012) and Wu *et al*. (2012) reported a substantial correlation of the TT genotype with response to anthracycline-based chemotherapy in patients of Chinese Han origin, while the correlation was not noteworthy in the Indian people cohorts, according to Chaturvedi *et al*. (2013) and George *et al*. (2009). A substantial linkage between populations in Germany and other Asian countries was noted by Kafka *et al*. (2003) and Ashariati *et al*. (2008). Furthermore, in Korea, Chang *et al*. (2009) observed in the metastatic BC population receiving taxane-based chemotherapy that the CT genotype was linked to lower rates of disease control and shorter overall survival. The divergent outcomes indicate the need to evaluate the 3435C>T in patients receiving AC-T chemotherapy. With respect to toxicity, individuals with the AG+GG genotype had greater incidences of the

following grade 3 and above toxicities (vomiting, fever, dysgeusia, peripheral neuropathy, myalgia, and hepatotoxicity) than those with the AA genotype while on the AC-paclitaxel regimen (OR > 1.0, $p < 0.05$). Abraham *et al*. (2012) also confirmed a noteworthy lower (OR = 0.83, 95% CI = 0.70-0.98, $p = 0.03$). There are other examples in the literature where toxicity was reported to be risked in a genotype-specific way. For example, Tsai *et al*. (2009) described the relationship of the CC genotype with anthracycline-induced leukopenia in Taiwanese females, while Kim *et al*. (2012) described a greater risk of taxane-induced diarrhea and neutropenia in TT genotype carriers in a Korean cohort. On the contrary, Chang *et al*. (2009) and Rizzo *et al*. (2010) did not find any positive and, in fact, found a significant negative association for 3435C>T genotypes with taxane-induced toxicity. Overall, these studies suggest that the effect of the rs1045642 variant associated with chemotherapy-related toxicity in a certain cohort is both genotype- and population-specific, and thus call for a more thorough, perhaps personalized, approach to toxicity assessment in BC patients on AC-T chemotherapy.

For the *ABCB1* 1236C>T (rs1128503) polymorphism, BC patients showed 25.70% AA wild-type, 50.60% AG heterozygotes, and 23.70% TT mutant homozygotes. The major (A) and minor (T) alleles have allelic frequencies of 51.00% and 49.00%, respectively, indicating that the study population has almost the same alleles. An Indian population study reported similar results to this study by Priyadarshini *et al*. (2019), demonstrating that the CC, CT, and TT genotype frequencies were 20.90%, 46.50%, and 32.60%, respectively, and their C and T allele frequencies were 44.20% and 55.80%, respectively. These South Asian populations share a similar distribution of alleles, and thus it is recommended that the *ABCB1* 1236C>T variant follows the same distribution pattern in the genetic sense due to the likely same ancestry and/or ethnic background. The studied genetic models indicate an association with a greater risk of BC with the *ABCB1* rs1128503 variant (OR > 1.0). Among the co-dominant 2, recessive, and allelic models, the risk is statistically significantly higher ($p < 0.05$) for BC, suggesting individual carriers of the mutant T allele may be at a higher risk. In our opinion, this confirms the rs1128503 variant as a plausible hereditary risk indicator for BC in the Bangladeshi population. Regarding chemotherapy response, the *ABCB1* 1236C>T variant was noted to be related with lower response rates to both AC-paclitaxel and AC-docetaxel regimens. The carrier patients (AG + GG) were noted to have substantial lower response rates (OR < 1.0; $p < 0.05$) to AC-paclitaxel as opposed to non-carrier patients (AA). Contrary to these findings, Priyadarshini *et al*. (2019) reported an upward trend (OR > 1.0) towards response to taxane-based chemotherapy amongst Indian patients, albeit these differences were not statistically noteworthy. Zhang *et al*. (2011) and Ji *et al*. (2012) similarly did not report a substantial relationship with this variant

and response to neoadjuvant anthracycline-based chemotherapy in Chinese individuals, and likewise Chaturvedi *et al*. (2013) revealed no substantial effect amongst Indian patients. These inconsistencies are likely due to ethnic or treatment-specific variations in *ABCB1*-mediated drug transport, and therefore population-specific pharmacogenetic assessments are warranted when predicting response to AC-T chemotherapy. The toxicity findings of our study indicate that while undergoing AC-paclitaxel treatment, carrier patients with the AG+GG genotype are more likely compared with the AA genotype to experience vomiting, fever, hepatotoxicity, and bone toxicity, all of which are higher grade (≥ grade 3) adverse effects (OR = 2.86-5.29; $p < 0.05$). This finding and list of adverse effects are also corroborated by Chaturvedi *et al*. (2013), where a noteworthy relationship was found for *ABCB1* 1236C>T and grade 2-4 toxicities (including anemia), specifically in Indian patients. In contrast, no comparable noteworthy correlation was discovered in Chinese populations by Zhang *et al*. (2011) and Ji *et al*. (2012), which again underscores the likely relevant importance of ethnic or population-specific aspects. Collectively, the results imply that *ABCB1* 1236C>T variant not only contributes to the incidence of BC but also has relevance for the effectiveness and toxicity of AC-T chemotherapy, which may have important implications for personalized therapy.

The *ABCB1* 129T>C (rs3213619) variant genotype distributions among BC patients were 84.34% normal homozygote (AA), 14.05% heterozygote (AG), and 1.61% (GG) mutant homozygote in this study. Also, Lévy *et al*. (2013) reported that the genotype frequencies for T-129C in BC patients were 87.13% wild-type homozygotes (TT), 11.88% heterozygotes (TC), and 0.99% mutant homozygotes (CC). In the case of genetic modelling, all genetic models of the SNP rs3213619 (with regard to co-dominant 1 and 2, dominant, recessive, over-dominant and allelic models) indicated a higher risk for BC (OR>1.0), but in any of the models, the association did not appear statistically noteworthy ($p>0.05$). Thus, it seems that the *ABCB1* 129T>C variant in this study failed to show a noteworthy correlation with BC development for the Bangladeshi studies. Conversely, no studies exist that would show an association of *ABCB1* 129T>C polymorphisms and BC risk. Regarding chemotherapy outcomes, carrier patients (AG+GG) exhibited greater clinical improvement with the AC-paclitaxel regimen, whereas they showed diminished clinical responses with the AC-docetaxel regimen, compared with non-carrier patients (AA). However, no correlations were statistically noteworthy ($p>0.05$). This supports Lévy *et al*. (2013), who reported no significant relationship between the *ABCB1* 129T>C polymorphisms and clinical responses in individuals with BC after doxorubicin- and docetaxel-containing neoadjuvant chemotherapy. Concerning toxicity, carrier patients (AG+GG) noticeably noted several greater-grade (≥3) toxicities following the AC-paclitaxel

regimen, including but not limited to diarrhea, fever, oral mucositis, altered taste (dysgeusia), and peripheral neuropathy (OR= 2.25 to 4.30; p<0.05), compared to non-carrier patients (AA). In the same way, Abraham *et al*. (2014) noted the CC (mutant homozygote) genotype of *ABCB1* 129T>C as a significant factor in taxane-associated sensory neuropathy among the BC patients in Europe. From all the results, the *ABCB1* 129T>C (rs3213619) variant is more likely to impact AC-T chemotherapy-induced toxicity than the chemotherapy response itself among BC patients in Bangladesh.

The frequency of the minor allele (A) was 9.84% for *ABCC2* rs17222723, which was consistent with previous findings of 7.0% in BC patients (Bosó et al., 2014). Although the various genetic models of *ABCC2* rs17222723 all demonstrated an elevated risk of BC, none were statistically noteworthy (p > 0.05). This finding implies a slight possibility of risk, which would be in line with the multitude of factors involved in BC risk. Importantly, Kiyotani *et al*. (2010) indicated that polymorphisms in *CYP2D6* and *ABCC*2 might be predictive factors for BC patients on tamoxifen, suggesting that *ABCC2* variants are clinically important even if the association was not noteworthy in our population. Concerning treatment response, carrier patients (TA+AA) showed decreased responses in both AC-paclitaxel and AC-docetaxel regimens, compared to non-carrier patients (TT). However, none of these associations showed statistical significance (p > 0.05). Consistent with these findings, Mistry *et al*. (2025) presented a noteworthy association (p<0.05) with *ABCC2* (58626AA) and treatment non-responsiveness, in relation to all genetic models, in BC patients treated with the AC-T regimen. In terms of toxicity, carrier patients (TA+AA) showed substantially greater occurrences of some high-grade (≥3) adverse events during AC-paclitaxel treatment in comparison to non-carrier patients (TT), such as nausea, vomiting, diarrhea, fever, dysgeusia, and hepatotoxicity. Similarly, *ABCC2* polymorphisms have been associated with gastrointestinal toxicities (stomatitis, nausea, vomiting, and diarrhea) and some hematological toxicities (anemia, neutropenia, and febrile neutropenia), as well as other sensory neuropathy, in individuals with BC who had taxane- or AC-T–based chemotherapy (Bosó et al., 2014; Kim et al., 2012; Abraham et al., 2014). To support the clinical importance, Sensorn *et al*. (2016) showed that the *ABCC2*-24CC genotype was linked to higher distant metastasis risk (P = 0.040; adjusted HR = 2.34; 95% CI, 1.04-5.27), and the combination of the *ABCC2*-24CC and *ABCB1* 3435 CT+TT genotypes had greater distant and also bone metastasis risk (P = 0.020; adjusted HR = 2.46; 95% CI, 1.15-5.26; and P = 0.040; adjusted HR = 3.70; 95% CI, 1.06-12.89, respectively). Our findings suggest that *ABCC2* rs17222723 is more prospective to have an influence on the risk of chemotherapy toxicity rather than BC risk or treatment response.

The *CYP2C8* rs11572080 genotype distribution in our Bangladeshi cohort included 84.74% CC, 13.65% CT, and 1.61% TT. These values are similar to those described in Egyptian women, in whom the genotype frequencies were 69.50% (CC), 30.50% (CT), and 0.00% (TT), respectively (Attia et al., 2024). In parallel, Lam *et al*. (2016) recorded the frequencies of genotypes as follows: wild-type homozygotes (GG) as 81.1%, heterozygotes (GA) as 18.9%, while mutant homozygotes (AA) were recorded at 0.00%. On the other hand, Seredina *et al*. (2012) described the mutant allele frequency of *CYP2C8*3* among BC patients to be 8.33%, which in other studies of Caucasian populations has been estimated to be around 13%. The allele has, however, been reported absent from Asian populations (Dai et al., 2001; Bahadur et al., 2002), signifying a possible ethnic variation. Also, in our study, all the genetic models of *CYP2C8* (rs11572080) showed a tendency of increasing the risk for BC, but none showed a statistically significant result ($p > 0.05$). Notably, 38.95% of our patients were premenopausal, which is in line with the findings from the Egyptian cohort, where Attia *et al*. (2024) reported a significant association between the *CYP2C8*3* variant and the risk of premenopausal BC. Further, Hertz *et al*. (2012) showed that the two linked SNPs of *CYP2C8* (rs11572080 and rs10509681) were fully concordant in the population of North Carolina, which highlights the significance of haplotype structure on the associations of population-specific genetics. Concerning the responses to treatment, the carrier patients (CT+TT) of our cohort reported higher response rates to the AC-paclitaxel regimen and lower response rates to the AC-docetaxel regimen, compared to non-carrier patients (CC). However, no relationships were statistically noteworthy ($p>0.05$). This aligns with the findings of Hertz *et al*. (2012), who reported that *CYP2C8*3* carriers had better complete responses to the neoadjuvant therapy with paclitaxel compared to wild-type homozygotes (OR = 3.92, 95% CI = 1.46-10.48, p = 0.046). Conversely, Tulsyan *et al*. (2014) noted that patients having the heterozygous GA genotype were at greater risk of being non-responders compared to the wild-type homozygous GG genotype (GA vs. GG: OR = 1.11, 95% CI = 0.27-4.47, p = 0.882) in taxane therapy, although this finding is also non-significant. Variations in population genetics, sample size, or treatment guidelines may account for such inconsistencies. With respect to toxicity, carrier patients (CT+TT) compared to non-carriers (CC) exhibited significantly greater incidences of some high-grade (≥3) toxicities following the AC-paclitaxel regimen, including vomiting, fever, myalgia, peripheral neuropathy, hepatotoxicity, lung toxicity, and bone toxicity ($OR>1.0$; $p<0.05$). This is in line with the findings of Attia *et al*. (2024), who noted that chemotherapy-induced peripheral neuropathy (CIPN) is one of the most frequently observed in the heterozygous carrier (CT) of the rs11572080 variant, especially in premenopausal women with

BC. CIPN, which affects 70% of patients, is one of paclitaxel's most common as well as devastating side effects, greatly reducing quality of life during and after treatment (Johnson et al. 2023). Moreover, according to Speck *et al*. (2013), the total drug dosage and older age are also independent risk factors for being more susceptible to CIPN. Likewise, Hertz *et al*. (2012) found *CYP2C8*3* carriers, which corroborates the findings of this study, as having more Grade (≥3) toxicities, which also included neuropathy, neutropenia, myalgia, hypersensitivity, fatigue, GI toxicity, and anemia. It appears that *CYP2C8* rs11572080 is more inclined to be linked to the risk of developing toxicities from chemotherapy rather than the BC risk or responding to BC treatment.

Taken together, the results underscore the complex toxicity profile associated with AC-T chemotherapy and the need for tailored surveillance and comprehensive management approaches that consider the specific patient's risk factors.

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Chapter-05
Limitations of the Study

Limitations of the Study

Since the study only focused on the pre-selected genetic variants, i.e., SLCO1B3 (rs4149117), *ABCB1* (rs1128503, rs1045642, rs2032582, rs3213619), *ABCC2* (rs17222723), and *CYP2C8* (rs11572080), it did not include several enzymes and haplotypes that could also contribute to the outcome of the chemotherapy. Key genes such as *SLCO1B1* and other *CYP2C8* alleles, as well as plasma drug levels and transporter activities, were not investigated. There is most likely a lack of laboratory validation, such as protein expression and enzymatic activity, and so the genetic associations are likely based solely on statistical correlation, which hinders mechanistic understanding. These limitations made it difficult to draw any correlation between the genetic differences to the drug exposures or the metabolic behaviour. Participants may have had recall bias, which may have influenced their self-reporting of non-haematological toxicities. Another limitation is that the study captures only a single homogeneous Bangladeshi population, so the findings may not be representative or relevant to other, more diverse populations. Regardless of these limitations, the study provides a preliminary and useful signal that could facilitate the identification of patients most likely to experience the adverse consequences and also advocates for tailoring chemotherapy to individual needs. This suggests a promising direction for future research, particularly in the genotyping of patients for *SLCO1B3*, *ABCB1*, *ABCC2*, and *CYP2C8* variants.

Chapter-06
Conclusion

Conclusion

This study represents the first cohort-based pharmacogenomic investigation evaluating the influence of genetic polymorphisms on treatment response and chemotherapy-related toxicities among breast cancer (BC) patients receiving the AC-T regimen in Bangladesh. Patients diagnosed with infiltrating or metastatic ductal carcinoma showed comparatively better responses to the AC-paclitaxel regimen. However, poorer responses to AC-paclitaxel were observed among patients who were underweight or had triple-negative tumors. Dysgeusia (78.71%) was the most prevalent non-hematological toxicity, anemia (61.45%) was the most frequent hematological toxicity, and hepatotoxicity (30.92%) was the most common organ-related toxicity. In the survival analysis of 249 patients, 27 (10.84%) died within 5 years of diagnosis, 18 (7.23%) died after the 5-years period during follow-up, and 204 (81.93%) were alive at the final follow-up. *SLCO1B3* rs4149117 was associated with a reduced risk of BC, while *ABCB1* rs1128503 and *ABCB1* rs1045642 showed an increased risk for BC. No risk associations were reported for BC for the following SNPs: *ABCB1* rs2032582, *ABCB1* rs3213619, *ABCC2* rs17222723, and *CYP2C8* rs11572080. In addition, carrier genotypes of *ABCB1* rs1128503 and *ABCB1* rs1045642 were associated with poorer responses to the AC-paclitaxel regimen, although the association for rs1128503 was marginal. None of the other SNPs we studied showed significant associations with the response to treatment with either AC-paclitaxel or AC-docetaxel. More pronounced associations were observed during AC-paclitaxel therapy, where certain polymorphisms were linked to increased risks of specific grade III-IV toxicities, including gastrointestinal, neurological, and hepatic adverse events. No significant associations between the investigated polymorphisms and toxicity were observed in patients treated with the AC-docetaxel regimen. Together, these findings provide initial insights into genetic and clinical predictors of AC-T chemotherapy outcomes among Bangladeshi BC patients. Before genotype-guided dosing may be implemented in oncology management in resource-constrained settings, such as Bangladesh, larger studies will be needed that include multi-ethnic validation cohorts and prospective trials.

Chapter-07
Future Aspects

Future Aspects

Personalized Therapy Development: Integrating pharmacogenetic screening (e.g., *ABCB1*, *SLCO1B3*) into everyday clinical practice is recommended to optimize chemotherapy regimens for breast cancer patients in Bangladesh.

Expanded Genetic Profiling: The addition of more SNPs and larger cohort sizes in future studies is recommended to confirm existing associations and identify additional genetic markers that impact treatment response and toxicity.

Mechanistic Studies: Biochemical and biological studies are needed to investigate the functional effects of the *ABCB1* rs1128503 and *ABCB1* rs1045642 polymorphisms on the response and toxicity to AC-T chemotherapy.

Toxicity Prediction Models: Using SNP data to create predictive models will yield powerful tools that can estimate the odds of various toxicities (i.e., hepatotoxicity, neuropathy, pulmonary, and bone) and drive the development of corresponding supportive care.

Guidelines and Implementation: Integrating these observations into national cancer treatment guidelines can improve precision medicine in low-resource settings such as Bangladesh.