CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

PMS2 Gene Expression Profiling in Peripheral Blood of Postmenopausal Women with Breast Carcinoma: A Pre & Post-Treatment Comparative Study

by

Asma

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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This thesis is dedicated to my beloved parents the two most influential figures of my life. Whose boundless love, unwavering support, and endless encouragement have been the cornerstone of my academic journey. I especially dedicated this work to my beloved father Abdul Razaq who have been my source of inspiration, guide and give me strength, when i thought of giving up, who continually provide his moral, spiritual, emotional and financial support.

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And lastly, I dedicated this book to our Almighty Allah that guides me, give strength, power of mind, protection and skills.

CERTIFICATE OF APPROVAL

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Abstract

Breast cancer are frequent neoplasms, with more than 4 million people worldwide diagnosed yearly. Breast cancer is particularly common, Among the malignancies that most commonly affect women. Many genes are involved in the development of breast cancer different studies show different genes. According to the data, approximately 178,388 new cases of breast cancer were registered in Pakistan in 2020. Current study goal is to investigate the expression of the PMS2 gene expression level in the blood of the postmenopausal women with breast cancer before and after treatment (neoadjuvant chemotherapy, Surgery). PMS2, a key gene in DNA mismatch repair, is linked to health conditions like Lynch syndrome. To find out the expression level of PMS2 gene level in the blood of postmenopausal women with breast cancer. The current study methodology includes collecting 47 peripheral blood samples from 27 older breast cancer patients pre- and post-tumor treatment (neoadjuvant chemotherapy).20 blood samples of breast cancer patients before the breast cancer diagnoses before treatment, and 20 blood samples after treatment and 7 were healthy women with no cancer consider as a control group. Some samples after 4 months of treatment (neoadjuvant chemotherapy), and some 6 months after treatment to assess the expression of PMS2 gene, the current study methodology include RNA extraction, cDNA formation, and nano Drop, reverse transcription (RT)-PCR analysis were performed to assess the PMS2gene expression. The study utilized SPSS for statistical analysis, which included descriptive statistics, correlation coefficient calculation, and paired sample t-tests. The demographic profile of 20 patients shows a significant decrease in gene expression level in blood of postmenopausal women with breast cancer after surgery. Paired sample T-test is performed further to support this observation with a significant mean difference $p = 0.042$ in pre- and post-tumor PMS2 expression levels. The paired T test reject the null hypothesis, and emphasizing the meaningful association between surgical intervention and alteration in PMS2 gene A higher prevalence of breast cancer among women aged 50-60, primarily diagnosed with IDC and grade 2 cancer. The survival rate of the postmenopausal women with breast cancer was remained same both before treatment and after treatment. There was a weak

negative association between pre- and post-tumor treatment of the PMS2 gene expression levels. The study indicates a significant correlation between surgical intervention and changes in PMS2 gene expression in older breast cancer patients, highlighting the potential of PMS2 as a biomarker for therapeutic efficacy.

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Abbreviations

BRCA1	Breast Cancer Gene 1
BRCA2	Breast Cancer Gene 2
LMICs	Low Middle-Income Countries
MSH 3-6	Mismatch repair protein at Position 3 and Position 6
PCNA	Proliferating Cell Nuclear Antigen
PMS2	Postmeotic Segregation Increase 2
PRC2	Polycomb Repressive Complex 2
RFC	Replication Factor C

Chapter 1

Introduction

1.1 Cancer

Cancer is a diverse, intricate disease with many different forms, each with unique features. It is caused by aberrant cells in the body growing and spreading out of control, which can result in the development of cancerous tumors. The term "metastasis" refers to the ability of these aberrant cells to spread to other areas of the body via the lymphatic and circulatory systems, as well as to infiltrate and destroy nearby healthy tissues. In 2016, approximately 7,046 neuro neoplasms were registered with 459 benign and 6,587 malignant cancers. Around 19.3 million new cancer cases and more than 10 million cancer deaths have been reported in 2020 [1].

1.2 Breast Cancer

Among the malignancies that most commonly affect women, breast cancer is particularly common. This disease is intricate and multidimensional, with aberrant cell proliferation resulting in the development of cancerous tumors. Throughout their lives, one in eight women will develop breast cancer. About 10% of cases are hereditary breast cancers, which are usually caused by genetic defects that are

passed down autosomally dominantly. It's noteworthy to note that while men can potentially develop breast cancer, being a woman is the primary risk factor. Breast cancer is around 100 times more common in women than in men [2]. Breast cancer is most likely to strike women with deleterious hereditary BRCA1 or BRCA2 mutations at some point in their lives. Up until the age of 70, the cumulative incidence of breast cancer for people with BRCA1 or BRCA2 mutations is 66%. Ages 30 to 50 are the highest risk for BRCA1 mutant carriers to get breast cancer, but age 40 to 60 is the highest risk for BRCA2 mutation carriers.

While BRCA2-associated breast cancers resemble sporadic tumors and are primarily hormone receptor-positive, BRCA1-associated breast cancers are aggressive and hormone receptor-negative. Women of different ages can be affected by breast cancer, however it's important to realize that up to 50% of those diagnosed with the disease are over 65 [3]. One problem, though, is that older people with breast cancer are frequently diagnosed when the disease has progressed. The diagnosis at the later stage may present more challenges for efficient management and therapy [4]. Research indicates that an individual's age increases the chance of dying from breast cancer, irrespective of the cancer's stage of progression. A person's overall survival odds are often reduced if they receive a breast cancer diagnosis beyond the age of 55. Age can have a different effect on how breast cancer behaves, but the reasons for these variations are still unclear. Nevertheless, a number of studies indicate that older women with breast cancer tend to exhibit certain traits that are generally less aggressive and indicate a better prognosis. These features include hormone receptor-positive tumors, HER2-negative tumors, low-risk tumors types, and tumors without involvement of neighboring lymph nodes. A better prognosis is usually indicated for older women with breast cancer when these symptoms are present. Triple-negative breast cancers are thought to account for 15–18% of cases in older people [5].

Breast cancer in elderly women has been found in numerous studies to be less aggressive and to react well to hormone therapy. This indicates that hormones frequently impact the growth of cancer, and in many situations, managing the

disease can be achieved by medicines that specifically target these hormonal pathways [6]. In the United States, 297,790 women are expected to receive an invasive breast cancer diagnosis in 2023, compared to 55,720 women who will receive a non-invasive (in situ) diagnosis. Interestingly, the incidence of invasive breast cancer in women has been increasing at a rate of about 0.5 percent each year since the mid-2000s. This increase is believed to be caused by a number of variables, such as an increase in women's excess body weight, shifting fertility rates, and an increase in the average age at which women give birth. It's crucial to keep in mind that 2,800 men in the US are expected to receive an invasive breast cancer diagnosis in 2023, despite the fact that the disease is more commonly associated with women. In 2023, it is projected that 43,700 Americans will pass away from breast cancer.

Breast cancer ranks as the second most common cause of death for women in the United States and the fifth most common cause of cancer globally, behind lung cancer. Thanks to advancements in therapy, greater awareness, and early identification, the number of fatalities from breast cancer has dropped by 43% since 2020, preventing almost 460,000 cases of the disease. Despite receiving fewer diagnoses, black women had a 40% higher death rate from breast cancer than white women. The median age of a breast cancer diagnosis is 63, while some women receive the diagnosis earlier Black women are more likely than white women to get the illness before turning 40 [7].

In low-to-middle-income countries (LMICs), including Pakistan and India, the prevalence of breast cancer is increasing while being more common in the industrialized world. Seventy percent of breast cancer-related mortality occur in low- and middle-income countries (LMICs) due to misdiagnosis, myths, cultural beliefs, lack of diagnostic methods, inadequate healthcare policy, and advancements in therapy. According to the provided data, approximately 178,388 new cases of breast cancer were registered in Pakistan in 2020. Misconceptions and myths play a part in the increase in Pakistani cases of breast cancer. False information concerning the clinical manifestation, diagnosis, and treatment of breast cancer

impedes timely detection, deteriorates the doctor-patient bond, and prevents efficient care [8].

FIGURE 1.1: Show a breast cancer [9]

When diagnosed with breast cancer, patients in low- to middle-income countries (LMICs) typically had advanced stages of the disease, in contrast to those in higher income nations [10]. The most typical indicator of breast cancer, which typically starts in the breast lobules or ducts, is the presence of painless lumps. In comparison to other Asian nations, Pakistan has a high incidence of breast cancer roughly 2.5 times greater than that of Iran and India. HER2 gene amplification is a significant predictor and prognostic factor for glycoprotein overexpression, affecting around 20% of individuals with breast cancer. An aggressive histological type, greater ER, PR negative, and HER2 positive are all linked to larger tumours, greater lymph node involvement, and higher tumor grade. Unlike in Europe and America, more than 60% of breast cancer patients in Pakistan present at an advanced stage of the disease. This suggests that Pakistani individuals' breast cancer has distinct biological behaviors [11]. When diagnosed with breast cancer, almost one-third of patients are 70 years of age or older. For women in this age group, the lifetime risk of breast cancer is 6.8%. This risk is higher than that of women under 49, who have a lower risk of 1.9%, and women 60–69, who have a risk of 3.5%. Older individuals with breast cancer are more likely to die from the disease than younger women. About half of all deaths related to breast cancer occur in women 70 years of age or older. Older women are more likely to have larger tumors. The

extremes of age <40 and >70 are associated with a worse prognosis for women with BC compared to middle-aged women between 40 and 69. People 65 years of age or older account for about 57% of breast cancer deaths. Women under 49 have a lower (0.2%) likelihood of dying from breast cancer, women 60–69 have a slightly greater (0.6%) risk, and women 70 and older have the greatest (2%). More than 80% of tumors identified in women 80–84 are hormone receptor positive. Older patients are more likely to have ductal histology, with lobular and well-prognosis carcinomas slightly overrepresented [12].

1.3 Risk Factors for Breast Cancer

FIGURE 1.2: Displays the breast cancer risk factors [13]

1.3.1 Breast Cancer Risk Factors Related to Lifestyle

An increased risk of breast cancer is specifically associated with an imbalanced diet. Physical inactivity has also been associated with an increased risk of breast cancer. Higher body mass index (BMI) postmenopausal women are more likely to develop breast cancer. This is related to the fact that breast fat tissue contains polycyclic aromatic hydrocarbons (PAH) the interaction between these PAHs and cellular estrogen receptors raises the possibility of developing breast cancer. Usage of alcohol is another cause of breast cancer as well. When alcohol is broken

down in breast tissue, chemicals are released that can disrupt the body's defense mechanisms and DNA repair processes, one of which is the BRCA1 gene's down-regulation.

1.3.2 Risk Factor for Breast Cancer: Reproductive History

Having a child after the age of thirty, getting married after the age of thirty, menarching early, and delaying menopause until after the age of fifty all increase the risk of developing breast cancer by altering hormonal fluctuations that occur during a woman's reproductive life. A study found that women who marry between the ages of 30 and beyond have a 7.0% risk while those who married younger have a 1.4% risk. Breast cancer incidence has been linked to early pregnancy and continued breastfeeding, with the majority of cases being both estrogen receptor (ER) positive and negative. Using hormonal contraceptives for an extended period of time can also raise your risk of breast cancer [14].

1.3.3 Radiation Exposure of Breast Cancer Risk Factor

The risk of breast cancer is increased by radiation exposure, particularly from radiation therapy. Particularly when it comes to the chest region, radiation treatments can have long-term consequences on breast health and raise the chance of breast cancer in later life.

1.3.4 Exposure to Diethylstilbestrol (DES) of Breast Cancer Factor

Women who were exposed to DES while still in utero had a greater risk of developing breast cancer. It has been demonstrated that exposure to the synthetic estrogen DES during pregnancy affects breast cancer risk factors and raises the chance of getting breast cancer later in life, especially in women over 40.

1.3.5 Dense Breasts as Known Risk Factor for Breast Cancer

Women who have dense breast tissue are more likely to get breast cancer. since a higher risk of breast cancer is associated with dense breast tissue.

1.3.6 Breast Cancer Risk Factor Based on Personal History

A personal history of breast cancer or certain non-cancerous breast illnesses may raise an individual's future risk of acquiring breast cancer.

1.3.7 Risk Factor for Breast Cancer in the Family History

A family history of breast cancer raises the risk of breast cancer. Genetic mutations that are inherited from generation to generation, such BRCA1 and BRCA2, can influence a person's susceptibility to breast cancer.

1.3.8 Metabolic Syndrome (MetS)

Metabolic Syndrome (MetS) causes a 52% increase in risk. higher blood glucose, triglycerides, decreased HDL cholesterol, and higher blood pressure are the main causes of MetS, which is characterized by high cholesterol, BP, and abdominal obesity. MetS alters hormones, such as insulin, estrogen, cytokines, and growth factors, which raises the risk of breast cancer.

1.3.9 Breast Cancer Factor: Hormone Replacement Therapy (HRT)

Extended usage of estrogen and progesterone-containing hormone replacement therapy, particularly postmenopausal hormonal therapy, may raise the risk [15].

1.4 Intervention of Breast Cancer

Multiple surgical operations may be necessary for the treatment of breast cancer, depending on the location of the tumors, the overall health of the patient, and the stage of the cancer. Here are some common surgical techniques used to treat breast cancer:

1.4.1 Lumpectomy

The surgical procedure during which doctors remove, just the tumors and a tiny portion of the surrounding healthy tissue. It seeks to protect the breast to the greatest extent feasible.

1.4.2 Mastectomy

The surgical procedure where doctors remove the entire breast

1.4.3 Types of Mastectomy

The mastectomy has three types such as

1.4.4 Total (Simple) Mastectomy

A procedure where surgeon remove the entire breast except the axillary lymph nodes.

1.4.5 Modified Radical Mastectomy

Removes some of the lymph nodes under the arm as well as the entire breast.

1.4.6 Radical Mastectomy

It removes the entire breast along with the surrounding chest muscles and lymph nodes.

1.4.7 Radiation Therapy

Radiation therapy procedure include internal radiation and external radiation therapy.

1.4.8 External Radiation Therapy

In this process the radiation machine utilizes outside of the body to deliver the targeted radiations to the damage breast.

1.4.9 Internal Radiation Therapy

This therapy involves inserting radioactive implants into or close to the tumor's location.

1.4.10 Chemotherapy

Employs medicine to eradicate cancer cells or halt its spread. It can be administered intravenously or orally, before or after surgery.

1.4.11 Hormone Therapy

Focuses on cancer cells' hormone receptors. Frequent in breast tumors that are hormone receptor-positive. Tamoxifen and aromatase inhibitors are two examples.

1.4.12 Targeted Therapy

Targets particular chemicals associated with the growth of cancer. Trastuzumab (Herceptin), a HER2-targeted treatment for HER2-positive breast cancer, is one example.

1.4.13 Immunotherapy

Encourages the immune system to identify and combat cancerous cells. Compared to some other cancer types, its application in the treatment of breast cancer is now more restricted.

1.4.14 Adjuvant and Neoadjuvant Therapies

1.4.14.1 Adjuvant Therapy

This therapy include Post-operative care aimed at lowering the likelihood of cancer return.

1.4.14.2 Neoadjuvant Therapy

Tumor-shrinking medication administered before to surgery.

1.4.15 Supportive Therapies

These include anti-nausea drugs, counselling, and pain management, among other therapies aimed at controlling side effects and enhancing the patient's general health. Studies reveal that treatment outcomes for elderly women with breast cancer differ greatly, highlighting the significance of delivering tailored and age-appropriate care while treating this population. One popular and perhaps effective treatment for breast cancer is surgery [16]. Contradictory findings are found in the research on the best course of treatment for older women with early-stage breast

cancer. According to some research, a women older than 70 may be more likely to have mastectomies or no operative treatment and less likely to have lumpectomies, a less invasive procedure. It might be chosen depending on patient desire, larger tumors, metacentric disease, or advanced malignancy [17].

1.5 Categorization of Breast Cancer

1.5.1 Non Invasive Breast Cancer Type

Incorporate both lobular and ductal cancer in situ.

1.5.2 DCIS, or Ductal Carcinoma Insitu

Since the aberrant cells are contained within the breast duct lining and have not metastasized, the cancer is considered non-invasive. Often observed during screening mammography. a good chance of healing but an increased risk of cancer recurrence.

1.5.3 Insitu Lobular Carcinoma (LCIS)

Is a non-invasive breast disease in which aberrant cells are discovered in the breast's lobules, which are small glands that produce milk. In situ refers to "in its original place," suggesting that the aberrant cells have not moved to neighboring breast tissue or other sections of the body beyond the lobules.

1.5.4 Types of Invasive Breast Cancer

Invasive ductal carcinoma and invasive lobular carcinoma are two types of invasive breast cancer.

1.5.4.1 IDC, or Invasive Ductal Carcinoma

Invasive ductal carcinoma (IDC) is the most common type of breast cancer, where cancer cells enter the duct and infiltrate the breast tissues nearby. frequently starts in the milk duct's cells. more prone to spread to other areas of the breast.

1.5.4.2 Lobular Carcinoma Invasive (ILC)

This type of cancer starts in the milk-producing lobules and can spread to other breast tissues. can be more challenging to locate through physical tests and imaging [18].

1.5.5 Classification of Breast Cancer Molecularly

1.5.5.1 Luminal A

Luminal A molecular subtype of breast cancer known as breast cancer is distinguished by particular genetic and molecular characteristics.

This subtype is included in the more general group of luminal tumors, which are usually hormone receptor positive. Luminal A tumors differ from other subtypes due to the following features

1.5.5.2 Hormone Receptor Status

Progesterone receptors (PR) and/or estrogen receptors (ER) are examples of hormone receptors that are positive in luminal A tumor. This suggests that the hormones causing these tumors are either estrogen or progesterone.

1.5.5.3 Expression of Ki-67

As a measure of cell proliferation, the protein Ki-67 is generally seen at lower levels in luminal A tumor. Slower cell growth is indicated by reduced Ki-67 expression.

1.5.5.4 HER2 Situation

Luminal A tumors typically do not overexpress the human epidermal growth factor and are HER2-negative. The prognosis for luminal A tumor is typically better.

1.5.5.5 Luminal B

Although Luminal B tumors also have positive hormone receptors, they also frequently exhibit elevated levels of Ki-67, indicating heightened cellular proliferation. Compared to Luminal A breast cancers, Luminal B breast cancers may have a marginally worse prognosis.

Progesterone and/or estrogen receptors are specific proteins that are searched for when breast cancer cells are removed during a biopsy or surgery. The progesterone and estrogens hormones' binding to these receptors promotes the progression of the cancer. Cancers are categorized as hormone receptor-positive or hormone receptor-negative based on the presence or absence of these receptors (proteins).

1.5.5.6 ER-Positive

Also known as ER+ cancers, these are breast tumors that include estrogen receptors.

1.5.5.7 PR-Positive

Progesterone receptor-positive (also known as PR+) breast tumors are those that have these receptors.

1.5.5.8 Hormone Receptor-Positive

This term, which also goes by the abbreviation HR+, describes a cancer cell that has one or both of the aforementioned receptors.

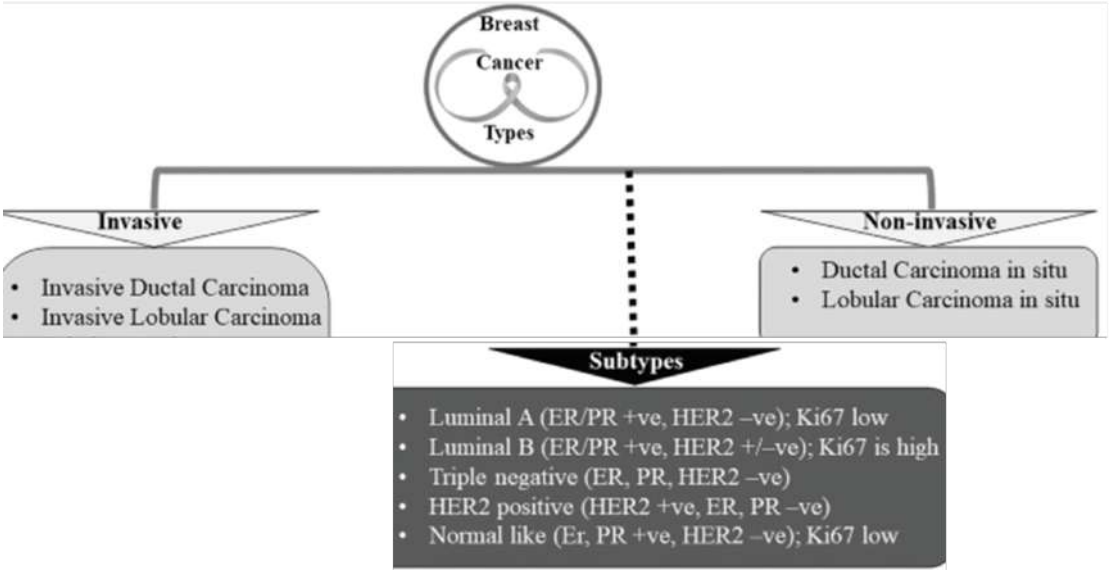

FIGURE 1.3: Show the classification of breast cancer [20]

1.5.5.9 Hormone Receptor-Negative

This word can also be used to describe a cancer cell that does not have an estrogens or progesterone receptor (HR-negative).

1.5.5.10 HER2-Positive

This type of tumor has an overexpression of HER2. Their hormone receptor status may be positive or negative. Targeted treatments like trastuzumab (Herceptin) are often effective in treating HER2-positive breast tumors.

1.5.5.11 Breast Cancer that is Triple-Negative (TNBC)

This subtype does not overexpress HER2 and does not express hormone receptors (PR and ER). Targeted therapy, such as hormonal or HER2-directed treatments, are ineffective against TNBC because of its tendency towards aggression. Chemotherapy is a common part of treatment [19].

1.6 Stages of Breast Cancer

TNM staging, widely used in breast cancer diagnosis, assigns a number to each tumour stage (0–4) according to its size, metastasis, and dissemination, indicating the degree of the malignancy.

1.6.1 Stage 0

Malignancy that is not invasive, limited to the ducts or lobules, and has not metastasized into the surrounding tissues

1.6.2 Stage I

Tiny, confined tumors. There is no evidence of cancer spreading to distant or adjacent lymph nodes. Possibly less than an inch.

1.6.3 Stage II

Larger tumor or cancer that has not progressed to other parts of the body but has affected the lymph nodes in the vicinity. Stage 2 is regarded as an early stage; it is more advanced than stage one. There are two subcategories within it: 2A and 2B.

1.6.3.1 Treatment Options

Include radiation therapy, surgery (such as a mastectomy or lumpectomy), and, in certain situations, systemic therapies including hormone therapy or chemotherapy.

1.6.3.2 Stage 2A

In stage 2A the size of a tumor is greater than 2 centimeters but not greater than 5 centimeters (not including a considerable number of lymph nodes). There is

no tumors in the breast, but there are tiny cancer cell clusters (known as micro metastases) in the lymph nodes. Even if the tumor may be smaller, more lymph nodes are involved.

1.6.3.3 Treatment

Treatment options for cancer may include surgery, radiation therapy, and possibly systemic treatments, depending on its specifics.

1.6.3.4 Stage 2B

The size of the tumor is greater; it is usually greater than 2 centimeters and may even exceed 5 centimeters. It's possible that the cancer has grown to encompass more lymph nodes in the vicinity. There may be evidence of invasion into adjacent structures or the chest wall.

1.6.3.5 Treatment

Similar to Stage 2A, treatment involves a combination of surgery, radiation therapy, and systemic treatments tailored to the individual characteristics of the cancer.

1.6.4 Stage III

Stage 3 is considered a more advanced stage of breast cancer staging than Stage 2. It suggests a more extensive and aggressive tumor that may have invaded adjacent structures and/or lymph nodes. Stage 3 is further broken into subcategories, such as 3A and 3B, which offer further information on the nature and scope of the illness. The stage 3 involve larger tumor size and may involve several lymph nodes, which could lead to the formation of bigger clusters and invasion of surrounding tissues, skin, or other structures, such as the chest wall. In most cases, the disease hasn't progressed to other organs.

1.6.4.1 Stage 3A

Tumor size varies, however it is often larger. Multiple lymph nodes may have been affected by the cancer. There may be an invasion into neighboring structures like the skin or chest wall.

1.6.4.2 Stage 3B

Involve a larger tumor size. Extensive involvement of the near lymph nodes. There could be a more sophisticated invasion of the skin or chest wall.

1.6.4.3 Therapy

Treatment for stage three breast cancer entails Treatment often consists of radiation therapy, surgery, and systemic medications such hormone therapy, chemotherapy, or targeted therapy. The specific treatment plan may include a multidisciplinary approach and is tailored to the specific characteristics of the cancer.

1.6.5 Stage IV

Known as metastatic breast cancer type, the cancer has spread to the brain, skin, lungs, liver, bones, and lymph nodes in the neck region near the collarbone.

1.6.6 Treatment for Stage IV Breast Cancer

1.6.6.1 Systemic Treatments

Chemotherapy, hormone treatment, targeted therapy, and immunotherapy are commonly used to target cancer cells throughout the body.

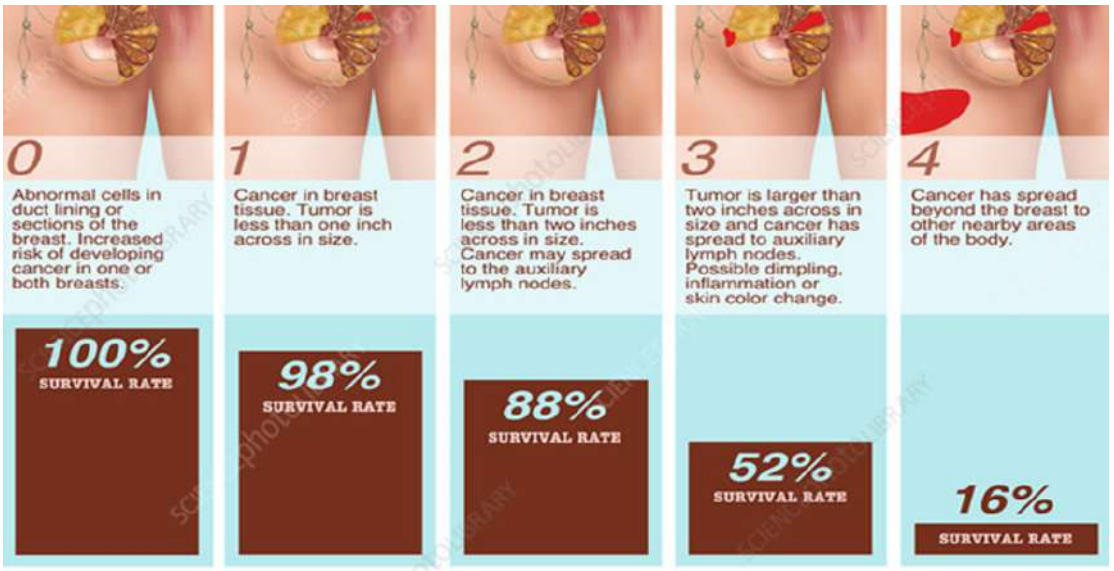

FIGURE 1.4: Shows stages of breast cancer [22]

1.6.6.2 Local Treatments

To address certain symptoms or issues brought on by metastatic lesions, local treatments including surgery or radiation therapy may be utilized in some situations [21].

1.7 Breast Tumor Grade

Grade of tumors are classified into

1.7.1 First Grade(I)

Strongly Differentiated

1.7.2 Second Grade(II)

Mildly differentiated

1.7.3 Third Grade(III)

A microscope-based evaluation of the appearance and properties of cancer cells yields a grade of 3 poorly differentiated (23).

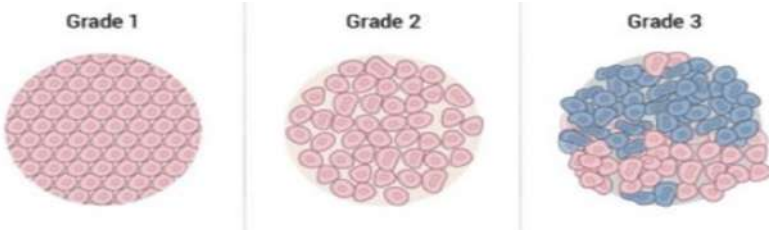

FIGURE 1.5: Shows tumor grades [23]

1.8 PMS2 Gene

Postmeotic segregation increase 2, or PMS2, a mismatch repairs gene with 38,125 base pairs, 15 exons, and an encoded protein of 862 amino acids. It is located at chromosomal segment location 22 on the short arm of chromosome 7 [24]. PMS2 is a nuclear protein with a mass of 95.8 kDa [25]. The primary function of this protein is to correct small DNA mismatches and insertions and deletions that may occur during homologous recombination and DNA replication. The MutL-alpha heterodimer is produced when the MLH1 gene product and the PMS2 protein interact to form heterodimers. When the MutL-alpha heterodimer detects mismatches and insertion/deletion loops, its endonucleolytic activity is triggered. The MutS-beta and MutS-alpha heterodimers aid in recognition. One type of LS called hereditary nonpolyposis is brought on by a heterozygous mutation in the PMS2 gene. The most common hereditary form of the disease is caused by inherited mutations in the MMR genes. The C-terminus of the protein produced by the PMS2 gene bears the DQHA (X) 2E (X) 4E motif [26]. This motif is a component of the endonuclease's active site and aids in the enzymatic activity that removes mismatched DNA. The letters DQHA (X) 2E (X) stand for particular amino acids, including D, Q, H, and A. such asD"is for aspartic acid (D), "Q" is for glutamine (Q), "H" is for histidine (H), and "A" is for alanine (A). These amino acids likely contribute to the structure or function of the motif. (X) 2: This indicates that

any two amino acids can occupy this position. The "X" represents any amino acid. This variability may allow for some flexibility in the structure while maintaining the overall function of the motif. E(X)4E: Similar to the previous part, "E" stands for glutamic acid (E), and "(X)4" indicates that any four amino acids can be present at these positions. Again, this allows for some variability while retaining the essential characteristics of the motif. The specific amino acids in this motif are likely involved in the catalytic activity of the nuclease, contributing to its ability to cleave nucleic acids during the repair process. The conservation of certain amino acids in this motif is important for maintaining the proper structure and function of the protein. Mutations in this motif could potentially affect the protein's activity and, in this case, may be associated with conditions like hereditary lunch syndrome also known as nonpolyposis colorectal cancer. And constitutional mismatch repair deficiency (CMMRD) syndrome [27].

1.9 The Mechanism of Mismatch Repairs and Cancer

DNA replication errors are corrected as part of the molecular mechanism of DNA mismatch repair (MMR) [28]. MMR is insufficient when it comes to fixing these mistakes. As a result, changes in microsatellite sequence length also increase the cell's mutational rate. Microsatellite instability (MSI) is the term used to describe the change in the lengths of the microsatellite repeats. During the replication of DNA in cells, the enzymes responsible for copying the genetic material, known as polymerases, aren't perfect. They make mistakes at a rate estimated to be around one error for every 100,000 building blocks (nucleotides) of DNA. This means approximately 100,000 errors occur in each round of cell division. These polymerase enzymes have their own built-in proofreading abilities to catch and fix mistakes. However, despite this proofreading, some mutations can still go unnoticed. To address these overlooked errors, the cell has a second line of defense: certain genes related to DNA repair, called MMR-related genes, step in to correct these mutations.

These genes play a crucial role in fixing mistakes that, helping to maintain the accuracy of the genetic code. The mismatch repair system was originally discovered in Escherichia coli [29]. DNA mismatch repair (MMR) pathway is crucial repair, recombination, and meiosis. Inactivation of MMR increases susceptibility to cancers [30]. The two mechanisms DNA mismatch repair and DNA polymerase proofreading are key mechanisms that ensure genome replication fidelity. Both mismatch repair and proofreading mechanisms play vital roles in maintaining genome stability by correcting errors in DNA replication, particularly misincorporated no of complementary nucleotides. When one or both of these pathways are impaired, there is an elevated rate of replicative mutagenesis. This increased mutational load can contribute to the development of hyper mutant [31].

1.10 Process of Recognition of Mismatch by Mismatch Repair Proteins

MMR-dependent harm reaction starts off as correcting a single error that the DNA copying process created. Specific proteins play a role in repairing these faults or flaws. They discover and correct mistakes made during the DNA copying process by interacting with other molecules. This aids in preserving the correctness of genetic data during replication. MSH2 heterodimerizes with MSH6 or MSH3 during the mismatch repair process to create MutSα or MutSβ, respectively. These play a role in identifying mismatch pairs and starting the repair process [32].

MutSα, a heterodimer of MSH2-MSH6, detects base-base mismatches in double-strand DNA. MutSα clings to the double-stranded DNA like a sliding clamp. In order to slide the MutSα clamp on the double-strand DNA in this stage, MSH2 needs ATP [33]. MutSα in its ATP-activated state can bind with MutLα, an MLH1-PMS2 heterodimer, to form a tetrameric complex [34]. Replication Factor C and proliferating cell nuclear antigen (PCNA) are recruited by the tetrameric complex as it finds a single-strand DNA gap on the nascent (daughter) strand while sliding up and down the double-strand DNA (RFC). Activated by PCNA,

MutLα can incise the nascent (daughter) strand [35]. The emerging (daughter) strand surrounding the mistake area is then eliminated by the recruitment of exonuclease 1 (Exo 1). Ligase 1 and DNA polymerase carry out the resynthesis stage [36]. MutSβ, a heterodimer of MSH2-MSH3, aids in the restoration of somewhat significant damages up to around ten nucleotide loops as well as minor loops. The major component of MutL function is MutLα, which is a heterodimer of PMS2 and MLH1 [37].

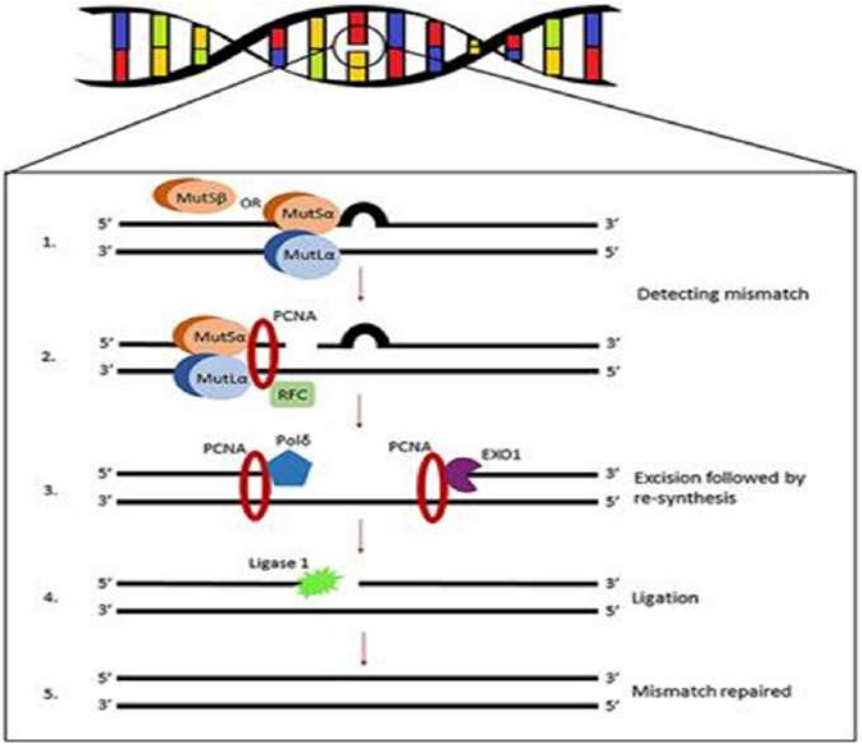

FIGURE 1.6: Shows mismatch repair process [37]

1.11 Aim of Study

The aim of current study is to explore the PMS2 gene expression in peripheral blood samples taken from postmenopausal women with breast cancer before and after treatment (neoadjuvant chemotherapy surgery). The main goal is to reveal the molecular changes link to the surgical procedure, offering information on possible biomarkers and treatment implications for this particular population.

1.12 Objectives

1. To determine the baseline PMS2 gene expression levels in blood samples of postmenopausal women with breast cancer before starting any medical treatments or therapies.

2. To investigate changes in PMS2 gene expression in blood samples obtained from the same individuals after treatment (neoadjuvant chemotherapy, surgery).

3. To compare the expression level of PMS2 gene expression prior to and following treatment in the blood samples of postmenopausal women with breast cancer.

1.13 Problem Statement

There is no information on blood levels of PMS2 gene expression before and after treatment in postmenopausal women with breast cancer. The goal of the current study is to close this information gap and investigate the molecular changes brought on by surgical procedures in this population of postmenopausal women with breast cancer.

1.14 Hypothesis

There is a significant difference in PMS2 gene expression in the blood of postmenopausal women with breast cancer before and after treatment (surgical intervention).

1.14.1 Null Hypothesis (H0)

There is no significant difference in PMS2 gene expression in the blood of postmenopausal women with breast cancer before and after treatment (surgical intervention).

1.15 Dependent Variable and Independent Variable

- Gene expression is Dependent variable.
- The Tumor excision, age, grade, stage and histological features are independent variable.

1.16 Inclusion Criteria and Exclusion Criteria

1.16.1 Inclusion Criteria

The study only included women in a specific age range 50 to 70 (postmenopausal women).The included Participants must have a confirmed diagnosis of breast cancer. The type and stage of breast cancer may also be specified. People with concurrent cancers or a history of breast cancer and other cancers also included.

1.16.2 Exclusion Criteria

The study does not include women who are younger than a specific age or whose don't reached to its menopause. People with no cancers history of breast cancer and other cancers are excluded.

Chapter 2

Review of Literature

2.1 Breast Cancer History

Humanity has been aware of breast cancer since prehistoric times. Nearly every historical era that has been documented has some mention of it. Medical professionals have been aware of lumps that develop into tumours since ancient times due to the obvious symptoms, particularly in the latter stages. Earlier, diagnosis and detection were uncommon due to social stigma and embarrassment. The pink ribbon, which has been a symbol of breast cancer since the 1990s, sparked a movement against the disease [38]. Breast cancer was identified in ancient Greece, where the famous physician Hippocrates attributed the term "cancer" to describe tumours using terminology like "karkinos" and "karkinoma," which are phrases related to a crab. Celsus, a Roman physician, later interpreted this phrase to mean "cancer." Hippocrates considered blood, phlegm, black bile, and yellow bile to be the body's humours, and he thought that an excess of black bile was the cause of breast cancer. Hippocrates implied that people with breast cancer should not have surgery [39]. A physician from the first to the second century A.D. named Archigenes of Apamea left behind meticulous surgical techniques that were recorded by Oribasius in the fourth century A.D. In cases of sepsis or certain carcinomas, his strategy called for the removal of damaged body parts; however, he also

recommended against surgery when early-stage disease could be controlled with treatment [40].

Around 200 A.D., Galen endorsed Hippocrates' hypothesis that breast cancer resulted from an overabundance of black bile building up in the body. He noticed that tumours had dark veins surrounding them, which added to their crab-like appearance. Unlike Hippocrates, Galen thought malignant tumors were more common in women and that they varied in their danger level. He noted a higher prevalence in non-menstruating females. Galen recommended a lumpectomy-like procedure involve excision around the tumor. In the 2nd century A.D., Leonides of Alexandria highlighted nipple retraction as a sign of breast cancer. Paulus Aagineta in the 7th century shared a similar belief that excessive black bile caused malignant tumors, particularly in female breasts or the uterus. He felt that surgery was not feasible for uterine cancer but deemed it necessary for breast cancer. Disagreeing with mastectomy [41].

2.1.1 17^{th} & 18^{th} Centuries, Breast Cancer

The historical development of beliefs and therapies for breast cancer up until the 17th century demonstrates a complicated and varied knowledge of the disease. Many medical professionals proposed theories regarding breast cancer, emphasizing ideas like humoral imbalance, the chemical change of lymphatic fluids, the function of nerve and glandular tissue, inactivity, the impact of sexual activity on lymphatic blockage, curdled milk, pus-filled inflammations, depressive mental illnesses, childlessness, and a sedentary lifestyle. Notably, Henri Le Dran, a French physician, suggested surgical removal of the tumour as a potential treatment for breast cancer in 1757.

On the other side, Nicolas Le Cat maintained that the primary method of treating breast cancer should be surgical therapy. Treatment philosophies were shaped by these concepts far into the 20th century. The radical mastectomy, a surgical technique that included the total removal of the breast as well as diseased lymph nodes from the armpits, was developed as a result of the trend towards surgical

procedures. For a long while, the radical mastectomy was a common therapy for breast cancer [42].

2.1.2 19^{th} and 20^{th} Century Breast Cancer

The condition known as breast cancer was well acknowledged in the 19th and 20th centuries. There were notable advancements in the knowledge and management of breast cancer during the 1800s. The foundation for the current use of hormone-based treatments and prevention was laid by surgeon Karl Thiersch, who proved that cancers metastasis by the spread of malignant cells and that estrogen encouraged breast tumours. In addition, the 19th century saw the introduction of the modern microscope, which helped identify cancer cells, and the discovery of x-rays, which paved the way for the use of radiation therapy to treat cancer. As we entered the 20th century, breast cancer patients were routinely treated with the radical mastectomy, which was created by Professor William Stewart Halsted and involved the removal of the breast, the underlying muscles, and the lymph nodes under the arm. In addition, the understanding of cancer has advanced with the identification of metastasis and the influence of persistent inflammation on the growth of cancer cells.

In addition to the introduction of mammography for early cancer detection, the 20th century witnessed the development of various therapeutics for breast cancer, including hormone treatments, surgeries, and biological therapies. Additionally, researchers identified the genes BRCA1, BRCA2, and ATM that cause breast cancer. Geneticist Mary-Claire King made the initial discovery of the BRCA1 gene in 1990. BRCA2 is the name of the second breast cancer susceptibility gene that was discovered in 1995 by a group of scientists headed by Professor Sir Mike Stratton and comprising Alan Ashworth. A trend towards more individualized care and focused radiation therapy methods occurred throughout time, leading to a decrease in invasiveness and toxicity. With a persistent focus on early detection as the strongest line of defense against breast cancer, these centuries have seen important advancements in the knowledge and treatment of breast cancer, opening the door to more specialized and potent treatment choices [43].

2.2 Molecular Aspects of Breast Cancer

Numerous molecular abnormalities occur in breast cancer, making it a complex disease. A number of molecular markers are used in breast cancer diagnosis, prognostic, and predictive research.

2.2.1 Most Often Implicated Genes in Breast Cancer

Many genes are involved in the development of breast cancer. The current study outlines a few of the most prevalent genes, including

2.2.2 BRCA1 and BRCA2

More than 20 years ago, it was first shown that the BRCA1 and BRCA2 genes were linked to an increased risk of breast and breast cancer. Approximately 20% of family breast cancer cases are linked to mutations in the BRCA1 and BRCA2 genes. The vulnerability to breast and ovarian cancer was initially associated with the tumor suppressor genes. The BRCA1 gene has over 1600 known mutations, many of which are single nucleotide polymorphisms in either the coding or noncoding regions, as well as deletions and insertions. More than 1800 BRCA2 mutations have been found to far. These mutations primarily cause premature truncation or non-functional protein and include frameshift insertions, deletions, and nonsense mutations. Aggressive breast cancer phenotypic development may be facilitated by BRCA1 [44]. With 22 exons, the BRCA1 gene is found on chromosome 17q21. It produces a nuclear protein of 1,863 amino acids. Breast and ovarian tissues are among the tissues in which BRCA1 is expressed. With 27 exons, the BRCA2 gene is found on chromosome 13q12-13. Although their exon architectures are similar, BRCA1 and BRCA2 do not exhibit sequence homology [45]. Tumour suppressor genes are involved in vital processes. Triple negative, high nuclear grade, medullary-like breast tumours are frequently linked to BRCA1 mutations. By the age of 70, carriers of the BRCA1 and BRCA2 mutations had an average risk of 65% and 45%, respectively, of developing breast cancer. When it comes to the

repair of DNA double-strand breaks by homologous recombination, the proteins that are encoded by BRCA1 AND BRCA2 are vital. These genes' loss of function changes lead to increased mutagenesis and genomic instability [46].

2.2.3 P53

As a form of cell commander, the P53 protein helps the cell respond to stress and injury and stops damaged cells from multiplying. It accomplishes this by turning on a number of genes that inhibit the formation of blood vessels, stop cell division, encourage cell death, fix DNA, and control the self-destruction of cells. Most P53 mutations affect the P53 protein and cause a loss of function. P53 functions as a switch for these activities by binding to particular DNA locations when it is activated. To safeguard the body, P53 might cause cell death if the harm to the cells is too great. Li-Fraumeni syndrome, a hereditary illness associated with early-onset malignancies such as those of the breast, adrenal gland, stomach, colon, pancreas, and soft tissue tumors, can be brought on by inherited mutations in the P53 gene [47].

2.2.4 PTENT

Breast cancer is associated with the tumor suppressor gene phosphatase and tensin homolog (PTEN). PTEN gene mutations play a significant role in aggressive subtypes such as HER2-positive breast cancer. Germline mutations in the PTEN gene produce Cowden syndrome, which is linked to an increased risk of breast cancer [48]. The main causes of PTEN anomalies in breast cancer cells are aberrant degradation, aberrant promoter DNA methylation, and deletion mutations [49].

2.2.5 MicroRNAs

MiRNAs can function as either oncogenes or tumour suppressors in the setting of breast cancer, particularly the inflammatory subtype, affecting several aspects of tumour growth, angiogenesis, metastasis, and apoptosis.

The tumour progression in inflammatory breast cancer is driven by the dysregulation of both oncogenic and tumour suppressor miRNAs and their corresponding targets [50].

2.2.6 HER2 (Human Epidermal Growth Factor Receptor 2)

It is an essential component of breast cancer because it controls cell survival, proliferation, and differentiation via a range of signal transduction pathways. Playing a significant role in cell division and proliferation, its overexpression has been linked to aggressive forms of breast cancer, especially the HER2-positive subtype. This subtype is characterized by HER2 gene amplification and/or overexpression of the HER2 receptor and is crucial to the start and progression of breast cancer. Understanding and targeting this pathway is crucial for the diagnosis and treatment of HER2-positive breast cancer.

HER2 functions as a mediator for two key pathways in breast cancer: the HER2/ER pathway and the HER2. route. The manner in which these pathways communicate and interact is crucial for the emergence of breast cancer. Moreover, HER2 is associated with early breast cancer metastasis and may enhance the motility of metastatic cells, which may account for the aggressive characteristics of HER2-positive breast cancer. By creating customized treatments based on knowledge of the interactions between these pathways, which sheds light on the underlying mechanisms of breast cancer growth, HER2-positive breast cancer can be efficiently treated [51].

2.2.6.1 Estrogen Receptor (ER) Signaling

It has a significant role in the onset and spread of breast cancer. The physiological effects of estrogens rely on this system, which is primarily mediated by the membrane receptor GPER (GT protein-coupled ER, also known as GPR30) and nuclear receptors ERα and ERβ. The complex interactions that occur within the

ER signaling system underscore its importance in comprehending the processes that propel breast cancer and offer prospective avenues for therapeutic therapies [52].The majority of breast cancers begin and progress due in large part to dysregulated estrogen receptor (ER) signaling. Endocrine medications primarily target this abnormal signaling system, highlighting the crucial role that ER regulation plays in the development of efficacious treatments for breast cancer. Improving outcomes and customizing therapy for patients afflicted by this common type of cancer has made addressing aberrant ER signaling a primary priority [53].

2.3 Mutations in PMS2

PMS2 (MLH1, MSH2, MSH6,) are mismatch repair genes that fix mismatches during DNA replication. Together with MSH2, MLH1 and PMS2 form a stable heterodimer that fixes minor mistakes involving damaged nucleotides that DNA polymerase introduces during DNA replication. Approximately 8 to 15% of cases worldwide have LS due to PMS2 mutations. The nation and the diagnostic techniques employed (PCR, microsatellite instability, IHC, or DNA sequencing) affect this occurrence differently [54]. Some PMS2 mutations are recurring [55]. Heterozygous PMS2 mutations are known to be the cause of five to fifteen percent of cases [56]. A malfunctioning MLH1 component causes both to deteriorate. whereas a defect in PMS2 results only in the degradation of PMS2 [57].

2.4 The Lynched Syndrome

The Lynch syndrome carries an 80% lifetime risk of developing several types of cancer, making it an autosomal dominant cancer predisposition condition. such as small bowel, pancreatic, prostate, endometrial, ovarian, urothelial, and colorectal cancers (CRC, EC, OC, UC), with the most prevalent cancers being CRC and EC [58]. when lynch syndrome is first described in 1913, the observation of the co-occurrence of gastric cancer and endometrial cancer led to the initial identification of these families, underlining the apparently diverse phenotype. Heterozygous

germline mutations in one of the four mismatch repair genes—MLH1, MSH2, MSH6, and PMS2 deletions—cause Lynch syndrome.

Less than 10% of people with MMR gene mutations will develop non-colorectal and non-endometrial malignancies by the time they are 70 years old. But when you contrast this with folks in the general population who do not have these mutations. The risk of getting these particular tumours is still increased in those who have the mutations. An analysis of the risk of developing specific tumours in individuals with PMS2 gene mutations was conducted. One study included 98 families from various regions of Europe that carried these mutations.

According to the study, people who have PMS2 mutations are more likely to get cancers other than colon tumours. Notably, there was a higher chance of developing malignancies, particularly in the breast, small intestine, ovaries, and renal pelvis. The prior study was the largest at the time, but it was unable to produce accurate estimates of the risk that a person with a PMS2 gene mutation would develop a particular uncommon cancer. This is due to the rarity of certain tumours, which makes it difficult to determine the risk with accuracy [59]. Regarding the risk of breast cancer in people with Lynch syndrome (LS), research has produced contradictory findings. the thorough examination of breast cancer in Lynch syndrome by researchers at different trials. They discovered that although some research demonstrated a large increase in risk, others did not (2–18 times more likely). Furthermore, distinct DNA repair-related genes (e.g., MLH1, MSH2, MSH6, and PMS2) have been associated with varying degrees of breast cancer risk relative to one another. Women are more likely to acquire breast cancer as they get older. The four MMR genes under investigation were found to have relatively equal risks. Constitutional mismatch repair deficiency (CMMRD) syndrome, which is brought on by detrimental alterations in both copies of specific genes involved in mismatch repair (MMR genes), may be associated with breast cancer, according to studies. Studies have shown that breast cancer in individuals with genetically inherited deficiencies in the mismatch repair genes (MMR faults) resembles normal breast cancer in terms of clinical pathological aspects, such as the average age of diagnosis being above 50. However, what's intriguing is that

mismatch repair deficit (dMMR) was present in over half of these breast cancer cases, suggesting a potential link to Lynch syndrome. (LS). In LS, harmful variations in PMS2 could be monoallelic (heterozygous) [60]. Alternatively, in the case of constitutional MMR deficiency syndrome, biallelic (homozygous) [61]. In 1994, PMS2 was discovered to be the cause of Lynch syndrome when it was cloned [62]. According to a study looking at people with PMS2 gene mutations associated with Lynch syndrome [63]. Researchers wanted to know if the severity of the condition varied depending on the type of gene mutation and whether it was inherited from the mother or father. They discovered that people with a particular type of gene mutation (loss of RNA expression) tended to develop colorectal cancer at a younger age than those without this specific mutation. Interestingly, there was no significant difference in cancer risk based on whether the mutation was inherited from the mother or father [64].

2.5 PMS2 Gene Significance

One mismatch repair gene involved in mismatch repair pathways is PMS2. Because it raises the chance of developing specific malignancies, such as colorectal and uterine cancer, the PMS2 gene is one of the major genes of concern [65]. There is a 10% cumulative risk for any cancer in both sexes among PMS2 mutation carriers when they are between the ages of 50 and 55. The risk of CRC is 5.2 times greater than in healthy individuals. People in their 50s had an age-related risk of 22% for colorectal cancer (CRC). According to a cohort research, endometrial cancer (in 8.1% of cases) and colorectal cancer (in 80% of cases) were the most common cancers associated with PMS2 mutation [66]. PMS2 deficiency is similarly linked to a higher risk of breast cancer, with a cumulative risk of 37.7% at 60 years of age [67]. According to reports, women with LS who have mutations in the PSM2 gene are more likely to develop breast cancer than those who have mutations in the MLH1, MSH2, or MSH6 genes. A different juvenile cancer syndrome is linked to balletic mutations in the PMS2 gene, which is not the same as the common

hereditary nonpolyposis colorectal cancer (HNPCC) linked to heterozygous PMS2 mutations [68].

2.6 PMS2 Gene Predisposition in Cancers

2.6.1 PMS2 Predisposition in Breast Cancer with (MSH3, MSH1, MSH6)

More over 4 million people worldwide receive a diagnosis of colorectal cancer (CRC) and breast cancer (BC) each year, making them common neoplasms [69]. In between 5% and 10% of instances of colorectal cancer (CRC) and breast cancer (BC), pathogenic mutations found within known genes associated to cancer susceptibility are the cause [70]. Hereditary cancer syndrome carriers have a higher chance of acquiring secondary primary tumors, like BC and CRC [71]. Hereditary colon cancer and several other cancers are caused by the lynched syndrome, which is also linked to mismatch repair problems in genes. However, there is ongoing dispute among researchers on the relationship between the lynched syndrome and breast cancer [72]. A study which aim was to determine the gene mutation that causes mismatch repairs in Lynch syndrome and the correlation between Lynch syndrome (LS) and breast cancer. Specifically, each LS gene was examined separately. 5000 individuals who have undergone genetic testing for hereditary cancer were reviewed retrospectively. 423 out of 5000 had mismatch flaws. A study was done on the personal and family history of cancer in 423 women who had germline mutations in MLH1, MSH2, MSH6, or PMS2 that were pathogenic or likely pathogenic. ..By comparing the study population's breast cancer frequencies with those of the general population, standard incidence ratios (SIRs) of breast cancer were determined. Accordingly, the study found no correlation between MLH1 and MSH2, while MSH6 and PMS2 were linked to a statistically significant risk for breast cancer (73). The research involved a sizable cohort of 8085 Chinese women with breast cancer, of which 15 had pathogenic germline variants in MMR genes, including PMS2 (n = 6), MSH6 (n = 5), MSH2 (n = 3), and MLH1 (n = 1).

Of these carriers, 40% had a history of HNPCC-related cancers in their families, with colorectal and gastric cancers being the most common. MMR variant carriers did not vary in age at diagnosis or family history of breast cancer, but they were more likely than non-carriers to have a positive family history of HNPCC-related malignancies. Patients with PMS2 gene breast cancer were more prevalent than those with other mismatch repair genes [74]. Lynch syndrome, which is brought on by one of five mismatch repair genes, has been linked to breast cancer, according to a research that was done to determine this. Data from more than 20,000 cancer patients who had genetic testing was examined in this study. A total of 20,110 patients were studied, and 272 (1.4%) of them had mutations in the DNA mismatch repair (MMR) gene, which was indicative of Lynch syndrome (LS). Thirteen (5%) of these LS patients had received a primary breast cancer diagnosis and had undertaken genetic testing using a panel designed to evaluate inherited genes associated with cancer susceptibility. The majority of the changes in these 13 BC patients with LS-related mutations were found in the MLH1 (15%), MSH2 (15%), MSH6 (46%), and PMS2 (23%) genes. The majority of them (54%) had only one cancer diagnosis, whereas some had numerous synchronous tumours or additional malignancies connected to LS. Eighty-four percent (84%) reported a family history of malignancies connected to LS. For these LS patients, the median age at BC diagnosis was 47 years old. This indicates that a small number of people with Lynch syndrome caused by MMR gene mutations also get breast cancer, sometimes at a young age, and that they frequently have a family history of diseases associated to Lynch syndrome [75]. A study that looked at the levels of mismatch proteins and microsatellite instability in patients with breast cancer revealed that various forms of the disease had varying expression levels of certain proteins. While some kinds had no protein loss and some had total loss of mismatch proteins, the luminal B-like type that had a limited life period showed a consistent loss of MMR proteins. However, a patient who had chemotherapy and had a long survival rate also showed protein decrease in their estrogens receptor-negative breast type. Patients with breast cancer had higher MMR lost protein loss compared to MSI.A study found that the MMR expressions varied according to the kind of breast cancer [76].

The purpose of the study was to investigate the role of microsatellite instability (MSI) and mismatch repair (MMR) in triple-negative breast cancer. Using tissue samples from 440 individuals with non-communicable diseases (NBC), they employed immunohistochemistry (IHC) to identify protein expression linked to MMR (MLH1, MSH2, MSH6, and PMS2). They also evaluated microsatellite instability in 195 samples by using MSI polymerase chain reaction (PCR) testing. With the exception of one sample that was determined to be MMR-deficient (dMMR) due to a particular protein loss, nearly all of the samples exhibited normal MMR protein expression. Remarkably, none of the samples displayed high-frequency MSI (MSI-H), and only a very tiny fraction (7.2%) had low-frequency MSI. The low-frequency MSI and potential gene loss were both detected in the DMMR sample with protein loss. A study found that TNBC had a noticeably low incidence of DMMR/MSI [77]. The study used a registry for hereditary colorectal cancer to examine the medical records of 188 women who had Lynch syndrome-related DNA mismatch repair (MMR) mutations. In people who had never had breast cancer before, the researchers calculated the risk of breast cancer using a prediction model called the Yurcer-Cuzick model. The kind of mutation had an impact on the incidence of breast cancer, according to the findings. Compared to women with MLH1, MSH2, and MSH6 mutations, which had diagnoses of 3%, 4%, and 9%, respectively, women with a PMS2 mutation had a diagnosis of 27% breast cancer. Women with a PMS2 mutation were diagnosed with breast cancer on average 46.7 years of age. According to the study's findings, those with Lynch syndrome who also have PMS2 mutations may be more likely to have breast cancer [78].

2.6.2 PMS2 Predisposition in Colorectal Cancer

In 2008, a significant cohort of 55 index patients and 55 relatives—carriers of pathogenic mutations in the PMS2 gene—was discovered. According to their research, male PMS2 carriers had a cumulative risk of 20% and female PMS2 carriers of 15% for colorectal cancer (CRC) at age 70. Compared to bearers of pathogenic mutations in other Lynch syndrome-associated genes (path_MLH1, path_MSH2, and path_MSH6), whose chances ranged from 25–75% for CRC, these

risks were noticeably reduced. Similar cancer rates were discovered in 98 PMS2 families in a follow-up research conducted in 2015. These findings provide more credence to the idea that people with PMS2-associated Lynch syndrome (PMS2-LS) had far lower cancer risks than those with other Lynch syndrome subgroups [79].Compared to the general population, people with a defective PMS2 gene have a higher lifetime risk of getting several forms of cancer at an earlier age. The elevated risk is especially noticeable for colorectal cancer, since the average age of diagnosis for individuals with a defective PMS2 gene is 61–66 years, compared to 68–72 years for the general population. Comparably, those with defective PMS2 genes are diagnosed with endometrial cancer at an average age of 49–50 years, compared to an average age of 60 [80].

Different from usual cases, Lynch syndrome patients have an increased risk of colorectal cancers (CRCs) due to particular gene mutations in mismatch repair genes. Because PMS2 gene variations are associated with fewer CRCs and older patients than CRCs caused by other gene variants, studying CRCs connected to these variants has proven challenging. A study compared many CRC forms using tissue samples from carriers of different gene variants and used a DNA platform to search these samples for certain genetic alterations. Interestingly, unlike other forms of CRCs, the ones linked to PMS2 mutations did not exhibit alterations in the C NNB1 gene. In addition, compared to other gene-related CRCs, certain KRAS mutations were less prevalent in PMS2-associated CRCs. This implies that Lynch syndrome, which is linked to PMS2, may have unique characteristics that contribute to its development and rarity [81]. Lynch syndrome, a prevalent hereditary type of colon cancer brought on by gene alterations, was the subject of this investigation. They examined 1706 Chinese individuals who underwent surgery for colorectal cancer. Of these individuals, around 11.8% reported issues with certain genes involved in fixing genetic errors. Of these, 3.1% alone had PMS2 gene abnormalities, and 8.3% had problems exclusively related to the PMS2 gene. They discovered that, in contrast to individuals without these gene problems, patients with PMS2 abnormalities showed distinct features in their malignancies. These variations included the kind of cancer, the location of the cancer in the colon, and a few characteristics pertaining to the cancer's size and stage. According to

the study, understanding and treating colorectal cancer in China may depend on figuring out these gene-related problems. It also emphasizes the necessity of more study to learn more about how to handle these particular situations . Certain gene mutations, such as those in the PMS2 gene, cause Lynch syndrome, which increases the risk of colon cancer in families. However, because it appears to vary from person to person, it is unclear how exactly persons with PMS2 mutations are at risk for cancer. This suggests that additional variables could be at play [82]. The full collection of genes in a family with a PMS2 mutation was examined by researchers using a technique known as whole exome sequencing. They evaluated these in a broader research including individuals with and without colorectal cancer and discovered a number of gene changes worth investigating further. They found a noteworthy alteration in the BAG1 gene. This gene may impact an individual's prognosis following a diagnosis and is linked to the growth of colorectal tumours. By increasing a specific cell activity, BAG1 appears to aid in the survival of Colorectal cancer cells [83]. In non-hereditary colorectal cancer (CRC), the study examined the relationship between PMS2 expression and tumor grade and clinical stage. Retrospective analysis was performed on samples from 67 colorectal resections, utilizing quantitative real-time PCR to measure mRNA expression and immunohistochemistry to evaluate PMS2 protein expression. As compared to paracancerous tissue, the results showed a substantial drop in PMS2 expression in CRC tissue, which was correlated with both tumor stage and differentiation grade. More advanced tumour stages were linked to lower PMS2 expression levels. According to the study's findings, PMS2 may be a useful biomarker for CRC categorization when examined using both mRNA and immunohistochemical techniques.

2.6.3 PMS2 Predisposition in Pancreatic Ductal Adenocarcinoma

The impact of diabetes on the expression of mismatch proteins in PDAC (pancreatic ductal adenocarcinoma) was examined in a study that focused on mismatch proteins specifically on PMS2. The tissues of 61 patients with surgically

resected PDAC were analyzed using techniques such as immunohistochemical analysis, which was used to assess MMR protein expression along with oxidative stress markers and immune cell infiltration. Additionally, PDAC cell lines were used in in vitro studies to mimic the effects of elevated glucose and palmitic acid on MMR protein production. Diabetes affects the PMS2 gene by causing dyslipidemia, or abnormal lipid levels, which lowers PMS2 gene expression. Diabetes induces PMS2 promoter hypermethylation. It's interesting to note that when various tumour types develop, oxidative stress levels rise and PMS2 protein and mRNA expression levels are downregulated. Therefore, via modifying PMS2 expression and the tumour immune milieu, which may be addressed by an immune checkpoint inhibitor, the various stages of diabetes have a significant effect on PDAC [84]. PMS2 expression was significantly reduced in investigations using streptozotocin-induced diabetic rat kidneys, however this suppression was mainly shown in the glomeruli rather than the tubules. The differential in PMS2 expression across various kidney structures raises the possibility that the effects of diabetes on PMS2 expression are tissue-specific, maybe as a result of variations in the tissues' susceptibilities to oxidative stress. This discovery suggests that mismatch repair (MMR) proteins, such as PMS2, may be degraded differentially in response to diabetes inside distinct kinds of carcinoma, suggesting tissue-specific responses to diabetic circumstances [85]. In comparison to MLH1 and MSH2 families, the prevalence of stomach, prostate, and breast cancers in PMS2 families is abnormally high. Additionally, the mean age of cancer onset is higher and the penetrance of colorectal and endometrial cancers is lower, similar to the circumstances in MSH6 families and there may be a genetic explanation for cancer risk reduction in families with PMS2 gene mutations. Similar to the MLH1 protein, another protein known as MLH3 also functions in concert with PMS2. Consequently, when PMS2 experiences a change that may impact its functionality, the MLH3 protein intervenes to assist. It can collaborate and work in part with MLH1 to accomplish the tasks that PMS2 typically completes. Even if having a fully functional PMS2 would be more efficient than this partnership, cancer is nevertheless prevented from forming more frequently. This may help to explain why certain cancer forms seem to be less common in these families. PMS2 mutations have been found more

frequently in families suspected of having Lynch syndrome (LS). The frequency of these PMS2 mutations is reported to be much more common, while the prevalence of mutations in the MLH1 and MSH2 genes is comparatively lower. Data from the InSiGHT database over the last couple of decades show that the occurrence of these mutations has increased from about 2-9% in some population groups [86].

2.6.4 PMS2 Predisposition in Oral Squamous Carcinoma

The purpose of the research was to determine if the PMS2 gene has a role in the advancement of oral squamous cell carcinoma (OSCC), and it focused on 76 instances of OSCC that were treated between 2011 and 2016. They used immunohistochemically labelling to investigate how patient prognosis was affected by PMS2 protein levels. In patients under 60 years of age, overexpression of PMS2 has been associated with decreased survival rates, particularly in those who underwent both surgery and chemotherapy. PMS2 overexpression may disrupt the cytotoxic signaling pathway, improving resilience to DNA damage and reducing response to chemotherapy. In addition, they discovered that PMS2 may be a viable prognostic protein marker in OSCC patients 60 years of age and younger. These findings imply that elevated PMS2 levels in younger individuals may increase the risk of mortality when paired with particular therapies [87].

2.6.5 PMS2 Predisposition in Ovarian Cancer

The goal of the study was to determine whether the PMS2 gene mutation in germline cells contributes to ovarian cancer. Five family members with Lynch syndrome (LS)-related cancers—colon, stomach, and ovarian—were found to have these diseases between the ages of 39 and 70. Three members of this family were found to have a particular PMS2 germline heterozygous mutation using gene sequencing. Additional verification through the use of qPCR, western blotting, and IHC methods verified the existence of this PMS2 mutation. Furthermore, structural alterations in the PMS2 protein were detected; one notable modification was the partial deletion of the C-terminal region inside an α-helix structure.

Possessing germline mutations in PMS2 increases one's risk of developing LS-associated malignancies significantly. To assist lower, the morbidity and death of LS-associated malignancies, careful clinical surveillance and Preventive surgery are therefore strongly advised [89].

2.6.6 PMS2 Predisposition in Prostrate Cancer

Research indicates that overexpression of the PMS2 gene is often observed in prostate cancer patients. When prostate cancer samples were examined using immunohistochemistry, a sizable portion of them revealed PMS2 and other DNA mismatch repair (MMR) genes to be expressed. Greater levels of PMS2 gene expression in tumours with advanced pathological tumour stage, high Gleason grade, nodal metastases, and early biochemical recurrence have raised the possibility of a link between PMS2 overexpression and aggressive features of prostate cancer [90].

Chapter 3

Materials and Methodology

The current study methodology is followed by a study which was conducted on the expression of HMGA1 gene in the blood samples of non-small cell lung cancer patients (91).

3.1 Blood Samples Collection

Using a venipuncture method, blood samples were taken from women who were suspected of having breast cancer.

1. A good-sized, easily visible vein was chosen, and the chosen skin area was cleaned for 30 seconds with a 70% alcohol swab.

2. About 5 milliliters of blood were extracted into a sterile syringe that was secured into the vein.

3. The blood sample was promptly moved to a vacutainer blood collection tube and appropriately marked.

4. Following patient blood collection, the blood specimens are taken to the lab for analysis.

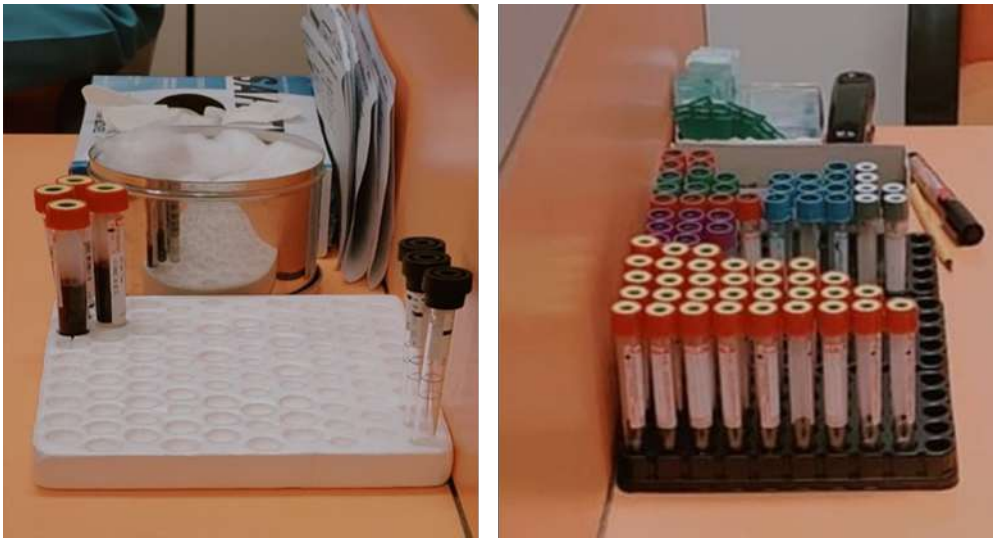

FIGURE 3.1: The image above displays the blood obtained from patients with breast cancer in vacuum-sealed tubes

3.2 RNA Isolation from Samples of Blood

The RNA extraction from fresh blood samples of breast cancer patients utilized the trizol method, a commonly employed RNA extraction technology, ensuring the isolation of high-quality and intact RNA for subsequent molecular analysis. The method involved taking fresh blood samples and adding 600 μL of reagent A (Trizol) per 300 μL of sample, followed by complete homogenization, a 5-minute incubation, and the addition of 400 μL of reagent B (Chloroform) with a subsequent 3-minute incubation. After centrifugation at 12000 rpm for 10 minutes at 4^{o}C, the aqueous layer was carefully transferred to a new eppendorf tube, and an equal volume of reagent C (Isopropanol) was added, followed by a 10-minute incubation at a horizontal position and a further centrifugation step. The supernatant was discarded, and the pellet was washed twice with 1 mL of reagent D (70% Ethanol) before centrifugation and air drying. Finally, 35 μL of reagent E (RNase-free Water) was added, and the RNA was stored at -80^{o}C. The storage conditions for each reagent were specified as follows: reagent A at 4^{o}C, reagent B at room temperature, reagent C at -20^{o}C, reagent D at room temperature, and reagent E at room temperature.

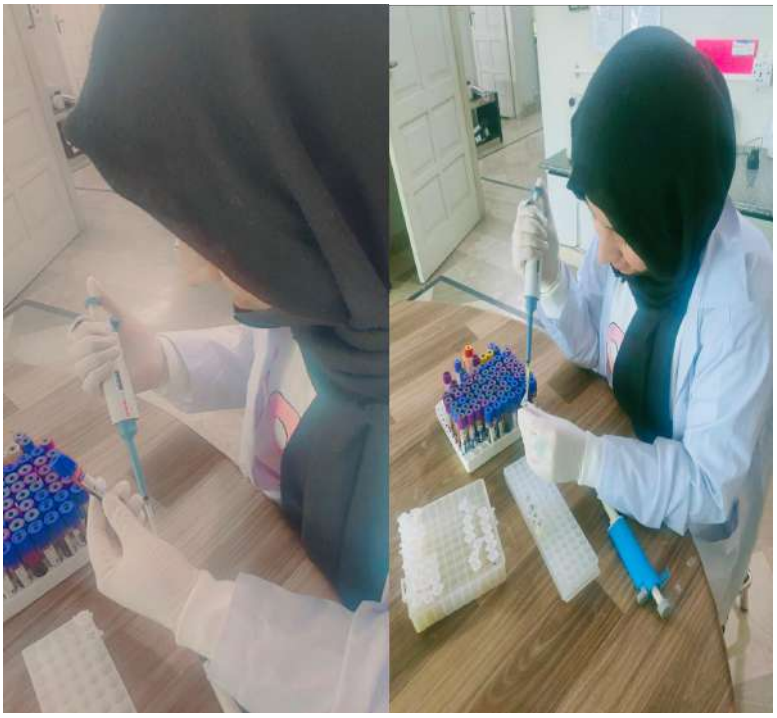

FIGURE 3.2: RNA from fresh blood of breast cancer patients

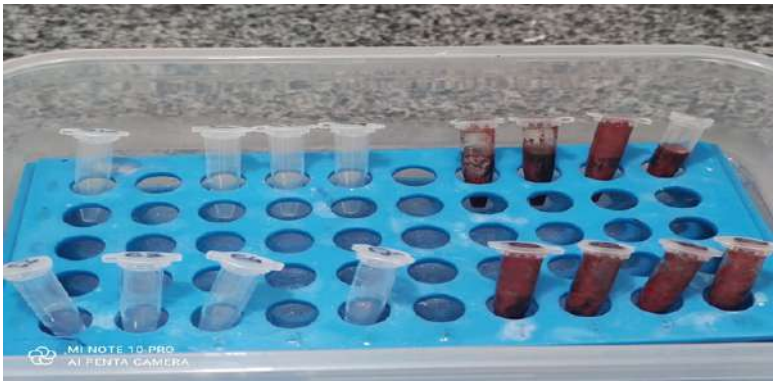

FIGURE 3.3: The Above showed effendorf tube having blood for RNA Extraction by Trizol Method

3.3 Gel Electrophoresis

The quality and integrity of the extracted RNA were assessed using 1.5% agarose gel electrophoresis following isolation from the blood sample. To prepare the gel, 30 mL of 1x TBE buffer and 0.45 g of agarose were meticulously mixed, then microwaved for one minute to dissolve the agarose. A brief cooling period ensued to prevent denaturation of the intercalating dye ethidium bromide, crucial for

visualizing nucleic acids. Subsequently, 5 μL of ethidium bromide was added, and the solution was poured into the gel mold, ensuring even distribution, and allowed to set. Precise measurements were maintained throughout. Loading dye (2 μL) was cautiously added to each well to aid in sample loading. The Fisherbrand Horizontal Mini Gel Electrophoresis System was set at room temperature for 30 minutes, facilitating electrophoresis with an electric field applied across the gel matrix, causing RNA molecules to migrate according to their sizes. After 30 minutes, the gel was placed in an ultraviolet transilluminator to visualize the bands.

FIGURE 3.4: Shows gel electrophoresis process

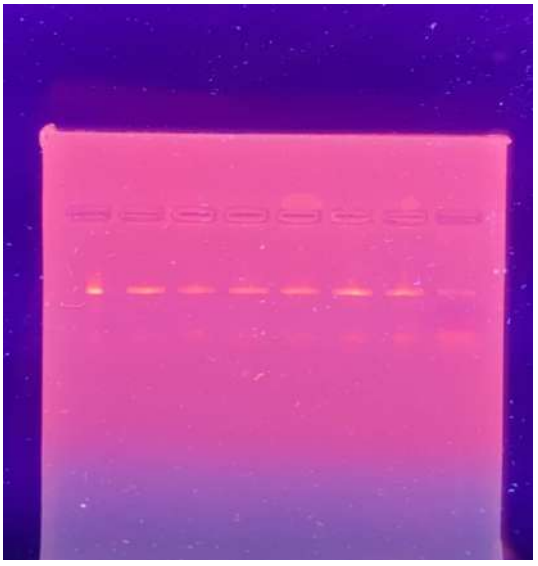

FIGURE 3.5: The band shows the successful extraction of RNA

3.4 CDNA Synthesis or Reverse Transcription

The total RNA extracted was reverse transcribed into complementary DNA (cDNA) using the flexible cDNA Synthesis kit with the fast and robust FIREScript reverse transcriptase kit protocol. In the method, a mixture consisting of 10 μl RNA template, 1 μl Oligo (dT) Primer, 1 μl random Primer, and 4 μl nuclease-free H2O (up to 16 μl) was incubated at 65^{o}C for 5 minutes and then cooled on ice. Subsequently, 2 μl of 10x rt reaction buffer with DTT, 0.5 μl of dNTP mix (20 mM of each), 1 μl of FIREScript® reverse transcriptase, and 0.5 μl of RiboGrip RNase Inhibitor were added. The temperature conditions for reverse transcription were set as follows: Primer annealing at around 25^{o}C for 5-10 minutes, reverse transcription at 50^{o}C for 15-30 minutes, and enzyme inactivation at 85^{o}C for 5 minutes.

FIGURE 3.6: The given figure show Formation of cDNA from RNA (Reverse Transcription)

3.5 Nanodrop

The concentration and purity of nucleic acid samples were measured in our research work using a nanodrop spectrophotometer. The nanodrop's cutting edge technology made it possible to analyze DNA and RNA samples precisely and quickly. This instrument was essential for quality control, making sure that our

genetic material met the standards needed for experiments further down the line. Its user-friendly interface and micro volume capability made it an invaluable tool in our laboratory. The nanodrop spectrophotometer significantly enhanced the accuracy and efficiency of our research. The cDNA was measured on nanodrop and showed the values (pr and po) for sample and control group is represented by C.

FIGURE 3.7: The CDNA was diluted 1:100

3.6 Primer Optimization

To ensure precise, specific, and effective amplification of the target DNA, primer optimization is pivotal within the polymerase chain reaction (PCR) process. This optimization process involves meticulous adjustments to the conditions and design of the primers utilized for amplifying specific DNA sequences regarding PMS2 primer optimization, the forward primer sequence is AGAGGCAGTGAGTTCCAG TC, while the reverse primer sequence is GTGTTTGGGGTTGCGAGATT, resulting in a product size of 312 base pairs. The annealing temperature for this primer was set is set was 52^{o}C to 58^{o}C.

Primers

The PMS2 gene sequence was taken from NCBI and then design a primer from Korea.

Table 3.1: Shows primer

SN	NAME	FORWARD	REVERSE	T(a)	PRODUCT SIZE
1	PMS2	AGAGGCAGTGA GTTCCAGTC	GTGTTTGGGG TTGCGAGATT	58	312bps

3.7 PCR for GAPDH

The reaction mixture for GAPDH amplification is composed of 10 μL of master mix, 2 μL of forward Primer, 2 μL of reverse Primer, 4 μL of PCR water, and 2 μL of template, resulting in a total volume of 20 μL. The cycling conditions for the PCR reaction involve a holding stage at 95^{o}C for 10 minutes, followed by denaturation at 95^{o}C for 30 seconds, annealing at 57^{o}C for 30 seconds, and extension at 72^{o}C for 90 seconds, repeated for 35 cycles. A final extension step is conducted at 72^{o}C for 10 minutes, and the reaction is then held at 4^{o}C.

3.8 Gel Electrophoresis

1.5% Agarose gel was used to run the purified PCR products. 30 mL of 1.5% gel was made by mixing 0.45 g of Agarose with 30 mL of 1x TBE buffer, heating the mixture for a minute in the microwave, letting it cool slightly, and then adding 5 μL of Ethidium Bromide. After adding 2μL of loading dye to the gel, 5μL of PCR-purified samples are put onto it.

3.9 Gradient PCR for PMS2 Gene

The optimization of primers for annealing temperature is conducted using the gradient PCR method as indicated earlier. The reaction mixture employed for PCR consists of 10 μL of master mix, 2 μL of forward primer, 2 μL of reverse

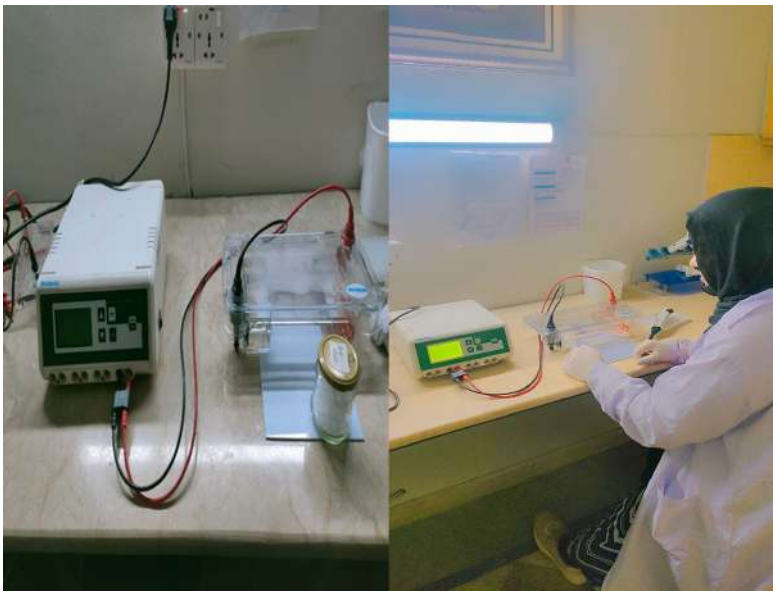

FIGURE 3.8: Shows gel electrophoresis process by Fisherbrand Mini Horizontal Gel System

primer, 4 μL of PCR water, and 2 μL of template, resulting in a total volume of 20 μL. The cycling conditions for this PCR protocol entail an initial holding stage at 95^{o}C for 10 minutes, followed by denaturation at 95^{o}C for 30 seconds. Subsequently, annealing occurs within a temperature gradient ranging from 48^{o}C to 68^{o}C for 30 seconds, repeated for 35 cycles. Extension takes place at 72^{o}C for 90 seconds, followed by a final extension step at 72^{o}C for 10 minutes.

FIGURE 3.9: Shows the gradient PCR running process for PMS2 gene

3.10 Gel Electrophoresis

1.5% Agarose gel was used to run the purified PCR products. 30 mL of 1.5% gel was made by mixing 0.45 g of Agarose with 30 mL of 1x TBE buffer, heating the mixture for a minute in the microwave, letting it cool slightly, and then adding 5 μL of Ethidium Bromide. After adding 2μL of loading dye to the gel, 5μL of PCR-purified samples are put onto it. The bands were shown at 56 and 58.

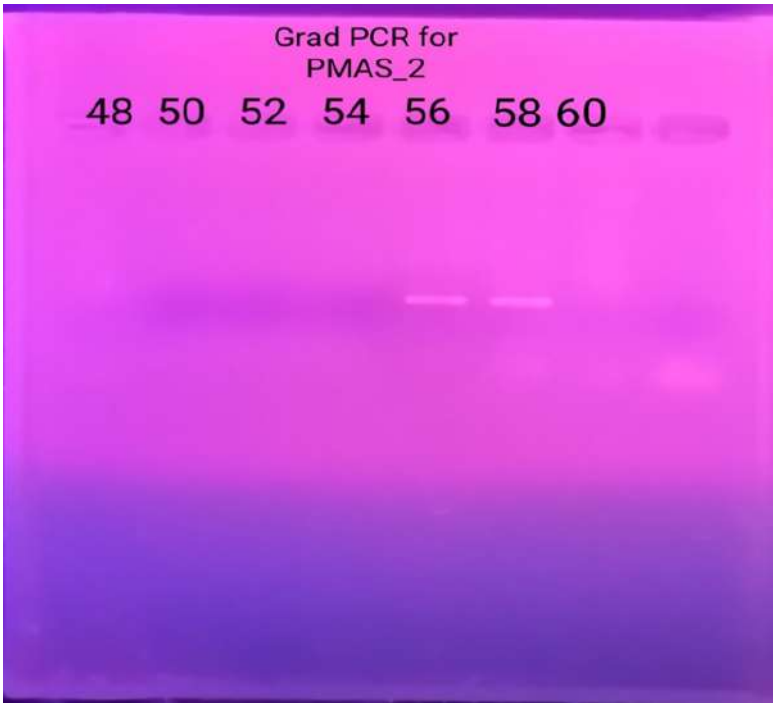

FIGURE 3.10: Shows gel bands

3.11 Real Time PCR for GAPDH

The PCR reaction mixture consists of 4 μL of PCR water, 1 μL of forward primer, 1 μL of reverse primer, 2 μL of cDNA, and 2 μL of Eva green, totaling a volume of 10 μL. Cycling conditions for this PCR protocol, involve an initial holding stage at 95^{o}C for 5 minutes, followed by denaturation at 95^{o}C for 30 seconds. Annealing is conducted at 56^{o}C for 30 seconds, repeated for 35 cycles, while extension occurs at 72^{o}C for 20 seconds.

FIGURE 3.11: Real time PCR for GAPDH

3.12 Real Time PCR for PMS2

The PCR reaction mixture comprises 4 μL of PCR water, 1 μL of forward primer, 1 μL of reverse primer, 2 μL of cDNA, and 2 μL of Eva green, totaling 10 μL. As detailed in section 3.13.1, the cycling conditions for this PCR protocol begin with a holding stage at 95^{o}C for 5 minutes, followed by denaturation at 95^{o}C for 30 seconds.

Annealing takes place at 58^{o}C for 30 seconds, repeated for 35 cycles, while extension occurs at 72^{o}C for 20 seconds.

FIGURE 3.12: The above figure showed RT-PCR tubes

FIGURE 3.13: Shows RT for PMS2

Chapter 4

Results and Discussion

4.1 Sample Collection

The current study includes 27 women with 47 blood samples, who visited Fuji Foundation Hospital Rawalpindi between February 2023 and September 2023.Out of 27, 20 where suspected to breast cancer and 7 where healthy women who consider as a control group. Blood were taken from 20 postmenopausal women with breast cancer before and after treatment to check the expression level of PMS2 gene in the blood of postmenopausal women with breast cancer and compare their expression levels. The range of the age was 50 to 60 and 60 to 70. All of the patients were female, and the mean age of the patients was 58.5 years.

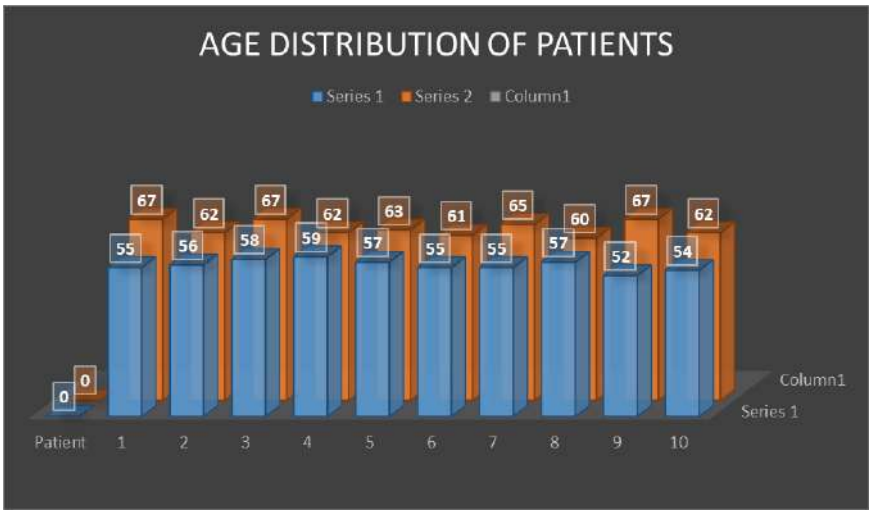

FIGURE 4.1: Suspected Patient's Age Distribution

4.2 RNA Isolation

A robust yield of total RNA from pre- and post-tumor excision samples was obtained by the Trizol method of RNA extraction; the average yield was X micrograms per microliter, and the A260/A280 ratio indicated high purity, ensuring that the extracted RNA was suitable for use in downstream applications. The extracted RNA was confirmed to be of high quality and integrity by the Agarose gel electrophoresis, which showed clear and intact RNA bands. Furthermore, the bio analyzer analysis revealed consistent RNA profiles, ensuring the validity of subsequent gene expression analyses.

4.3 Creation of cDNA

Effective cDNA synthesis from the extracted RNA was produced by the reverse transcription process; this was demonstrated by the presence of distinct and strong qPCR signals for the PMS2 gene.

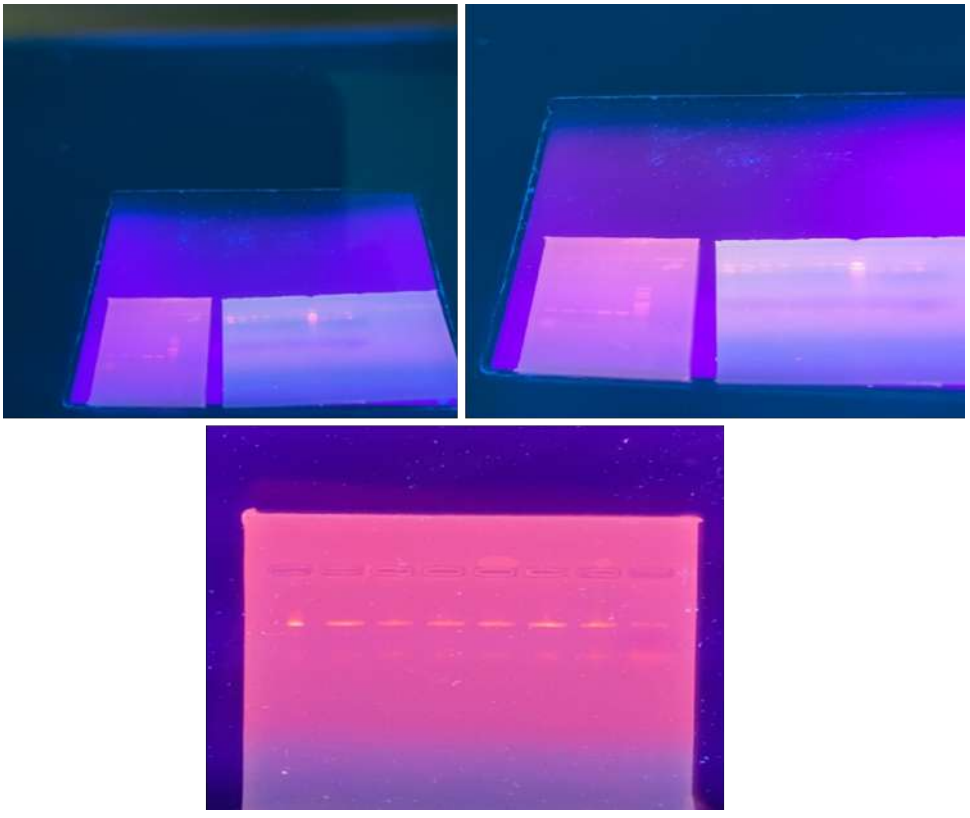

FIGURE 4.2: Show the bands of successfully conversion RNA in to cDNA

Pre- and post- treatment samples showed consistent cDNA formation efficiency, which guaranteed the validity of subsequent gene expression analyses. The synthesized cDNA showed high integrity and purity; this was demonstrated by consistent amplification curves and the lack of non-specific amplification. These findings suggest that the cDNA pool accurately represents the original RNA and provide valuable insights into the dynamics of PMS2 gene expression.

4.4 Nanodrop

For RT-PCR, dilution is required. Dilutions are made based on the amount of RNA or DNA in the sample. Since the amount of cDNA in the samples varies, using the same amount will not explain the results. We then find the lowest amount of cDNA in the samples—7.3 in this case—and divide all the values by this number. The divided value indicates the dilution (more cDNA means more dilution). Therefore, the number represents the amount of PCR water and 1 is DNA, i.e., 1µl cDNA with 4µl of PCR water in sample S1 and so on.

Table 4.1: Table given shows the Dilution value of cDNA in Nanodrop

Sample	**CDNA ng/μl**	**Factor**	**Dilution**
Pr1	34.3	5	1:5
Pr2	54.5	7	1:7
Pr3	384	55	1:55
Pr4	260	37	1:37
Pr5	177.9	25	1:25
Pr6	291	41	1:41
Pr7	264	38	1:38
Pr8	804	114	1:114
Pr9	177.9	25	1:54
Pr10	59.6	8	1:8
Pr11	128.2	18	1:18
Pr12	196.2	28	1:28
Pr13	433	63	1:63

Sample	**CDNA ng/μl**	**Factor**	**Dilution**
Pr14	297	38	1:38
Pr15	100	14	1:14
Pr16	457	65	1:65
Pr17	125	18	1:18
Pr18	272	38	1:38
Pr19	348	49	1:49
Pr20	114.9	16	1:16
C1	33.3	5	1:5
C2	34.3	7	1:7
C3	54.5	55	1:55
C4	384	37	1:37
C5	260	25	1:25
C6	177.9	41	1:41
C7	291	38	1:38
PO1	264	114	1:114
PO2	35.3	7	1:7
PO3	7.3	8	1:8
PO4	59.6	18	1:18
PO5	128.2	28	1:28
PO6	196.2	63	1:63
PO7	433	38	1:38
PO8	297	14	1:14
PO9	100	65	1:65
PO10	457	18	1:18
PO11	125	38	1:38
PO12	272	49	1:49
PO13	348	16	1:16
PO14	59.6	8	1:8
PO15	128.2	18	1:18
PO16	196.2	28	1:28
PO17	433	63	1:63

Sample	CDNA ng/μl	Factor	Dilution
PO18	297	38	1:38
PO19	100	14	1:14
PO20	457	65	1:65

4.5 RT (Real Time)-PCR Cycle

This molecular analysis aimed to elucidate the molecular transformations associated with breast cancer progression and the impact of surgical intervention on PMS2 gene expression. The RT-PCR analysis was performed to quantify the expression levels of the PMS2 gene in peripheral blood samples collected from older breast cancer patients both before and after treatment.

4.6 PMS2 Gene Expression Level in Blood Samples

Current study includes the total of 47 blood samples of women. In which 7 women are healthy and consider as control group. The 20 blood samples were taken before treatment from a woman who's were diagnose from Breast Cancer and having Tumor. Almost patients have the colon, breast, ovarian cancer in their family history and 20 Blood samples were taken after treatment and in post blood sample 10 blood samples were taken after 4 months of treatment and 10 were taken after 6 months of treatment of Breast Cancer patients. Current study focused on expression level of PMS2 Gene in the blood of post-menopausal women with breast cancer. The expression profiling of the PMS2 gene was showed by using RT (Real time) PCR. The obtained Ct values were subsequently utilized to calculate the expression levels of PMS2. This quantitative approach allowed for a precise assessment of the gene's transcript abundance in the peripheral blood, providing valuable insights into its potential involvement in breast cancer progression. The application of RT-PCR not only enabled a sensitive detection of PMS2 expression

but also facilitated the comparative analysis of pre and post-treament samples, shedding light on dynamic changes in gene expression associated with the surgical intervention. The Table give below represent the 2^-△△Ct values of gene expression obtained from RT-PCR.

TABLE 4.2: Shows expressional values of pre –post, control participants

Patients No.	**PMS2 control**	**PMS2 Pre Tumor (pr)**	**PMS2 Post Tumor (po)**
	2ˆ-△△Ct value	2ˆ-△△Ct value	2ˆ-△△Ct Value
1	0	50.56264	.0005921
2	0	670.18187	.005563
3	0	281700.11	.08912
4	0	54.94819	.005921
5	0	113000000	.005563
6	0	0	0
7	0	72.00374	.00891
8		113000000	.005921
9		5000.56	0
10		50.56264	.0891
11		50.56264	.00593
12		670.18187	.0059
13		2817.11	.00891
14		54.94819	.00592
15		113000000	.00556
16		720.00374	0
17		54.94819	.0055
18		720.00374	.00592
19		.002559	0
20		113000000	.000891

4.7 Expression of PMS2 Gene in Pre- Tumor Treatment & its Association with Selected Clinicopathological Characteristics

PMS2 gene expression in the context of breast cancer, we investigated the relationship between PMS2 gene expression and important clinicopathological features in our study of postmenopausal women with breast cancer. In this section, we concentrate on the expression level of PMS2 gene in pre-tumor treatment and their correlation with particular clinical characteristics. The Table presents a detailed data of the relationship between PMS2 expression in pre- tumor resection and parameters like histological factor, stages (TNM), grade, and patient age. The goal of this investigation is to identify potential connections between molecular changes at the gene expression level and the clinicopathological profile of postmenopausal women with breast cancer patients, providing important information about the prognostic. There we observe the high PMS2 gene expression level in stage 3B with Grade 3 and in stage 3A with Grade 2.

TABLE 4.3: Shows expressional values of pre –post, control participants

PMS2 expression level in pre-tumor resection patients	**2ˆ-△△Ct**	**Age**	**Histological Features**	**Grade**	**Stage(TNM)**
1	50.56264	55	ILC	2	3B
2	670.18187	56	IDC	2	2
3	281700.11	58	ILC	2	3A
4	54.94819	67	ILC	2	1
5	113000000	59	IDC	3	3B
6	0	62	IDC	3	2
7	72.00374	57	IDC	2	2A
8	113000000	55	IDC	2	3a
9	5000.56	55	ILC	2	4
10	50.56264	67	ILC	2	2

PMS2 expression level in pre-tumor resection patients	**2ˆ-△△Ct**	**Age**	**Histological Features**	**Grade**	**Stage(TNM)**
11	50.56264	62	ILC	2	3B
12	670.18187	63	ILC	3	2
13	2817.11	57	ILC	2	3A
14	54.94819	52	ILC	2	1
15	113000000	54	IDC	3	3B
16	720.00374	61	IDC	2	2
17	54.94819	65	IDC	2	2A
18	720.00374	67	IDC	2	3A
19	0.002559	60	IDC	3	4
20	113000000	62	IDC	2	2

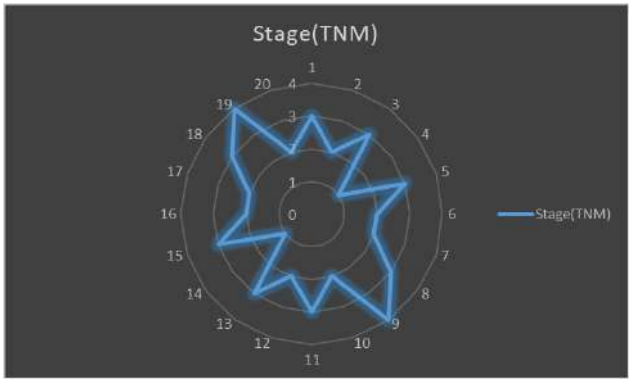

FIGURE 4.3: Shows the graphical representation of the breast cancer stages before surgery

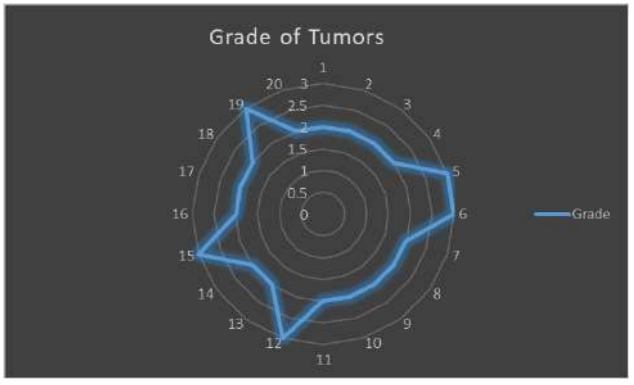

FIGURE 4.4: Shows the graphical representation of the breast cancer grades before surgery

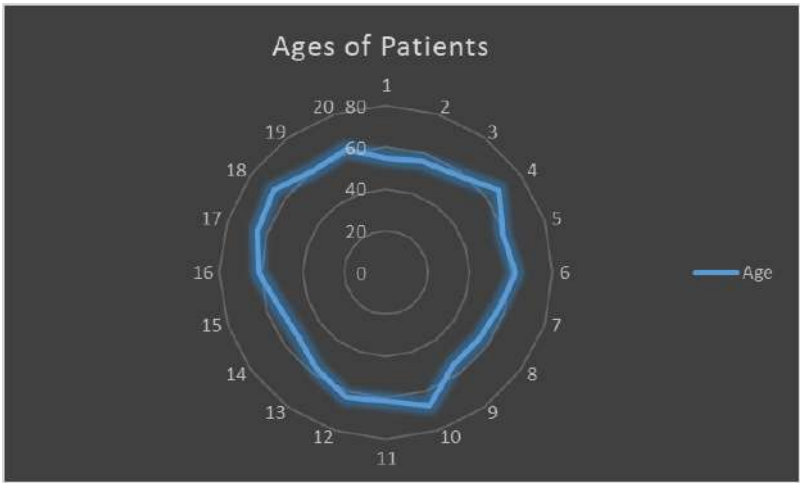

FIGURE 4.5: Shows the graphical representation of the breast cancer ages before surgery

4.8 Expression of PMS2 Gene in Post Tumor Treatment and its Association with Selected Clinicopathological Characteristics

The expression of the PMS2 gene contributes significantly to our understanding of molecular changes that occur after tumor treatment in post-menopausal patients with breast cancer. In this section, we examine the relationships between post-tumor treatment. PMS2 gene expression and specific clinicopathological features in an effort to uncover potential implications for prognosis and treatment response. The Table presents a comprehensive analysis of the relationships between post-tumor treatment. PMS2 gene expression levels and important clinical parameters, such as grade, stage (TNM), histological features, and patient age.

TABLE 4.4: Show correlation between a few clinicopathological characteristics and post tumor resection PMS2 gene expression

PMS2 expression level in post-tumor resection patients	**PMS2 Tumor (pot) Patients 2ˆ-△△Ct**	**Age**	**Histological Features**	**Grade**	**Stage(TNM)**
1	0.0005921	55	ILC	2	3B
2	0.005563	56	IDC	2	2

PMS2 expression level in post-tumor resection patients	**PMS2 Tumor (pot) Patients 2ˆ-△△Ct**	**Age**	**Histological Features**	**Grade**	**Stage(TNM)**
3	0.08912	58	ILC	2	3A
4	0.005921	67	ILC	2	1
5	0.005563	59	IDC	3	3B
6	0	62	IDC	3	2
7	0.00891	57	IDC	2	2A
8	0.005921	55	IDC	2	3a
9	0	55	ILC	2	4
10	0.0891	67	ILC	2	2
11	0.00593	62	ILC	2	3B
12	0.0059	63	ILC	3	2
13	0.00891	57	ILC	2	3A
14	0.00592	52	ILC	2	1
15	0.00556	54	IDC	3	3B
16	0	61	IDC	2	2
17	0.0055	65	IDC	2	2A
18	0.00592	67	IDC	2	3A
19	0	60	IDC	3	4
20	0.000891	62	IDC	2	2

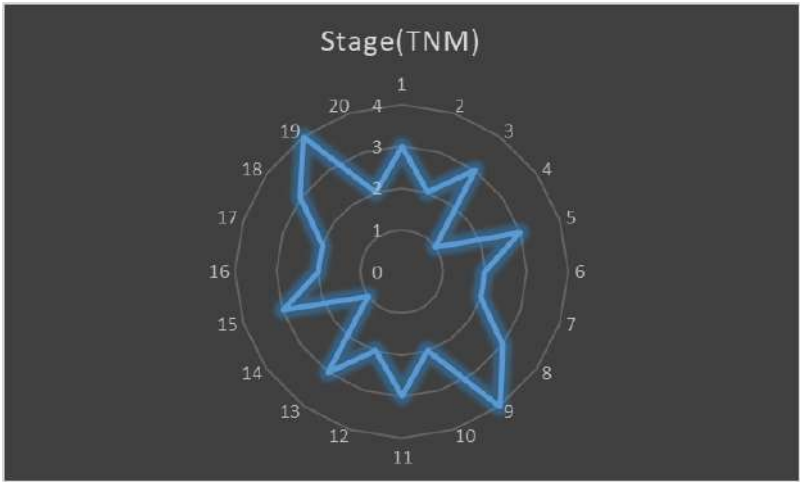

FIGURE 4.6: Shows the graphical representation of the breast cancer stages after tumor removal

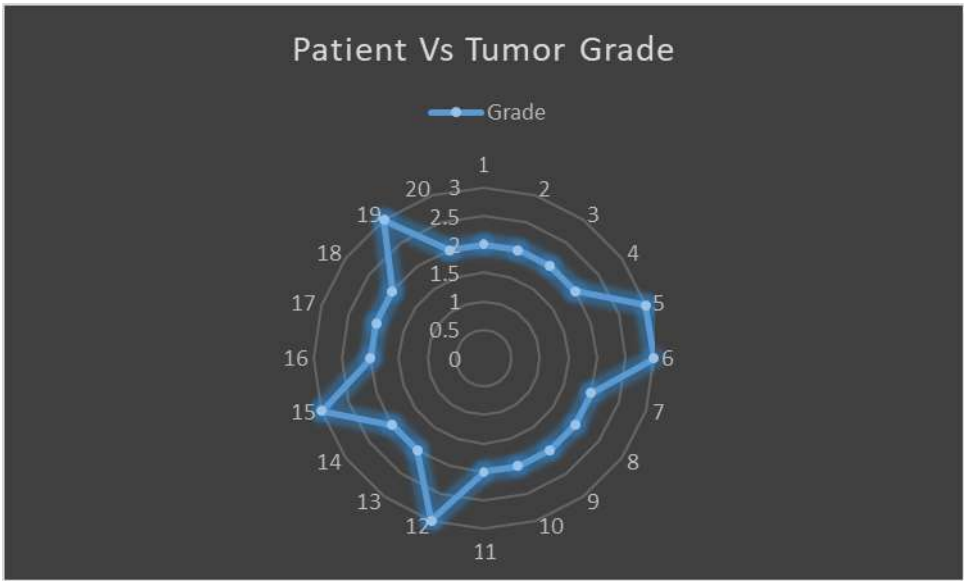

FIGURE 4.7: Shows the graphical representation of the breast cancer grades after tumor removal

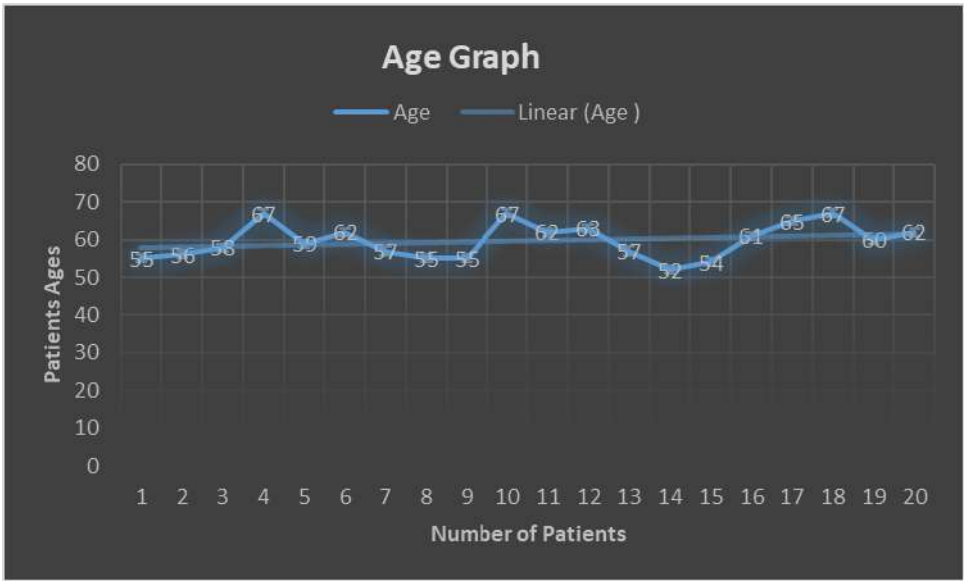

FIGURE 4.8: Shows the graphical representation of the breast cancer ages after surgery

4.9 Control Group Women

The control group was 7 included in current study who undergone for clinical breast cancer examination, mammography, ultrasound, all their reports results were negative.

TABLE 4.5: Shows control group 2ˆ-ΔΔCt values and ages

Control group	**2ˆ-ΔΔCt**	**Age**
C1	0	57
C2	0	64
C3	0	59
C4	0	67
C5	0	62
C6	0	58
C7	0	69

4.10 Survival Rate of the Breast Cancer Women Before Treatment

The table below define the survival rate of the patients before treatment at the time of diagnosis. All the patients were alive with no other events or accidents occurring prior to treatment.

TABLE 4.6: Shows survival rate of BC patients before treatment

PMS2 expression level in pre-tumor resection patients	**2ˆ-ΔΔCt**	**Blood Collection at time of diagnosis**	**Survival Time**
1	50.56264	Before treatment	Survived
2	670.18187	Before treatment	Survived
3	281700.11	Before treatment	Survived
4	54.94819	Before treatment	Survived
5	113000000	Before treatment	Survived
6	0	Before treatment	Survived
7	72.00374	Before treatment	Survived
8	113000000	Before treatment	Survived
9	5000.56	Before treatment	Survived
10	50.56264	Before treatment	Survived
11	50.56264	Before treatment	Survived

PMS2 expression level in pre-tumor resection patients	**2ˆ-ΔΔCt**	**Blood Collection at time of diagnosis**	**Survival Time**
12	670.18187	Before treatment	Survived
13	2817.11	Before treatment	Survived
14	54.94819	Before treatment	Survived
15	113000000	Before treatment	Survived
16	720.00374	Before treatment	Survived
17	54.94819	Before treatment	Survived
18	720.00374	Before treatment	Survived
19	0.002559	Before treatment	Survived
20	113000000	Before treatment	Survived

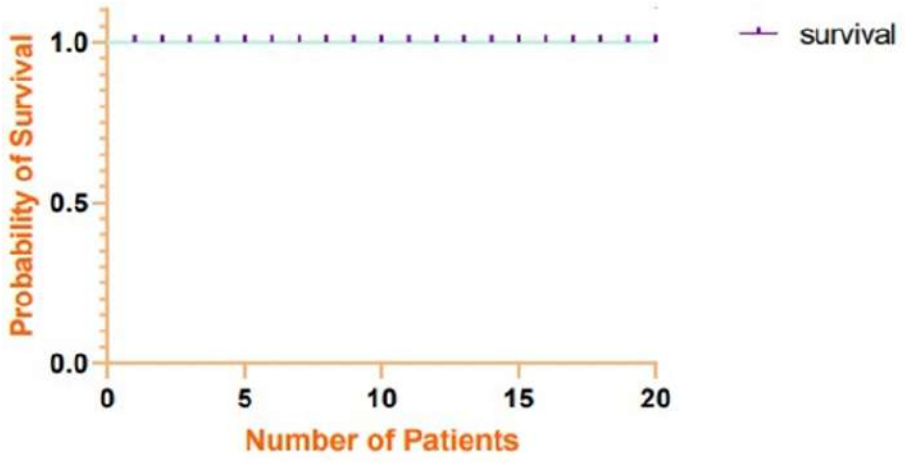

FIGURE 4.9: Shows the graphical representation of survival rate of breast cancer women before treatment

4.11 Survival Rate of the Breast Cancer Women After Treatment

The table below shows the survival rate of breast cancer patients after treatment. All the patients included in current study were alive whose blood were taken after treatment as well.

TABLE 4.7: Shows the survival rate of women breast cancer after treatment

PMS2 expression level in post-tumor resection patients	**2ˆ- ΔΔCt**	**Blood Collection Time**	**Survival Rate**
1	0.0005921	4 months After Treatment	Survived
2	0.005563	4 months After treatment	Survived
3	0.08912	6 months After Treatment	Survived
4	0.005921	6 months After Treatment	Survived
5	0.005563	6 months After Treatment	Survived
6	0	4 months After Treatment	Survived
7	0.00891	6 months After Treatment	Survived
8	0.005921	6 months After Treatment	Survived
9	0	4 months After Treatment	Survived
10	0.0891	4 months After Treatment	Survived
11	0.00593	4 months After Treatment	Survived
12	0.0059	4 months After Treatment	Survived
13	0.00891	6 months After Treatment	Survived
14	0.00592	6 months After Treatment	Survived

PMS2 expression level in post-tumor resection patients	**2ˆ- ΔΔCt**	**Blood Collection Time**	**Survival Rate**
15	0.00556	6 months After Treatment	Survived
16	0	4 months After Treatment	Survived
17	0.0055	6 months After Treatment	Survived
18	0.00592	6 months After Treatment	Survived
19	0	4 months After Treatment	Survived
20	0.000891	4 months After Treatment	Survived

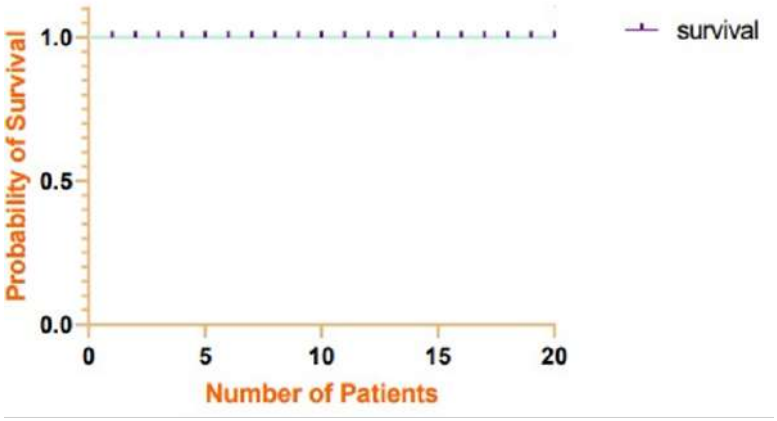

FIGURE 4.10: Shows the graphical representation of survival rate of breast cancer women after treatment

4.12 Discussion

This study's main goal is to assess the expression levels of the PMS2 gene in the blood of postmenopausal women with breast cancer both before and after the

tumor is treatment. The survival rates of the patients both before and after treatment were another major focus of this investigation. There are 27 individuals in the current study and 47 blood samples. To assess the PMS2 gene transcripts, blood samples were obtained from each of the twenty patients with breast cancer both before and after the tumor treatment. The study employed a number of techniques, including RNA extraction, cDNA synthesis, Nano Drop analysis, and PCR methods, to profile the PMS2 gene expression. A mismatch repair gene known as PMS2 (post meiotic segregation increased 2) genes for a protein that fixes errors in DNA replication [88]. It is now widely recognized that MMR inactivation in human cells is linked to instability across the genome, including damage to microsatellites or DNA, and a susceptibility to specific cancer types [89]. Correct any little insertions or deletions that may occur during DNA replication and homologous recombination. The MutL-alpha heterodimer is formed when the PMS2 protein and the MLH1 gene product interact to form heterodimers. The endonucleolytic activity of the MutL-alpha heterodimer is activated upon detection of mismatches and insertion/deletion loops. Recognition is aided by the heterodimers MutS-beta and MutS-alpha. Constitutional mismatch repair deficit (CMMRD) syndrome is caused by mutations in PMS2 [25].

Lynch syndrome and instability of microsatellites with an 80% lifetime chance of developing several forms of cancer, Lynch syndrome is an autosomal dominant cancer predisposition condition. such are pancreatic, prostate adenocarcinoma, ovarian, small bowel, colorectal, and endometrial cancers (CRC and EC being the most prevalent malignancies) [90]. Thorough the examination of breast cancer in Lynch syndrome by researchers at different trials. They discovered that whereas some research demonstrated a large increase in risk, others did not (2–18 times more probable). Furthermore, distinct DNA repair-related genes (e.g., MLH1, MSH2, MSH6, and PMS2) have been associated with varying degrees of breast cancer risk relative to one another. Women are more likely to acquire breast cancer as they become older [57]. A PMS2 deficiency also raises the risk of breast cancer, with a cumulative risk of 37.7% at 60 years of age. According to reports, women with LS who have mutations in the PSM2 gene are more likely to develop breast cancer than those who have mutations in the MLH1, MSH2, or MSH6 genes [59].

In the current investigation, the PMS2 gene was shown to be highly expressed in the bloodstreams of postmenopausal women prior to neoadjuvant chemotherapy, surgery and less expressed following the procedure. The overexpression of the PMS2 gene were seen in oral squamous carcinoma [85]. And its overexpression was seen in prostate [87]. Most of the studies shows that deficiency of the PMS2 leads to cancers such as colon, breast, pancreatic ductal adenocarcinoma, ovarian cancer but some studies shows its high expression such as prostrate, oral squamous carcinoma.

The current study shows the high expression of PMS2 gene before a treatment such as neoadjuvant chemotherapy and surgery and less expression of PMS2 gene after the treatment in the blood of postmenopausal women with breast cancer. To clarify the current study results, a paired sample t-test, correlation analysis, and a variety of descriptive statistics were carried out using statistical analysis using SPSS. The demographics of research participants indicate that women between the ages of 50 and 70 are most commonly diagnosed with stage 2 breast cancer, with the majority of these women falling between the 50 and 60 age range. The most prevalent type of cancer diagnosed in the majority of patients was invasive ductal carcinoma (IDC), or grade 2 malignancy. In the study, there were two time periods: four months and six months, with the same number of participants in each group. All of the patients survived both the pre- and post-tumor phases, indicating a high likelihood of survival. Pre- and post-tumor treatment showed a weakly negative connection, according to the correlation analysis. PMS2 gene expression levels, suggesting a relationship between lower post-tumor treatment expression and greater pre-tumor treatment gene expression. This implies that PMS2 gene expression levels in the blood of breast cancer patients may drop following surgical treatment. The paired sample t-test revealed a significant change in PMS2 gene expression before and after surgical intervention, which lends more credence to this idea. Pre-tumor treatment was associated with greater expression levels than post-tumor treatment, with a statistically significant mean difference in gene expression levels. This discovery validates the theory that surgical intervention affects breast cancer patient's expression of the PMS2 gene.

Chapter 5

Statistical Analysis

The data was entered in SPSS for further analysis of the present study. Descriptive statistics were utilized to analyze the demographics of N=20 patients. To highlight the expression of PMS 2 gene level in breast cancer patients, the Correlation Coefficient between pre and post-tumor was also computed along with a paired sample t-test. The details of these tests are given below.

5.1 Descriptive Statistics for Demographic Variables

Table 5.1: Highlighting Descriptive statistics (N = 20)

Variables	**Categories**	**F**	**%**
Age	50 to 60 years	11	55.0%
	60 to 70 years	9	45.0%
Histological Factor	ILC	9	45.0%
	IDC	11	55.0%
Grade	2	15	75.0%
	3	5	25.0%
Stage (TNM)	Stage 1	2	10.0%
	Stage 2	6	30.0%

Variables	Categories	F	%
	Stage 2A	2	10.0%
	Stage 3A	4	20.0%
	Stage 3B	4	20.0%
	Stage 4	2	10.0%
Time Duration	4 months	10	50.0%
	6 months	10	50.0%
Survival Rate (Pre-Tumor)	Survived	20	100.0%
	Not-Survived	0	0%
Survival Rate (Post-Tumor)	Survived	20	100.0%
	Not-Survived	0	0%

Note: f = frequency, % = percentage, ILC= Invasive lobular carcinoma, IDC= Invasive ductal carcinoma.

The demographic variables comprised breast cancer grade and stage, its underlying histological factors (stage), age and survival frequency. To analyze age, two categories were made. The least reported age was 52, and the highest reported age was 67. The frequency of females with breast cancer was higher in women within the age group of 50 to 60 years.

A percentage of 55.0 with a frequency of 11 was noted in this age group. Whereas the other category of age group has a frequency of 9 and a percentage of 45.0. The most common breast cancer type reported is Invasive ductal carcinoma (IDC) is more with a frequency of 11 and a percentage of 55.0 (55.0%). Most of the patients were diagnosed with grade-2 cancer, with a frequency of 15 and a percentage of 75.0 (75.0

The least reported breast cancer is stages (1, 2A and 4) with a frequency of 2 and a percentage of 10.0. Whereas the most reported stage is stage 2 cancers with a percentage of 30.0 and a frequency of 6. The other largest reported stage is noted as stage 3A and 3B with 20.0 percent and a frequency of 4. The data set of the patients was taken after 2 categories of time of 4-month and 6-month. Out of two

experimental groups (Control and experimental), the control group comprised of (N=7) patients whereas the experimental group comprised of N=20 patients.

To measure the survival rate in both pre-tumor treatment and post-tumor treatment phases, two categories of survival were brought under consideration. All patients survived in both phases.

5.1.1 Explanation of Data

The most reported breast cancer stage among females of 50 to 70 years of age was stage 2, grade 2. Most of participants of this study fall within the age range of 50 to 60 years. The most common breast cancer type among the patients was reported as IDC known as Invasive ductal carcinoma.

For the time duration, an equal number of patients were noticed in the 4-month and 6-month groups.

TABLE 5.2: Highlighting Descriptive statistics – Control Group (N = 7)

Variables	**Categories**	**F**	**%**
Age	50 to 60 years	3	42.9%
	60 to 70 years	4	57.1%
Gene Expression	Nil	7	100%
Family History	YES	0	0%
	NO	7	100%

Note: f = frequency, % = percentage

Table 5.2 shows the control group difference in the study participants. The control group was taken after getting excessive complaints of breast pain (due to other conditions).

The gene expression for the control group participants was 0, with no family history of cancer. Whereas the age range taken was similar age group of patients diagnosed with breast cancer.

5.2 Descriptive Statistics (b)

TABLE 5.3: Descriptive Statistics - (B) (N = 20)

Variables	**N**	**Min**	**Max**	**Mean**	**SD**
Age	20	1	2	1.45	.510
Histological Factor	20	1	2	1.55	.510
Grade	20	1	2	1.25	.444
Stage	20	1	6	3.40	1.603
Time Duration	20	1	2	1.50	.513
Survival Rate (pre-tumor)	20	1	1	1.00	.000
Survival Rate (post-tumor)	20	1	1	1.00	.000

Note: The data highlighted above represents N (total number of participants, Min (minimum), Max (maximum), Mean (average), and SD (standard deviation). The lowest age was 52 and the highest was 67. The mean for age is 1.45 whereas the standard deviation is noted as .510. The mean for histological factors is noted as 1.55 with a standard deviation of 510.The least reported cancer stages are 1, 2A and 4 whereas the most reported is stage 2. The mean value for stage is 3.40, with standard deviation 1.603. The mean value of Grade is 1.25 and a standard deviation of .444. The mean value for time duration is 1.50 with a standard deviation of .513. The survival rate was high in both conditions (pre- and post). The mean value for the pre-and-post survival rate is 1 with a standard deviation of .000 depicting no variability in data (all patients have the same survival rate throughout the study).

TABLE 5.4: Descriptive Statistics - (B) Control Group (N = 7)

Variables	**N**	**Min**	**Max**	**Mean**	**SD**
Age	7	1	2	1.57	.535
Gene Expression	7	0	0	0	0
Family History	7	2	2	2	0

Note: The data highlighted above represents N (Total Number of Participants, Min (minimum), Max (maximum), Mean (Average), and SD (Standard Deviation)

The min. age range was 57 and the max. age range was 69, with a mean of 1.5 and a SD of .53. The gene expression was noted as zero. The family history of the participants in the control group was with no cancer history.

5.3 Correlation Analysis

Table 5.5: Correlation Coefficient (N=20)

Variables	**1**	**2**
PMS2 Pre-tumor	-	-0.161
PMS2 Post-tumor	-0.161	-

To test the hypothesis **Ha**: "There is a significant difference in PMS 2 gene expression in the blood of older women with breast cancer before and after surgical intervention. The observed variations are not due to random chance and there is a meaningful association between the surgical procedure and alterations in PMS 2 gene expression." To highlight the expression of PMS 2 gene level in both conditions, the Pearson correlation coefficient was calculated based on the pre-tumor and post-tumor recorded observations. The correlation is negative between the two experimental conditions. -0.161. The negative sign highlights a weak linkage between the conditions (PMS 2 pre-tumor (pr) ct and PMS 2 post-tumor (pr) ct). A higher level in the first experimental condition (pre-tumor) is weakly associated with the second experimental condition (post-tumor). It shows that the level of gene fluctuates downwards in the post-tumor condition- after 4 to 6 months of surgical treatment of tumor.

5.3.1 Explanation of Data

Pearson's correlation coefficient (with the negative sign) highlighted the fluctuation of PMS 2 gene levels in Breast cancer patients. The higher PMS 2 level in pre-tumor stage is associated with the lower PMS2 level in the post-tumor stage.

This represents patients who had higher levels of the PMS2 gene were seen as having lower levels of the PMS2 gene after tumor removal.

5.4 Paired Sample T-test

To test the hypothesis **Ha**: "There is a significant difference in PMS2 gene expression in the blood of older women with breast cancer before and after surgical intervention. The observed variations are not due to random chance and there is a meaningful association between the surgical procedure and alterations in PMS2 gene expression." The researcher proceeded with a paired sample t-test with the two experimental conditions. The detailed description is given next:

TABLE 5.6: Paired Sample T-Test (N=20)

Variables	Pre-test (n=20)		Post-test (n=20)		t	P	Cohen's d
	M	SD	M	SD			
Pair 1							
PMS2 Pre-tumor (pr) ct – PMS 2 Post-tumor (po) ct	22614634.33	46366756.27	.01	.026	2.181	0.042	0.812

Note: M = mean, SD = standard deviation, t = t-statistics, p = p-value, d = Cohen's d value.

To test the alternate hypothesis, a paired sample t-test was used. The mean value of pre-tumor PMS 2 gene is 22614634.33 with a standard deviation of 46366756.27. On the contrary, the mean value of post-tumor PMS 2 gene is .01 with a standard deviation of .026. The 95% confidential level lower limit (LL) is 914324.402, whereas the Upper limit (UL) is 44314944.24. The p-value is noted as 0.042. The t-statistics is 2.181 with 19 degrees of freedom. It indicates that the mean difference is significant at 0.05 level. The Cohen's d value is 0.812.

5.5 Interpretation of Results

The results support the rejection of the null hypothesis indicating a significant difference in the PMS 2 gene level before and after surgical treatment. The paired sample t-test and Pearson's correlation coefficient provide supporting evidence for the acceptance of the alternative hypothesis. The gene level gradually decreases in the post-tumor phase as indicated by a weak negative correlation. For the paired sample t-test, the mean value difference for the pair of the PMS 2 gene is 22614634.32 and the standard deviation value is 46366756.28. The t-value is (2.181), and the p-value is (0.042) with a 95% confidence interval further reinforcing these findings, and favoring the alternative hypothesis. These results suggest a substantial difference in PMS 2 gene expression in the blood of older women with breast cancer following surgical intervention. The observed variations are unlikely to be attributed to random chance, indicating a meaningful association between the surgical procedure and alterations in PMS 2 gene expression.

5.6 Results

5.6.1 The Expression Level of PMS2 Gene

The statistical analysis for this study was conducted using SPSS, focusing on the expression level of the PMS 2 gene in 20 patients diagnosed with breast cancer. Two experimental groups were defined, as illustrated in Figure 1. The control group comprised 7 normal individuals (with gene expression zero, and zero family history of cancer diagnosis), whereas the experimental group consisted of 20 breast cancer patients, spanning stages 1 to 4. The paired sample t-test was done to compare the pre and post-test scores. Among 20 participants (patients diagnosed with Breast cancer), the blood samples of 10 patients were collected after 6 months while the blood samples of the other 10 patients were collected after 4 months (demonstrated in Figure 2). The findings revealed a decrease in the concentration level of the PMS 2 gene in the post-tumor observations (illustrated in

Figure 3), with higher gene expression levels observed during pre-tumor observations. The calculated mean difference (22614634.32), t-value (2.181), and p-value (0.042) were found to be significant, leading to the rejection of the Null Hypothesis. These results indicate a meaningful difference in the PMS 2 gene expression levels following surgical intervention in breast cancer patients.

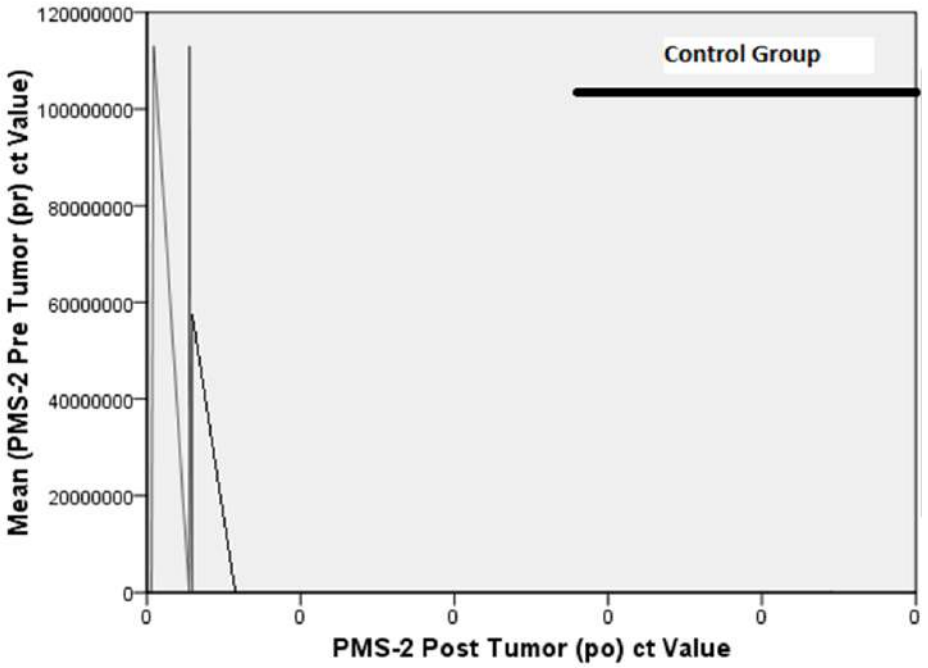

FIGURE 5.1: Variation in Control Group and Experimental Group (Pre-tumor and Post-tumor)

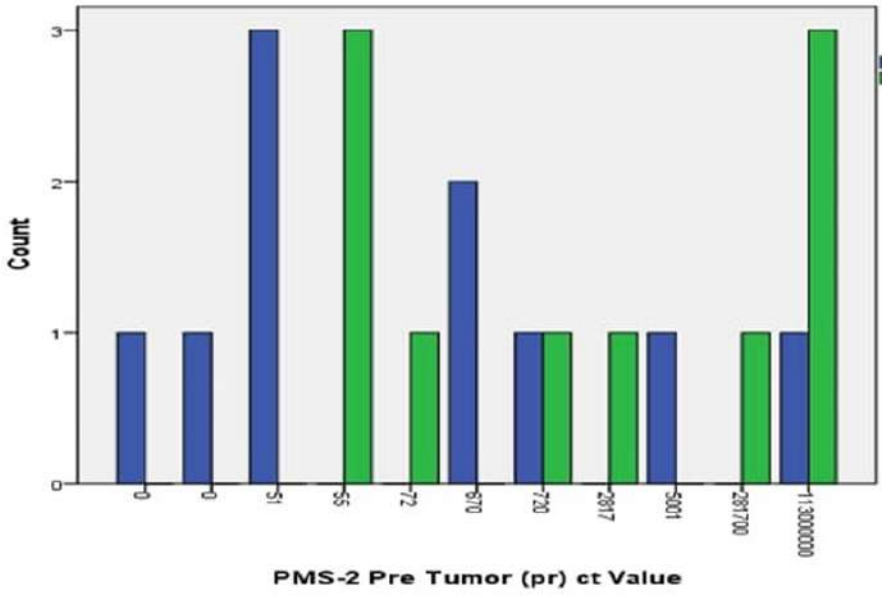

FIGURE 5.2: Expression of PMS2 Gene with time variation in Patients with tumor

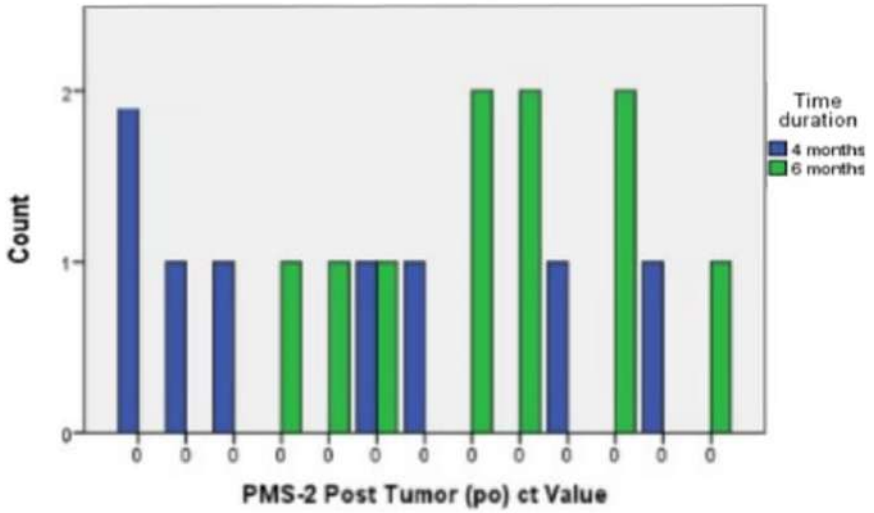

FIGURE 5.3: Expression of PMS2 Gene with time variation in Patients after tumor removal

Chapter 6

Conclusion

The PMS2 gene is one of the mismatch repairs gene which help to correct the errors occur during DNA replication. Mutation in PMS2 gene cause lynched syndrome which is leads to cause colorectal cancer (CRC), endometrial cancer (EC), ovarian cancer (OC), urothelial cancer (UC), and small bowel cancer, pancreas, prostate adenocarcinoma. PMS2 gene mutation in breast cancer is debating topic some studies show the involvement of PMS2 gene in breast cancer on another hand other some studies show that there is no role of PMS2 gene in breast cancer but there is no specific study in breast cancer on the expression of the PMS2 gene in the blood of postmenopausal women with breast cancer. The current study aim is to investigate the expression level of the PMS2 gene in the blood of postmenopausal women with breast cancer before and after treatment (neoadjuvant chemotherapy, surgery) and compares the expression level of the PMS2 gene by using different methods. The correlation analysis revealed a weak negative correlation between pre-tumor treatment and post-tumor treatment. PMS2 gene expression levels, indicating that higher pre-tumor treatment gene expression was associated with lower post-tumor treatment expression. This suggests that the overexpression of the PMS2 gene can also leads to breast cancer in postmenopausal women with breast cancer and following a treatment overcome its expression levels in the blood of postmenopausal women with breast cancer. To support this notion, the paired sample t-test indicated a significant difference in PMS2 gene expression before and after treatment (surgical intervention). The mean difference in gene

expression levels was statistically significant, with a higher expression observed pre-tumor treatment compared to post-tumor treatment. This finding strengthens the hypothesis that surgical intervention influences PMS2 gene expression in breast cancer patients. There for the current study conclude that the PMS2 gene is overexpression before treatment (neoadjuvant chemotherapy, surgery) and decrease after treatment (neoadjuvant chemotherapy surgery in postmenopausal women with breast cancer who's having a family history of other cancers. All the patients survive before treatment (neoadjuvant chemotherapy, surgery) and as well after treatment (neoadjuvant chemotherapy surgery). The current study is conducted on small population. To validate the results further the study should be conducted on large sample size.

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