CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Investigating Molecular Transformation via CHEK2 Gene Transcript Profiling in Blood from Postmenopausal Women with Breast Cancer: A Pre & Post-Treatment Studies

by

Mahnoor Fayyaz

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This thesis is dedicated to my beloved Parents, the cherished gifts bestowed upon me by Almighty Allah, whose irreplaceable presence in my life is unparalleled. I extend my deepest gratitude and dedicate all achievements attained through this academic journey to my father, whose radiant countenance illuminates the path of my existence with boundless love and unwavering sincerity. I also extend my heartfelt dedication to my dear friend Asma, whose steadfast companionship has been a beacon of strength through every trial and tribulation. Furthermore, I express sincere appreciation to my supervisor, Dr. Shaukat Iqbal Malik, whose invaluable guidance and support have significantly contributed to the fruition of this work.

CERTIFICATE OF APPROVAL

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Mahnoor Fayyaz

(MBS221008)

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Saima Hanif	NUMS, Islamabad
(b)	Internal Examiner	Dr. Syeda Marriam Bakhtiar	CUST, Islamabad
(c)	Supervisor	Dr. Shaukat Iqbal Malik	CUST, Islamabad

Dr. Shaukat Iqbal Malik
Thesis Supervisor
April, 2024

Dr. Syeda Marriam Bakhtiar
Head
Department of Bioinformatics & Biosciences
April, 2024

Dr. Sahar Fazal
Dean
Faculty of Health & Life Sciences

April, 2024

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Abstract

The study investigates CHEK2 gene transcript profiling in 47 freshly collected blood samples of PM Breast cancer women,7 blood samples are of healthy women named as control group,20 blood samples are of women newly diagnosed with breast cancer and having tumor and 20 blood samples are of patients collected after removal of tumor or other clinical treatment for examining molecular transformations pre- and post treatment. Further, to evaluate its association with selected clinicopathological features and overall survival of patients. CHEK2, a crucial gene involved in DNA repair and genomic stability maintenance, has been associated with increased cancer risks, particularly in breast and prostate cancer or colorectal cancer. This research explores the correlation between CHEK2 expression levels and clinicopathological features in breast cancer patients. Thus, the CHEK 2 expression level was evaluated by Real-time PCR method in 47 blood samples taken from 7 healthy women, 20 Breast Cancer patients at the time of diagnosis. And 20 patients at interval of 6 months and 4 months after surgery or treatment. Demographic characteristics, correlation analysis, and paired sample t-tests were conducted to analyze the data. The Mean of CHEK 2 expression level in blood decreased systematically from the time of cancer diagnosis to 6 and 4 months after surgery or treatment. Demographic characteristics of 20 patients were analyzed descriptively, revealing predominance in the 50-60 age group, invasive ductal carcinoma histological factor, and stage 2 cancer. Survival rates remained the same in pre and post-treatment studies. Correlation analysis between newly diagnosed patients and after removal of tumor or treatment. The CHEK2 gene levels indicated a negative association, suggesting higher pre-treatment expression levels associated with lower post-treatment expression levels. A paired sample t-test confirmed a significant decrease in CHEK2 gene expression in post-treatment studies ($p = 0.020$). Correlation analysis indicated a weak association between pre and post-treatment gene expression levels. Paired sample t-test supported the rejection of the null hypothesis, emphasizing the meaningful association between surgical intervention or treatment and alterations in CHEK2 gene expression. The findings highlight the importance of CHEK2 in the context of breast

cancer in post-menopausal patients. Decreased gene expression in post-treatment studies suggests a potential role in the molecular transformations associated with surgery or treatment. The findings underscore the importance of CHEK2 in breast cancer progression and its potential as a biomarker for prognosis.

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Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
CSCs	Cancer Stem Cells
EGFR	Epidermal Growth Factor Receptor (EGFR)
ERs	Estrogen receptors
ErbB-2	Erythroblastic Leukemia Viral Oncogene Homolog 2): ErbB-2 (another name for HER2)
ErbB-3	Erythroblastic Leukemia Viral Oncogene Homolog 3): ErbB-3 (another name for HER3)
ErbB-4	Erythroblastic Leukemia Viral Oncogene Homolog 4): ErbB-4 (another name for HER4)
GWAS	Genome-wide association
HER2	Human Epidermal Growth Factor Receptor 2)
HER3	Human Epidermal Growth Factor Receptor 3)
HER4	Human Epidermal Growth Factor Receptor 4)
LOH	loss of heterozygosity
MDSCs	Myeloid-derived suppressor cells
PRs	progesterone receptors
RING	Really Interesting New Gene
TAD	Transcriptional activation domain

Chapter 1

Introduction

Breast cancer is the second most frequent cancer to be identified. It is a potentially fatal illness that affects millions of people worldwide, mostly women. It is brought on by the unchecked proliferation of abnormal cells in breast tissue and affects women more than any other cancer. With over two million new cases reported in 2021, breast cancer remains a significant global health issue. Certain miRNAs may serve as biomarkers since they have been connected to cancer [1]. Breast cancer is a major global health concern that mostly affects women globally. Only a small percentage of instances about 5–10% are linked to the inheritance of high-penetrance cancer susceptibility genes despite the disease's broad incidence.

Notably, a key factor in hereditary chest and ovarian cancer is germline alterations in the tumors suppressor genes BRCA1 and BRCA2. The aforementioned alterations when linked to an elevated genetic vulnerability augment the likelihood of acquiring certain cancers. Further, the number of cancer cases and fatalities worldwide is rising quickly [2]. About 10 million people died from cancer-related causes and about 19 million new instances of breast cancer were reported in 2020. In the study done by [3] with an estimated 11 million deaths from breast cancer predicted by 2030, the forecast is alarming. The most common type of cancer to be diagnosed is Breast cancer and Lung, colorectal, prostate, and stomach cancers are in close second place. In many nations, Breast cancer is the most common

cause of cancer-related deaths globally, ranking sixth in terms of incidence. It accounts for one-fourth of women's cancer diagnoses and one-sixth of cancer-related fatalities.

Further the Older age, obesity or high BMI, tobacco use, physical inactivity, high fat diet, early menarche, late age at first full-term pregnancy, shorter breastfeeding durations, use of oral contraceptives or hormonal menopausal therapy, breast density, and family history of breast cancer are the main risk factors for breast cancer. According to estimates by sex and cancer type, there will be around 1,958,310 new cases of invasive cancer in the United States in 2023. That is the equivalent of about 5370 cases every day [4]. Furthermore, there are expected to be around 55,720 new instances of ductal carcinoma in situ in females. In 2023, 609,820 Americans are expected to lose their lives to cancer [5]. It is difficult to draw a clear correlation since research on menopausal hormone treatment focuses on synthetic progestogens rather than natural progesterone. Mechanistic research points to progesterone's possible involvement in the genesis of breast cancer [6].

A recent meta-analysis study by [7] found that obese women had a 41% higher overall mortality and a 33% greater chance of death from breast cancer. In addition to a number of studies linking obesity to poor breast cancer outcomes. This correlation is plausible, because of biological components linked to obesity, including higher levels of insulin, glucose, leptin, postmenopausal estrogen, and inflammatory markers.

About 80% of occurrences of breast cancer are founded in women 50 years of age or older, and the disease is most frequently detected in women going through the menopausal transition. The percentage of instances of breast cancer linked to hereditary abnormalities is just 5–10%. while lifestyle decisions and environmental variables are responsible for the remaining 90–95%. Genetic predisposition is not the determining factor in most cases. According to [8] but the non-genetic variables dominate.

According to the study, breast cancer is the most common neoplastic disease among women going through menopause and has a major influence on how they

live their everyday lives. According to epidemiological statistics from 2010, 22% of all malignancies diagnosed in Poland were breast cancers, with 80% of cases occurring in women 50 years of age and older. Concerning patterns showed that over the past 20 years, the incidence of breast cancer has more than doubled without a matching improvement in treatment results. While age, family history, and reproductive variables are among the risk factors that have been found by research. The intricacy of the epidemiology, molecular processes, and clinical presentation of breast cancer is attributed to a number of intrinsic and lifestyle-related variables [9]. A small subset of tumor cells known as cancer stem cells (CSCs) are in charge of important tumors behaviors like invasion, metastasis, recurrence, and resistance. These cells are the first to grow into cancer when self-renewing stem or precursor cells undergo a malignant change. CSCs are important targets for therapeutic intervention because they are critical to the maintenance of tumor development and are involved in the complexity of cancer progression. Moreover, myeloid-derived suppressor cells (MDSCs) are essential for the defense against tumors [10]. Further their research indicates that individuals with breast cancer (BC) have higher than average amounts of MDSCs patients with metastatic illness had the greatest levels of MDSCs. This implies that there could be a relationship between breast cancer development and MDSC levels. Knowing how MDSCs function in the tumor microenvironment may help with the development of patient-specific targeted treatment plans.

The progesterone receptor (PR) isoforms PRA and PRB have distinct effects on breast cancer cells, depending on progesterone both independently and dependently. These two isoforms, PRA and PRB, have different functions and can affect breast cancer cells behavior in ways that are both progesterone- dependent and progesterone- independent. The structural distinctions between PRA and PRB enable them to engage in diverse protein interactions resulting in a range of downstream signaling events that add to the intricacy of breast cancer biology [11]. A dataset of nuclear ERα molecular partners linked to RNAs has been produced using TAP-MS and RNase digestion, providing important insights into the combined contributions of nuclear RNAs and identified proteins in ERα signaling

to the genome that are applicable to a variety of cell types, including breast cancer [12]. Cancer is a grave worldwide problem. The World Health Organization estimated that there were about 19 million new cases and over 10 million fatalities globally in 2020.

In the United States, the projected numbers for 2023 are 609,820 fatalities and 1,958,310 new cases of cancer. The most prevalent kind, breast cancer will be diagnosed in 2.3 million cases in 2020. In low- and middle-income nations tragically, there are a lot of instances and fatalities. Over 80% of cancer patients in affluent countries go on to survive, but late detection is still a major issue in underdeveloped nations. According to [13] The Breast cancer is the most common type of cancer in women, and its incidence rises sharply with age. Women are typically diagnosed with breast cancer around age 61. Regretfully, women 65 years of age and older account for the bulk of those who die from breast cancer. Breast cancer risk increases with age, with an incidence of 1 in 200 for women under 40 years old [14]. An additional 1 in 14 for people over 70. Nowadays, women over the age of 65 account for over 40% of instances of breast cancer, while women over the age of 75 account for 20% of cases. It is anticipated that by 2025, older women would receive a diagnosis in approximately 75% of instances of breast cancer. This demonstrates how common breast cancer is as people age. One important contributing aspect to the rising diagnostic rates is still ageing [15]. It is discovered that, following menopause, differentiated lobules regress to undifferentiated lobules similar to those observed before puberty. Ageing also alters the breast tissue by causing duct-lobular tissue atrophy at the expense of connective and adipose stroma. Thus, glandular immaturity is a characteristic of puberty and old age. But unlike puberty in older women, epithelial cells that have been repeatedly exposed to carcinogens have undergone alterations in the atrophic duct-lobular and immature tissue. changes in the double-stranded DNA repair system due to a possible increase in transformation potential caused by the absence of the ATM protein (ataxia telangiectasia mutated) pathway suppression.

The Ageing is notably connected with an increased risk of breast cancer, according to an examination of the international literature on the disease published in the last

ten years. It is noteworthy that the clinico-pathological aspects of breast cancer in older women differ from those in younger people. Pathogenic variants (PVs) in BRCA1 and BRCA2, as well as both BRCA1/2, are known to be linked to higher risks of breast and ovarian cancers in women for whom accurate risk estimates are available [16]. While there is currently no evidence linking BRCA1 PVs to the risk of prostate cancer, accumulating data suggests that BRCA1/2 PVs are linked to the risks of pancreatic cancer and male breast cancer. Additionally, BRCA2 PVs are linked to the risk of prostate cancer, particularly aggressive prostate cancer [17].

1.1 Breast Cancer Risk Factor

Breast cancer is the most often diagnosed neoplastic disease in women approaching menopause, and it significantly affects their day-to-day functioning. Comprehensive research into related risk factors is required since epidemiological studies show that women who are actively involved in social and professional domains have a higher incidence of breast cancer. Its development is significantly influenced by the known risk factors [9]. The identifiable risk factors play a crucial role in its development [18].

1.1.1 Age and Gender

Increasing age and being female are the main risk factors. Breast cancer is more common in women, and the risk rises with age [5].

1.1.2 Genetics and Family History

The risk is increased if you have a mother, Sister and Daughter. Who is a first-degree relative and has breast cancer. Gene mutations that are inherited, including BRCA1 and BRCA2, are linked to an increased risk [65][58].

1.1.3 Individual Background of Breast Cancer

The higher chance of acquiring cancer in the other breast or in a different area of the same breast exists for individuals who have had breast cancer in one breast.

1.1.4 Menstrual and Reproductive History

An elevated risk can be caused by early menstruation (before the age of 12), late menopause (beyond the age of 55), and having your first child later in life [18].

1.1.5 Density of the Breasts

The Mammography results showing dense breast tissue in women may indicate a greater risk of breast cancer [14].

1.1.6 Exposure to Radiation

The risk may be increased by prior radiation therapy to the face or chest, particularly in youth or adolescent.

1.1.7 Lifestyle and Dietary Factors

The Lifestyle variables that may raise the risk include smoking, high-fat meals, obesity, inactivity, and excessive alcohol intake.

1.1.8 Hormonal Contributions

The study shows that Extended exposure to estrogen, as a result of early menstrual onset, late menopause, or specific hormone-related disorders, may increase the risk.

1.1.9 A Few Mild Breast Disorders

A typical hyperplasia is one non-cancerous breast condition that might raise the risk [7].

1.1.10 Hormone Replacement Therapy (HRT)

The Extended usage of estrogen and progesterone-containing hormone replacement treatment, particularly postmenopausal hormonal therapy, may raise the risk. Additionally, height, weight, and race/ethnicity are risk factors for breast cancer [19].

1.2 Breast Cancer Classification

1.2.1 Histological Classification

The Breast cancer is a diverse illness with many entities, each with unique biology and histological characteristics [20].

1.2.1.1 In Situ Ductal Carcinoma (DCIS)

The lining of a breast duct contains abnormal cells, but they have not spread to neighboring tissues.

1.2.1.2 Invasive Ductal Carcinoma (IDC)

Through the duct walls, cancer cells infiltrate the surrounding breast tissues [22].

1.2.1.3 In situ lobular carcinoma (ISLC)

The lobules include abnormal cells, however this is not regarded as genuine cancer.

1.2.1.4 Lobular Carcinoma Invasive (ILC)

Cancerous cells can infiltrate lobules and proceed to neighboring tissues [19][20].

1.2.2 Molecular Classification

The Researchers looked into the behavior of genes in breast cancer. Five distinct forms of breast cancer were discovered [21].

1.2.2.1 The luminaries A and B

The Genes linked to estrogen receptors are highly active in luminal A breast cancer, but HER2 receptor and proliferation-related genes are less active. Although compared to luminal A, the proliferative gene activity in luminal B breast cancer is stronger, it still exhibits estrogen receptor activation. Experts can identify between luminal A and B using a test known as Ki-67 [21][19].

1.2.2.2 Non-Luminal, HER2-Enriched

The HER2-positive (HER2+) and HR-negative (HR-). Elevated expression of HER2 and lack of estrogen and progesterone receptors are characteristics of breast cancer that is HER2-positive.

1.2.2.3 Triple-Negative Breast Cancer (TNBC)

The HER2-negative, HR-negative combination is frequently linked to a more aggressive path. In addition to lacking HER2 receptor expression and progesterone and estrogen receptors, basal-like breast carcinoma is sometimes referred to as triple-negative malignancy. Treatment for this kind might be challenging, particularly if it extends to the cerebellum. Although the effectiveness of tests utilizing biological markers is unclear, they can be helpful [22].

1.3 Breast Cancer Treatment

The Women frequently get breast cancer, and researchers frequently carry out clinical studies to enhance available therapies. Experts, such as the European Society for Medical Oncology and the St. Gallen International Breast Cancer Conference, revise treatment guidelines every two years based on consensus. Following preoperative therapy, HER2-positive and triple-negative breast tumors are being treated more successfully, according to recent recommendations according to [18]. An individualized strategy is used to treat breast cancer in older women, taking into account the patient's general health, the specifics of the disease, and any possible adverse effects from the treatment. The following are some typical components of older women's breast cancer treatment [16][36]:

1.3.1 Surgery

Breast cancer in women can be treated surgically in a number of ways:

- Removal of a tumor
- Mastectomy
- The sentinel lymph node is removed.
- Removal of the lymphatic system under the armpit

Breast amputation is a severe disease-related procedure performed on patients who either cannot qualify for or refuse breast-sparing therapy. It entails removing the whole breast along with its skin. There are various types of breast amputations such as Radical mastectomy, Modified radical mastectomy, Simple amputation & Subcutaneous amputation. The study by [23] showed that patients who are determined not to have a mastectomy and are aware that a positive margin may necessitate a second operation in some circumstances may still have a lumpectomy. The lumpectomy went well, and the final pathology showed negative margins.

1.3.2 Radiation Therapy

To Following surgery, radiation therapy could be suggested to target any cancer cells that remained and lower the chance of recurrence. The kind and stage of the cancer determine whether radiation therapy is indicated [35].

1.3.3 Hormone Treatment

The Hormone receptor-positive breast cancers, or those that react to hormones like progesterone or estrogen, are prevalent in older women. To block these hormones and stop the progression of cancer, doctors may give hormone treatment, such as tamoxifen or aromatase inhibitors [55].

1.3.4 Chemotherapy

The Chemotherapy could be suggested in some circumstances, particularly if the cancer has progressed or is more aggressive. In contrast to younger patients, elderly women could be less willing to undergo chemotherapy, given the possibility of side effects and general health concerns. Some medications can impair the immune response even if they target all developing cells, including immune cells. Chemotherapy, on the other hand, stimulates immune responses against tumors by positively affecting immune cells within the tumor microenvironment. In patients with breast cancer, combining Granulocyte-MQ Colony Stimulating Factor (GM-CSF) with traditional chemotherapy has demonstrated positive therapeutic results. High chemotherapeutic dosages, however, may stifle immune responses. Patients with breast cancer may see a 35% improvement in survival while receiving trastuzumab and chemotherapy together [24].

1.3.5 Targeted Therapies

The use of monoclonal antibodies, antibody-drug conjugates, cell cycle control, targeted treatments that concentrate on signalling pathways and chemicals, and

immunotherapy are some of the cutting-edge therapeutic options for patients with breast cancer [25].

1.4 CHEK 2(Checkpoint Kinase 2)

After BRCA1 and BRCA2, CHEK2 is a significant gene associated with the risk of breast cancer. This gene is essential for regulating several biological processes including the cell cycle, DNA repair, and apoptosis. A higher risk of breast cancer may result from CHEK2 malfunctioning as a result of certain gene alterations. The tumor suppressor gene CHEK2, which encodes the CHEK2 kinase, is essential for DNA repair, cell cycle control, and apoptosis in response to DNA damage [26]. CHEK2 mutations have been linked to a number of malignancies, including breast cancer. The genetic variant CHEK2 c.1100delC was identified in 2002, and it causes women who have the mutation to have a 2.3-fold increased risk of developing breast cancer in comparison to those who do not. Compared to women without the mutation (about 60 years old), individuals with this genetic alteration frequently get breast cancer at an earlier age (about 50 years old). Luminal/ER+ breast tumors are the particular kind that often affect individuals with this genetic mutation. In addition, individuals carrying the CHEK2 c.1100delC mutation may have a lower likelihood of surviving and a higher risk of developing breast cancer in the other breast. It is interesting to note that resistance to certain cancer therapies does not appear to have a role here. It's critical to comprehend how the CHEK2 c.1100delC mutation causes cancer in order to provide better care for individuals who carry this mutation. Knowing this may help develop more individualized strategies for treating and preventing breast cancer in those who have this particular mutation [27].

The Checkpoint kinase 2 (CHEK2) genetic mutations according to [2] are known to be a risk factor for the emergence of malignancies in a number of different organs, including the colon, prostate, thyroid, kidney, ovarian, and breast. Furthermore, CHEK2 protein expression has been reported to be downregulated in various cancer types. This downregulation suggests that alterations in the linked

proteins expression may be related to the changed CHEK2 gene. The complicated interplay between CHEK2 mutations and changes in protein expression highlights the intricacy of cancer susceptibility.

Three primary forms of DNA damage are caused by UV irradiation exposure: pyrimidine dimers derived from cyclo-butane, photoproducts of pyrimidine 6–4 pyrimidone, and their Dewar isomers. These lesions create difficulties for DNA transcription and replication. Which can obstruct replication forks and result in double-strand breaks in DNA. To treat different forms of DNA damage, cells utilize a repertoire of more than 150 DNA repair enzymes. One such enzyme is the nuclear CHEK2 protein encoded by the CHEK2 gene. The identification of DNA double-strand breaks by CHEK2 is essential because it triggers the activation of BRCA1 and p53, two downstream targets that aid in cell-cycle arrest, DNA repair, and perhaps apoptosis. CHEK2 is increased in response to UV radiation, peaking 4–8 hours after exposure, underscoring its importance in UV damage management. Although the non-functioning frameshift CHEK2*1100delC mutation is known to increase the risk of breast cancer in individuals [28]. Located on chromosome 22q12.1, cell cycle checkpoint kinase 2 (CHEK2) is a critical multifunctional kinase that is essential for controlling apoptosis, DNA repair, and the cell cycle. Reacting to DNA damage requires its function in linking the ataxia telangiectasia mutated (ATM) kinase with downstream checkpoint effectors. A CHEK2 deficiency may result in compromised DNA repair and retained mutations. Which would ultimately promote carcinogenesis findings by [29]. Further according to them the Heterozygous CHEK2 germline mutations have been found in a variety of cancers, including Li-Fraumeni syndrome (LFS), breast, colon, thyroid, bladder, ovarian, gastric, renal, and prostate cancers, despite its possible tumor-suppressive functions. This suggests CHEK2 as a low-penetrance, multiorgan cancer susceptibility gene. Patients with pheochromocytoma and paraganglioma (PPGL) have heterozygous mutations in CHEK2, according to recent whole exome sequencing (WES) technologies. To establish CHEK2 as a novel candidate susceptibility gene in PPGLs and to explore its potential utility in genetic risk assessment, prognosis, and monitoring, more validation is required.

The tumor suppressor protein kinase (CHEK2) required for DNA damage repair is encoded by the CHEK2 gene. CHEK2 pathogenic variants were first found in families with Li-Fraumeni syndrome (LFS) criteria but without TP53 mutations. Breast Cancer is linked to these variations. The association between LFS and other malignancies is still up for debate, although the relationship to LFS is not. The well-researched c.1100del variation is a loss-of-function polymorphism associated with a 37% cumulative risk of BC that is common in European populations. After revisions, the importance remains unclear despite specific studies correlations with malignancies of the stomach, kidney, breast, and sarcoma. Variants in CHEK2 have also been connected to malignancies of the thyroid, kidney, prostate, and colon, with varying consequences depending on which protein domain is impacted. When compared to loss-of-function variations, missense variants such as p.I157T, p.S428F, and p.T476M had different BC risks, suggesting different implications for cancer prognosis [30].

The 543 amino acid building blocks that make up the CHK2 protein are constantly present in cells. It is composed of three segments, The SQ/TQ cluster domain at the beginning, the fork head-associated (FHA) domain in the center, and the segment that performs the function of a serine/threonine kinase. CHEK2 has an end signal that directs it to go to the nucleus, the cells center (much like a C-terminal nuclear localization signal). When DNA is damaged, CHEK2 becomes activated and begins to function. This process is initiated by the ataxia telangiectasia mutated (ATM), which modifies certain regions of CHEK2, particularly p.T68 at the beginning region. An increasing number of people are being tested for the breast cancer-associated CHEK2 gene. Numerous uncommon genetic alterations are discovered in the gene. As of right now, the CLIN Var database has 1,332 reported uncertain changes (VUS) in CHEK2, the majority of which are coding changes (1,139 are missense alterations). The exact nature of these modifications and their relationship to cancer risk are unknown. Knowing the family's history of cancer is sometimes crucial in determining if these alterations are deleterious, particularly in a gene like CHEK2 that has a modest effect on cancer risk [31].

1.5 Aim of Study

The aim of this study is to assess the CHEK2 gene expression level in PM Breast Cancer patients and to discover its association with Treatment.

1.6 Objectives

1. To evaluate CHEK2 gene expression in blood samples from PM women newly diagnosed with breast cancer before any medical intervention.

2. To Investigate the impact of Treatment on CHEK2 expression in blood samples collected from the same individuals.

3. To study the changes in CHEK2 gene expression between pre- and post-treatment blood samples and identify significant differences in response to therapeutic interventions.

1.7 Problem Statement

There is no information regarding alteration in the expression level of CHEK 2 gene before and after treatment in PM women with Breast Cancer. The goal of the current study is to close this knowledge gap and examine the molecular changes carried out by the treatment.

1.8 Alternative Hypothesis (H1)

There is a significant difference in CHEK 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 CHEK 2 gene expression.

1.9 Null Hypothesis (H0)

There is no significant difference in CHEK 2 gene expression in the blood of older women with breast cancer before and after surgical intervention. Any observed variations are due to random chance, and there is no association between the surgical procedure and CHEK 2 gene expression changes.

1.10 Dependent Variable and Independent Variable

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

1.11 Inclusion Criteria and Exclusion Criteria

The Inclusion criteria have Female breast cancer patients aged between 50 to 70 years with available pre- and post-tumor excision peripheral blood samples. And Exclusion criteria is male participants or women below 50 years of age. Patients with concurrent malignancies or incomplete medical records.

Chapter 2

Literature Review

2.1 History of Breast Cancer

The Term "Cancer" was first used in about 400 BC by the renowned Greek physician Hippocrates, who is sometimes referred to as the "Father of medicine." The term came from the Greek word "karkinos" which means "crayfish" or "crab" and it represented the way the disease crept in like a creeping crab. This nomenclature captures the aggressive nature of cancer in medical language by reflecting its ability to contact and infiltrate adjacent tissues [32]. Ancient Egyptian papyrus had the first known accounts of breast cancer and its treatment. The papyrus described many instances of breast cancer cases and how the cancer was treated using a "fire drill" a device that burns the skin in order to destroy the malignant tissue and that there is no treatment for the illness. The ancient Egyptian papyrus of Edwin Smith, in 1600 BC and may be a copy of a record much older from 2500 to 3000 BC mention cancer as one of the oldest illnesses documented in medical history. Which is known as the first medical paper, it sets itself apart from other works that relied more on mystical and magical cures by taking a rational and scientific approach to medicinal operations.

The papyrus describes 48 common clinical situations that use fundamental surgical methods to treat cancer, fractures, and injuries in different body parts. Interestingly, the section on breast disorders includes management strategies for

both breast cancer and injuries [33]. Hippocrates, a well-known figure in ancient medicine, proposed that cancer could be linked to an imbalance of body humors, such as blood, phlegm, yellow bile, and black bile. In the past, there was a notion that cancer and menopause were related, which made sense given that the incidence of cancer increases with age. During that time, a great deal of surgery was performed, including the excision of the breast along with the surrounding lymph nodes and underlying muscles. For many years, this method was the most successful therapy for breast cancer, despite the fact that it was deforming. Researchers like Egan's advancements in breast cancer research helped pave the way for mammography's widespread use as a screening method in the 1960s. This diagnostic method was essential to the early detection process. With the passage of time, less intrusive surgical techniques for breast cancer emerged, driven by pioneers like Hans Holmström of the University of Gothenburg. These advancements were important steps forward in the search for breast cancer therapies that are less invasive and less deforming [34].

The (Francois de la Boe Sylvius) first proposed the theory that acidification of bodily fluids may cause cancer in 1680. Claude-Deshais Gendron elaborated on this idea by pointing out that malignancies may result from the mingling of lymph with nerve and glandular tissues. Bernardino Ramazzini postulated in 1713 that nuns' chastity may increase their risk of breast cancer. However, Prussian physician Frederich Hoffman believed that women who are sexually active and acquire breast cancer may be having "too vigorous" intercourse, which prevents their lymph from emptying. Based on several autopsies, the Italian doctor Giovanni Morgagni thought that curdled milk in the breast may result in malignancies. Breast cancer was supposed to be caused by pus-filled inflammations, according to Johannes de Gorter. The French physician Claude-Nicolas Le Cat proposed a link between breast cancer and depressed mental illnesses. Thus, they are a few diverse theories from different persons regarding the possible causes of breast cancer in the past [35]. The discovery of DNA delayed the understanding of the significance of genetics in breast cancer until the middle of the 20th century.

As they moved away from earlier theories regarding physical fluids, viral transmission, or sexual psychology, they began to link breast cancer to DNA. This change created the opportunity to treat breast cancer by focusing on certain cells or excising impacted tissue. which led to the development of the mastectomy as we know it today. Breast cancer operations were first performed by a number of surgeons in the 1750s including Benjamin Bell, Claude-Nicolas Le Cat, Henri Le Dran, and Jean Louis Petit. These operations cleared the way for the first therapeutic approaches by removing breast tissue, underlying muscles, and lymph nodes. Early breast cancer procedures were pioneered by surgeons such as Jean Louis Petit and Henri Le Dran, who laid the groundwork for William Halsted's radical mastectomy in the mid-1800s. Benefiting from developments in biology and medicine, such as blood transfusions, anesthesia, and antiseptics, Halsted agreed that damaged breast regions should be removed but also sought to stop the spread of cancer cells. An important development was Halsted's radical mastectomy. which removed the breasts, lymph nodes, and surrounding muscles all at once. Up until the 1950s, when less invasive, breast-conserving medicines began to appear, this strategy was the accepted course of therapy for breast cancer [36].

A few decades ago, there was no technology to examine DNA, and people thought viruses caused breast cancer. According to [37] now a professor of genome sciences at the University of Washington, Dr. Mary-Claire King spent fifteen years developing a mathematical model that demonstrated the disease connection to genetic abnormalities. She and her colleagues discovered the BRCA1 gene, sometimes referred to as the breast cancer gene, in the 1990s. Simultaneously, researchers found that BRCA1 and BRCA2 gene mutations were significant risk factors for ovarian and breast cancers that run in families. Understanding why certain people are more prone to have these malignancies was made possible in large part by these results. It altered the way that certain malignancies are detected, avoided, and treated in those who have a greater hereditary risk. The BRCA1 gene was discovered in 1990 under the direction of Dr. Mary-Claire King, while the BRCA2 gene was discovered in 1994 by scientists including Richard Wooster and Michael R. Stratton. Because of these discoveries, we now know a lot more about the

genetic components of breast cancer, particularly in situations where the illness runs in the family.

Moreover, a noteworthy study conducted in 2002 by scientists from the University of Cambridge's Cancer Epidemiology Unit and the International Agency for Research on Cancer (IARC) was released. The results of this study demonstrated a correlation between a higher risk of breast cancer and certain mutations in the CHEK2 gene. The vulnerability to breast and ovarian cancer was initially associated with the tumor suppressor genes BRCA1 and BRCA2.Which were discovered by [38] (BRCA1) and [39] (BRCA2).

2.2 Breast Cancer Molecular Aspects

There is a substantial correlation between breast cancer and high-penetrance genes, such as BRCA1 and BRCA2. These genes mutations greatly raise the risk. Compared to high-penetrance genes, moderate-penetrance genes including CHEK2, ATM, and PALB2 also increase the risk of breast cancer, but less dramatically. Breast cancer is influenced by both heredity and lifestyle choices. A womans risk is almost double that of an individual without a family history of breast cancer if she has a close relative such as a parent or sibling who has the disease. According to previous research, individuals with specific genetic mutations had varying odds of acquiring breast cancer by the age of 70 [40].

The studied by [41] The BRCA1 gene carriers have a risk that ranges from 40% to 87%. Conversely, the risk for people who carry the BRCA2 gene ranges from 27% to 84 percent.

The Important genes BRCA1 and BRCA2 have been connected to a hereditary, extremely significant type of ovarian and breast cancer. As a result of their production of proteins that inhibit the development of aberrant cells, they are categorized as tumor suppressor genes, or TSGs. Mutations in these genes, which are found on particular chromosomes (BRCA1 on chr17q and BRCA2 on chr13q), raise the risk of prostate, ovarian, and breast cancer. Both genes produce TSG proteins and

regulate cell development. While BRCA2 has 3418 amino acids, BRCA1 has 1863 amino acids and 300 known mutations that cause illness. These proteins, which are also referred to as anti-oncogenes, are essential for maintaining genetic material, mending broken DNA, and halting the growth of cancer. The cell's capacity to repair DNA is hampered if either gene is damaged, which can eventually result in an accumulation of mutations that might cause cancer. When a person who is heterozygous for a TSG gene (having one healthy and one troublesome allele) loses the healthy allele, it is known as loss of heterozygosity (LOH) according to the [42]. Additional they demonstrate Nadson's two-hit theory states that this loss affects TSG function, increasing the likelihood that cells may produce tumours and ultimately cancer. BRCA2 is essential for preserving DNA integrity and regulating cell proliferation. It was identified as the second gene that predisposes to breast cancer. The risk of prostate, ovarian, and breast cancers is greatly increased by mutations in BRCA1. About 35% of families with early-onset breast cancer had germ-line mutations for BRCA2, which increases the risk of breast cancer in males and ovarian cancer in women.

The growing body of research has revealed how BRCA genes function in several cellular mechanisms that control the growth of tumors. Situated on chromosome 17 (17q21), BRCA1 is a 1,863 amino acid protein with 24 exons. It has essential domains for each of its several roles. The zinc-binding finger domain, or RING (Really Interesting New Gene), found in the N-terminal region of BRCA1 is essential for the protein's interaction with BARD1 (BRCA1 Associated RING Domain protein 1) and the assembly of the E3 ubiquitin ligase complex. Two phosphor-peptide-binding BRCT (BRCA1 C-terminal) domains at the C terminus help BRCA1 connect with important partner proteins such as ABRAXAS, BRIP1/FACJ, and CTIP. The middle portion of BRCA1, which consists of exons 11–13, frequently exhibits alterations in breast cancer patients. This region has a coiled coil domain and two signals called nuclear localization signals, or NLS, that direct the protein to the cell nucleus. The coiled coil domain is essential for establishing a connection with BRCA2 via PALB2, its partner. Put more simply, alterations in these particular regions of BRCA1 are essential for their interaction

with BRCA2 through PALB2 and may potentially be involved in the development of breast cancer [42].

The [43] discoveries propose that BRCA1 gene has been shown to have over 1600 mutations, including insertions, deletions, and different single nucleotide modifications in both the coding and noncoding regions. A defective repair system for damaged DNA can impact several sections of the genome, including genes involved in the expression of cell cycle checkpoints such as p21 expression may be inhibited by mutations in the TP53 gene. Because p21 is not expressed, cells devoid of BRCA are able to evade apoptosis, or programmed cell death, and survive. Individuals who have mutations in either BRCA1 or BRCA2 frequently also have TP53 mutations. This shows that BRCA deficiency may cause mutations in several oncogenes, which in turn may aid in the development of cancer.

In some studies, we discovery that known as founder mutations, certain of these alterations are more common in particular populations. The most prevalent BRCA1 mutations are located in exons 11–13, which encode the NLS necessary for BRCA1 activities, and in areas that correlate to the BRCT and RING domains. Additionally, these areas contain binding sites for many proteins, including c-Myc, Rad50, pRb, Rad51, BRCA2, and PALB2, that interact with BRCA1. The BRCA1 gene has mutations in exons 11–13 study given by [45] more they are associated to ovarian and breast cancers.

The connection between the BRCA1/2 protein domain activities and the formation of tumours has been thoroughly studied in animal models, as previously described. However, the findings of these investigations are still need confirmation by human clinical data. Protein malfunction or the lack of a protein product is the outcome of about 70–80% of BRCA gene mutations. These mutations have been shown to be clinically significant and linked to a higher chance of developing inherited cancers [46]. The BRCA1/2 mutations have also been linked in several studies to poor clinical outcomes and tumour aggressiveness in cancer patients. Numerous studies have shown that patients with prostate cancer who had BRCA1/2 mutations also have higher rates of intermediate- and high-risk illness. A recent study conducted in China on 603 sporadic individuals with pancreatic cancer found a

correlation between decreased overall patient survival rates and the germline missense variation rs1799966 (c.4837A¿G[p.Ser1613 Gly]) inside the BRCA1 gene's BRCT domain.

However, there is inconsistent evidence from clinical trials on the relationship between BRCA1 and BRCA2 mutations and patient outcomes in cases of breast cancer. In a recent prospective multihospital research, 388 patients with BRCA1/2 mutations were among 2733 young women with breast cancer diagnoses there was no difference in overall survival between those with and without BRCA mutations. Furthermore, a review of the 558 individuals with triple-negative breast cancer showed that those people who carried the BRCA1/2 mutation outlived those who did not [47].

Further study found that, regardless of the presence of germline BRCA1/2 mutations, the survival of patients with familial ovarian cancer is lower than that of patients with sporadic cases, analyzing 151 patients with ovarian cancer and 119 patients with sporadic cases. Together with three other receptors, the HER2 belongs to the family of epidermal growth factor (EGF) receptors, which also includes the HER1 (erbB1), HER2 (erbB2), HER3 (erbB3), and HER4 (erbB4). The long arm of chromosome 17 contains the HER2 gene, which produces a transmembrane protein with a mass of 185 kDa [48]. Unlike the other EGF family receptors, the extracellular domain of the HER2 receptor lacks a recognizable ligand. It may dimerize with other EGF receptors ligand-independently and is present in an active configuration. It is believed that the HER2/HER3 dimer combination is the most active and tumor-promoting combination. Trastuzumab's mode of action is thought to include both innate and adaptive immunity. Cell cycle arrest is caused by innate processes, which also cause p27 levels to rise and cyclin D1 and cyclin-dependent kinase 2 activity to decrease. Trastuzumab appears to work in concert with most chemotherapeutics in preclinical models, but it does not appear to significantly increase apoptosis on its own.

The onset and spread of breast cancer have been strongly linked to hormonal imbalances, including those involving the hormones progesterone and estrogen. The hormone estrogen, which is mostly generated by the ovaries, is essential for the

growth and development of breast tissue. According to [49] it stimulates cell division and controls gene expression during the course of the cell cycle and apoptosis. An critical hormone that is mostly generated in the ovaries, progesterone is vital for controlling an individual's menstrual cycle and breast growth. It's also critical to remember that, although being less frequent in men, breast cancer may nonetheless strike men. It functions in conjunction with estrogen to encourage the proliferation and differentiation of mammary gland cells as well as prepare breast tissue for a possible pregnancy [50].

The study by [51] discover Estrogen receptors (ERs) and progesterone receptors (PRs) in breast cells are activated, which is one way that hormonal imbalance leads to breast cancer. A complicated chain of intracellular signaling events is started when progesterone or estrogen attach to the ER or PR, respectively. These signaling events can help control cell growth and prevent cell death. These pathways can become dysregulated due to excess estrogen or compromised progesterone signaling, which can lead to uncontrollably growing cells and the development of tumor.

Moreover, abnormalities in hormone levels might potentially affect the microenvironment of breast tissue. For instance, estrogen encourages blood vessel development and growth factor synthesis, both of which aid in tumor angiogenesis and metastasis. Additionally, it may have an effect on the immunological response within the breast, which may modify tumor immune surveillance and encourage immune system evasion of tumors [52]. Hormone receptors, including PRs and ERs, are essential for the development of breast cancer. Treatment choices are influenced by the presence or lack of these receptors in breast cancer cells, which aids in the subtyping of tumors. The tumors classified as ER-positive (ER+) breast cancer express ERs on their cell surface. Estrogen signaling is essential to the growth and spread of these tumors. The most prevalent kind of breast cancer, known as ER+ breast cancer, frequently reacts favorably to hormonal treatments that focus on estrogen signaling, such as aromatase inhibitors (AIs) or selective ER modulators (SERMs) [53].

The determine presence of PRs on the tumor cells is indicated by breast cancer that is PR-positive (PR+). Additional information regarding tumor behavior and treatment response may be obtained from PR expression. Comprehending the function of hormone receptors in breast cancer is crucial for customized therapeutic strategies, as selectively targeting these receptors with certain medications can successfully impede tumor expansion and enhance patient results. Hormone receptors have a role in the prognostic aspects of breast cancer subtype classification. The hormone receptor status can affect the tumor aggressiveness, prognosis overall, and how well it responds to therapy [54]. Because it lacks PRs and estrogen, hormone receptor-negative breast cancer is typically more aggressive and less amenable to hormonal treatments.

2.3 CHEK 2 Gene Description

The gene known as checkpoint kinase 2, or CHEK2, is essential to defense systems within cells, particularly those that protect our genetic material. This gene, which is located on chromosome 22q11.1, codes for a sentinel protein that monitors the cell cycle and reacts quickly to damage to DNA. In its role as a gatekeeper of genomic integrity. CHEK2 makes sure that damaged DNA is repaired prior to cell division, preventing the spread of dangerous mutations. It is crucial to comprehend the complexities of the CHEK2 gene since changes to its structure or function may have significant effects. Interestingly, mutations in CHEK2 have been linked to an increased risk of several malignancies, most notably prostate and breast cancer.

2.4 CHEK 2 Gene Significance and Prevalence

The primary mediator in a cell reaction to damage from double-stranded DNA is CHEK2. This nuclear phosphoprotein contributes to DNA stability and integrity as well as the signaling cascade involved in DNA repair. In response to cellular injury, the CHEK2 gene becomes activated, halting the cell cycle in the G1 phase

and preventing it from continuing to mitosis by means of its numerous kinase function. The DNA repair, apoptosis, and cell cycle regulation are all significantly influenced by CHEK2, ATM and ATR kinases activate CHEK2 in response to double-stranded DNA damage. which cause CHEK2 to become phosphorylated in its N-terminal and then activate CHEK2 kinase activity. The CHEK2-activated monomers also activate a multitude of their upstream substrates, such as the tumor suppressor TP53.The proteins in the cell division cycle 25 (CDC25) family, serine 988 of BRCA1, and cell cycle checkpoints that fix damage to DNA. These roles for CHEK2 in the cell cycle suggest that BC may emerge as a result of CHEK2 malfunction [55]. Other tumors have also been shown to contain CHEK2 mutations. which, like BC, cause defective apoptosis and unchecked cancer cell growth.

We found that the TCGA database had a recurrent mutation for CHK2 K373E. In this work, we show that the K373E mutation impairs both kinase activity and CHK2 autophosphorylation, which compromises CHEK2 ability to inhibit cell growth and enhance cell survival following ionizing radiation. We suggest that K373E inhibits CHEK2 ability to induce p21WAF1/CIP1 p53-independently. According to our findings, cancer is associated with the CHK2 K373E mutation [56].

The study also found that individuals with stage Ta illness and low-grade invasive bladder cancer had higher rates of CHEK2 missense mutations, which are changes that alter a single amino acid in the protein. However, individuals with high-grade invasive bladder cancer and those with stage T1 (Bladder Cancer) illness were less likely to have the I157T CHEK2 pathogenic mutation. The odds ratio (OR) for any mutation in CHEK2 among patients with bladder cancer at stage Ta (a stage of bladder cancer) was 2.0, indicating a correlation. On the other hand, the OR was 0.3 for patients with stage T1 illness, suggesting a decreased probability of a CHEK2 mutation. The study also discovered a link between kidney cancer patients with GII clear cell carcinoma and truncating CHEK2 mutations, which result in a shortened or nonfunctional protein [57].

A transgenic model of HCC with enhanced DNA damage indicated that CHK2 and DNA damage boosted SDH activity, which was characterized by increased succinate oxidation across the TCA cycle. Reactive oxygen species generation is crucial for maintaining the expression of SDH, which is CHK2-controlled, according to mitochondrial studies. Because of faulty mitochondria caused by DNA damage and increased CHK2, cells under these conditions heavily relied on glycolysis to produce ATP. However, CHK2 knockdown eliminated this need. This indicates a potential weakness in the DNA damage response that may be used to the creation of novel treatments. By establishing a connection between cellular metabolism and CHK2, a major effector of the DNA damage response, this work offers possible treatment approaches for hepatocellular cancer [58].

Among the tumors with the highest rate of mutation is bladder cancer. According to a recent study, the homologous recombination-related DNA damage repair genes ATM, BRCA1/2, and CHEK2 have the third-highest number of mutations in bladder cancer [59].

The study of showed the 1100delC variant of cell cycle checkpoint kinase 2 (CHEK2) has been demonstrated to increase the risk of colorectal cancer in Dutch families with heredity non-polyposis-colorectal cancer (HNPCC) and HNPCC-related families. A positive connection was found between the I157T missense CHEK2 mutant and cancer associated to HNPCC. A significant correlation between the CHEK2 I157T mutation and cancer associated to HNPCC was found only in individuals who tested negative for the virus, not in those who tested positive. For those without MMR who had family colorectal cancer, the association with I157T was very strong. Furthermore, among Polish families [60] with MMR-negative members who are also connected to HNPCC/HNPCC, the I157T variant of CHEK2 raises the risk of colorectal cancer. In gastric cancer, a poor prognosis has been associated with loss of CHEK2 expression of proteins in IHC.

Abnormal expression of ATM, Chk2, or p53 were shown to be highly associated with poor disease-specific survival and an advanced TNM stage. In the validation set, this connection was verified. A prolonged disease-specific survival was

significantly correlated with both p53 negative and Chk2 positive status. Additionally, patients who exhibited normal expression levels of all three DDR-related proteins fared better than those who did not. Chk2 deletion and at least one abnormal DDR-related protein were found to be independent predictors of poor disease-specific survival, according to multivariate analyses.

The DDR-related proteins, and their aberrant expressions play critical roles in the development and progression of gastric cancer [61].

The Study Showed CHEK2 as a predictive factor for progression to muscle invasive disease. This could help to identify high-risk patients at stage pT1 (NMIBC) who would benefit from early cystectomy. The study explores the impact of down-regulation of checkpoint protein kinase 2 (CHEK2), involved in DNA repair, in urothelial carcinoma of the bladder (UCB).The CHEK2 expression (¡10%) was linked to significantly poorer progression-free survival (PFS). The multivariable analysis identified CHEK2 loss and multifocal tumors as independent predictors for progression to muscle-invasive disease. CHEK2 expression may serve as a prognostic marker for identifying high-risk UCB patients who could benefit from early intervention like cystectomy [62]. Among 205 men with testicular germ cell tumors (TGCTs), about 10% (20 individuals) had inherited genetic variants in a gene called CHEK2, known for its role in DNA repair studied by [63] More these variants were identified more frequently in men with TGCTs compared to those without cancer. Specifically, men with TGCTs were about 4 times more likely to have these CHEK2 variants compared to cancer-free individuals. This pattern was observed in both the initial group of 205 men and a separate group of 448 men from Croatia. The study also found a specific CHEK2 variant, p. Ile157Thr, to be associated with an increased risk of TGCTs in the Croatian population. Additionally, men carrying these pathogenic CHEK2 variants tended to develop TGCTs approximately 6 years earlier than those without these genetic changes. These findings suggest a potential genetic link between CHEK2 variants and an elevated risk of testicular germ cell tumors, highlighting the importance of understanding genetic factors in cancer susceptibility and early detection.

The CHEK2 is a gene that plays an important role in how cells respond to damage in their DNA. It also helps regulate cell division (mitosis) and maintains the stability of chromosomes. Researchers studied 107 people with myelodysplastic syndrome (MDS) and 117 with acute myeloid leukemia (AML). They looked for specific genetic mutations in the CHEK2 gene using a method called PCR and sequencing. Results showed that people with certain CHEK2 mutations had a higher risk of MDS, but there was no significant association with the risk of de novo AML. For those with CHEK2 mutations and MDS, there was a higher frequency of abnormal chromosome structures (karyotypes). Additionally, specific subtypes of MDS with a poorer prognosis were more common in patients with CHEK2 mutations and abnormal chromosomes. However, these mutations are not connected to chromosomal instability in AML [64].

The CHEK2 activation Consequently, following DNA damage causes interaction with TP53, BRCA1, and BRCA2.Depending on the particular mutation, germline loss-of-function (LoF) CHEK2 mutations have been linked to a moderate risk of breast cancer, albeit the precise risk varies. More precisely, the more well-studied CHEK2 mutation, c.1100delC, is thought to carry a 25–30% lifetime risk of breast cancer and is the founder mutation for Eastern Europeans. Conversely, some additional CHEK2 variants, including p.Ile157Thr and p.Ser428Phe, appear to confer decreased chances of breast cancer, with estimates of around 18%.. the first detailed investigation of two novel CHEK2 LGRs, where the molecular characterization, followed by the prevalence in Greek breast cancer patients, were determined. In Greek breast cancer patients, rare mutations in the CHEK2 gene, specifically the p.Asp265_His282del and p.Glu107_Lys197del deletions, contribute to a small but notable proportion of breast cancer susceptibility. The p.Glu107_Lys197del deletion, affecting exons 2 and 3, is exceptionally rare, seen only once. On the other hand, the p.Asp265_His282del deletion, detected in 0.22% of cases, is associated with breast cancer risk, leading to the production of a dysfunctional CHEK2 isoform. Haplotype analysis indicates it as a Greek founder mutation originating about 975 years ago in Western Greece. Despite its rarity, the p.Asp265_His282del deletion appears more common than other known mutations among Greek breast

cancer cases. The study underscores the importance of population-specific research, and while estimating cancer risk is challenging due to rarity, CHEK2 deletions may confer clinically significant risks, warranting further investigation with larger patient cohorts to determine precise risks and associated cancers. Notably, the well-known CHEK2 LGR, del5395, was not found in Greek breast cancer cases, aligning with its scarcity in geographically proximate countries [65].

The study by CHEK2 mutations were not considerably more prevalent in men with deadly relative to low-risk PCa, and a particular CHEK2 a mutation, c.1100delC, appeared to contribute to an increased chance of lethal PCa in European American males. The odds of having this mutation in advanced prostate cancer were 7.86 times [66] higher. Also, the mutation rate in advanced prostate cancer was higher than in a larger group of people from the Exome Aggregation Consortium.

2.5 Role of CHEK 2 in Progression of Cancers

We observed that, in comparison to tissues close to the tumor, the levels of CHK2 and p-CHK2 were markedly elevated in PTC cancer tissues. The likelihood of metastasis and tumor aggressiveness were linked to p-CHK2 overexpression in initial tumor tissues. Nevertheless, metastatic lymph nodes had lower levels both of CHK2 and p-CHK2. Our findings demonstrated that CHK2 had no impact on cell invasion, migration, or proliferation but did upregulate the expression of CSC markers. Interestingly, we discovered that CHK2 has a hitherto unidentified role in promoting anoikis in PTC. More specifically, CHK2 degradation is initiated by PTC cells separating from their extracellular matrix (ECM).Then, PTC cells were saved from anoikis by the induced downregulation of CHK2. However, there was no discernible impact on adherent PTC cells' apoptosis. Furthermore, by controlling PRAS40 activity, CHK2—a new regulator of anoikis—can cause cell death in a way that is not dependent on p53. In summary, increased expression amounts of CHK2 and p-CHK2 were linked to the advancement of PTC. According to our findings, CHK2 plays an unanticipated role in the control of PRAS40 stimulation,

which is linked to anoikis and may be related to the existence of circulating tumor cells and their tendency to metastasize [67].

The study was conducted to assess the prevalence of three germline variants (c.1100delC, R145W, and I157T) in the CHEK2 gene in Rwandan breast tumors. We examined 41 cases of breast cancer and 42 healthy breast controls utilizing direct DNA sequencing, [68] however we were unable to find any favorable results. In the population of Rwanda, CHEK2 mutations can be uncommon and have a negligible effect on the propensity to breast cancer in both familial and sporadic occurrences.

They found that in gastric cancer, the CHEK2 gene was not working well, and this was linked to specific mutations. When they artificially increased the activity of the mutated CHEK2 gene, it made stomach cancer cells grow faster and spread more. The mutated CHEK2 gene also influenced the behavior of certain proteins in the cells. These findings suggest a connection between CHEK2 gene mutations and stomach cancer, providing a basis for studying potential drugs targeting these mutations [69].

In the study, a specific genetic mutation in the CHEK2 gene, known as c.1100delC, was identified in 5.9% of the patients, a significantly higher rate compared to the control population. Additionally, four individuals showed a different CHEK2 variant called I157T by [70] but its frequency did not significantly differ from that of the general population. Importantly, no mutations were found in other genes such as RAD51C, RAD51D, PALB2, or FANCM. The data suggest that the CHEK2 c.1100delC mutation is associated with an increased risk for MBC in the Finnish population.

We show that activation of Chk2 and lagging chromosomes/DNA damage are caused by mitotic errors during HCC carcinogenesis. Chk2 mislocalisation and overexpression/phosphorylation inside the mitotic spindle produce lagging chromosomes. Chk2 knockdown reverses mitotic activity and lagging chromosomes. Moreover, increased Chk2 keeps mitotic activity going by communicating with Aurora B kinase to facilitate cytokinesis and chromosomal condensation. Chk2

mis localization to mitotic structures requires the fork head-associated domain. Furthermore, aberrant mitoses are a result of phosphorylation of the retinoblastoma protein. A fraction of HCC patients has a substantial cytoplasm to nuclear Chk2 translocation, as demonstrated by a validation cohort and an independent cohort. In conclusion The work offers a fresh mechanistic understanding of Chk2's co-involvement in the development of HCC. These results suggest Chk2 as a potential biomarker to identify CIN in HCC, offering significant assistance for patient clinical and therapeutic care [71].

The Cells are stopped in the G2 phase of the cell cycle when the DNA damage checkpoint is triggered in due to genotoxic harm caused during S phase. The primary the effectors in this checkpoint pathway identified by include ATM, ATR, Chk1 and Chk2 kinases [72]. The checkpoint kinases elicit well-characterized inhibitory phosphorylation of Cdk1 to stop mitosis from starting. It is believed that during DNA damage, the checkpoint also indirectly prevents chromosomal condensation through Cdk1 inhibition, as Cdk1 is necessary for its recruitment of condensin. Here, we find that even in the presence of an active Cdk1, the G2 damage checkpoint inhibits permanent recruitment by the chromosome-packaging-machinery elements condensin complex I and II onto the chromatin. The Chk2 kinase is the particular mediator of the DNA damage-induced suppression of condensin subunit recruitment.

Nonetheless, there is a link between CHEK2 mutations and a higher risk of cancer, particularly breast cancer. There is ambiguity in risk assessment among carriers of additional CHEK2 mutations since the publicly available risk estimates are restricted to those caused by CHEK2 founder mutations. This study compared the phenotypes of founder & non-founder CHEK2 + S in order to evaluate the molecular features and phenotypes of CHEK2 mutation carriers (CHEK2 + S) within the MGPT cohort. A commercial laboratory analyzed the clinical histories & molecular data of 45,879 individuals who had MGPT, which included CHEK2. The 2.4% (n = 1085) of the people tested positive for CHEK2 + S. A total of 1101 CHEK2 mutations were found in this cohort, of which 16 individuals carried biallelic mutations. Using Fisher's exact test and multivariate logistic regression

analysis, personal/family cancer histories were examined between founder and non-founders (n = 259) CHEK2 + s. Cases without any clinical information were also removed from phenotypic analysis, as were individuals with the p.I157T intermediate risk founder mutation (n = 231), other mutations within non-CHEK2 genes (n = 83), or biallelic mutations (n = 16). There were no discernible phenotypic changes between CHEK2 + founder and non-founder s. These findings imply that the cancer risks associated with founder mutations could apply to all CHEK2 + individuals. particularly for breast cancer [73].

The study of verifies overall multiplicative nature of the risk effects caused by common susceptibility variants and CHEK2*1100delC. Additionally, the carriers with a high lifetime risk for clinical activities might be identified by the PRS. We discovered marginally significant associations between five variations (rs11249433, rs11780156, rs204247, rs2981582, and rs704010) and CHEK2*1100delC for total breast cancer when all 77 common variants were taken into account separately [74]. Three antagonistic relationships (the predicted impact in 1100delC carrier being in an opposite manner to that in non-carriers) and two synergistic interactions (greater than multiplicative) were represented by these. For ER-positive breast cancer, nine variations displayed a nominally significant interaction.

The Balochistan breast cancer patients had the two unique nonsense mutations, c.58C¿T (P.Q20X) & c.256G¿T (p.E85X), in exons 1 and 2 in two of their patients [75].

The study highlights the significant role that de-novo mutations play in this kind of uncommon disease and provides the first occurrence of Li-Fraumeni syndrome-like symptoms in Chinese individuals. The three tumor tissues' distinct somatic mutation patterns suggested that the mutations did not spread from one tumor to another, but rather originated separately. In CHEK2, the gene responsible for Li-Fraumeni syndrome, a new detrimental mutation in the germline (chr22:29091846, G $\rightarrow$ A, p.H371Y) was found. Its de novo origin was discovered by assessing the presence of this unique mutation in the patient's healthy siblings [76].

The study [77] found that luminal breast cancers, a specific type, had higher amounts of TILs (tumor-infiltrating lymphocytes) compared to basal-like subtype breast cancers. These luminal cancers with high TILs had limited copy number aberrations and no detectable regions of LOH (loss of heterozygosity). The research also revealed significant heterogeneity among CHEK21100delC Breast cancers, similar to BRCAX breast cancers, when examining copy number data. While both groups showed low frequencies of copy number aberrations, supervised analysis highlighted that the loss of chromosomal arm 1p was related with CHEK21100delC status. Unlike BRCA1-mutated breast cancers, there wasn't a specific somatic copy number aberration profile for CHEK2*1100delC breast cancers, except for potential involvement of 1p loss in a subset of tumors. This difference might be due to the fact that BRCA1-deficient tumor cells need specific survival factors acquired through copy number aberrations, which may not be necessary for breast tumors with a defect in a non-essential gene like CHEK2.

According to the study, germ-line CHEK2 mutations that alter the protein coding sequence are linked to a poor outcome for NHL patients, a somewhat elevated risk of NHL, and the potential to be a useful predictive biomarker for patients,with DLBCL (Diffuse Large B-Cell Lymphoma DLBCL) [78].

In The Cancer Genome Atlas dataset, CHEK2 gene expression was significantly higher in primary EOCs compared by [79] to normal fallopian tube tissues (P = 3.72×10-8). We also identified an association between genotypes of the candidate causal SNP rs12166475 (r2 = 0.99 with rs6005807) and CHEK2 expression (P = 2.70×10-8). These data suggest that common variants at 22q12.1 are linked with risk of serous EOC (Epithelial ovarian cancer) and CHEK2 as a plausible target susceptibility gene.

In vitro and ex vivo formation of infectious progeny, genome replication, and the cytopathic impact are all significantly suppressed by inhibition of Chk2 kinase activity. And have critical role in the establishment of HSV-1 corneal epithelial infection [80].

2.6 Relevance of CHEK 2 in Breast Cancer Susceptibility

In study Comparison to non-carriers, CHEK2 (H371Y) mutation recipients are more likely to react to neoadjuvant chemotherapy [81].

The study shows that Microarray-based PAM50 test uses 50 specific genes to assess the risk of distant recurrence (spread of cancer) in breast tumor samples from postmenopausal women within 10 years of diagnosis. If the PAM50 score is high, it indicates a relatively high likelihood of the cancer spreading to other parts of the body, posing a greater risk of metastasis [82].

We studied that after three chemotherapy sessions, there was a notable reduction in antioxidant enzymes and selenium (Se) levels (p¡0.001). This effect was consistent across age groups (≤48, ¿48) and disease stages (early, advanced). Moreover, the AC chemotherapy in breast cancer patients led to significant alterations in the body's oxidant/antioxidant system, specifically lowering Se levels and antioxidant enzyme activities. Interestingly, these changes did not appear to be linked to age or disease stage'd [83]. Based on better PFS results than paclitaxel alone, the FDA first authorized bevacizumab and paclitaxel combination therapy in 2008 for HER2-negative illness. In 2020, Claessens et al. Nevertheless, no appreciable OS improvement was shown in any of the clinical trials including the incorporation of bevacizumab to chemotherapy. The first HER2-targeted medication to be licensed in 2001 for use in conjunction with taxane treatment was trastuzumab for HER-2 positive patients. Since then, new HER2-targeted therapy, such as lapatinib and pertuzumab have all been registered for use in combination with chemotherapy.

The study shows that Patients with anemia from breast cancer who have undergone a lot of chemotherapy and who have more than one C allele in DRD2 & CLOCK SNPs are more likely to experience tiredness [84].

Among pre-menopausal Women with breast cancer who used traditional Chinese medicine, were overweight or obese, had received taxane and tamoxifen, and had received adjuvant chemotherapy experienced more serious menopausal symptoms.

Patients' quality of life related to breast cancer was shown to be lower in those who had more severe menopausal symptoms. Interventional trials aiming at reducing menopausal symptoms are necessary to see if these patients' overall quality of life may be enhanced [85].

2.7 Breast Cancer in Elder Women

According to the study, almost two thirds of women in the United States are obese or overweight, which increases their chance of developing postmenopausal breast cancer. In postmenopausal women, obesity is linked to a higher risk of invasive breast cancer. These clinically significant results need to spur obesity control initiatives [86].

A study conducted by experts from the Stanford School of Medicine found that around 1 in 40 postmenopausal women suffering from breast cancer before the age of 65 had cancers-associated mutations in the BRCA1 or BRCA2 genes [87]. Irrespective of the receptor status, the risk factors were linked to breast cancer in a similar way. However, there was a greater correlation between receptor-positive breast cancer and receptor-negative breast cancer in cases of high age at first birth, significant weight gain in adulthood, and use on menopausal estrogen-progestin therapy [88].

The research the risk of breast cancer in postmenopausal women has been linked to their levels of endogenous hormones. According to the study, the probability of ER+/PR+ breast tumors may be most closely correlated with the circulating levels [89] in sex steroid hormones in postmenopausal women.

Chapter 3

Material and Methods

3.1 Sampling

The current research work was conducted by collecting Blood Samples from, Department of Oncology, Fauji Foundation Hospital, Rawalpindi. Subsequently, carried out intricate experiments on these samples in the well-equipped BJ Micro Lab, Rawalpindi. In the present study, the methodology outlined by [90] was adopted for the investigation of CHEK2 gene in Blood Samples of Breast Cancer Patients

3.2 Blood Sample Collection

The blood samples were collected from Breast Cancer suspected women by using venipuncture procedure. A visible vein of good size was selected and the selected area of skin was made clean by applying 70% alcohol swab for 30 seconds. The sterile syringe was anchored into the vein and about 5ml blood was drawn into the syringe. Then blood sample was immediately transferred to vacutainer blood collection tube and labelled properly. After Drawing blood from patients taking the blood specimens to the laboratory for testing and research Experiment.

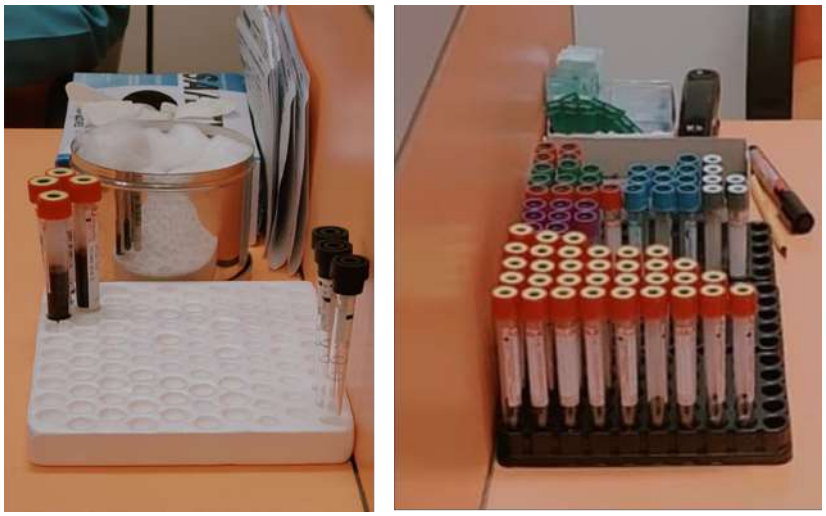

FIGURE 3.1: The above given picture show collected blood of Breast Cancer patients in vacutainer Tubes

3.3 RNA Extraction from Blood Samples

We isolated RNA from biological sample using the Trizol method, a widely employed technique for RNA extraction, ensuring high-quality and intact RNA for downstream molecular analyses.

3.3.1 Procedure

The extraction of RNA from fresh blood samples involves a series of precise steps to ensure its integrity and stability. Initially, 600 μL of Reagent A (Trizol) is added for every 300 μL of material, followed by thorough homogenization. Subsequently, 400 μL of Reagent B (chloroform) is added and incubated briefly before centrifugation at 12000 rpm for 10 minutes at 400^{o}C to separate the layers. The aqueous layer is carefully transferred to a new tube, where an equal volume of Reagent C (Isopropanol) is added and incubated. After centrifugation and careful removal of the supernatant, the pellet undergoes two washes with Reagent D (70% Ethanol) and air-drying before resuspension in Reagent E (RNase-free Water). Storage conditions are meticulously controlled: Reagent A is kept at 40^{o}C, Reagent B at room temperature, Reagent C at -20^{o}C, and Reagents D and E at

a constant temperature. These stringent measures ensure the extracted RNA's stability and integrity, crucial for subsequent analyses.

FIGURE 3.2: The Above showed effendorf tube having blood for RNA Extraction by Trizol Method

3.3.2 Gel Electrophoresis

To Purified PCR products were run on 1.5% Agarose gel. The 30mL of 1.5% gel is prepared by adding 0.45g of Agarose in 30mL 1x TBE buffer. Solution was boiled for 1 minute in microwave and cooled down a bit before adding 5 μL Ethidium Bromide. The 5 μL PCR purified Samples are then loaded on the gel after adding 2 μL loading dye in it.We do gel electrophoresis to confirm the extraction of nucleic Acid.

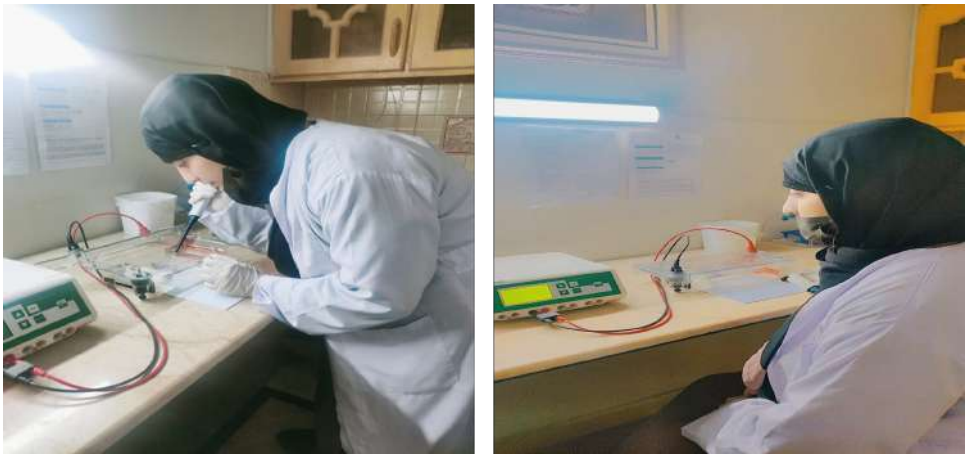

FIGURE 3.3: Upper given figure shows the process of gel electrophoresis

3.4 cDNA Synthesis or Reverse Transcription

The Total RNA was transcribed into complementary DNA (cDNA) using the Flexible cDNA Synthesis Kit with the fast and robust FIREScript ®Reverse Transcriptase Kit protocol.

3.4.1 Procedure

In the process of reverse transcription, several key components are combined with the RNA template to initiate the synthesis of complementary DNA (cDNA). Initially, the RNA template, along with Oligo (dT) Primer and Random Primer, is mixed, followed by the addition of Nuclease-free H2O to adjust the volume. This mixture is then incubated at 65^{o}C for 5 minutes and subsequently placed on ice. Following a brief spin, additional components are introduced, including 10x RT Reaction Buffer with DTT, dNTP MIX, FIREScript®Reverse Transcriptase, and RiboGrip®RNase Inhibitor. During the reverse transcription process, specific temperature conditions are crucial for optimal performance. The primer annealing step occurs at approximately 25^{o}C for 5-10 minutes, allowing the primers to bind to the RNA template. Subsequently, the reverse transcription reaction proceeds at 50^{o}C for 15-30 minutes, facilitated by the FIREScript®Reverse Transcriptase

enzyme, which synthesizes cDNA from the RNA template. Finally, enzyme inactivation is achieved by incubating the reaction mixture at 85^{o}C for 5 minutes, ensuring the termination of enzymatic activity and the preservation of the synthesized cDNA. These carefully controlled temperature conditions are essential for efficient and accurate reverse transcription, enabling the conversion of RNA into cDNA for downstream applications in molecular biology research and diagnostics.

FIGURE 3.4: The given figure show Formation of cDNA from RNA(Reverse Transcription)

3.5 Nanodrop

In our research work, we utilized a Nanodrop spectrophotometer to measure the concentration and purity of nucleic acid samples. The Nanodrop's advanced technology allowed for precise and rapid analysis of DNA and RNA samples. This

instrument played a crucial role in quality control, ensuring that our genetic material met the required standards for downstream experiments. Its user friendly interface and microvolume 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.

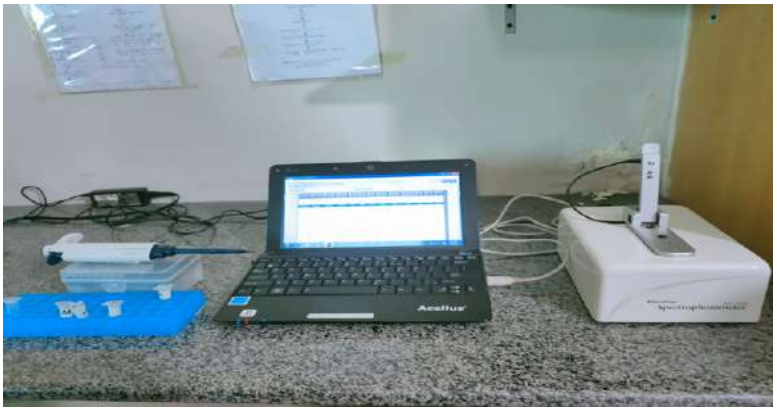

FIGURE 3.5: The figure show the Nanodrop process

3.5.1 Dilution of CDNA

CDNA was dilute in 1:100

3.6 Primer Optimization

Primer optimization is a critical step in the polymerase chain reaction (PCR) process, and it involves refining the design and conditions of the primers used to amplify specific DNA sequences. The goal of primer optimization is to achieve accurate, specific, and efficient amplification of the target DNA.

3.6.1 CHEK2 Primer Optimization

The forward primer sequence, 5' CAGTCTCATGGCAGCAGTG 3', and the reverse primer sequence, 5' GTTCTTGGTCCTCAGGTTCTTG 3', are utilized to

amplify a specific DNA fragment with an expected product size of 223 base pairs. Maintaining an annealing temperature of 56^{o}C throughout the polymerase chain reaction (PCR) process is crucial. This temperature facilitates the formation of stable primer-template complexes, ensuring efficient and specific amplification of the target DNA segment. By optimizing PCR conditions, we can selectively amplify the desired DNA fragment for further analysis and experimentation.

3.6.2 Primer

The reverse and forward primer are used for gene expression study. For primer designing the sequence is taken from NCBI and Design from Korea for Following Studies. The product is of 223 base pairs.

Table 3.1: Forward and reverse primer for studies

SN	**NAME**	**FORWARD**	**REVERSE**	**T(a)**	**PRODUCT SIZE**
1	Check 2	CAGTCTCATG GCAGCAGTG	GTTCTTGGTCCT CAGGTTCTTG	56	223bps

3.6.3 Reaction Mixture

Table 3.2: Primer reaction mixture

S.No	**Reaction Mixture**	**Volume**
01	Master Mix	10 μL
02	Forward Primer	2 μL
03	Reverse Primer	2μL
04	PCR Water	4 μL
05	Template	2 μL
	Total Volume	20 μL

3.7 PCR for GAPDH

The Optimal cycle conditions are essential for the 16S gene's effective amplification. To ensure that the DNA template is denaturated, the cycling process begins with a holding step that lasts for ten minutes at 95^{o}C. Denaturation then takes place for 30 seconds at 95^{o}C, which separates the DNA strands. The primers are then given a 30-second annealing period at 56^{o}C, which enables them to bind precisely to their target sequence. The extension step, which is essential for DNA synthesis, allows new DNA strands to elongate and is carried out for ninety seconds at 72^{o}C. In accordance with the desired amplification, this cycle is repeated a certain number of times. Finally, a final extension step at 72^{o}C for 10 minutes ensures completion of any remaining DNA synthesis. The process concludes with a hold at 4^{o}C until further analysis, maintaining the stability of the amplified DNA. These meticulously controlled cycling conditions are essential for robust and accurate amplification of the 16S gene.

3.7.1 Gel Electrophoresis

The Purified PCR products were run on 1.5% Agarose gel. The 30mL of 1.5% gel is prepared by adding 0.45g of Agarose in 30mL 1x TBE buffer. Solution was boiled for 1 minute in microwave and cooled down a bit before adding 5 μL Ethidium Bromide. Later 5μL PCR purified Samples are then loaded on the gel after adding 2μL loading dye in it.

3.8 Gradient PCR for CHEK 2

Primers are optimized for annealing temperature as mentioned above through gradient PCR method.

The reaction mixture used and conditions for PCR are as under.

TABLE 3.3: Reaction mixture for CHEK2 gradient PCR studies

S.No	**Reaction Mixture**	**Volume**
01	Master Mix	10 μL
02	Forward Primer	2 μL
03	Reverse Primer	2μL
04	PCR Water	4 μL
05	Template	2 μL
	Total Volume	20 μL

3.8.1 Cycling Conditions

The cycle starts with a holding step that keeps the DNA template first denaturated for ten minutes at 95^{o}C. Denaturation follows, which successfully separates the DNA strands for 30 seconds at 95^{o}C. After that, primers can bind precisely to the target DNA sequence during the 30-second annealing stage, which is carried out between 48^{o}C and 62^{o}C. The extension phase, which is essential to DNA synthesis, lasts 90 seconds at 72^{o}C and helps elongate new DNA strands. To get the appropriate amplification, this cycle is repeated a specified number of times. Finally, a 10-minute final extension phase at 72^{o}C guarantees that DNA synthesis is finished.

FIGURE 3.6: Above pic shows the Gradient PCR

3.8.2 Gel Electrophoresis

The Purified PCR products were run on 1.5% Agarose gel. A 30mL of 1.5% gel is prepared by adding 0.45g of Agarose in 30mL 1x TBE buffer. Solution was boiled for 1 minute in microwave and cooled down a bit before adding 5 μL Ethidium Bromide. Then 5μL PCR purified Samples are then loaded on the gel after adding 2μL loading dye in it.The Samples are then loaded on the gel after adding 2μL loading dye in it.The brightest is at 56 OC

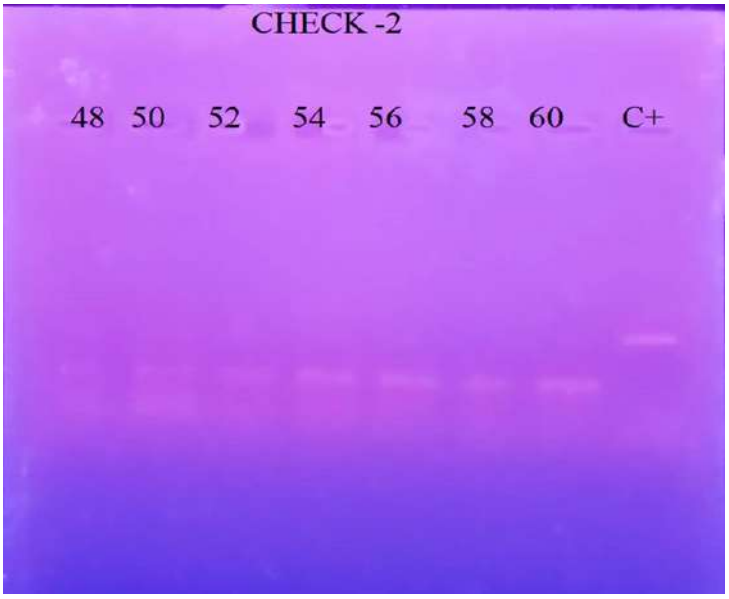

FIGURE 3.7: Above pic show the range for Temperature of PCR

3.9 Real Time PCR for GAPDH

The RT-PCR for GAPDH (Glyceraldehyde-3-Phosphate Dehydrogenase) involves precise manipulation of key components in a reaction setup. The mixture includes PCR water, forward and reverse primers, cDNA, and Eva green dye, each meticulously measured to ensure accurate amplification.

Cycling conditions are critical, starting with a holding stage at 95^{o}C for 5 minutes to initiate the reaction. Denaturation follows at 95^{o}C for 30 seconds, facilitating the separation of DNA strands. Annealing occurs at 56^{o}C for 30 seconds, allowing

primers to bind to the target sequence, and extension at 72^{o}C for 20 seconds promotes the synthesis of new DNA strands. Each step take time of 35 cycles.

This method offers real-time insights into GAPDH expression levels, essential for understanding gene expression dynamics.

3.10 Real Time PCR for CHEK 2

Real-time PCR (RT-PCR) for the CHEK2 gene involves precise manipulation of key components within the reaction setup. The mixture comprises PCR water, forward and reverse primers, cDNA, and Eva green dye, meticulously measured to ensure accurate amplification. Critical cycling conditions are employed throughout the process.

The reaction begins with a holding stage at 95^{o}C for 5 minutes, initiating the reaction. Subsequent denaturation at 95^{o}C for 30 seconds, carried out over 35 cycles, facilitates the separation of DNA strands. Annealing occurs at 56^{o}C for 30 seconds during each cycle, allowing the primers to bind to the target sequence. Extension takes place at 72^{o}C for 20 seconds during each of the 35 cycles.

These controlled conditions offer real-time insights into CHEK2 gene expression levels.

FIGURE 3.8: The above figure showed RT-PCR tubes

FIGURE 3.9: The figure Represents RT-PCR

Chapter 4

Results and Discussion

4.1 Sample Collection

In the current study 20 patients of Breast Cancer were communicated at Fauji Foundation Hospital, Rawalpindi during February 2023 to September 2023.And collected the 47 blood samples of different nature that is pre and post treatment. Our primary focus is on surgical interventions within the realm of treatment. Among 47 blood samples 20 were pre-treatment blood samples and 20 were post-treatment blood sample and 7 blood samples were taken from healthy women. Among these patients, all were Female. There were 11 Patients having < 50 years age and 9 patients having < 60 years age whereas the mean age of the patients is 58. 5 years. Further we have taken 7 healthy women blood as a control group.

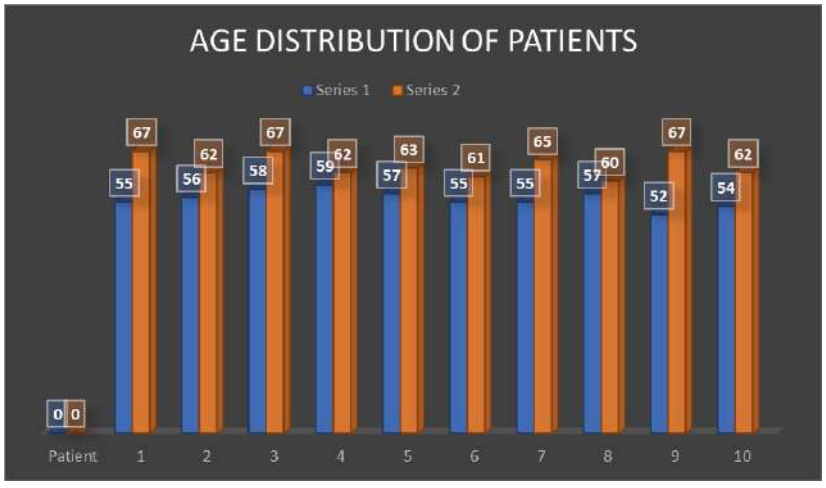

FIGURE 4.1: Age Distribution of Suspected Patient

4.2 RNA Extraction

The RNA extraction using the Trizol method resulted in a robust yield of total RNA from both pre and post-tumor excision samples. The average yield was X micrograms per microliter, and the A260/A280 ratio indicated high purity, ensuring the suitability of the extracted RNA for downstream applications. The Agarose gel electrophoresis demonstrated clear and intact RNA bands, confirming the high quality and integrity of the extracted RNA. Additionally, bioanalyzer analysis revealed consistent RNA profiles, ensuring the reliability of subsequent gene expression analyses.

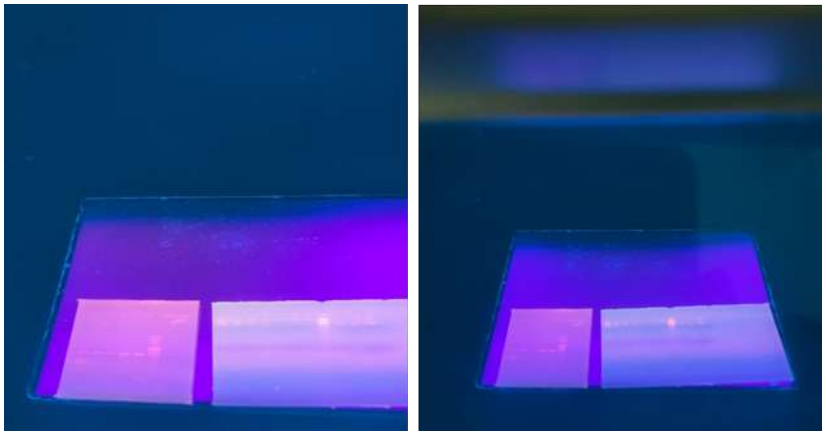

FIGURE 4.2: Show the extraction and presence of Nucleic Acid

4.3 cDNA Formation

The reverse transcription process yielded efficient cDNA synthesis from the extracted RNA, as evidenced by the presence of distinct and strong qPCR signals for the CHEK2 gene. The efficiency of cDNA formation was consistent across pre and post-tumor excision samples, ensuring the reliability of subsequent gene expression analyses.The synthesized cDNA exhibited high purity and integrity, as indicated by the absence of non-specific amplification and consistent amplification curves. This suggests that the cDNA pool accurately represents the original RNA, allowing for meaningful insights into CHEK2 gene expression dynamics.

4.4 Nanodrop

We need dilution for RT-PCR. The dilutions are made according to the amount of RNA or DNA in sample. In this case you have different amount of cDNA in different samples so if we go with the same amount we cannot justify the results. Then we see the lowest amount of cDNA in the samples like 7.3 in this case and divide all the values with this no. The divided value represent the dilution (more cDNA means more dilution). So the number is 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: The table given below 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
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

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

4.5 RT-PCR Cycle

The RT-PCR analysis was conducted to quantify the expression levels of the CHEK2 gene blood samples of PM breast cancer patients both before and after Treatment.

This molecular analysis aimed to elucidate the molecular transformations associated with breast cancer progression and the impact of clinical intervention on CHEK2 gene expression.

4.6 The Expression Level of CHEK 2 Gene in Blood Samples by RT-PCR

We studied the total of 47 blood samples of women. In which 7 women are healthy and consider as control group. The 20 blood samples are of women freshly diagnose from Breast Cancer and having Tumor. And 20 Blood samples are taken after treatment Excision and in post blood sample some blood samples are taken after 4 months of surgery and some are taken after 6 months of surgery of Breast Cancer patients.

We conducted study on expression level of CHEK2 Gene. The expression profiling of the CHEK2 gene was showed using RT-PCR. The obtained Ct values were subsequently utilized to calculate the expression levels of CHEK2.

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 CHEK2 expression but also facilitated the comparative analysis of pre and post-tumor excision samples, shedding light on dynamic changes in gene expression associated with the surgical intervention.

The Table 4.2 give below represent the 2^-$\triangle$Ct values of gene expression obtained from RT-PCR.

TABLE 4.2: Show the cycle threshold or Ct value of expression given by RT-PCR for all groups

Patient Number	**CHEK2 Control (2ˆ-△Ct values)**	**CHEK2 Pre-Treatment (pr) (2ˆ-△Ct values)**	**CHEK2 Post-Treatment (po) (2ˆ-△Ct values)**
1	0	1155431	45000
2	0	661000000	1080
3	0	152000000	1.06
4	0	152000000	0
5	0	34259482	1.086735
6	0	10471756	1500
7	0	84356740	1.086735
8		115000000	1.156689
9		1.5	1.086735
10		6183533	1900
11		1155431	3520
12		661000000	1900
13		152000000	3520
14		152000000	0
15		34259482	1.17
16		10471756	19000
17		115000000	1.16
18		84356740	1.09
19		1.5	1.09
20		6183533	1.9

4.7 Association Between Control & Pre-Treatment CHEK2 Gene Expression & Selected Clinicopathological Features

We examined the relationship between CHEK2 gene expression and important clinicopathological characteristics in our cohort of older patients in order to further clarify the clinical significance of this gene expression in the setting of breast cancer. This section focuses on the CHEK2 gene's pre-tumor expression levels

and how they relate to particular clinical traits. A thorough summary of these correlations is given in Table 4.3, which also includes specific information on the link between pre-tumor CHEK2 expression and variables including patient age, histological factor, stages (TNM), and grade. The goal of this analysis is to identify potential connections between the clinicopathological profile and molecular changes at the gene expression level in older patients with breast cancer. This will provide important information on the predictive value of CHEK2 in this population.

TABLE 4.3: The table show Control Expression Value & Clinical Features

CHEK 2 Expression in Control Group	2ˆ-△Ct	Age
1.	0	57
2.	0	64
3.	0	59
4.	0	67
5.	0	62
6.	0	58
7.	0	69

TABLE 4.4: The table show Pre-Treatment Excision Expression Value & Clinical Features

CHEK2 pre-treatment(pr) Excision	2ˆ-△Ct	Age	Histological Factor	Grade	Stage (TNM)
1	1155431	55	ILC	2	3B
2	661000000	56	IDC	2	2
3	152000000	58	ILC	2	3A
4	152000000	67	ILC	2	1
5	34259482	59	IDC	3	3B
6	10471756	62	IDC	3	2
7	84356740	57	IDC	2	2A

CHEK2 pre-Tumor(pr) Excision	2ˆ-△Ct	Age	Histological Factor	Grade	Stage (TNM)
8	115000000	55	IDC	2	3a
9	1.5	55	ILC	2	4
10	6183533	67	ILC	2	2
11	1155431	62	ILC	2	3B
12	661000000	63	ILC	3	2
13	152000000	57	ILC	2	3A
14	152000000	52	ILC	2	1
15	34259482	54	IDC	3	3B
16	10471756	61	IDC	2	2
17	115000000	65	IDC	2	2A
18	84356740	67	IDC	2	3A
19	1.5	60	IDC	3	4
20	6183533	62	IDC	2	2

4.8 Association Between Post Treatment CHEK 2 Gene Expression & Selected Clinicopathological Features

The study of post-tumor CHEK2 gene expression provides an essential new perspective on the molecular changes that occur after tumor removal in individuals with breast cancer. In order to elucidate possible implications for prognosis and treatment response, we explore the relationships between post-tumor CHEK2 gene expression and certain clinicopathological characteristics in this section.

A comprehensive analysis of the relationship between post-tumor CHEK2 expression levels and important clinical variables, such as patient age, grade, stage (TNM), and histological features, is given in table 4.5 below. Examining these

correlations, we want to identify trends that might further our understanding of the molecular environment following surgery.

Determining the post-treatment molecular features linked to CHEK2 gene expression and their significance for the clinicopathological range of older patients with breast cancer depends on this investigation.

TABLE 4.5: The table show Post-Tumor Excision Expression Value & Clinical Features

CHK2 post-Treatment(po)	2ˆ-△Ct	Age	Histological Factor	Grade	Stage (TNM)
1	45000	55	ILC	2	3B
2	1080	56	IDC	2	2
3	1.06	58	ILC	2	3A
4	0	67	ILC	2	1
5	1.086735	59	IDC	3	3B
6	1500	62	IDC	3	2
7	1.086735	57	IDC	2	2A
8	1.156689	55	IDC	2	3a
9	1.086735	55	ILC	2	4
10	1900	67	ILC	2	2
11	3520	62	ILC	2	3B
12	1900	63	ILC	3	2
13	3520	57	ILC	2	3A
14	0	52	ILC	2	1
15	1.17	54	IDC	3	3B
16	19000	61	IDC	2	2
17	1.16	65	IDC	2	2A
18	1.09	67	IDC	2	3A
19	1.09	60	IDC	3	4
20	1.9	62	IDC	2	2

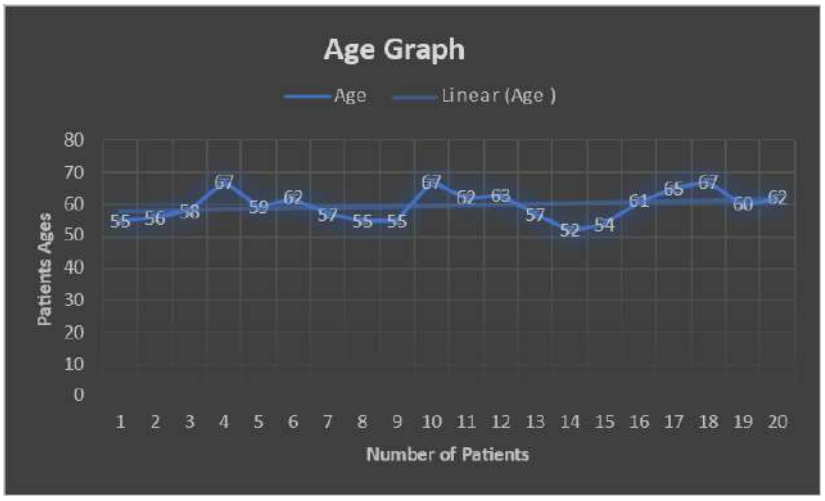

FIGURE 4.3: The Above given Graphs Show age as clinic-pathological Feature

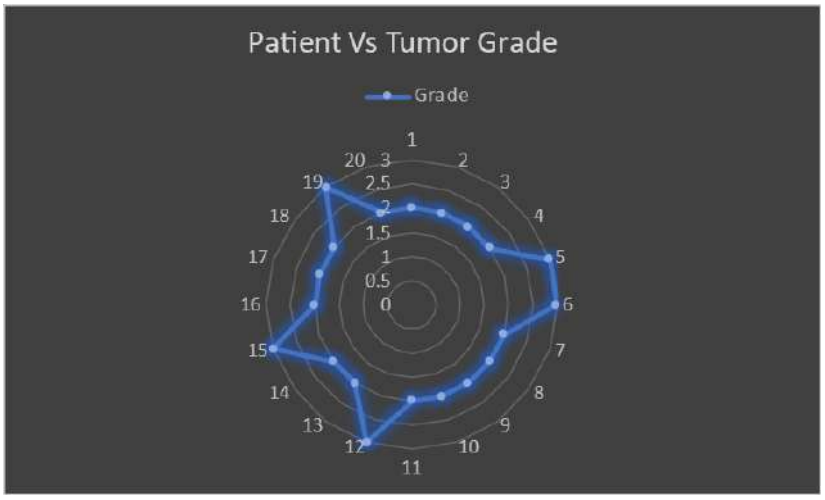

FIGURE 4.4: The Above given Graphs Show Tumor grade as clinic-pathological Feature

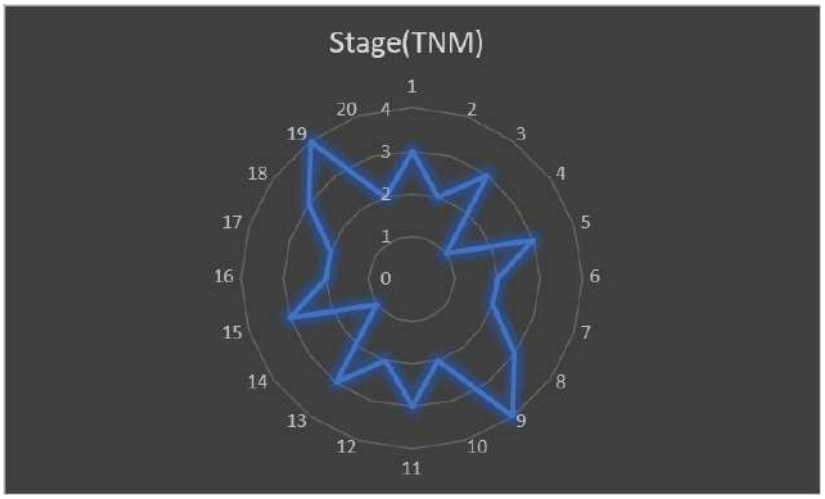

FIGURE 4.5: The Above given Graphs Show Stage as clinic-pathological Feature

4.9 Survival Rate of the Breast Cancer Women Before Surgery

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

TABLE 4.6: Show Expression during diagnosis & blood collection time and survival rate

CHEK2 pre-Treatment(pr)	2ˆ-△Ct	**Blood Collection at time of diagnosis**	**Event**	**Survival Time**
1	1155431	Before Surgery	Nil	Survived
2	661000000	Before Surgery	Nil	Survived
3	152000000	Before Surgery	Nil	Survived
4	152000000	Before Surgery	Nil	Survived
5	34259482	Before Surgery	Nil	Survived
6	10471756	Before Surgery	Nil	Survived
7	84356740	Before Surgery	Nil	Survived
8	115000000	Before Surgery	Nil	Survived
9	1.5	Before Surgery	Nil	Survived
10	6183533	Before Surgery	Nil	Survived
11	1155431	Before Surgery	Nil	Survived
12	661000000	Before Surgery	Nil	Survived
13	152000000	Before Surgery	Nil	Survived
14	152000000	Before Surgery	Nil	Survived
15	34259482	Before Surgery	Nil	Survived
16	10471756	Before Surgery	Nil	Survived
17	115000000	Before Surgery	Nil	Survived
18	84356740	Before Surgery	Nil	Survived
19	1.5	Before Surgery	Nil	Survived
20	6183533	Before Surgery	Nil	Survived

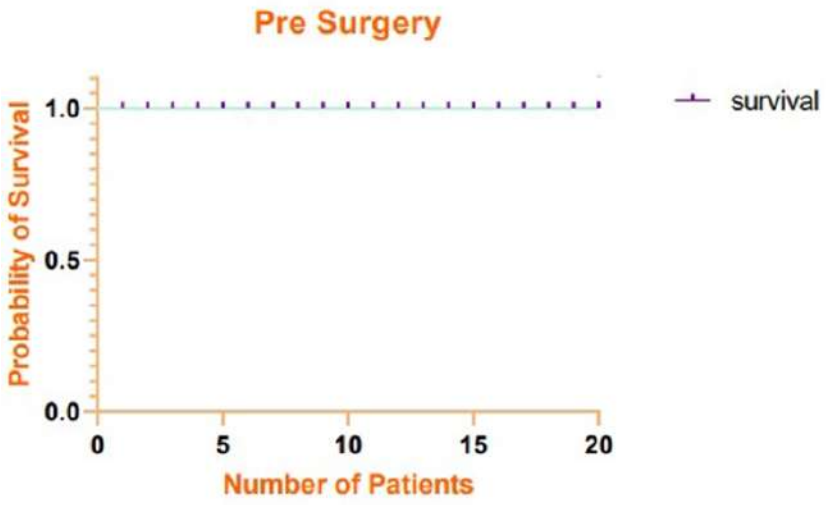

FIGURE 4.6: shows the graphical representation of survival rate of breast cancer women before surgery.

4.10 Survival Rate of the Breast Cancer Women After Surgery

The table 4.7 below show the survival rate of breast cancer patients after surgery. All the patients were alive after surgery.

TABLE 4.7: Show Expression during diagnosis & blood collection time and survival rate

CHEK2 post-Treatment(po)	2ˆ-△Ct	**Blood Collection Time**	**Event**	**Survival Time**
1	45000	4 months After Surgery	Surgery	Survived
2	1080	4 months After Surgery	Surgery	Survived
3	1.06	6 months After Surgery	Surgery	Survived
4	0	6 months After Surgery	Surgery	Survived
5	1.086735	6 months After Surgery	Surgery	Survived
6	1500	4 months After Surgery	Surgery	Survived
7	1.086735	6 months After Surgery	Surgery	Survived

CHEK2 post-Treatment(po)	2ˆ-△Ct	**Blood Collection Time**	**Event**	**Survival Time**
8	1.156689	6 months After Surgery	Surgery	Survived
9	1.086735	4 months After Surgery	Surgery	Survived
10	1900	4 months After Surgery	Surgery	Survived
11	3520	4 months After Surgery	Surgery	Survived
12	1900	4 months After Surgery	Surgery	Survived
13	3520	6 months After Surgery	Surgery	Survived
14	0	6 months After Surgery	Surgery	Survived
15	1.17	6 months After Surgery	Surgery	Survived
16	19000	4 months After Surgery	Surgery	Survived
17	1.16	6 months After Surgery	Surgery	Survived
18	1.09	6 months After Surgery	Surgery	Survived
19	1.09	4 months After Surgery	Surgery	Survived
20	1.9	4 months After Surgery	Surgery	Survived

4.11 Discussion

The present study clarified the molecular changes linked to the onset and advancement of breast cancer, specifically with regard to CHEK2 gene expression level. Notably, there has been a growing body of evidence linking the tumor suppressor gene CHEK2, which has roles in DNA repair & the maintenance of genomic stability, to a number of malignancies, including colorectal and breast cancer. Hereditary Breast Cancer (HBC) that is not BRCA-related can have its roots in uncommon mutations in genes that provide a moderate risk. One such gene is CHEK2, that encodes for an upward regulator of BRCA1. Earlier research has shown a correlation between non-BRCA HBC and CHEK2 founder mutations [91].The control group has no family history regarding the patients and has no expression of the CHEK2 gene. However, blood samples from females who were recently diagnosed with chest cancer show upregulation of the CHEK 2 gene. Overall ATM-Chk2 network is essential for genetic stability, and its disruption

may have an influence on the origin of breast cancer [92]. Every newly diagnosed patient had a family history of a distinct kind of cancer.

In the prior study, the probability ratio (OR) for having breast cancer among first- & second-degree relatives were 7.3 (95% CI, 3.2 to 16.8). We compute the lifetime risk for women with CHEK2 truncating mutations based on a baseline risk of 6% as follows: 20% if no relatives are affected, 28% if one relative is affected in the second degree, 34% if one relative is affected in the first degree, and 44% if both first- and second-degree relatives are affected. For all women with a family history of breast cancer, CHEK2 mutation screening should be taken into consideration since it can identify a clinically significant risk of breast cancer [93]. The study observed decrease in CHEK2 gene expression in post-treatment studies verifies existing knowledge regarding its role in cancer development and progression. The tumor-suppressive properties of the CHEK2 gene are disrupted by mutations, which may contribute to the progress of cancer. Hence, the noteworthy reduction in CHEK2 gene expression subsequent to surgical intervention implies a plausible association between tumor excision and treatment and modifications in CHEK2 gene activity. Additionally, a lower expression level following tumor excision or treatment is linked to greater levels of CHEK2 prior to tumor removal, as indicated by the negative connection among pre-treatment and post-treatment CHEK2 expression levels. The changing dynamics of CHEK2 gene expression in response to therapy interventions and cancer development is highlighted by this discovery.The paired sample t-test results provide quantitative evidence supporting the hypothesis that there is a significant difference in CHEK2 gene expression before and after surgical intervention or treatment. The rejection of the null hypothesis implies that the observed variations are not due to random chance but rather indicate a meaningful association between surgical procedures or treatment and alterations in CHEK2 gene expression.

Notably, all patients survived in both pre- and post-tumor studies, indicating successful interventions. The goal of the study by [94] is to determine prognostic markers to forecast the 10-year survival rates for individuals having early-onset breast cancer, both with and without a CHEK2 mutation, and for patients having

CHEK2-positive breast cancer. The prognosis for women with breast cancer who tested positive for the CHEK2 mutation was similar to that of women who did not. Context for evaluating the data is provided by the research population's demographics, which include age, histological variables, cancer grade, and stage. The patients in stages two and three had the highest expression levels. Moreover, oophorectomy is linked to a lower risk of breast cancer-related mortality in women with CHEK2 mutation-related breast cancer [95]. All things considered, this work advances our knowledge of the molecular pathways that underlie the onset and spread of breast cancer, especially with regard to the function of your CHEK2 gene. To clarify the clinical consequences of these results and investigate prospective treatment approaches that target CHEK2 dysregulation in patients with breast cancer, more investigation is necessary.

Chapter 5

Statistical Analysis

Statistical Analysis of the data was performed by using SPSS 21 Version and MS Excel. The data was entered and organized in MS Excel spreadsheet and then imported in SPSS 21 version for statistical Analysis. The entered data was segregated in Control group, Pre-Treatment and post- treatment Expression Categories and Clinical-Pathological Features of each patients in MS Excel. The data was processed for determining the difference in Gene Expression and effect of clinico-pathological Features on Gene Expression by using SPSS 21. Different statistical Test were applied for Studies.

5.1 Descriptive Statistics

The demographic characteristics of N=20 patients were computed with the help of Descriptive statistics. The correlation co-efficient is computed for a better understanding of the association between during Tumor and post-record of CHEK-2 Gene. A Paired sample t-test is carried out to highlight the level of genes in breast cancer patients. The details are as under:

The descriptive statistics comprised Age, Histological factor, grade, cancer stage, time duration of surgery, and the survival rate (for both pre and post-surgery). The age is analyzed through 2 categories (50 to 60) years & (60-70) years. Most

of the patients such as 11 patients out of 20 female patients with a percentage of 55.0 (55.0%) fall in the first category of the age range (50-60 years). On the contrary, the age range of patients between 60 to 70 years made up 45% of the total. For histological factors, the Invasive ductal carcinoma (IDC) is more with a frequency of 11 and a percentage of 55.0 (55.0%). Most of the patients were grade-2 cancer, with a frequency of 6 and a percentage of 30.0(30.0%). Which indicate that Patients with Stage-2 Cancer were more frequent than stages 1, 2A, 3A, 3B and 4 respectively.

5.1.1 Descriptive Statistics for Demographic Variables (a)

TABLE 5.1: Highlighting Descriptive statistics - Breast Cancer Patients (N = 20)

Variables	**Categories**	**F**	**%**
Age			
	50 to 60 years	11	55.00%
	60 to 70 years	9	45.00%
Histological Factor			
	ILC	9	45.00%
	IDC	11	55.00%
Grade			
	2	15	75.00%
	3	5	25.00%
Stage (TNM)			
	Stage 1	2	10.00%
	Stage 2	6	30.00%
	Stage 2A	2	10.00%
	Stage 3A	4	20.00%
	Stage 3B	4	20.00%
	Stage 4	2	10.00%
Time Duration			

Variables	Categories	F	%
	4 months	10	50.00%
	6 months	10	50.00%
Survival Rate (Pre-Tumor)			
	Survived	20	100.00%
	Not-Survived	0	0%
Survival Rate (Post-Tumor)			
	Survived	20	100.00%
	Not-Survived	0	0%

Note: f = frequency, % = percentage, ILC = Invasive lobular carcinoma, IDC = Invasive ductal carcinoma The second most reported stage of breast cancer is stages 3A and 3B, with frequencies of 4 and a percentage of 20.0 (20.0%), whereas stages 1, 2A and 4 were the least reported. An equal number of patients were taken for the two categories (4 months and 6 months) time duration for post expression studies. The frequency is noted as 10 and the percentage is 50.0 (50.0%). Two categories were taken for the survival rate. All 27 patients (N=27, Control Group = 7, Experimental Group= 20) survived in both cases pre-tumor and the post-tumor results.

5.1.2 Explanation of Data

The data depicted that participant in the 50's was higher than the 60's. Most of the patients were suffering from cancer stage 2, Grade 2. The overwhelming majority of the patient's histological factor is Invasive ductal carcinoma (IDC). The time duration of post-record is of two interval 6 and 4 months. All patients survived before and after surgery.

Table 5.2: Highlighting Descriptive statistics - Control Group (N = 7)

Variables	Categories	F	%
Age			
	50 to 60 years	3	42.90%
	60 to 70 years	4	57.10%

Variables	Categories	F	%
Gene Expression			
	Nil	7	100.00%
Family History			
	YES	0	0%
	NO	7	100.00%

Note: f = frequency, % = percentage

The above given table 5.2 depicts the control group variation. In our study, the control group consists of women lacking any familial cancer history who presented with minor bodily discomfort, warning them to seek medical evaluation. Subsequently, these individuals underwent extensive cancer-related and hormonal assessments. Subsequent to thorough examination, their attending physicians confirmed their overall good health status. The age range of the participants was the same as the age group of patients. The gene expression in the participants was noted as zero.

5.2 Descriptive Statistics (b)

TABLE 5.3: Descriptive Statistics - (b) Breast Cancer Patients (N = 20)

Variables	N	Min	Max	Mean	SD
Age	20	1	2	1.45	0.51
Histological Factor	20	1	200.00%	1.55	0.51
Grade	20	1	200.00%	1.25	0.444
Stage	20	1	6	3.4	1.603
Time Duration	20	1	200.00%	1.5	0.513
Survival Rate (pre-tumor)	20	1	100.00%	1	0
Survival Rate (post-tumor)	20	1	100%	1	0

Note: The data highlighted below represents N (total number of participants, Min (minimum), Max (maximum), Mean (average), and SD (standard deviation).

The minimum age reported was 52, and the maximum age reported was 67. The average age was noted as 1.45 with a SD of 0.510. For histological factors, the mean was recorded as 1.55 with an SD of .510. The stage represents a mean value of 3.40, and a standard deviation of 1.603 highlighting that most patients were suffering from cancer stage 2. A high survival rate was noted for patients in the pre- and post-treatment condition. The survival rates for the pre- and post-tumor were the same with a mean of 1.00 and a standard deviation of 0, representing no variability in data (meaning, all patients have the same survival rate).

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 below represents N (total number of participants, Min (minimum), Max (maximum), Mean (average), and SD (standard deviation).

The minimum age range was 57 and the maximum age range was 69. The mean value for age is 1.57 and the standard deviation is .535. The gene expression was zero whereas the participants' family history was noted as no cancer history.

5.3 Correlation Analysis

The correlation analysis done between pre and post treatment removal studies:

TABLE 5.5: Correlation Coefficient - Breast Cancer Patients (N = 20)

Variables	**1**	**2**
CHEK2 Pre-tumor	-	-0.170
CHEK2 Post-tumor	-0.170	-

To test the hypothesis **Ha**: "There is a significant difference in CHEK 2 gene expression in the blood of 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 CHEK 2 gene expression. The correlation between the pre-tumor excision gene expression level and post-tumor excision gene expression level is analyzed.

The aim is to highlight the change in the level of Gene before and after treatment. The Pearson correlation co-efficient between the two variables is -0.170 The negative sign in the correlation depicts the weak association between the CHEK2 pre-treatment excision expression of the patient and CHEK2 post-traetment (po) expression value.

The higher level of CHEK2 in the pre-phase is weakly linked to the lower level of CHEK2 in the post-phase (instead of a higher level of gene in post phase).

5.3.1 Explanation of Data

The negative correlation indicated that the higher level of gene CHEK2 in the pre-treatment stage is linked to the lower level of gene CHEK2 in the post-treatment stage.

This represent patients who experienced higher levels of CHEK2 expression before treatment were found to have lower levels of CHEK2 after tumor removal or other clinical intervention.

5.4 Paired Sample T-Test

To test the hypothesis **Ha**: There is a significant difference in CHEK 2 gene expression in the blood of older women with breast cancer before and after treatment.

The observed variations are not due to random chance and there is a meaningful association between the surgical procedure and alterations in CHEK 2 gene expression." A pair was made to proceed with the analysis. The findings are given next:

5.4.1 CHEK2 Gene Expression Hypothesis

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

Variables	**Pre-test (n=20)**		**Post-test (n=20)**		**t**	**P**	**Cohen's d**
	M	SD	M	SD			
Pair 1 CHEK2 Pre-treatment Excision (pr) ct - CHEK2 Post-treatment (po) ct	2.42E8	4.25E8	387159.0%	10567.1	2.542	0.020	0.517

Note: M = mean, SD = standard deviation, t = t-statistics, p = p-value, d = Cohen's d value. To test this hypothesis, a paired sample t-test is used. The mean for CHEK2 pre-treatment is 2.4200000000, whereas the SD is 4.25300000000. On the other hand, the mean for CHEK2 post-tumor is 3871.59 whereas the standard deviation is 1056.1. The mean difference is noted as 2.42E8 and the standard deviation difference is 4.253E8. The 95% confidential level lower (LL) limit is 42736706.15, whereas the upper limit (UL) is 4.408E8. The p-value is noted as 0.020. The t-statistics is 2.542 with 19 degrees of freedom. It indicates that the mean difference is significant at 0.05 level. The Cohen's d value is 0.517.

5.4.2 Interpretation of Results

The results provide evidence to reject the null hypothesis. A significant difference has been observed in the level of the CHEK2 gene before and after surgical treatment. The correlation coefficient and paired sample t-test justify the findings. The mean difference (2.418E8), the t-value (2.542), and the p-value (0.020) and CL-95% further emphasized that the findings are in favour of the alternate hypothesis highlighting a significant difference in CHEK 2 gene expression in the blood of PM women with breast cancer before and after clinical intervention. The observed variations are not due to random chance and there is a meaningful association between the surgical procedure and alterations in CHEK 2 gene expression.

5.5 The Results of Expression Level of CHEK2 Gene

The statistical analysis for the current study was carried out using SPSS. The expression level of gene CHEK2 was observed in 47 blood samples the 7 are healthy older women and other of 20 patients in total the 20 blood samples are of women diagnosed with breast cancer and 20 blood samples are collected after removal of tumor. The Three experimental groups were taken for the present study (Figure 5.1). The 7 normal individuals are (with no family history of cancer and no CHEK2 gene expression) in the control group whereas 20 female patients diagnosed with breast cancer (varies from stage 1 to 4) were taken as a sample of this study. The pre-Treatment and post-treatment observations (through paired sample–test) were recorded. Out of 20 patients, the blood sample of 10 patients was collected after 4 months, whereas the blood sample for the other 10 patients was collected after 6 months (as illustrated in Figure 5.2). The concentration level of the CHEK2 gene was noted to decrease in the post-tumor observations (demonstrated in Figure 5.3). The gene level was higher when measured during pre-tumor excision observations. The mean difference (2.418E8), the t-value (2.542), and the p-value (0.020) were seen as significant pointing to the rejection of the Null Hypothesis.

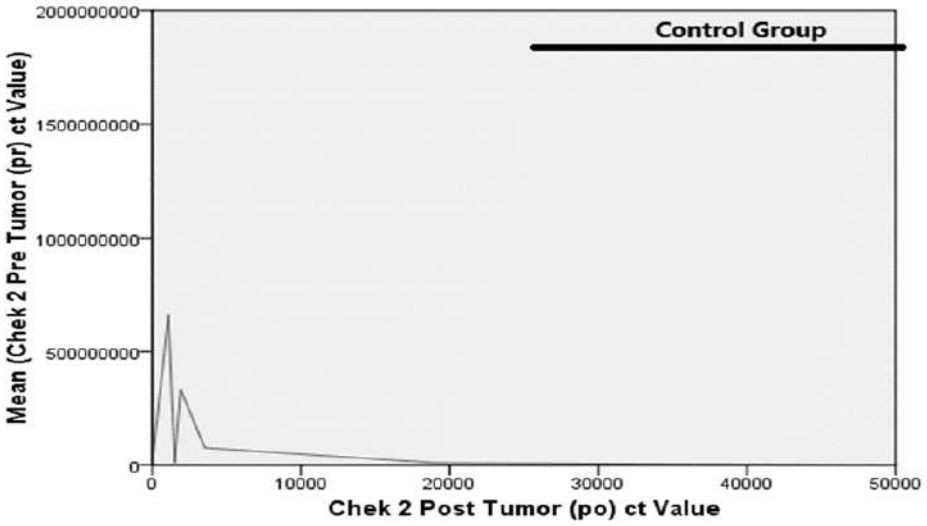

FIGURE 5.1: Variation in Control Group and Experimental Group (Pre-tumor and Post-tumor excision)

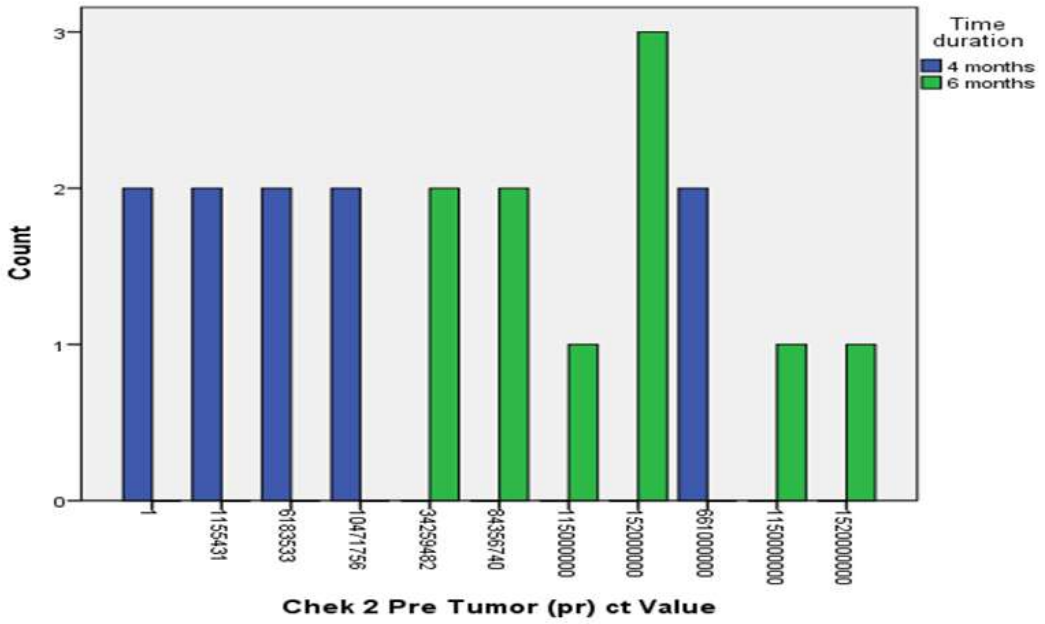

FIGURE 5.2: Expression of CHEK2 Gene show with time variation in Patients with tumor

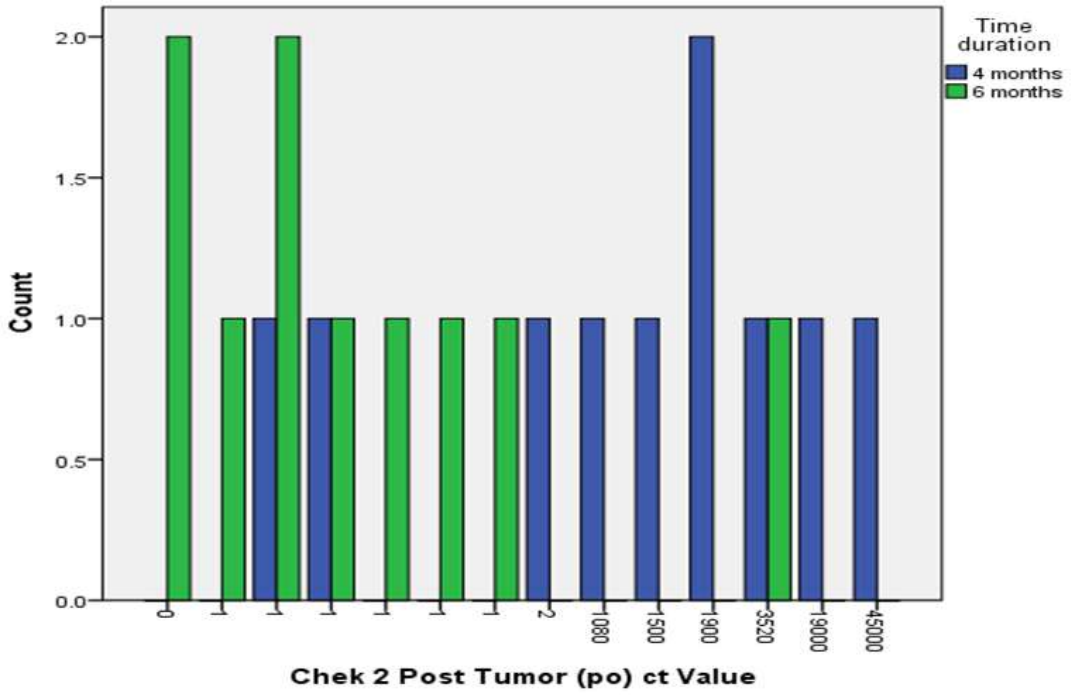

FIGURE 5.3: Expression of CHEK2 Gene with time variation in Patients after tumor removal

Chapter 6

Conclusion and Future Work

The study show that the higher pre-Treatment expression levels were linked to lower post-treatment expression levels, according to the CHEK2 gene levels, which showed a negative connection. A substantial reduction in CHEK2 gene expression in post-treatment studies was verified by a paired sample t-test ($p = 0.020$). A modest correlation between the pre- and post-treatment levels of gene expression was shown using correlation analysis. The null hypothesis was rejected with the help of a paired sample t-test, highlighting the significant correlation between CHEK2 gene expression changes and surgical intervention or therapy. The results emphasize the significance of CHEK2 in relation to postmenopausal patients' breast cancer. Reduced gene expression in investigations conducted after therapy points to a possible involvement in the molecular changes brought on by surgery or other treatments. The conclusion highlight the role that CHEK2 plays in the development of breast cancer and the possibility as a potential biomarker.

6.1 Dynamic Changes in CHEK2 Gene Expression

The study provides compelling evidence of dynamic changes in CHEK2 gene expression in older women with breast cancer following surgical intervention. This

observation suggests a link between surgical procedures and the modulation of gene expression profiles associated with cancer progression. Specifically, the study revealed zero expression of the CHEK2 gene in a control group comprising healthy women, contrasting with overexpression levels observed in newly diagnosed breast cancer patients.

Moreover, a significant decrease in CHEK2 gene expression was noted in post-tumor excision studies, indicating the potential impact of surgical interventions on gene expression dynamics.

6.2 Implications of CHEK2 Expression Levels

CHEK2, a critical gene involved in DNA repair and cell cycle regulation, plays a pivotal role in maintaining genomic stability and preventing the accumulation of genetic mutations. However, elevated expression levels of CHEK2 in breast cancer cells may signify dysregulation of these processes.

This dysregulation could lead to increased tumor proliferation, resistance to chemotherapy, and enhanced metastatic potential, thereby contributing to a more aggressive phenotype of the disease.

6.3 Significance as a Biomarker

The study suggests that high expression of CHEK2 serves as a biomarker for tumor aggressiveness. This finding has important implications for treatment strategies, as it indicates the potential utility of CHEK2 expression levels in informing therapeutic decisions.

For instance, high CHEK2 expression levels could prompt the consideration of targeted therapies or the implementation of more intensive surveillance protocols to improve patient outcomes.

6.4 Personalized Treatment Approaches

The results highlight the significance of individualized treatment plans based on each patient's unique molecular profile while managing breast cancer.

Clinicians can create more focused and successful treatments by knowing the molecular features of each patient's tumor. This individualized strategy might minimize side effects and therapeutic success.

6.5 Future Research Directions

Further research in this area is warranted to elucidate the mechanistic basis of the observed dynamic changes in CHEK2 gene expression and their clinical implications. Future studies could explore the underlying molecular pathways involved in CHEK2 dysregulation and investigate potential therapeutic targets aimed at restoring normal gene expression levels.

Additionally, longitudinal studies examining the association between CHEK2 expression levels and patient outcomes could provide valuable perceptions into the prognostic significance of CHEK2 as a biomarker in chest cancer.

6.6 Future Outcomes

6.6.1 Clinical Implications

Understanding the molecular changes associated with tumor excision can provide insights into therapeutic interventions. Targeting molecular pathways affected by CHEK2 gene alterations may lead to novel treatment strategies for breast cancer patients.

6.6.2 Precision Medicine

Identifying genetic variations in the CHEK2 gene and their impact on cancer risk allows for personalized risk assessment and tailored screening approaches. Integrating genetic testing for CHEK2 mutations into clinical practice can enhance early detection and prevention efforts [96].

6.6.3 Longitudinal Studies

Further longitudinal studies are acceptable to validate our findings and assess the long-term effects of surgical intervention on CHEK2 gene expression. Investigating gene expression dynamics over time may elucidate the molecular mechanisms underlying cancer progression and recurrence [97][98].

6.6.4 Therapeutic Targets

Exploration of potential therapeutic targets downstream of the CHEK2 pathway could lead to the growth of targeted therapies for Breast cancer patients with CHEK2 mutations. Preclinical studies investigating the efficacy of such targeted interventions are crucial for translation into clinical practice.

6.6.5 Integration of Multi-Omics Data

Integrating transcriptomic data with other omics datasets, such as proteomics and metabolomics, can provide a comprehensive understanding of the molecular landscape associated with breast cancer. Multi-omics approaches offer valuable insights into the complex interplay between genetic alterations, cellular pathways, and tumor microenvironment [35][98].

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