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Research Article

EXPRESSION ANALYSIS OF CHEK2 GENE IN BLOOD OF POSTMENOPAUSAL WOMEN WITH BREAST CANCER: A COMPARATIVE CASE-CONTROL STUDY

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Abstract

Background: Breast cancer remains a leading cause of cancer-related mortality among postmenopausal women, with genetic and hormonal factor playing a crucial role in disease progression and prognosis. The CHEK2 gene, a key regulator in DNA damage repair, has been implicated in increased cancer susceptibility. This study investigates the expression profile of CHEK2 in postmenopausal breast cancer patients to evaluate its potential as a prognostic biomarker. **Methods:** A total of 47 blood samples were analyzed, including 7 from healthy controls, 20 from newly diagnosed breast cancer patients, and 20 from patients' post-treatment (4 to 6 months post-surgery or therapy). RT-PCR analysis was carried out to measure the expression levels of the CHEK2 gene in these blood samples, providing insights into the genetic changes linked with breast cancer progression and the effects of clinical interventions. The resulting Ct values were used to calculate the expression levels of CHEK2, allowing precise evaluation of its transcript levels in peripheral blood. This enabled sensitive detection and comparative analysis of gene expression between healthy Control/Cases and post-surgery samples. **Results:** CHEK2 expression exhibited a highly significant difference in between Group 1 healthy control show absence expression and Group 2 Cases show upregulation of gene CHEK2 ($P<0.001$)A down-regulation of gene expression is seen between Cases to Group 3 post-treatment ($P = 0.020$, paired t-test), suggesting its potential involvement in cancer pathogenesis. The majority of patients aged 50–60were diagnosed with stage 2 invasive ductal carcinoma. This systematic regulation in CHEK2 expression highlights its role in disease progression, evolution and therapeutic response. **Conclusion:** The findings underscore CHEK2 as a promising biomarker for breast cancer prognosis and treatment monitoring. The down regulation of expression in post-treatment suggests its potential utility in tracking molecular changes and guiding therapeutic interventions in post-menopausal breast cancer patients. Further studies are warranted to elucidate its mechanistic role in tumor progression and treatment response.

Keywords: CHEK2, Breast Cancer, Invasive Ductal Carcinoma, Prognostic Biomarker, Gene Expression, Postmenopausal Women.

INTRODUCTION

The most frequently occurring type of cancer is Breast Cancer with a high mortality rate reported. There is a wide range of histopathological aspects associated with Breast Cancer, including metastatic patterns and genetic polymorphism, and prognostic outcomes (Siegel *et al.,* 2024; Siegel *et al.,* 2023, Feng *et al.,* 2018). Breast cancer is identified as the second most frequently occurring type of cancer. It is a fatal illness that affects millions of people worldwide. It occurs due to the unchecked proliferation of abnormal cells in breast tissue. Breast cancer is diagnosed more frequently in women than any other cancer. Breast cancer remains a significant global health issue, with two million cases reported in 2021. Various miRNAs may serve as biomarkers since they have been connected to cancer (Altalebi *et al.,* 2023). Luminal- like breast cancer is considered crucial in making of 60 to 70 percent of breast tumor (Johnson *et al.,* 2021). Luminal A is reported as 40 percent whereas Luminal B is reported as 20 percent of all breast cancers (Johnson *et al.,* 2021). A study conducted by Midlenko and colleagues (2023) depicted an estimation of 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.

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In various 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. A recent meta-analysis carried out by Goodwin *et al.,* (2020) found that obese women had a 41% higher overall mortality and a 33% greater chance of death from breast cancer, in addition to several studies linking obesity to poor breast cancer outcomes. This correlation is plausible, because of biological components linked to obesity, including higher insulin levels, glucose, leptin, postmenopausal estrogen, and inflammatory markers. 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. About 80% of occurrences of breast cancer are found in women 50 years of age or older, and it 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%. at the same time, lifestyle decisions and environmental variables are responsible for the remaining 90–95%. Genetic predisposition is not the determining factor in most cases. According to Kolak *et al.,* (2017), the non-genetic variables dominate. Age, family history, and reproductive variables are among the risk factors found by research. The intricacy of the epidemiology, molecular processes, and clinical presentation of breast cancer is attributed to some intrinsic and lifestyle-related variables (Kaminska *et al.,* 2015). The Checkpoint kinase 2 (CHEK2) genetic mutations according to Aksoy *et al.,*

(2022), are known to be a risk factor for the emergence of malignancies in several 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 protein 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. 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 (Bychkovsky *et al.,* 2022). 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 (Hallamies *et al.,* 2017). However, its frequency did not differ significantly 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.

MATERIAL AND METHODS

Blood Sample Collection

Blood samples were collected from Female Breast cancer patients at the Department of Oncology, Fauji Foundation Hospital, Rawalpindi. A Venipuncture was carried out to collect 5 mL of blood into vacutainer tubes, ensuring proper labelling for further testing. A total of 47 blood samples were gathered, encompassing Control=7, Cases/Pre-treatment group =20 and post-treatment group = 20 samples. Their ages are between40 to 60 years. The mean age noted is 58.5 years. These samples were processed at BJ Micro Lab, Rawalpindi.

RNA Extraction

RNA extraction was conducted using the Trizol method, considering its efficiency in isolating high-quality RNA. The process comprised of adding 600 µL Trizol to 300 µL of sample, followed by 400 µL chloroform and centrifugation at 12,000 rpm for 10 minutes at 4°C. After the separation of the aqueous layer, RNA was precipitated with isopropanol. The extraction was washed with 70% ethanol and resuspended in RNase-free water for storage. The temperatures were strictly controlled to ensure the stability of RNA for further analysis. Gel Electrophoresis was performed to check the RNA extraction. It was carried out using 1.5% agarose gel in 1x TBE buffer. Agarose gel electrophoresis highlighted the quality and stability of RNA, ensuring the reliability of upcoming study results.

cDNA Synthesis

The isolated RNA was transcribed into complementary DNA (cDNA) using the FIREScript® Reverse Transcriptase Kit. The reverse transcription procedure showed an effective cDNA synthesis for the extracted RNA. Obtained cDNA was stored at− 20 °C until analysis. To check cDNA synthesis efficiency, PCR for GAPDH gene was conducted.

Quantification by Nanodrop

Dilution was necessary for RT-PCR. Dilutions of cDNA were prepared at 1:100 for further analysis.

Quantitative Real Time PCR (qRT-PCR)

The differential Expression of gene was performedby RT-PCR. The CHEK2 gene primers were designed using NCBI sequences and synthesized from Korea. The forward primer sequence5'CAGTCTCATGGCAGCAGTG3', and the reverse primer sequence was 5' GTTCTTGGTTCTCAGGTTCTTG 3'. Optimization of the PCR reaction was achieved by maintaining an annealing temperature of 56°C, ensuring efficient amplification of a 223 bp DNA fragment. 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°C / 5 minutes, initiating the reaction. Subsequent denaturation at 95°C / 30 seconds, carried out over 35 cycles, facilitates the separation of DNA strands. Annealing occurs at 56°C / 30 seconds during each cycle, allowing the primers to bind to the target sequence. Extension takes place at 72°C / 20 seconds during each of the 35 cycles. These controlled conditions offer real-time insights into CHEK2 gene expression levels.

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 data was processed for determining the difference in Gene Expression and effect of clinicopathological Features on Gene Expression by using SPSS 21.

Table 1. Descriptive statistics for Demographic variables (a)

Variables	Categories	F	%
Age			
	50 to 60 years	11	55.0%
	60 to 70 years	9	45.0%
Histological Factor			
	ILC	9	45.0%
	IDC	11	55.0%
Grade			
	2	15	75.0%
	3	5	25.0%
Stage (TNM)			
	Stage 1	2	10.0%
	Stage 2	6	30.0%
	Stage 2A	2	10.0%
	Stage 3A	4	20.0%
	Stage 3B	4	20.0%
	Stage 4	2	10.0%
Time Duration			
	4 months	10	50.0%
	6 months	10	50.0%
Survival Rate (Pre-Tumor)			
	Survived	20	100.0%
	Not-Survived	0	0%
Survival Rate (Post-Tumor)			
	Survived	20	100.0%
	Not-Survived	0	0%

Different statistical Test were applied for Studies. The Expression comparison is done by using paired sample T -Test between Groups. The p – value between healthy control and cases is P<0.001 and between Cases and post-treatment P = 0.020 respectively

Table 2. Descriptive statistics for control group Demographic variables (b)

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

RESULTS

Expression Analysis of CHEK2 Gene in control and cases

Quantitative Real Time PCR (qRT-PCR) was used to determine the expression of CHEK2 in healthy controls and Cases female diagnose with breast cancer. We observed nearly absent expression in healthy control and highly significant up regulated expression P<0.001 in Breast cancer patients. The upregulated expression level showed CHEK 2 role in breast cancer progression.

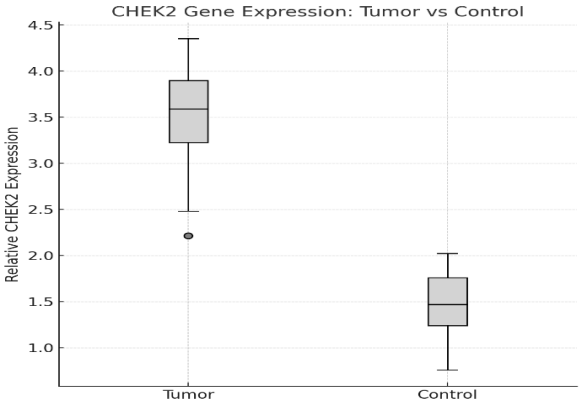

Fig. 1. Show expression of CHEK2 in Healthy control and in Breast Cancer patients:

Comparison of Expression Analysis of CHEK2 Gene in Participants

We observed the expression is down regulated after the surgery and the expression value showed significant difference in expression after tumor excision P=0. 020.

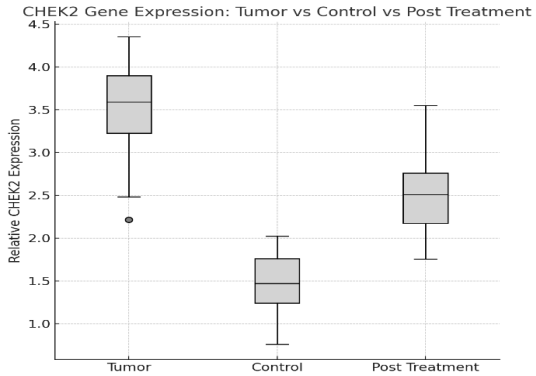

Fig. 2. Show expression of CHEK2 in Healthy control,Tumor and post treatment:

The significant value indicate that upregulation during tumor is associated with pathogenesis. The given fig 2 indicate the expression regulation across all the samples respectively.

Paired Sample T-test

The null hypothesis is rejected. A significant difference has been observed in the Expression level of the CHEK2. The correlation coefficient and paired sample t-test justify the findings. The mean difference (2.418), the t-value (2.542), and the p value for control and Cases is P<0.001 with 99.9% CI and SD 4.253and cases Vs post treatment p-value (0.020) and CL-95% and SD 1.056 the findings support the alternate hypothesis highlighting a significant difference in CHEK 2 gene expression in the blood of PM women with breast cancer, control and after clinical intervention. The observed variations are not due to random chance there is a meaningful association between the surgical procedure and CHEK 2 gene expression alterations.

Pearson Correlation Coefficient of Breast Cancer Patients

The value of correlation coefficient is -0.170 and negative sign in the correlation depicts the weak association between the gene expression of the control / cases group and the CHEK2 post-treatment expression value. The higher level of gene in the pre-phase is weakly linked to the lower level of CHEK2 in the post-phase.

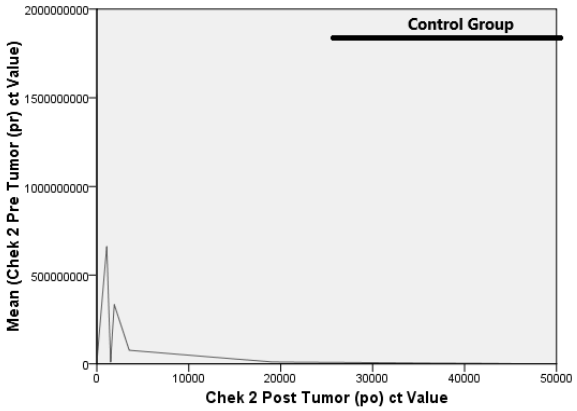

Fig. 3.Variation in Control Group and Experimental Group (Tumor and Post-tumor excision)

DISCUSSION

RT-PCR was conducted to assess the expression levels of the CHEK2 gene in breast cancer patients before and after treatment. The expression values were compared using 2^-ΔΔCt. The results showed dynamic changes in gene expression post-surgery, suggesting a potential link between surgical intervention and CHEK2 gene activity. The study further examined the correlation between CHEK2 gene expression and clinic-pathological features such as age, histological factors, grade, and stage. This analysis aimed to understand how the gene's expression relates to disease characteristics in breast cancer patients, especially in older populations. Before and after surgery, all patients survived, reflecting successful intervention. The tables provided show the survival rates and CHEK2 expression levels pre- and post-treatment, with graphical representations indicating age, tumor grade, and stage. 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 cancer-associated mutations in the BRCA1 or BRCA2 genes. Irrespective of the receptor status, the risk factors were similarly linked to breast cancer. 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 of menopausal estrogen-progestin therapy (Rosenberg *et al.,* 2006). 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 of sex steroid hormones in postmenopausal women (Missme *et al.,* 2004). There is no information regarding alteration in the expression level of the CHEK 2 gene before and after treatment in PM women with Breast Cancer. The goal of the current study was to close this knowledge gap and examine the molecular changes carried out by the Treatment. Cancer is specified on the basis of clinical physiological features that entails, tumor size, and cancer stage, these conditions help highlight the involvement of various breast cancer subtypes on the basis of which hormone receptors depend (including progesterone and estrogen) (Vuong *et al.,* 2014; Acs *et al.,* 2021). Various assessment is used in the treatment of breast cancer based on clinical factors like tumor diameter, histological grade and lymph node invasion (Ultimescu *et al.,* 2024). 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 clinicopathological 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 (Lodi *et al.,* 2017). 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 (Li *et al.,* 2022). 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. The study confirms the involvement of the CHEK2 gene in breast cancer progression. The decreased expression of CHEK2 after surgery aligns with its known role in tumor suppression, suggesting its regulation in cancer. A significant association between surgical treatment and changes in CHEK2 gene expression was noted, supported by a paired t-test. CHEK2 gene mutations are increasingly linked to hereditary breast cancer and other malignancies. This gene is crucial for genomic stability, and its mutation can lead to cancer progression. The study observed significant gene expression in pre-treatment samples but reduced levels post-treatment, reinforcing the tumor-suppressive role of CHEK2. 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.

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