RESEARCH ARTICLE

Significance of CHEK2 expression in pathogenesis of breast cancer

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Abstract

Objective: To compare the histo-morphological features and checkpoint kinase-2 expression in breast cancer versus benign breast disease.

Method: The cross-sectional study was conducted at the Department of Anatomy, Basic Medical Sciences Institute, in cooperation with the Department of Surgery, Jinnah Postgraduate Medical Centre, Karachi, from March 31, 2021, to March 30, 2022, and comprised women admitted to surgical units with a history of breast tumour. The subjects were allocated to fibroadenoma group A and breast cancer group B. After gross anatomical examination, the breast tissue was processed with haematoxylin and eosin staining for histomorphology and immunohistochemical analysis for checkpoint kinase-2 expression. Data was analysed using SPSS 23.

Results: Of the 80 women, 40(50%) were in group A with mean age 30.05±6.4 years, while 40(50%) were in group B with mean age 43.139±8.3 years. The most common presenting complaint of group A was pain 15(37.5%) compared to painless lump in group B 35(87.5%). In both groups tumours had affected the upper outer quadrant of the left side of the breast. On microscopy 38(95%) group B patients had invasive ductal carcinoma; 26(65%) having grade II and 22(55%) having stage II cancer. Necrosis (p=0.55), calcifications (p=0.41), ductal carcinoma in situ component (p=0.09) and lymphocytic infiltration (p= 0.26), were significantly noticeable in group B. Immunohistochemical expression of checkpoint kinase-2 was significantly higher in group B compared to group A (p=0.001).

Conclusion: There was a significantly higher expression of checkpoint kinase-2 in breast cancer patients, and this could be considered an important diagnostic marker in breast cancer pathogenesis.

Keywords: Breast cancer, Fibroadenoma, Chekpoint kinase-2, Immunohistochemistry, Histomorphology.

(JPMA 76: 1454; 2026) **DOI: https://doi.org/10.47391/JPMA.30493**

Introduction

Breast carcinoma is the second most common cause of cancer-related deaths in women around the world. Numerous genetic mutations, epigenetic biomarkers and sporadic alteration are linked with disease progression, but their utility as dynamic and vigorous indicators able to differentiate among a variety of breast tumours at the cellular level still remains ambiguous.[1]

In Pakistan, one in every nine women suffers from breast cancer, which in global terms represents one of the highest breast cancer mortality rates. Breast cancer has surpassed lung cancer, and it is estimated that in 2040, about 28.4 million cancer cases may occur globally.[2] The incidence of breast cancer has significantly increased among younger women aged <40 years, with more aggressive and less responsive tumours being diagnosed that are also associated with higher tumour grade, unfavourable histological variables and higher death rates compared to older women.[3]

There are numerous determinants for breast cancer development, with gene mutations in breast cancer (BRCA) gene 1 and 2 (BRCA1 & 2) having been identified as the main factors. Checkpoint kinase-2 (CHEK2) is the most common non-BRCA pathological variant. The CHEK2 is a moderate cancer susceptibility gene that encodes serine/threonine kinase. It acts as a cell cycle checkpoint regulatory protein that is involved in various cellular pathways, such as cell cycle, deoxyribonucleic acid (DNA) repair and apoptosis. In response to DNA damage, CHEK2 maintains chromosomal stability between cellular DNA repair pathway and apoptosis through transcription factors.[4] Depending upon family history, individuals with CHEK2 mutations carry lifetime risk of cancer development, ranging 28-37%. Although CHEK2 is a moderate penetrant gene, its prevalence varies worldwide.[5] To classify the genetic markers causing breast cancer, numerous genome-based trials have been conducted. Due to the complexity of breast lesions, the diagnosis is contingent upon the specific features of

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Submission complete: 15-03-2025 **First Revision received:** 15-07-2025

Acceptance: 13-05-2026 **Last Revision received:** 12-05-2026

each case. The assessment of genetic features and its association with histological findings by immunohistochemistry (IHC) is a cost-effective method and plays an invaluable role in identifying molecular expression pattern of biomarkers in breast cancer.[6]

The current study was planned to compare the histomorphological features and CHEK2 expression in breast cancer versus benign breast disease.

Patients and Methods

The cross-sectional study was conducted at the Department of Anatomy, Basic Medical Sciences Institute (BMSI), in cooperation with the Department of Surgery, Jinnah Postgraduate Medical Centre (JPMC), Karachi, from March 31, 2021, to March 30, 2022. After approval from institutional ethics review board, the sample size was calculated using OpenEpi[7], with 95% confidence interval (CI) and 5% margin of error based on the prevalence rate mentioned in an earlier study.[8] The sample was raised using non-probability consecutive sampling technique. Those included were females aged 18-50 years admitted to surgical units with a history of breast tumour. Those with a history of previous breast cancer surgery, hypertension and menopause at any age were excluded.

After obtaining written informed consent from all the subjects, they were divided into fibroadenoma group A and breast cancer group B. The groups were later subdivided on the basis of younger and older subjects, with those aged 18-34 years in subgroups A1 and B1, and those aged 35-50 years in subgroups A2 and B2. Demographic and clinical data, such as age, marital status, parity, family history of breast cancer and ethnicity, was collected.

The surgically removed breast tumour specimens were stored in 10% buffer neutralised formalin (BNF) for 24 hours. After gross morphological examination, lesion's location was noted by serial cutting of specimen from medial to lateral at 1cm interval. Representative sections from the site of lesion were processed to paraffin-embedded tissue blocks, and 5µm thick sections were cut and mounted on glass slides. The slides were stained with haematoxylin and eosin (H&E) stain for histomorphological assessment. Ten slides per specimen and 3 areas per section were observed for histomorphological changes, including necrosis, calcifications, ductal carcinoma in situ (DCIS) component and lymphocytic infiltration. Nottingham modification of the Bloom-Richardson system was used for grading the tumour. The American Joint Commission on Cancer (AJCC), Tumour-Node-Metastasis (TNM) pathological staging for breast carcinoma was used for staging.[9]

Immunohistochemical (IHC) staining for the expression of CHECK2 marker was performed on formalin-fixed paraffin sections using Thermo Scientific (Life Tech) kit. CHECK2 Recombinant Rabbit Monoclonal Antibody (SC604; CAT # MA5-32220) was diluted at 1:100, Goat anti-Rabbit immunoglobulin G (IgG) (H+L) Secondary Antibody, horseradish peroxidase (HRP) CAT # 65-6120) and Stable 3,3'-Diaminobenzidine (Dab) (CAT # 750118) were used as per the manufacturer's recommendations. Image J Fiji software was used to measure expression intensity of CHEK2 by assessing percentage of optical density (OD) that measured average darkness of DAB-stained image.[10] The intensity scoring system was used to for the assessment of expression analysis (absent: 0.01-0.09; low positive: 0.10-0.19; positive: 0.20-0.29; high positive: >0.30).[11] The prepared slides were observed under digital camera microscope (Nikon Eclipse 5oi; Japan) connected to video link digitalising board system (Digital sight second-generation stand-alone Live camera control unit-DS-L2).

The obtained results were compared between and within groups. SPSS Version 23 was used for Statistical analysis. For Quantitative variable mean ± SD values were computed and compared for their mean differences by Fisher's exact test and considered significant at $p \leq 0.05$. For an estimate of range of percentages in the population on the basis of sample, their 95% confidence intervals (CI) were also calculated.

Results

Of the 80 women, 40(50%) were in group A with mean age 30.05±6.4 years, while 40(50%) were in group B with mean age 43.139±8.3 years. There were 27(33.75%) women aged 18-34 years, while 53(66.25%) were aged 35-50 years. Primarily, the Urdu-speaking ethnic group was

Table 1: Histomorphological variables of breast carcinoma cases based on age 18-34 years (B1) and 35-50 years (B2).

Variables	Age group		Total %	Test statistic
	B1(n=03)	B2 (n=37)		
Necrosis				
Present	0	7	17.5	Fisher's exact test =1.0 p=0.55
Absent	3	30	82.5	
Calcification				
Present		10		Fisher's exact test =0.56 p=0.41
Absent	3	27	75	

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DCIS component				
Present	2	2	20	Fisher's exact test =0.096 p=0.09
Absent	1	31	80	
Lymphocytic infiltration				
Mild	0	10	25	chi-square=2.654 p=0.26
Moderate	3	19	55	
Severe	0	8	20	

DCIS: Ductal carcinoma in situ.

affected; in group A, 27 (67.5%) related to group B, 17 (42.5%); the most common presenting complaint of group A was pain, 15 (37.5%), compared to the painless lump in group B, 35 (87.5%). In both groups tumour had affected the upper outer quadrant (UOQ) of the left side of the breast. On microscopy, 38(95%) group B patients had invasive ductal carcinoma (IDC); 26(65%) having grade II and 22(55%) having stage II cancer.

There was a higher prevalence of DCIS in subgroup B1 compared to B2 (p=0.096) (Table 1). Necrosis and calcifications were more apparent in subgroup B2 compared to B1 (Figure 1).

IHC expression of CHEK2 was significantly higher in group B compared to group A (p=0.001) (Figure 2).

OD showed increased expression of CHEK2 in group A compared to group B (p=0.001). Subgroup analysis also

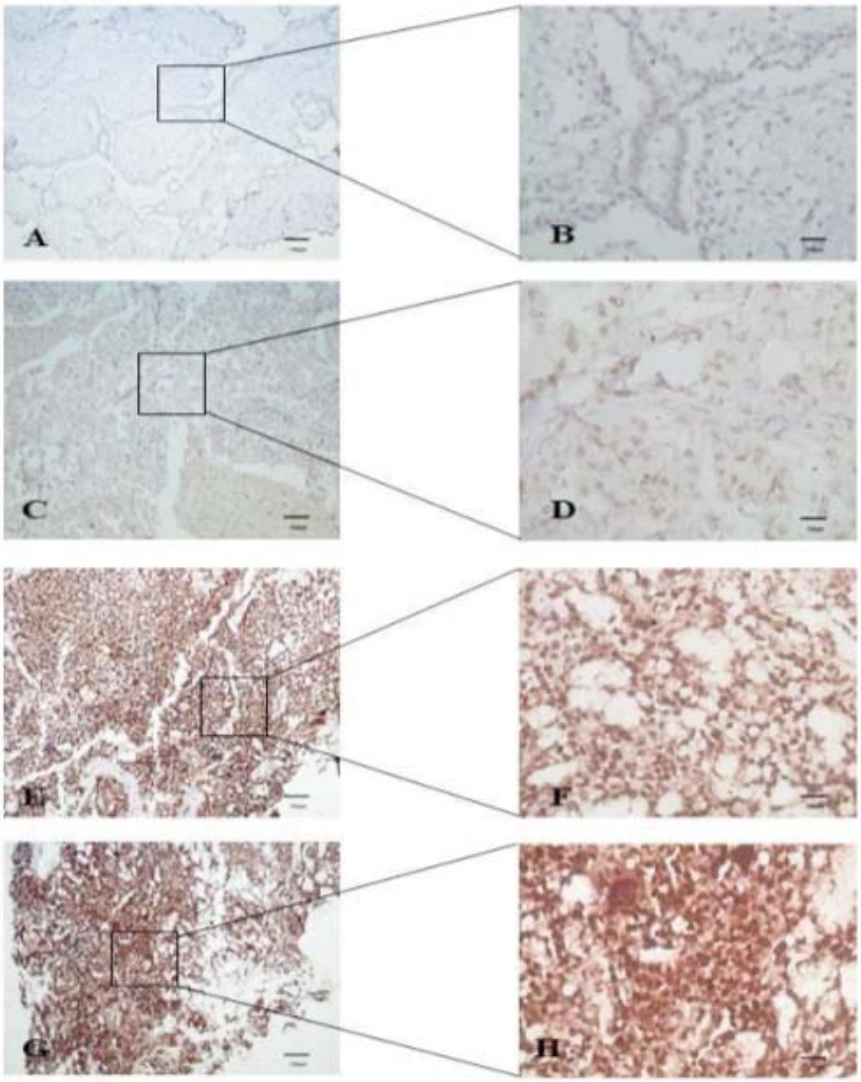

Figure-1: Immunohistochemical expression of CHEK2 in Benign as absent (A, C) 100X & low positive (B, D) 400X and in Malignant as positive (E, G) 100X & high positive (F, H) 400X.

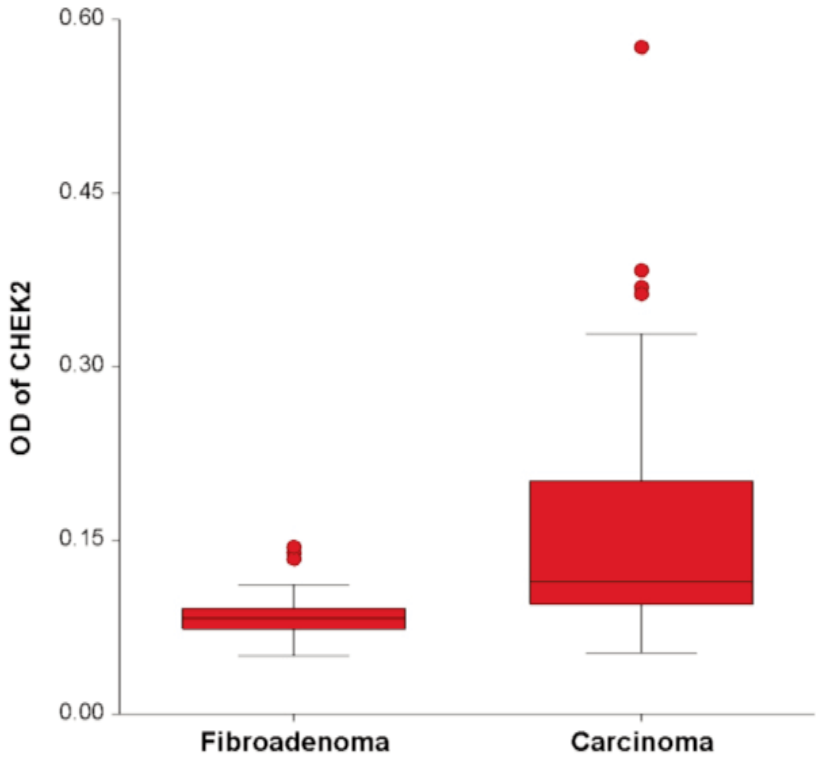

Figure-2: Mean optical density (OD) of CHEK2 expression in Fibroadenoma versus Carcinoma., p= 0.001 (Student t test)

Table-2: Comparison of CHEK2 expression among groups and age-based sub-groups.

Age Groups	Groups	Number	Mean	SD	p-value
18-34 years (n=27)	A1	24	0.08	0.02	0.003
	B1	3	0.18	0.15	
35-50 years (n=53)	A2	16	0.08	0.02	0.006
	B2	37	0.16	0.11	
Total (n=80)	A	40	0.08	0.02	0.001
	B	40	0.16	0.11	

SD: standard deviation.

showed statistically significant differences (Table 2).

Discussion

Over the past few years, there has been a steady increase in young onset breast cancer cases worldwide, which is partly because of a rise in diagnosis by mammography screening, but may also suggest that current preventive methods have become ineffective due to drug resistance in tumour cells.[12] It has recently been reported that genomic instability due to defected cell cycle, DNA repair pathway and apoptotic deregulation were linked with genetic events that gradually leads to tumour development.[13]

It is essential to identify the contributing factors in breast cancer development as the incidence rate is rising rapidly worldwide. As with many other tumours, methods for detection of breast cancer remain inadequate and limited. In Pakistan, women usually present with breast cancer before the age of 40, with a high tumour grading, and have a low prevalence of lymph node involvement.[14] In the current study, a substantial proportion of younger individuals were affected by breast tumour, which was in agreement with an earlier study.[15] Overall, in the present study, Urdu-speaking ethnic group was primarily affected. This ethnicity-specific expression may be useful for preliminary screening of these high-risk patients. The difference in breast cancer incidence rates between most ethnic groups has been largely explained by variations in risk factor distribution.[16] The current study observed that group A experienced pain as presenting complaint, whereas group B presented with painless lump. These findings are in accordance with literature.[17] UOQ was the most affected region in both the study groups. Even though the mechanism remains uncertain, data from literature indicates that increased breast density in UOQ influenced by hormones and growth factors may lead to an increased risk of benign and malignant lesions.[18]

Studies have tried to find histopathological indicators that could predict progression of breast cancer, and have reported that the presence of calcifications and necrosis was associated with aggressive forms of tumour and unfavourable outcomes[19, 20], while tumours with DCIS component had good prognostic outcomes.[21] One study described that the presence of increased levels of

lymphocytic tumour infiltrating cells had a good predictive role, and patients turned out to be more responsive to chemotherapy.[22]

In the current study, further evaluation was done to analyse IHC expression of CHEK2 that was significantly expressed in group B compared to A. Though histo-morphological factors were not related to the elevated expression of the marker, the subjects with high positive tumours were older women having grade II and stage II breast carcinoma. These results are supported by a study.[23] Previously, a Chinese study suggested that CHEK2 mutation was significantly associated with increased breast cancer risk in the Asian population, and early diagnosis would help in managing and designing suitable treatment regimens.[24] However, a Pakistani study of 145 patients with negative BRCA1 and BRCA2 breast cancer proposed that CHEK2 mutations may not significantly contribute to breast cancer risk development.[25]

The current study has some limitations. As this was a single-centre study with a small sample size group of population, the findings may not be generalised. Due to financial limitations, a single gene marker was used instead of multiple genes for the comparison of expression and analysis in breast carcinoma.

Conclusion

CHEK2 expression in breast cancer cases showed remarkable connotation with pathogens of breast carcinoma, while those with fibroadenoma exhibited the reverse. To achieve individualised targets for treatment, CHEK2 genetic marker assessment could be of great significance in clinical management and prognostication.

Acknowledgement: We are grateful to Prof (Dr) Shahid Rasul and to the staff of Surgical Wards 3 and 21, JMPC, Karachi, for facilitating the study.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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AUTHORS' CONTRIBUTIONS:

TF: Research plan, experiment conduction, data analysis, drafting and final approval.
ZH: Design, critical review, data interpretation, drafting and final approval.
SA: Drafting, critical review and data analysis.
SH: Data collection, literature review, experimental work, editing and review.
SZ: Data collection, literature review, experimental work and review.
FN: Data collection and literature review.