Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 12, 2023

Role of Immunohistochemistry of Cyclooxygenase –2 Expression in Breast Carcinoma

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Abstract

Background: In 21st century, Cancer has emerged as the one of the major public health problem across the globe. It exerts a significant impact on everyone involved ranging from individual affected to their families, communities and healthcare systems. According to GLOBOCON 2020, female breast cancer has surpassed the lung cancer as the most common cancer across worldwide with an estimate of 2.3 million new cases (11.7%) being diagnosed followed by lung cancer (11.4%). **Objectives:** The present study was conducted to study the overexpression of cyclooxygenase-2 (COX-2) in the patients of breast cancer and its association with the clinical, pathological data, including age, size, site of tumor, histological type, grade, lymph node metastasis and stage of tumor. **Material and Methods:** This was a Prospective Study carried out in a Tertiary Care Centre situated at western part of India. The study duration was 1 year, and the period was from November 2022 to November 2023 with the department of Pathology after obtaining clearance from the institutional ethics committee and written informed consent from the study participants. All patients diagnosed with breast carcinoma attending Tertiary Care Centre were included. Exclusion criteria included tru-cut biopsy, breast excisions without axillary evacuation, lumpectomies without axillary evacuation, cases with no residual invasive carcinoma after pre-surgical (neoadjuvant) chemotherapy or with massive necrosis or fibrosis. A p-value of <0.05 was considered statistically significant. **Results:** Out of 20 patients, 85% of patients were females and rest 15% males. ER positivity was seen in 85% of cases, while PR positivity was observed in 75% of cases. HER-2/neu overexpressed was seen in 20% while COX-2 overexpression on immunohistochemistry was seen in 10% patients. The association between COX-2 overexpression and ER negative was statistically significant ($p = 0.016$). **Conclusion:** It can be concluded from the present study that the inhibition of COX-2 expression may lead to retard tumour progression and thus, limiting breast carcinogenesis which may have therapeutic implications for the prevention and treatment of breast cancer in such patients with high risk.

Keywords: COX-2, Breast Cancer, HER2/neu, Cancer.

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Introduction

In 21st century, Cancer has emerged as the one of the major public health problem across the globe. It exerts a significant impact on everyone involved ranging from individual affected to their families, communities and healthcare systems. Apart from these, cancers are one of the leading cause of morbidity and mortality among the general population. Rising trends of cancers could be contributed to many factors such as changing demographics of population, lifestyle changes such as unhealthy diet, physical inactivity, tobacco and alcohol use, environmental pollution and exposure to different carcinogens agents.[1]

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According to GLOBOCON 2020, female breast cancer has surpassed the lung cancer as the most common cancer across worldwide with an estimate of 2.3 million new cases (11.7%) being diagnosed followed by lung cancer (11.4%).[2] Female breast cancer is also fourth leading cause of death among all cancers. In India, female breast cancer is also the most common cause of cancer across all ages and gender, newly diagnosed cases contributing 13.5% of total cases.[3] The incidence of breast cancer is almost 1-in-9 women, requiring tissue removal, chemotherapy, radiotherapy and hormone therapy most of the time.[4]

Breast cancer is a type of tissue cancer that mainly involves inner layer of milk glands or lobules, and ducts (tiny tubes that carry the milk).[5] Age,[6] high hormone levels,[7] race, economic status, and iodine deficiency in diet,[8,9,10] are major risk factors that are associated with breast cancer. The prognosis of breast cancer depends on various biological and molecular factors.[11,12] The enzymes in the cyclooxygenase (COX) family play a crucial role in the transformation of arachidonic acid into prostaglandins. In most tissues, cyclooxygenase-1 (COX-1) is constitutively expressed at a level that is constant throughout the cell cycle. The inducible isoform, Cyclooxygenase-2 (COX-2), is often overexpressed in breast cancer.[13]

Because of HER-2/neu gene amplification, 20–30% of breast cancer tumors overexpress the human epidermal growth factor receptor type 2 (HER-2).[14] In colorectal cancer cells, HER-2/neu stimulates COX-2 expression through the action of mitogen-activated protein kinase (MAPK).[15] This same mechanism of action is observed when HER-2/neu is transfected into breast cancer cells. (16,17) ER-negative breast cells may be stimulated to produce COX-2 either the RAS/MAPK pathway or through the protein kinase C (PKC) pathway. (18) In human breast cancer, COX-2 expression has been associated to angiogenesis and lymph node metastasis.[19]

The present study was conducted to study the overexpression of cyclooxygenase-2 (COX-2) in the patients of breast cancer and its association with the clinical, pathological data, including age, size, site of tumor, histological type, grade, lymph node metastasis and stage of tumor. Apart from this, the association between COX-2 and expression of ER, PR and HER-2/neu status.

Methodology

Patient Selection

This was a prospective study carried out in a Tertiary Care Centre situated at western part of India. The study duration was 1 year, and the period was from November 2022 to November 2023 with the department of Pathology after obtaining clearance from the institutional ethics committee and written informed consent from the study participants. The sample size was 20, all patients diagnosed with breast carcinoma attending tertiary care centre were included. Exclusion criteria included tru-cut biopsy, breast excisions without axillary evacuation, lumpectomies without axillary evacuation, cases with no residual invasive carcinoma after pre-surgical (neoadjuvant) chemotherapy or with massive necrosis or fibrosis.

Specimen Processing

Specimens were received from surgical department post mass radical mastectomy (MRM) or lumpectomy in 10% buffered formalin.

Staining Methods: The staining method used to stain specimen included hematoxylin and eosin stain (H&E) and immunohistochemistry (IHC) staining for establishing ER, PR, HER2/neu and COX-2 status of specimen.

Statistical Analysis

Collected data was entered into MS-Excel and was analyzed using Statistical package for Social Sciences (SPSS) version 25.0. Categorical data was expressed as frequency or proportions and quantitative data as mean and standard deviation. Chi-square test or Fisher's exact test were used as test of significance for categorical variables and unpaired t-test was

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used for mean and standard deviation. A p-value of <0.05 was considered statistically significant.

RESULTS

The mean age (mean ± standard deviation) of the patients was 52.45 ± 10.71 years with range 27 -68 years and median age was 55.50 years. Majority of patients were female (85%) and rest were males (15%). Out of total 20 patients, 95% (19) of patients had invasive ductal carcinoma and only 1 patient (5%) had infiltrating ductal carcinoma. ER-positive tumors constituted 85% of total tumors examined; the remaining 15% were ER-negative tumours. Similarly, tumours expressing progesterone receptor (PR) were found to be expressed in 75% of samples while 25% were PR negative.

HER-2/ neu expression was positive in only 20% of the patients while 80% were negative for same. Similarly, COX2 overexpression was seen in 10% of the samples and 90% of the sample had no overexpression. The histological grades were measured by the Modified Bloom-Richardson Grading Scheme. Majority of tumours (90%) were of Grade 1 and remaining 5% were Grade 2 and not otherwise specified (NOS). (Table 1)

Table 2 shows the test of association between COX-2 status and different clinicopathological characteristics. The association between ER status being positive and COX-2 being negative was found to be statistically significant (p = 0.016). Similarly, for HER-2/neu status being negative with COX-2 negative was found to be associated and was statistically significant (p = 0.032). However, the association between PR and COX-2 was not found to be statistically significant (p = 0.053).

Figures 1 to 5 show different microscopic slides examined showing different types of positivity seen in our study. Figure 1 and 2 shows the cells being positive for ER while figure 3 shows the positive status for PR seen on microscopic examination in our study. Figure 4 shows the membrane positive status for HER-2/neu expression in cancerous cells examined. Figure 5 depicts the positivity of cytoplasmic COX-2 seen in the one case examined in the present study.

Table 1: Clinicopathological details

Clinicopathological Variables	Frequency (n)	Percentage (%)
Age		
≤ 50 years	07	35
> 50 years	13	65
Gender		
Females	17	85
Males	03	15
Histology Type		
Invasive Ductal Carcinoma	19	95
Infiltrating Ductal Carcinoma	01	05
ER Status		
Positive	17	85
Negative	03	15
PR Status		
Positive	15	75
Negative	05	25
HER-2/ neu		
Positive	04	20

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Negative	16	80
COX-2 Overexpression		
Positive	02	10
Negative	18	90
Histological Grade		
Grade 1	18	90
Grade 2	01	05
NOS	01	05

Table 2: Association between COX-2 and different Clinicopathological variables

Clinicopathological Variables	**COX-2**		**p-value***
ER status	Positive	Negative	
Positive	00	17	0.016
Negative	02	01	
PR Status			
Positive	00	15	0.053
Negative	02	03	
HER-2/ neu			
Positive	02	02	0.032
Negative	00	16	

** p-value <0.05 statistically significant; Fisher's Exact test used*

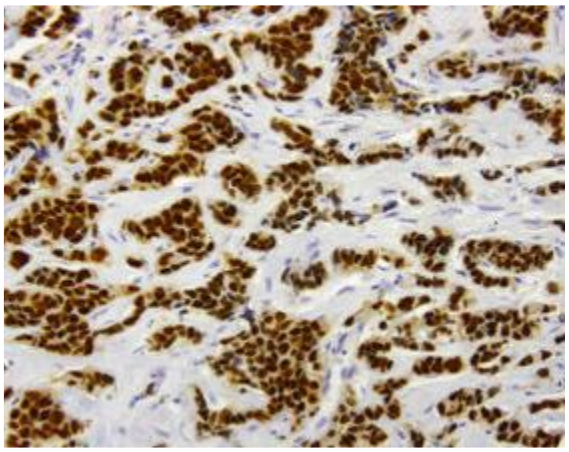

Figure 1: ER Positive staining showing Nuclear Staining (40X)

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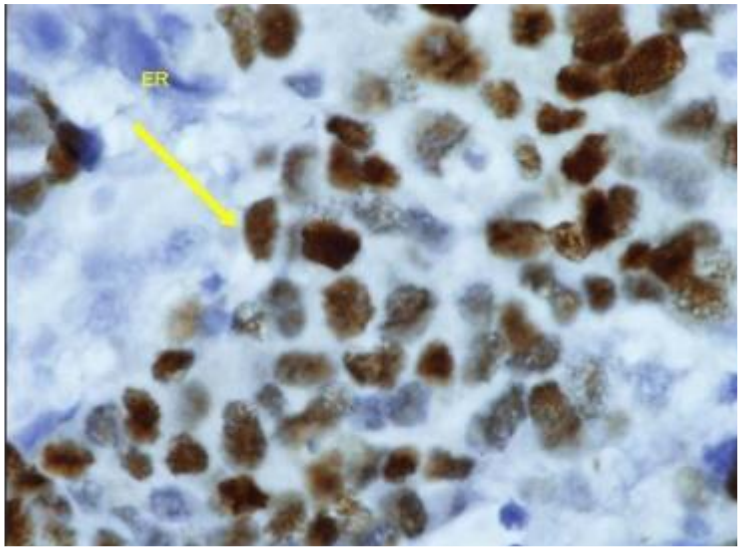

Figure 2: ER Positive Staining showing Nuclear staining (on Higher Magnification)

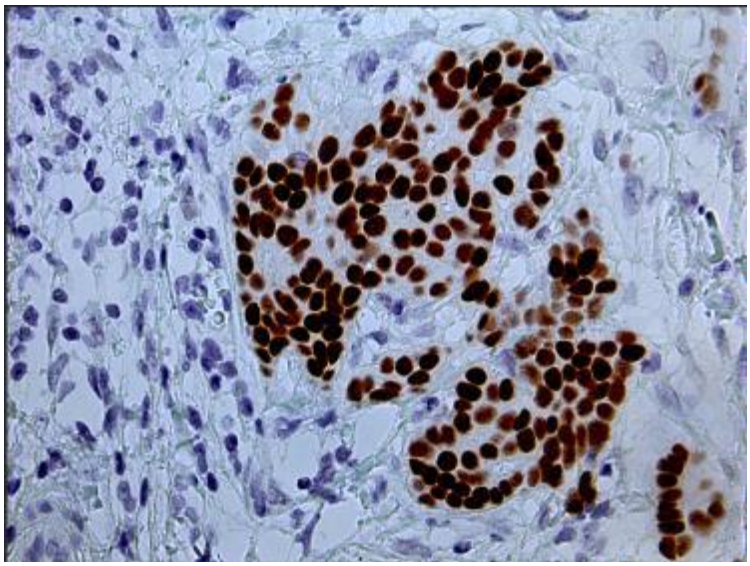

Figure 3: PR Positive Staining showing Nuclear Staining (40X)

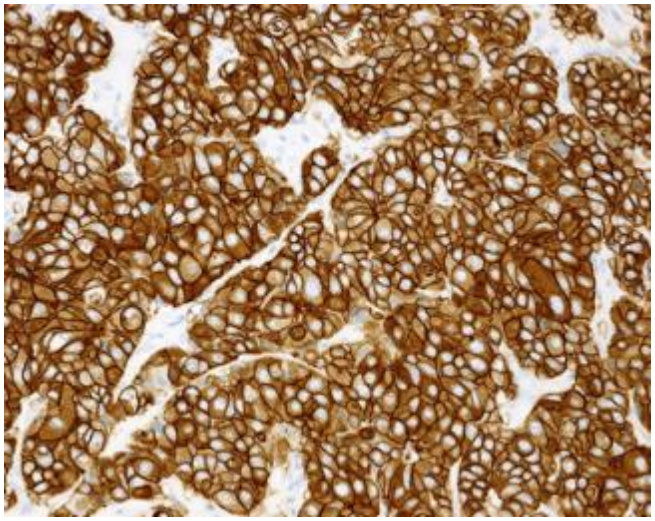

Figure 4: HER-2/neu showing Membrane Positive (40X)

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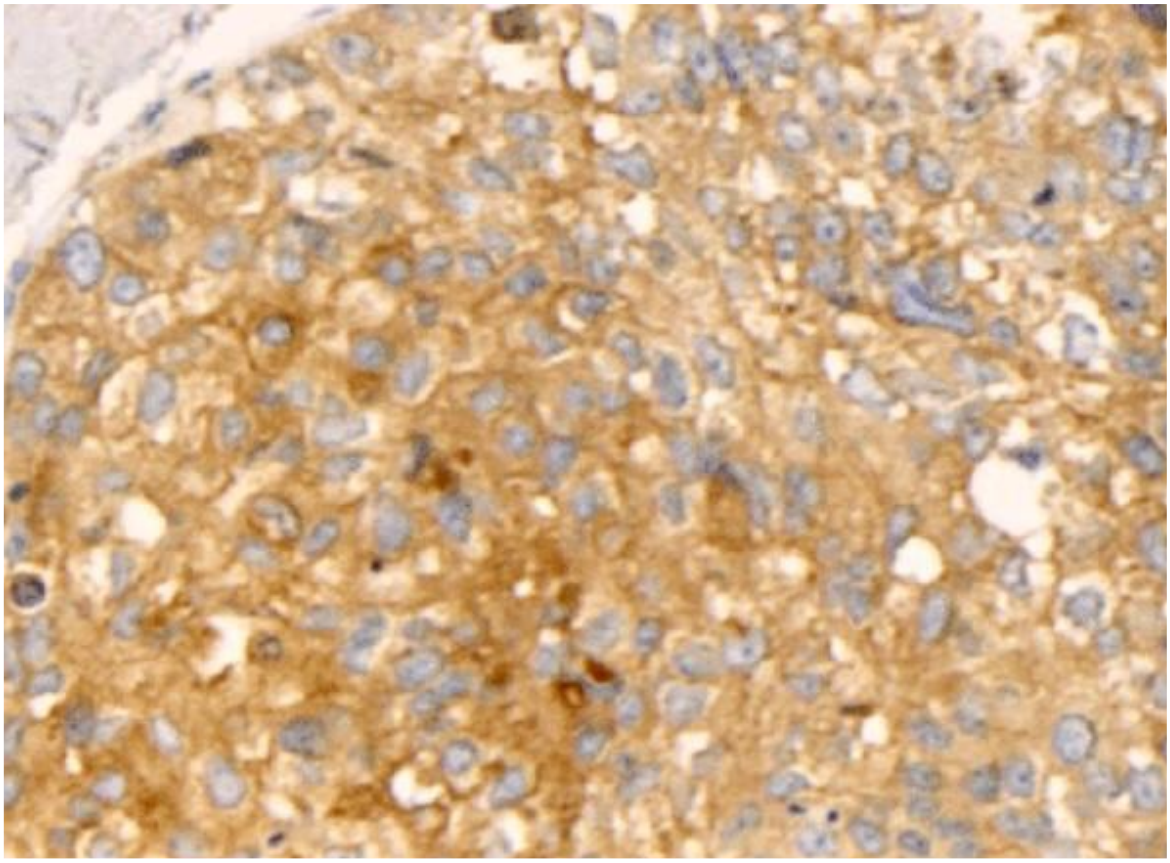

Figure 5: Ductal Carcinoma in situ, showed strong cytoplasmic COX-2 expression in tumor cells (on higher magnification)

DISCUSSION

Several studies have reported that COX-2 has been shown to be expressed in ductal carcinoma in situ and invasive ductal carcinoma but not in the normal breast tissues.[20-22] In our study, we found that COX-2 overexpression was seen in patients with ER and PR negative cell lines while it was highly correlated with HER-2/neu overexpression. Similar results were also obtained by Jana et al,[23] in whose study it was found that COX-2 overexpression was highly correlated with HER-2/neu overexpression and absence of ER and PR expression. HER-2/neu is known to be a poor prognostic factor in breast cancer, and its relationship to the regulation of COX-2 expression has also been suggested in some studies.[15,23]

Cyclooxygenase (COX) are group of enzymes that are responsible for conversion of arachidonic acid to prostaglandins which plays an important role in mediation of inflammatory process throughout the human body. COX-2 plays an important part in the regulation of tumour growth, invasion and metastasis in breast cancer. Our study showed that the association between COX-2 expression in human breast cancer against the expression of ER, PR and HER-2/neu clinicopathological parameters.

If COX-2 expression is an early initiating event in the development of breast cancer, then the different and novel therapeutic strategies can be considered in the treatment of such individuals. No study is totally complete in itself and therefore, the present study also has its own limitations. Firstly, the sample size of the present study was too small and therefore, to establish a stronger association between COX-2 expression and prognosis, a study with larger sample size needs to be carried out. Secondly, the results can not be generalized as the sample size is too small.

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CONCLUSION

It can be concluded from the present study that the inhibition of COX-2 expression may lead to retard tumour progression and thus, limiting breast carcinogenesis which may have therapeutic implications for the prevention and treatment of breast cancer in such patients with high risk. Selective COX-2 inhibitors may be useful in the chemoprevention and adjuvant therapy of breast cancer.

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