

Immunohistochemical Expression of Cyclooxygenase-2 (COX-2) in Breast Cancer

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ABSTRACT

Background: breast cancer (BC) is the most common type of diagnosed cancers in women. It is still the second leading cause of cancer-related death among women after lung cancer all over the world. Breast cancer is the first of top ten cancers in Egypt. It ranks as the first malignancy affecting females, contributing 30% of all female cancers. It affects 1 in 14 women during their life time.

Aim: This study investigated the association between cyclooxygenase-2 (COX-2) expression in female breast cancer versus the expression of ER, PR, as well as its association with other established prognostic indicators like age, tumor size, lymph nodal status, stage, grade, lymphovascular invasion, insitu component and histological subtype, and aims to validate the role of overexpression of COX-2 as a prognostic marker in female patients with breast cancer in Egypt.

Results: High significant correlation was found between lymph node metastasis, negative ER and PR cases and COX-2 expression. No significant correlation could be detected between age, tumor size, site, histologic type, grade, insitu component, LVI and COX-2 expression.

Conclusion: Cyclooxygenase-2 has poor prognostic parameter in breast cancer, as it is over expressed in majority of breast carcinoma, especially with lymph node metastasis, ER and PR negative hormone receptor.

Keywords: Breast cancer, Cyclooxygenase-2, prognostic marker, immunohistochemistry (IHC), ER\PR receptor, clinicopathological data.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the most common cause of cancer death among women. It accounts for nearly (23%) of all female cancers worldwide, with more than a million new cases each year. Although BC incidence rates in Egypt are substantially lower than the rates in the United States and other developed countries, breast cancer is the most common cancer among women in Egypt. Prognosis of breast cancer depends on different factors as tumor size, histologic type, stage, and molecular profile ⁽¹⁾.

Cyclooxygenase (COX) group of enzymes are important for the conversion of arachidonic acid to prostaglandins. Cyclooxygenase-1 (COX-1) is constitutively expressed at a constant level throughout the cell cycle in most of the tissues. The inducible isoform, Cyclooxygenase-2 (COX-2), is often over expressed in breast cancer ⁽²⁾.

Cyclooxygenase-2 derived metabolites may contribute to maintenance of premalignant hyperproliferation, transformation, tumor viability, growth, invasion and metastatic spread ⁽³⁾. Cox-2 and its products, particularly prostaglandin E₂ (PGE₂), act via classical cancer signaling pathways in primary tumor cells to promote tumorigenesis. Evidence has shed a spotlight not only on the tumor cell itself, but also the tumor microenvironment, especially macrophages in the tumor ⁽⁴⁾. Over-expression of COX-2 has been detected in several malignancies including breast cancer, and contributes to carcinogenesis by stimulating cancer cell proliferation, inhibiting apoptosis, increasing invasiveness and modulating inflammation and im-

munity ⁽⁵⁾. Clinical studies have noted a reduced risk for breast, lung, prostate, and colon cancers after treatment with non-selectively COX-2 inhibition by non-steroidal anti-inflammatory drugs (NSAIDs) or selective COX-2 inhibition with COX-2 inhibitors ⁽⁶⁾.

It has been reported that elevated COX-2 expression was more common in larger size breast cancer tumors with axillary lymph node metastasis ⁽⁷⁾. More studies are needed to confirm the potential role of COX2 in breast cancer prognosis and studying the role of COX2 inhibitors in reducing the risk of breast cancer ⁽⁸⁾.

AIM OF THE WORK

To study the expression of (COX-2) in breast cancer and the relationship of its expression with the clinicopathological data of the studied cases, including (age, size, site of tumor, histological type, grade, lymph node metastasis, stage of tumor, estrogen and progesterone positive or negative tumor).

MATERIAL AND METHODS

This retrospective study was carried out on 50 cases of Egyptian (BC) patients. These cases were selected from archives of Pathology department, Faculty of Medicine, Assiut University, Aswan University and private laboratories from January 2014 till reaching the target of cases. Cases were collected as paraffin embedded blocks. The selected cases included modified radical mastectomy (MRM) and conservative breast surgery either lumpectomy or wide excision with axillary evacuation.

The study was approved by the Ethics Board of Aswan University and an informed written consent was taken from each participant in the study.

The following cases with breast cancer were not suitable for this study: true cut biopsy, breast excisions without axillary evacuation, lumpectomies without axillary evacuation, cases with no residual invasive carcinoma after pre-surgical (neoadjuvant) chemotherapy, cases consisting predominately of CIS with microinvasion, cases with massive necrosis or fibrosis and male patient.

Clinical data: Clinical data were collected from pathology reports including (age, site, size of tumor and type of surgery). Two serial sections from each tissue block were cut at a 4 microns thickness to be used as follows: One section was stained with routine Haematoxylin and Eosin (H & E) to evaluate the histopathologic diagnosis and the other one was stained with COX2 antibodies.

Histopathological examination: All cases were examined using a light microscope. The following items were recorded: Histopathological type: according to WHO classification ⁽⁹⁾. IDC (NST), ILC, mixed ductal and lobular carcinoma, mucinous carcinoma, medullary carcinoma. Histological grade: Nottingham histological score ⁽¹⁰⁾. In situ component. Lymph node metastasis. Lympho vascular invasion. Staging of breast carcinoma (T.N.M classification for staging of breast cancer is provided before).

ER & PR stained slides: All cases were examined for their ER & PR status according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) ⁽¹¹⁾ guideline recommendations for IHC testing of ER& PR in breast cancer.

Uninterpretable: if finding that no tumor nuclei are immune reactive, and that internal control or normal epithelial elements present in the sample lack any nuclear staining.

Immunohistochemical staining: Sections of 4 µm thickness, from each case were stained for Cyclooxygenase 2 (COX-2). Negative control slide was run by omitting the primary antibody. Positive control was colorectal carcinoma. The method used for immunostaining was streptavidin-biotin amplified system. The primary antibodies used is Rabbit monoclonal antibody against COX-2, cat. #RM-9121-S0 (Thermo scientific, Fremont, CA 94538-6406, USA), 0.1 ml concentrated with dilution of 1:100.

Evaluation of COX-2 immunostaining:

For the evaluation of cytoplasmic staining results for COX-2, a predefined scoring system based on the product of staining intensity and percentage of positive tumor cells was used ⁽¹²⁾. Immunoreactivity for COX-2 in tumor cells was assessed using a scoring system based on staining intensity:

Staining 0 and 1+ were classified as "COX-2 negative", whereas staining 2+ and 3+ as "COX-2 positive" ⁽¹³⁾.

Confidentiality of the data:

No encroach on private data of the patient, as blocks of tumor tissue are going to be signed with serial code numbers instead of the name of the patient. Also, the results of the research will be used only in scientific aims.

Statistical analysis of the data

Data were collected, tabulated and statistically analyzed using a personal computer with Statistical Package for Social Science (SPSS) version 20.0. (Armonk, NY: IBM Corp) ⁽¹⁴⁾. Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level ⁽¹⁵⁾.

The used tests were:

- 1 - **Chi-square test (X²- test):** was used to compare between qualitative data such as different tumor grade and stage.
- 2 - **Fisher's Exact or Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5
- 3 - **Student t-test:** Used in comparing one quantitative variable and one qualitative variable if they were normally distributed.
- 4 - **F-test (ANOVA):** For normally distributed quantitative variables, to compare between more than two groups
- 5 - **Mann Whitney test:** For abnormally distributed quantitative variables, to compare between two studied groups
- 6 - **Kruskal Wallis test:** For abnormally distributed quantitative variables, to compare between more than two studied groups.

Probability (P- value) ⁽¹⁶⁾: difference considered as follow:

Statistically significant (S) when (P < 0.05).

Highly significant (HS) when (P < 0.01). Not significant (NS) when (P > 0.05).

RESULTS

- Assessment of IHC expression of COX-2 was done in malignant epithelial cells for staining status, pattern and intensity.
- All positive cases showed cytoplasmic pattern of expression. 43 cases out of 50 (86.0%) showed positive expression of COX-2, while 7 cases (14.0%) showed negative expression (table 2).
- No significant correlation could be detected between age, tumor size, site, histologic type, grade, in situ component, LVI and COX2 expression (table 3).
- High significant correlation was found between lymph node metastasis (P=0.009), negative ER and PR cases (P ≤ 0.001) and COX2 expression (table 4)

Table (1): Distribution of the studied group as regard age, Site of tumor, histologic type, histologic grade, tumor size, in situ component, T.N.M and Lymphovascular invasion

	No.	%
Age (years)		
<50	17	34.0
≥50	33	66.0
Total	50	100.0
Min. – Max.	25.0 – 77.0	
Mean ± SD.	52.18 ± 12.21	
Median	54.0	
Site of tumor		
UOQ	31	62.0
UIQ	4	8.0
LOQ	3	6.0
LIQ	5	10.0
Retro-areolar	7	14.0
Diagnosis		
Invasive duct carcinoma (NST)	35	70.0
Invasive lobular carcinoma	9	18.0
Medullary carcinoma	3	6.0
Mucinous carcinoma	2	4.0
Metaplastic carcinoma	1	2.0
Histologic grade		
I	1	2.0
II	37	74.0
III	12	24.0
Tumor size		
<2	6	12.0
2 – 5	36	72.0
>5	8	16.0
Min. – Max.	1.0 – 8.0	
Mean ± SD.	3.73 ± 1.67	
Median	3.50	
In situ component		
No	17	34.0
Yes	33	66.0
Pathologic stage		
T1	8	16.0
T2	31	62.0
T3	8	16.0
T4	3	6.0
N0	14	28.0
N1	9	18.0
N2	12	24.0
N3	15	30.0
Lymphovascular invasion		
No	34	68.0
Yes	16	32.0

UOQ : Upper outer quadrant
quadrant

LOQ : Lower outer quadrant

UIQ: Upper inner quadrant LIQ: Lower inner

Table (2): Distribution of the studied cases according to ER expression, PR expression, Cox2 expression

	No.	%
ER		
Negative	30	60.0
Mild positive	4	8.0
Moderate positive	6	12.0
Strongly positive	10	20.0
PR expression		
Negative	32	64.0
Mild positive	6	12.0
Moderate positive	3	6.0
Strongly positive	9	18.0
Cox2		
Negative	7	14.0
Positive	43	86.0

Table (3): Relation between Cox2 expression and age, Site of tumor, histologic type, histologic grade, in situ component and Lymphovascular invasion

		Cox2				Test of Sig.	P
		Negative (n = 7)		Positive (n = 43)			
		No.	%	No.	%		
Age (years)	<50	2	12	15	88	$\chi^2=$	^{FE} p=
	≥50	5	15	28	85	0.107	1
	Min. – Max.	32.0 – 70.0		25.0 – 77.0		t =	0.97
	Mean ± SD.	52.0 ± 11.68		52.21 ± 12.43		0.042	
	Median	55		54			
Site of tumor	UOQ	5	16.1	26	83.9	4.723	0.22
	UIQ	2	50	2	50		
	LOQ	0	0	3	100		
	LIQ	0	0	5	100		
	Retroareolar	0	0	7	100		
Diagnosis	Invasive duct carcinoma NST	6	17.1	29	82.9	0.957	0.66
	Invasive-Lobular carcinoma	0	0	9	100	1.787	0.33
	Medullary carcinoma	0	0	3	100	0.52	1
	Mucinous carcinoma	1	50	1	50	2.243	0.26
	Met aplastic carcinoma	0	0	1	100	0.166	1
	c²(^{MC}p)	4.337(0.336)					
Histologic grade	I	0	0	1	100	0.864	1
	II	5	13.5	32	86.5		
	III	2	16.7	10	83.3		
In situ component	No	3	17.6	14	82.4	0.285	0.68
	Yes	4	12.1	29	87.9		
Lymphovascular invasion	No	4	11.8	30	88.2	0.441	0.67
	Yes	3	18.8	13	81.3		

χ^2 , p: χ^2 and p values for **Chi square test** ^{FE}p: p value for **Fisher Exact** for Chi square test

^{MC}p: p value for **Monte Carlo** for Chi square test t, p: t and p values for **Student t-test**

- No significant statistical association could be detected between COX2 expression clinicopathological parameter such as age, site of tumor, size, grade, insitu component and lymphovascular invasion.

Table (4): Relation between Cox2 expression and lymph node status, Pathologic stage, ER expression and PR expression.

	Cox2				χ^2	^{FE} p
	Negative (n = 7)		Positive (n = 43)			
	No.	%	No.	%		
Lymph node status						
Negative	6	31.6	13	68.4	7.962*	0.009*
Positive	1	3.2	30	96.8		
Pathologic stage						
Stage T						
T1	0	0.0	8	100.0	1.719	0.693
T2	6	19.4	25	80.6		
T3	1	12.5	7	87.5		
T4	0	0.0	3	100.0		
Stage N						
N0	1	7.1	13	92.9	8.537*	0.010*
N1	1	11.1	8	88.9		
N2	5	41.7	7	58.3		
N3	0	0.0	15	100.0		
ER expression						
Negative	0	0.0	30	100.0	13.824*	0.001*
Mild	1	25.0	3	75.0		
Moderate	2	33.3	4	66.7		
Strongly	4	40.0	6	60.0		
PR expression						
Negative	0	0.0	32	100.0	21.165*	<0.001*
Mild	0	0.0	6	100.0		
Moderate	2	66.7	1	33.3		
Strongly	5	55.6	4	44.4		

χ^2 , p: χ^2 and p values for **Chi square test** ^{MC}p: p value for **Monte Carlo** for Chi square test

*: Statistically significant at $p \leq 0.05$ ^{FE}p: p value for **Fisher Exact** for Chi square test

High significant correlation was found between lymph node metastasis ,negative ER and PR cases and COX2 expression.

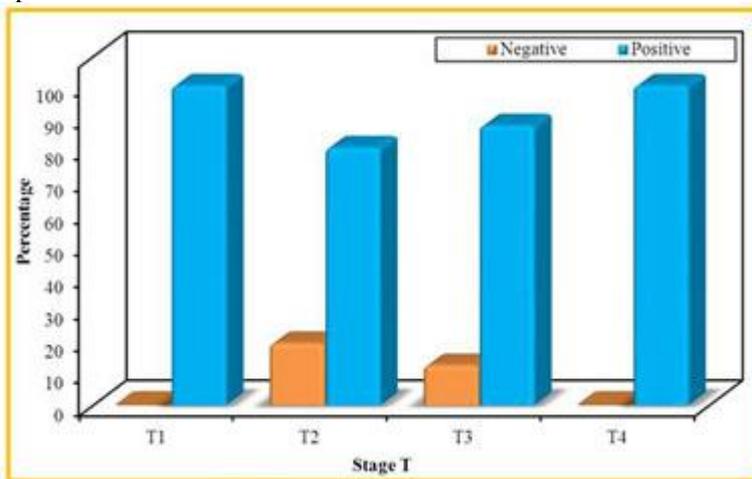


Figure (1): Relation between COX2 expression and stage T.

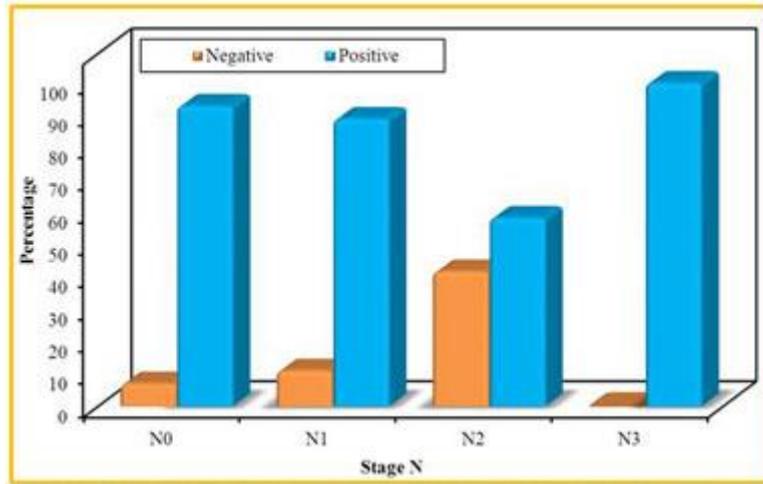


Figure (2): Relation between Cox2 expression and stage N.

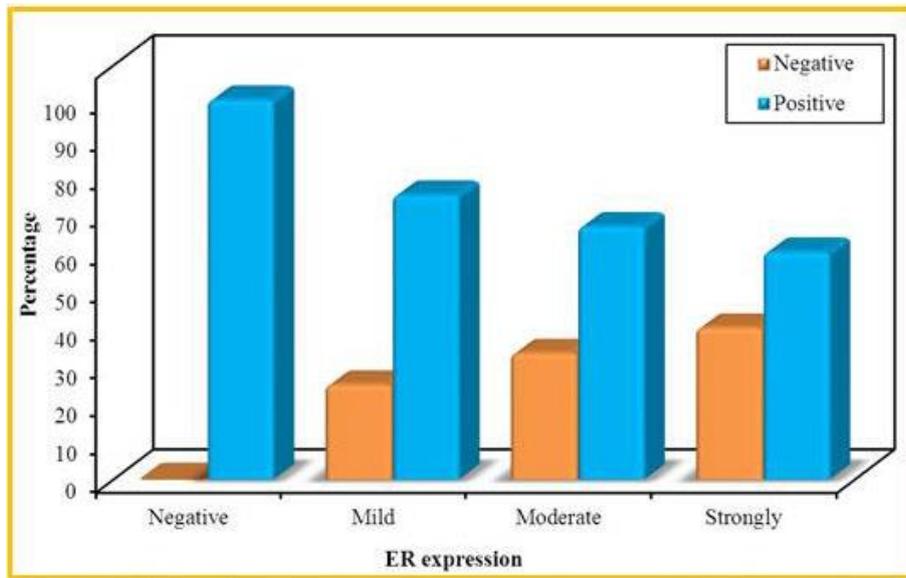


Figure (3): Relation between Cox2 expression and ER expression.

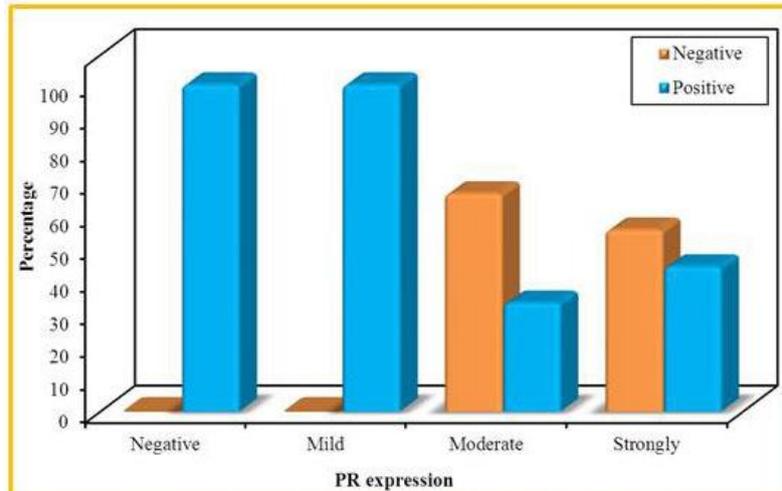


Figure (4): Relation between Cox2 expression and PR expression.

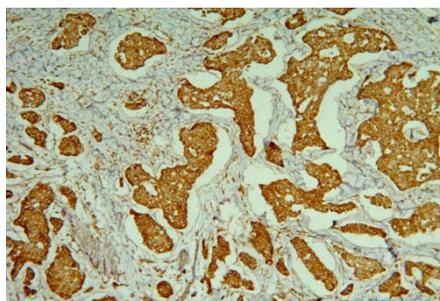


Figure (5): invasive duct carcinoma, showed strong cytoplasmic COX2 expression in tumor cells (IHC X 100).

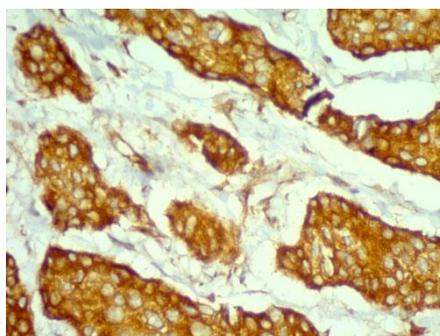


Figure (6): High power of previous case shows strong cytoplasmic expression of COX2 in tumor cells (IHC X400).

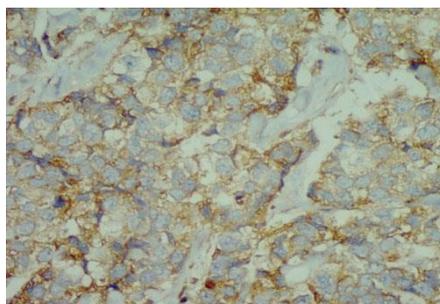


Figure (7): IDC, shows mild cytoplasmic expression of COX2 in tumor cells (IHC X400).

DISCUSSION

Breast cancer is the second leading cause of cancer-related death among women after lung cancer all over the world ⁽¹⁷⁾. Breast cancer represents 38.8% of cancers occurring in Egyptian females in 2014 and it increases linearly as population increase ⁽¹⁸⁾.

Evidence indicates a role for inflammation in the development of different steps of cancer progression; the incidence of several cancers is tightly associated to inflammation such as colon, breast, and prostate cancers ⁽¹⁹⁾.

The aim of the current study was to evaluate IHC expression of COX-2 in tumor cells and their effect on prognosis of breast cancer.

In this study, cases were females of age ranging from 25 to 77 years. The mean age of the cases studied was 52 years, while the median age was 54 years. These results are comparable to those reported by *Jana et al.* ⁽²⁰⁾ who made studied Cox2 in 123 female of primary breast carcinoma. They reported that the mean age was 50.78 ± 8.81 years with range of 37–75 years and the median age was 50 years.

For histologic type, the current study denotes that most of studied cases were IDC (NST) (70.0%). This agrees with *Rakha et al.* ⁽²¹⁾ who reported that the

majority (60% to 75%) of breast cancers have no special type of characteristics.

In this study, about two thirds of the cases showed in situ component (DCIS/LCIS) (66%). DCIS, the preinvasive form of breast cancer, forms a major part of breast surgery practice because of its liability to detection via screening mammography *Half et al.* ⁽¹²⁾.

For tumor grade, grade II (moderately differentiated) tumors were found to represent the highest percentage (74.0%) of cases in the current research. Tumor grade is an important determinant of breast cancer outcome and complementary to lymph node (LN) stage through the ability to influence the outcome of patients in different LN stage categories. Studies have also demonstrated that grade is an independent prognostic factor in specific subgroups of breast cancer patients ⁽²²⁾.

The tumor size in this study ranged from 1 to 8 cm with a mean of 3.73 cm and a median of 3.5 cm. For node-negative patients, tumor size is the most powerful prognostic factor and is routinely used to make adjuvant treatment decisions ⁽²³⁾.

About one third of the studied cases showed LVI invasion (32.0%). The existence of LVI may help identify who is at increased risk for axillary lymph

node and distant metastasis ⁽²⁴⁾. Although LVI has been accepted as an important prognostic factor in patients with lymph node-negative invasive breast cancer ⁽²⁵⁾, LVI is controversial regarding prognostic value in patients with lymph node-positive breast cancer ⁽²⁶⁾.

In the current study, Cox2 expression using immuno-histochemical staining was detected in the majority of the studied cases (86%) regardless their histological type with /without in situ component. Some studies reported its over expression ranged from 63-95% ⁽²⁷⁾. On the other hand, other studies showed lower percentage that ranged from 37-56% ⁽¹⁷⁾ and these differences between various studies may be due to different cutoffs and variations or differences in scoring systems ⁽²⁸⁾.

On correlation of COX-2 expression with clinicopathological variables, no significant statistical association between COX-2 expression in tumor cells and other clinicopathologic parameters including age, tumor size, tumor location, histologic type, tumor grade and lymphovascular invasion. These results agree with *Kelly et al.* ⁽²⁹⁾ who studied the expression of COX-2 by immunohistochemistry in 106 primary breast carcinoma specimens and found that no statistically significant correlation between cox2 and tumor size, grade, histology and nodal status. Also, *Ranger et al.* ⁽³⁰⁾ observed a significant association between COX-2 overexpression and distant metastasis, but no significant association with any other clinical or pathological variable.

In contrast, some of these studies showed that COX-2 expression was associated with larger tumor size, high nuclear grading, poor differentiation, and lymphovascular invasion ⁽²⁸⁾. *Shim et al.* ⁽⁷⁾ observed COX-2 over expression in 46 out of 64 (72%) breast cancers and studied the correlation of COX-2 expression with various clinic pathologic parameters, including tumor size, lymph node status, stage, ER and PR expression, and nuclear and histologic grade. Larger size and advanced stage in tumors were significantly correlated with COX-2 over expression.

In this study no significant statistical correlation could be detected between COX2 expression and insitu component. This result agrees with *Kelly et al.* ⁽²⁹⁾ and *Ranger et al.* ⁽³⁰⁾. Also, the study by *Jana et al.* ⁽²⁰⁾ found that the histology of lobular carcinoma and ductal carcinoma in situ did not show any significant difference in COX-2 expression.

However, in the present work majority of cases reported in situ component showed COX2 expression (87.9%). Two third of the cases with in situ component expressed COX 2 with score 4. The study by *Half et al.* ⁽¹²⁾ hypothesized that COX-2 up-regulation is an early event in carcinogenesis and thus is overexpressed in pre-invasive lesions, Women with initial DCIS lesions that express COX-2 have a higher risk for developing subsequent invasive cancer in compari-

son to those with COX-2 negative DCIS ⁽³¹⁾. The discrepancy between different results may be due to different methods used for evaluation of COX-2 expression and COX-2 scoring as well as small number of studied cases.

The present work revealed that high significant correlation between lymph node positive cases and COX-2 expression (P = 0.009). Also, high significant association was detected between COX-2 expression and pathologic stage of lymph node (P= 0.01).

Several studies show significant correlation between COX2 expression and lymph node metastasis. For example, *Costa et al.* ⁽³²⁾ indicated that significant association could be found between COX2 expression and lymph node metastasis, which may suggest that COX-2 over expression is associated with aggressive features and poor prognostic parameters in breast cancer.

According to the current work, there is high significant association between COX2 over expression and ER negative cases (P=0.001) and PR negative cases (P< 0.001). These results agree with *Kargi et al.* ⁽³³⁾ and *Jana et al.* ⁽²⁰⁾.

The study of *Jana et al.* ⁽²⁰⁾ found that high levels of COX-2 expression correlated with absence of ER and PR expression. COX-2 expression in ER negative cell lines is also associated with mutated RAS. Increased expression of this protein has been associated with reduced estrogen dependence in breast cells. Both PKC and mutated RAS have been associated with an increased metastatic potential in cell lines ⁽²⁰⁾.

It is established that PR negative, ER negative cases of breast cancer are known to be of bad prognosis and that agree with the bad prognostic behavior of COX-2 in breast cancer ⁽²⁰⁾.

CONCLUSION

In this study expression of COX2 is not statistically significant with clinico-pathological parameters; the age, histologic type, tumor size or grade. However it is highly significant with cases of negative ER &PR as well as lymph nodal metastasis. This indicates it is associated with poor prognosis. This observation may points to the need to consider anti COX2 treatment modalities in treating the patient.

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