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Immunohistochemical Cyclooxygenase-2 (COX-2) and P53 Expression in Breast Carcinoma with Correlation to Clinico-Pathological Parameters

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Abstract

Background/Aims: Breast cancer is the most common cancer in Egyptian women.

This Study Aims to:

1- Evaluate COX-2 and p53 expressions in the successive steps of breast carcinogenesis.

2- Determine the correlation between COX-2 and p53 with the clinico-pathological parameters in ductal breast carcinoma.

Patients and Methods: This study included 74 specimens of breast lesions. Data about patient's age, tumor size and local aggressive changes, history of recurrence and/or presence of distant metastasis were obtained from clinical sheets. Hematoxylin and Eosin (H&E) stained sections were evaluated for histopathological tumor type, tumor grade, presence or absence of normal, hyperplastic, in situ component, lymphocytic infiltration, lymphovascular invasion, and axillary lymph node status. COX-2 and p53 immunostaining was done to detect their expressions using the avidin-biotin peroxidase method.

Results: COX-2 and p53 expressions increased with increasing grade of ductal carcinoma in situ (DCIS) and invasive ductal carcinomas (IDC) ($p<0.05$ and $p<0.002$ respectively for COX-2) and ($p<0.01$ and $p<0.002$ respectively for p53). COX-2 and p53 expressions increased progressively along the continuum of neoplastic changes from normal breast epithelium to IDC ($p<0.01$ for each). There was significant correlation between either COX-2 or p53 and tumor size ($p<0.05$ for each), tumor grade ($p<0.002$ for each), lymphovascular invasion ($p<0.03$ & <0.02 respectively). There was significant correlation between COX-2 and lymph node metastasis ($p<0.02$). There was significant correlation between p53 and lymphocytic infiltration ($p<0.03$). There were positive correlations between COX-2 and p53 in DCIS and in IDC ($p<0.000$ for each).

Conclusion: Both COX-2 and p53 were increased with poor prognostic parameters; tumor size, tumor grade, lymphovascular invasion, lymph node metastasis and lymphocytic infiltration. P53 is likely to be involved in the regulation of COX-2 expression in ductal breast cancer. These findings might imply for new therapeutic strategies in order to prevent progression of DCIS to IDC and to improve breast cancer therapy.

Key Words: *Breast cancer – DCIS – IDC – COX-2 – P53.*

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Introduction

BREAST cancer is the most common type of cancer and the most common cause of cancer-related mortality among women worldwide [1]. According to the Egyptian National Cancer Institute (NCI), breast cancer is the most common cancer in Egyptian women, representing 18.9% of total cancer cases; 35.1% in women and 2.2% in men [2]. The age-adjusted rate was 49.6 per 100 000 population [3].

Mammary carcinogenesis is a multistep process with transformation of normal ductal epithelial cells → benign proliferative breast disease → DCIS → IDC [4]. Women with benign breast disease could be prevented from developing invasive breast carcinoma (IBC) if we can exactly identify patients with which subtype of benign lesions will subsequently develop IBC and treat them.

COX-2 is a prostaglandin synthetase enzyme that converts arachidonic acid into pro-inflammatory prostaglandins, induced in inflammation and cancer [5]. It is not expressed constitutively like COX-1, and is not normally present

Abbreviation:

Cyclooxygenase-2 (COX-2)	: Invasive ductal carcinomas.
(IDC)	: Ductal carcinoma in situ.
(DCIS)	: National Cancer Institute.
(NCI)	: Invasive breast carcinoma.
(IBC)	: World Health Organization.
(WHO)	: Hematoxylin and eosin.
(H&E)	: Immunoreactive score.
(IRS)	: Quantity score.
(QS)	: Intensity score.
(IS)	: Non-specific type.
(NST)	: Atypical ductal hyperplasia (ADH).

or is present at very low amounts, but COX-2 is rapidly induced by growth factors, cytokines, tumors promoters, hypoxia, ionizing radiation and carcinogens [6].

COX-2 seems to be involved in the processes of malignant transformation and tumor progression by affecting cell proliferation, mitosis, cell adhesion, apoptosis, immune surveillance, and angiogenesis. An elevated COX-2 level has been shown to correlate with a worse prognosis for patients with some types of tumors including breast cancer [7]. However, there have been only a few studies dealing with the association between COX-2 expression and tumor progression in breast cancer.

P53 is a tumor suppressor gene that maintains genomic stability either by inducing cell cycle arrest or apoptosis. In malignant cells, its function can be compromised by various mechanisms; mutations, alteration of p53 regulators, alteration of p53 target genes [8]. In ductal carcinomas, p53 gene is mutated with subsequent overexpression of p53 protein [9].

Studies in normal and cancer cells suggest that the p53 gene might play a role in the regulation of COX-2 expression [10]. The correlation between COX-2 expression and expression of p53 in breast carcinomas has been studied recently, but the results are still controversial [7].

The purpose of this study was to evaluate COX-2 and p53 expressions in the successive steps of breast carcinogenesis and to correlate them with the clinic-pathological parameters of breast cancer.

Patients and Methods

A total of 74 breast specimens, 4 with normal breast tissue, 5 with typical ductal hyperplasia, 11with DCIS, and 54 with IDC, were selected from the files of the Department of Pathology, Sohag University Hospital, Egypt in the period from 2010-2011. Availability of adequate tissue material and clinical data was the only criterion for selection of patients with DCIS and/or IDC.

Immunohistochemistry:

After reviewing hematoxylin and eosin (H&E) stained slides, a representative blocks was chosen for the study. Serial sections from each block were used for immunohistochemistry. Five micron tissue sections mounted on sialinized glass slides were deparaffinized and rehydrated through descending graded alcohols to water. Tissue sections were incubated in hydrogen peroxide for 10 min to block endogenous peroxidase activity. Then slides were treated with antigen retrieval solution (citrate buffer; 10 mmol sodium citrate buffer solution, pH (6.0). The buffer was allowed to boil in microwave at 750 Watt for 15 min divided into 3 cycles. Non specific protein binding was blocked with 10 min exposure to 10% normal goat serum.

Sections were then incubated with Rabbit polyclonal for COX-2, ready to use (Catalog; Cat # RB-9072-R7, LABVISION Corporation, Fremont, USA) and mouse monoclonal antibody for p53, ready to use (Cat # MS-738-R7, LABVISION corporation, Fremont, USA) for one hour at room temperature. Then biotinylated goat polyvalent was applied on each section for10 min with Streptavidin peroxidase. DAB (14- diaminobenzidine and 0.06% H_2O_2) chromogen was applied to each tissue section for 10 min then washed in distilled water. Universal staining kit (Cat # TP-015-HD, LABVISION Corporation, Fremont, USA) composed of: Hydrogen peroxide block, Biotinylated goat anti-polyvalent, Streptavidine peroxidase, DAB chromogen, DAB subsrate was used. Tissue sections were counterstained with Mayer's hematoxylin, dehydrated alcohols, cleared in xylene, and cover slipped.

Positive control: Sections from colon cancer for COX-2 and for p53 were used as positive control. COX-2 and p53 showed brownish cytoplasmic and nuclear and staining respectively.

Negative control: The negative control slides were stained in parallel, but with omission of the primary antibody.

Assessment of COX-2:

According to Spizzo et al. [11] the immunoreactive score (IRS) was determined by combining an estimate of the percentage of immunoreactive cells; quantity score (QS) with an estimate of the staining intensity; intensity score (IS). QS was calculated as follows: No staining is 0, 1-10% stained cells was scored as 1, 11-50% stained cells was scored as 2, 51-80% stained cells was scored as 3, and 81-100% stained cells was scored as 4. IS was calculated as follows: On a scale of 0-3, where 0 was no staining, 1 was weak staining, 2 was moderate staining, and 3 was strong staining. IRS was measured by multiplying QS by SI [12]. An IRS of 0-4 was considered weak, 5-8 was moderate, and 9-12 was considered strong [13].

Assessment of p53:

Nuclear staining is only considered [16], and its expression in 5% of tumor cells was considered as the threshold of positive staining [17]. QS was estimated as follows: Negative=<5% stained cells, 1=5-25% stained cells, 2=26-50% stained cells,

3=51-75% stained cells and 4=76-100% stained cells. SI was scored on a scale of 0-3: 0=no staining, 1=weak staining, 2=moderate staining and 3=strong staining. IRS was measured by multiplying QS by SI [12]. An IRS of 0-4 was considered weak, 5-8 was moderate, and 9-12 was considered strong [13].

Statistical analysis: ANOVA test (Analysis of variance) and Pearson's Correlation Coefficient tests were used with p-value <0.05 was considered statistically significant.

Results

Clinical and histopathological findings of ductal carcinomas:

The mean age of the patients was 53 years (range 26-77 years). The tumor size was >2cm and ≤5cm in 26/54 (48%) cases, and >5cm in 28/54 (52%) of cases. Axillary lymph nodes were positive in 36/54 (67%) patients. IDC of non-specific type (NST) was the most common histological pattern 41/54 (76%). Lymphovascular invasion was observed in 21/54 (39%) cases. Lymphocytic infiltrate was prominent in 14/54 (26%) of cases. Desmoplasia was prominent in 35/54(65%) of cases. Local aggressive manifestations were present in 7/54 (13%) of cases of IDC; peau d'orange appearance in two cases, nipple retraction in three cases, skin ulceration and fungation in one case, and fixation to chest wall in one case. A summary of these data was presented in (Table 1). (Fig. 1) shows H&E in DCIS and IDC.

According to World Health Organization (WHO) criteria [14], DCIS was graded into, 2/11 (18%) low grade, 3/11 (27%) intermediate grade, and 6/11 (55%) high grade. IDC were classified according to Elston and Ellis grading system [15] into 6/54 (11%) low grade, 29/54 (54%) intermediate grade, and 19/54 (35%) high grade. All patients with carcinomas were treated by modified radical mastectomy.

COX-2 expression and its relationship to clinicpathologic features:

COX-2 immunoreactivity was brown granular cytoplasmic stain of the epithelial and myoepithelial cells. Some peritumoral mononuclear inflammatory and stromal cells also displayed cytoplasmic COX-2 staining. COX-2 expression was weak in 3/4 (75%) and moderate in 1/4 (25%) cases of normal breast tissue, whereas it was weak in 2/5 (40%), and moderate in 3/5 (60%) of cases of typical ductal hyperplasia of the breast. COX-2 was weak in 3/11(27.3%), moderate in 6/11(54.5%), and strong in 2/11(18.2%) of cases of DCIS. There was an increase in COX-2 expression with increasing grade of DCIS (p<0.05) (Table 2). COX-2 expression was weak in 11/54 (20.4%), moderate in 19/54 (35.2%), and strong in 24/54 (44.4%) cases of IDC. There was an increase in COX-2 expression with increasing grade of IDC (p<0.002) (Table 3). (Fig. 2) shows COX-2 expression in DCIS and IDC.

Weak expression of COX-2 was often observed in normal-appearing lobular acini and ductal epithelium adjacent to IDC. When DCIS and IDC coexisted, COX-2 immunostaining in DCIS was usually less or equal to the staining of the corresponding IDC. COX-2 expression appeared to increase progressively along the continuum of neoplastic changes from normal breast epithelium to IDC (p<0.01) (Table 4).

Table (1): Clinical and histopathological findings of ductal carcinomas.

Paremeter	No. of cases
Histological types:	
IDC NOS	42 (77.8%)
Medullary carcinoma	5 (9.3%)
Neuroendocrine carcinoma	2 (3.7%)
Micropapillary carcinoma	2 (3.7%)
Papillary carcinoma	1 (1.9%)
Tubular carcinoma	1 (1.9%)
Mucinous carcinoma	1 (1.9%)
Tumor grade of IDC (54):	
Grade I	6 (11%)
Grade II	29 (54%)
Grade III	19 (35%)
Lymphovascular invasion:	
Absent	33 (61%)
Present	21 (39%)
Lymphocytic infiltrate:	
Minimal	40 (74%)
Prominent	14 (26%)
Desmoplasia:	
Minimal	19 (35%)
Prominent	35 (65%)

Table (2): COX-2 expression in DCIS.

Tumor grade	COX-2 expression (IHCS)							IHCS (X±SD)
	Weak			Moderate		Strong		
	0	2	4	6	8	9	12	
Grade I (2)	0	1	0	1	0	0	0	4±2.8
Grade II (3)	0	1	1	1	0	0	0	4±2
Grade III (6)	0	0	0	2	2	0	2	8.7±2.73
p-value	<0.05 *							

ANOVA test is used. * Significant.

Table (3): COX-2 expression in IDC.

Tumor grade	COX-2 expression (IHCS)							IHCS (X±SD)
	Mild			Moderate		Strong		
	0	2	4	6	8	9	12	
Grade I (6)	1	1	2	1	1	0	0	4±2.8
Grade II (29)	1	2	2	5	8	6	5	7.6±3.1
Grade III (19)	0	0	2	2	2	6	7	9.2±2.7
p-value				<0.002**				

ANOVA test is used. **Highly significant.

Table (4): COX-2 expression in different breast lesions.

Histological stage	COX-2 expression (IHCS)							IHCS (X±SD)
	Weak			Moderate		Strong		
	0	2	4	6	8	9	12	
Normal breast (4)	0	3	0	1	0	0	0	3±2
Hyperplasia (5)	1	2	0	0	2	0	0	4±3.7
DCIS (11)	0	2	1	4	2	0	2	6.5±3.4
IDC (54)	2	3	6	8	11	12	12	7.74±3.3
p-value				<0.01*				

ANOVA test is used. * Significant.

There was statistically significant correlation between COX-2 expression and tumor size ($p<0.05$), tumor grade ($p<0.002$), lymphovascular invasion ($p<0.03$), and lymph node metastasis ($p<0.02$). However, no significant correlation between COX-2 and the age, lymphocytic infiltration, or desmoplasia was found (Table 5).

P53 expression and its relationship to clinicopathological features:

P53 expression was negative in 3/4 (75%), and it was weak in 1/4 (25%) of normal breast tissue. P53 expression was negative in 4/5 (80%) and it was weak in 1/5 (20%) of typical ductal hyperplasia. P53 was negative in all 2/2 (100%) grade I, positive in 2/3 (66.7%) grade II, and in all 6/6 (100%) grade III DCIS ($p<0.01$) (Table 6). P53 was positive in 3/6 (50%) grade I, 20/29 (69%) grade II, and in 17/19 (89.5%) grade III IDC ($p<0.002$) (Table 7). P53 expression appeared to increase progressively along the continuum of neoplastic changes from normal breast epithelium to IDC ($p<0.01$) (Table 8).

There was statistically significant correlation between p53 expression and tumor size ($p<0.05$), tumor grade ($p<0.002$), lymphovascular invasion ($p<0.02$), and lymphocytic infiltration ($p<0.03$). However, there was no significant correlation between p53 expression and age, lymph node metastasis or desmoplasia (Table 9). (Fig. 3) shows p53 expression in DCIS and IDC.

Relations between the estimated biological markers in DCIS and in IDC of the breast:

Pearson's Correlation revealed positive correlation between COX-2 and p53 in DCIS and in IDC ($r=0.888, p<0.000$ & $r=0.894, p<0.000$ respectively).

Table (5): COX-2 expression in IDC in relation to clinicopathological parameters.

Clinicopathological Parameter	No.	COX-2 expression			IHCS (X±SD)
		Low IHCS 4 (11 cases)	Moderate 6≤IHCS≥8 (19 cases)	High IHCS >8 (24 cases)	
Age:					0.1 (NS)
<50	19	7	5	7	
>50	35	4	14	17	
Tumor size:					0.05*
2-5	23	7	9	7	
>5	31	4	10	17	
Tumor grade:					0.002**
Grade I	6	4	2	0	
Grade II	29	5	13	11	
Grade III	19	2	5	13	
Lymphovascular invasion:					0.03*
Absent	33	9	13	11	
Present	21	2	6	13	
Lymphocytic infiltration:					0.9 (NS)
Minimal	40	8	14	18	
Prominent	14	3	5	6	
Desmoplasia:					0.6 (NS)
Absent	19	4	5	10	
Present	35	7	14	14	
Lymph node status:					0.02*
Negative	18	7	6	5	
Positive	36	4	13	19	
Local aggressive manifestations:					0.2 (NS)
Absent	47	10	18	19	
Present	7	1	1	5	

ANOVA test is used. NS: None significant. * Significant. ** Highly significant.

Table (6): P53 expression in DCIS of the breast according to grade.

Tumor grade	No. of positive cases	P53 expression (IHCS)								IHCS mean (X)
		Weak				Moderate		Strong		
		1	2	3	4	6	8	9	12	
Grade I (2)	0/2	0	0	0	0	0	0	0	0	0±0
Grade II (3)	2/3	0	1	0	0	1	0	0	0	2.7±3.1
Grade III (6)	6/6	0	0	0	1	1	1	2	1	8±2.8
p-value		< 0.01*								

ANOVA test is used. * Significant.

Table (7): P53 expression in IDC of the breast.

Tumor grade	No. of positive cases	P53 expression (IHCS)								IHCS mean (X)
		Weak			Moderate			Strong		
		1	2	3	4	6	8	9	12	
Grade I (6)	3/6	0	2	0	0	0	1	0	0	2±3.1
Grade II (29)	20/29	0	2	0	4	4	6	1	3	4.7±4.1
Grade III (19)	17/19	0	0	0	1	0	9	1	6	8.3±3.6
p-value		< 0.002**								

ANOVA test is used. ** Highly significant.

Table (8): P53 expression in different breast lesions.

Tumor grade	No. of positive cases	P53 expression (IHCS)								IHCS mean (X)
		Weak				Moderate		Strong		
		1	2	3	4	6	8	9	12	
Normal breast (4)	1/4	1	0	0	0	0	0	0	0	0.3±0.5
Hyperplasia (5)	1/5	0	1	0	0	0	0	0	0	0.4±0.9
DCIS (11)	8/11	0	1	0	1	2	1	2	1	5.1±4.2
IDC (54)	40/54	0	4	0	5	4	16	2	9	5.7±4.3
p-value		< 0.01*								

ANOVA test is used. ** Highly significant

Table (9): P53 expression in IDC of the breast in relation to clinicopathological factors (No=54).

Clinicopathological Parameter	No. of cases	P53 expression					*p*<
		Positive cases (40 cases)	Negative cases (14 cases)	Weak IHCS 4 (9 cases)	Moderate 6≤IHCS≥8 (20 cases)	High IHCS>8 (11 cases)	
Age:							0.5 (NS)
<50	19	15	4	3	9	4	
>50	35	25	10	6	11	7	
Tumor size:							0.05*
2-5	23	16	8	6	6	3	
>5	31	24	6	3	14	8	
Tumor grade:							0.002**
Grade I	6	3	3	2	1	0	
Grade II	29	20	9	6	11	3	
Grade III	19	17	2	1	8	8	
Lymphovascular invasion:							0.02*
Absent	33	27	12	6	10	5	
Present	21	13	2	3	10	6	
Lymphocytic infiltration:							0.03*
Minimal	40	30	13	7	14	6	
Prominent	14	10	1	2	6	5	
Desmoplasia:							0.1 (NS)
Absent	19	12	7	5	4	3	
Present	35	28	7	4	16	8	
Lymph node status:							0.3 (NS)
Negative	18	10	6	3	7	2	
Positive	36	30	8	6	13	9	
Local aggressive manifestations:							0.1 (NS)
Absent	47	34	13	8	19	7	
Present	7	6	1	1	1	4	

ANOVA test is used. NS: None significant. * Significant. ** Highly significant.

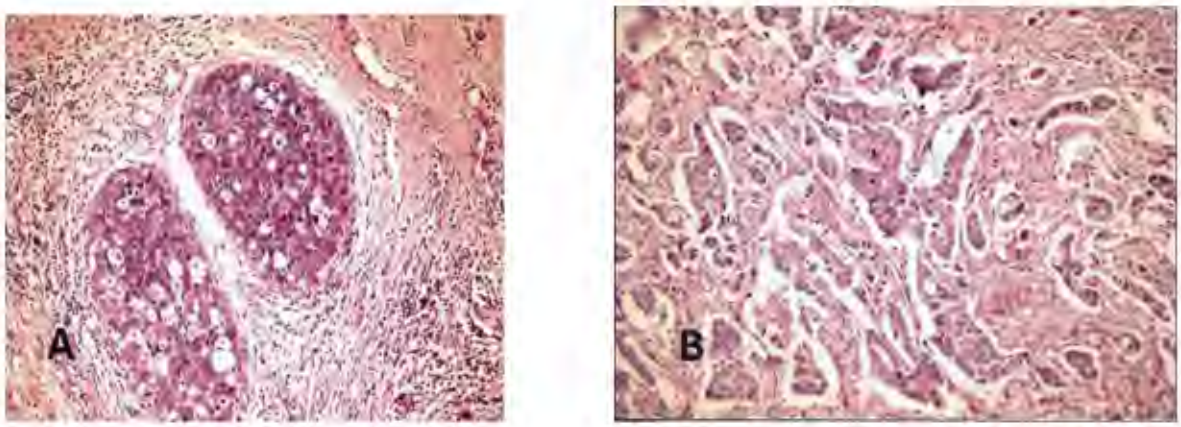
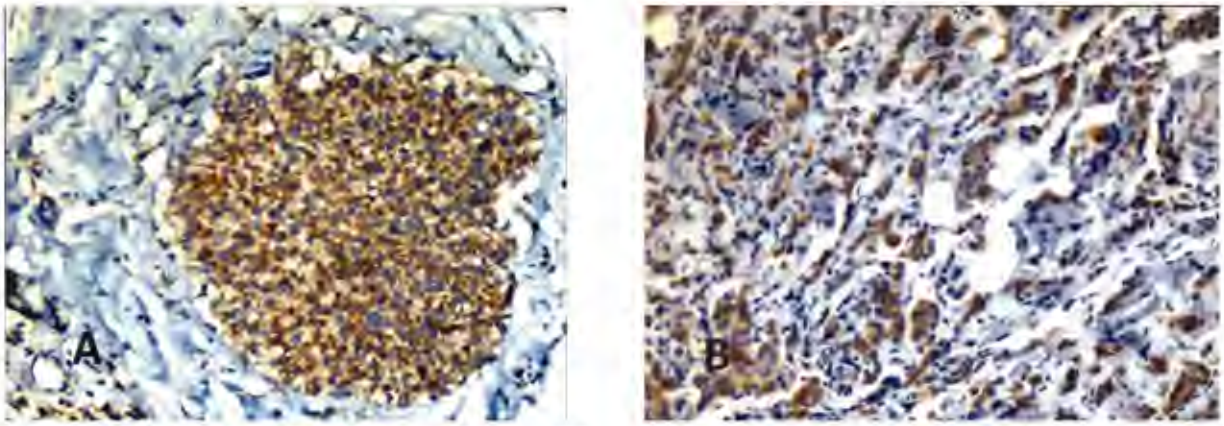


Fig. (1): H&E stained sections of A. DCIS, B. IDC. Magnifications X 200 (A,B).



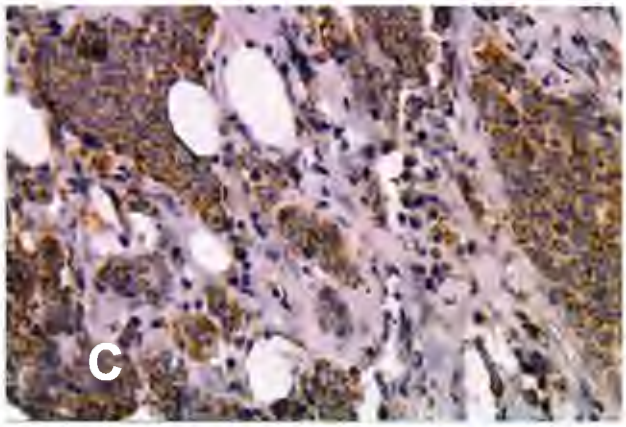


Fig. (2): Moderate cytoplasmic COX-2 immunostain in A. DCIS, B. IDC grade II; Strong granular cytoplasmic COX-2 immunostain in C. Magnifications X 200 (A), X400 (B,C).

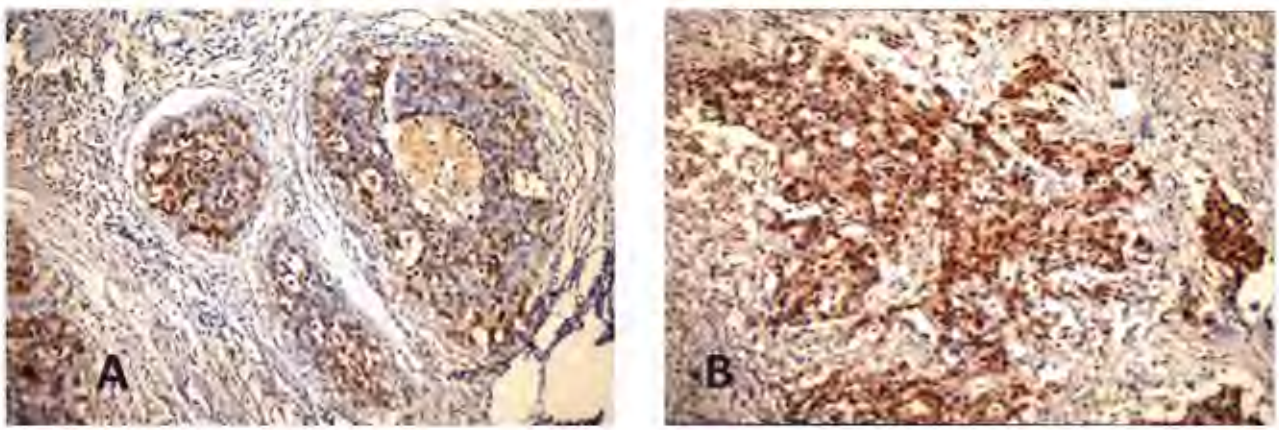


Fig. (3): Moderate nuclear p53 expression in F. DCIS, strong nuclear p53 expression in IDC grade II. Magnifications X 200 (A,B).

Discussion

Breast cancer is a malignant tumor threatening women's health with an increasing incidence worldwide. There are still many deaths due to relapse or metastasis [18]. Age is an independent prognostic factor for overall survival and disease free survival in breast cancer patients [19]. In our study, patient ages ranged from 26-77 years, with the highest age incidence between 45-59 years (mean age was 53 years) consistent with Nouh et al. [20] who reported that the mean age was 47 years, in a larger study made in Upper Egypt. In developed countries, however there is decrease in breast cancer incidence in women over 40 years due to decrease in using hormone replacement therapy [21].

Tumor size is one of the most powerful predictors of tumor behavior in breast cancer. It constitutes the basis of major staging systems [22]. Our data showed that in 52% of the patients tumor size was more than 5cm in diameter, consisting with the National Cancer Institute (2003), which stated that the mean tumor diameter in Egyptian patients was 4.5cm [3,23]. While, in developed countries higher percentages of small sized tumors are present due to periodic examination and early detection using screening mammography [24].

The current hypothesis of tumorigenesis in humans suggests that cancer cells acquire their hallmarks of malignancy through the accumulation of gene activation and inactivation events over long periods of time. For breast cancer, this multistep process may manifest itself as a sequence of pathologically defined stages. It is widely held that breast cancer initiates as the pre-malignant stage of atypical ductal hyperplasia (ADH), progresses into the pre-invasive stage of DCIS, and culminates in IDC [25].

In our study in situ component was evident only in 6/54 (11%) cases. This percentage is much lower than the findings of Tavassoli [26], who found foci of associated DCIS in up to 80% of cases of IDC. This marked difference may be explained at least in part by late discovery of cases in our locality. Omar et al. [23], found even smaller percentage of carcinoma in situ component (1.5%), in breast cancer patients in Upper Egypt.

IDC NST is the most commonly encountered form of IDC [27]. In our series most of the specimens were IDC NST (77.8%). This ratio looks near to that found by Li et al. [28], Denkert et al. [29], and El-Gendi & Abdel-Hadi [30] who found that the frequency of IDC NST was 72.8%, 80% and 85%, respectively.

Tumor grade has been a highly valuable prognostic factor for breast cancer, as poorly differentiated lesions are associated with significantly poorer clinical outcome [31]. Histological grade may also provide useful information with regard to response to chemotherapy and, therefore, be a predictive factor as well as a prognostic indicator [22].

In our study; 6/54 (11%) of IBC were grade I, 29/54 (54%) were grade II, and 19/54 (35%) were grade III. Which is consistent with the findings of Omar et al. [23], who found that pathological grading of IDC in Egyptian patients showed a low incidence of grade I tumors (5.4%), while grades II and III tumors were 66.0% and 28.6% respectively, and away from Cho et al. [7] who found that in IDC; 21.2% were grade I, 43.4% were grade II and 35.4% were grade III. This could be explained by presence of certain genetic or environmental carcinogens which may lead to the development of aggressive tumor phenotypes in our locality.

Axillary lymph node is the most important prognostic factor affecting local control, disease-free and overall survival [3,32]. In our study axillary lymph nodes were involved in 67% (36/54). In agreement with Jatoi et al. [33] who found axillary lymph node involvement in 63.3% (1.068/1.696) of studied cases, and the finding of El-Bolkainy. [34], on Egyptian patients who reported that the frequency of axillary lymph node metastases in breast cancer was 75%. This finding is away from that of Silverstein et al. [35], Denkert et al. [29], and Cho et al. [7]; who reported axillary lymph node metastasis in 36% (680 /1891), 52% (108/ 208), and 49.5% (49/ 99) respectively. This marked difference may be explained at least in part by the late discovery of cases in our locality.

Invasion of the lymphovascular channels is a necessary gateway to the metastatic process and is an independent prognostic indicator in breast cancer [36]. In our study, lymphovascular invasion was present in 39% (21/54) of breast cancer patients Similar incidence was found by Cho et al. [7], Mohammed et al. [37] and Zhang et al. [38], who found lymphovascular invasion in 42.4% (42/99), 31.6% (56/177) and 35.7% (25/70) respectively. However, higher incidence 78% (54/69) of lymphovascular invasion was found by Ito et al. [39]. This is most likely due to the use of lymphatic endothelial markers; D2-40 and podoplanin in the latter study; useful markers in accurate detection of lymphovascular invasion by tumor cells.

There is no definitive conclusion regarding the efficacy of T cell-dependent immune mechanisms

or regarding the correlation between the extent and type of lymphocyte infiltration and tumor progression in most subtypes of breast carcinoma [40]. Some studies noting an adverse effect on clinical outcome [41], and others observing either no significant effect [4], or a beneficial effect [42]. Our study showed that lymphocytic infiltration was prominent in 26% (14/54) of breast cancer patient consistent with Demaria et al. [43], who found that lymphocytic infiltrate was minimal in the majority of patients and showed no relationship with the clinical response.

Desmoplastic reaction is characteristic of IDC of the breast, and the intensity of this reaction can be different from case to case. The interactions between the tumoral stroma and the neoplastic cells are very important, and the tumor stroma can act as a regulator of neoplastic behavior [44]. In our study, desmoplastic stroma was evident in 65% (35/54) of cases, in agreement with Ferrini and Rossi [44], who found a ratio of 74% of tumors with prominent desmoplasia.

COX-2 is recognized as a promising pharmacological target for the prevention and treatment of many human cancers, on the basis of epidemiology, expression patterns, and preclinical studies. Several epidemiological studies reported an inverse correlation between breast cancer incidence and regular use of non-steroidal anti-inflammatory drugs including aspirin. In rodent tumor models, it has been shown that treatment with COX-2 inhibitors reduces the occurrence and growth of breast carcinomas [7].

COX-2 over-expression has been described in human breast cancer [11,45]. These studies found that the role of COX-2 in breast tumor progression was limited. In the current study COX-2 was often weakly expressed in normal appearing lobular acini and ductal epithelium. Weak expression of COX-2 was seen in 75% (3/4) cases, while moderate expression was observed in one case (25%) in agreement with Cho et al. [7], who found that out of 15 normal breast cases, COX-2 expression was weak in 14 and moderate in one case. In contrast Leo et al. [46], found that in normal breast epithelium, 54% exhibited moderate or strong COX-2 expression, whereas 46% were negative for COX-2. This may be due to differences in methods, antibody used, scoring system, and protein expression cut-off levels.

COX-2 protein expression was weak in 40% (2/5) and moderate in 60% (3/5) of hyperplastic lesions. Although the number of patients studied is small but this finding is supported Cho et al. [7], who found that COX-2 expression was strong in 1/15 (7%), moderate in 5/15 (33%), and weak in 60% 9/15 (60%). Also Visscher et al. [47], found that 23 (10%) showed no COX-2 staining, 107 (46%) showed weak, 71 (30%) showed moderate and 34 (14%) showed strong COX-2 staining.

COX-2 was weak in 27.3% (3/11), moderate in 54.5% (6/11), and strong in 18.2% (2/11) of DCIS, and its expression correlated positively with higher tumor grade ($p<0.05$). This observation is comparable with that of Cho et al. [7], who found weak COX-2 expression in 11/30 (37%), moderate expression in 14/30 (47%), and strong expression in 5/30 (17%) of DCIS. But it was highly different from that of Leo et al. [46], who found moderate or strong COX-2 staining in 55% of DCIS, whereas 45% had negative COX-2 expression. This may be due to the smaller number of patients in our study.

In this study, COX-2 expression was weak in 11/54 (20.4%) of cases, moderate in 19/54 (35.2%), and strong in 24/54 (44.4%) of cases of IDC. Slightly different ratios for COX-2 expression was reported by Cho et al. [7] who found weak COX-2 expression in 32/ 99 (32.3%), moderate expression in 35/99 (35.4%), and strong expression in 32/99 (32.3%). Leo et al. [46] found that COX-2 expression was weak in 41% of cases, while moderate and strong expression was found in 55% of lesions collectively. This difference is most likely due to variable proportions of different tumor grades in those studies.

COX-2 expression showed progressive increase along the continuum of neoplastic changes from normal breast epithelium to IDC ($p<0.01$). This is supported by the findings of Ciris et al. [48]. These results suggest that COX-2 expression may be involved in the progression of breast cancer, and may provide a clinically useful biomarker for estimating tumor aggressiveness. COX-2 may be a useful target for chemoprevention or increased therapeutic effectiveness in breast cancer.

We found positive correlation between COX-2 expression and tumor grade of IDC ($p<0.002$). This was elucidated previously by several studies e.g. Ristimaki et al. [49], Singh and Lucci [50], Schmitz et al. [51], Ciris et al. [48], and Kim et al. [52]. These findings indicate that up-regulation of COX-2 expression is common in advanced breast carcinomas. In the contrary other studies revealed no significant correlation between COX-2 expression and tumor grade e.g. Nam et al. [53], Cho et al. [7], Leo et al. [46], Dillon et al. [54], and Zhang et al. [38].

In this study, no correlation was present between COX-2 expression and age of the patients ($p<0.1$). This is consistent with the findings of Nam et al. [53], Cho et al. [7] and Zhang et al. [38].

Tumor size is one of the strongest predictive factors for local recurrence, and tumors greater than 2cm leads to decreased disease free survival [55]. We found positive correlation between COX-2 expression in IDC and tumor size ($p<0.05$). This is consistent with the finding of Cho et al. [7]. While other studies revealed no significant correlations between COX-2 expression in IDC and tumor size e. g. Nam et al. [53], Cho et al. [7], Leo et al. [46], Dillon et al. [54], and Zhang et al. [38].

We found positive correlation between COX-2 expression in IDC and lymphovascular invasion ($p<0.03$). This is consistent with the findings of Zhang et al. [38]. These data suggest that elevated COX-2 expression in breast carcinoma may reflect a more aggressive biological behavior. In the contrary other studies of Cho et al. [7], and Leo et al. [46], found no significant correlation between COX-2 expression and lymphovascular invasion.

Axillary lymph node metastasis is an important prognostic fac-tor, and metastasis occurs through the lymphatic route [55]. Current study showed positive correlation between COX-2 protein expression and lymph node positivity ($p<0.02$), consistent with Nam et al. [53], Zhang et al. [38], and Dillon et al. [54]. In the contrary Cho et al. [7], and Leo et al. [46] found no significant correlation between COX-2 protein expression and lymph node status.

Our results showed that COX-2 overexpression is a significant unfavorable prognostic factor in breast cancer, and provide selective criteria for anti-COX-2 combinations in IBC therapy. But we found no significant correlation between desmoplasia ($p<0.6$), lymphocytic infiltration ($p<0.9$), and local aggressive manifestations ($p<0.2$). To the best of our knowledge, no previous studies discussing these relations.

Nearly one-third of breast cancers have mutations in the p53 gene [56]. Immunohistochemical assays generally detect nuclear accumulation of p53 protein, which is often related to conformational alterations and a prolonged half-life of the encoded protein [57,58].

In the current study p53 was expressed in 1/4 (25%) of normal breast tissue adjacent to IDC. P53 was expressed in one case of typical ductal hyperplasia adjacent to IDC too. This finding is supported by Rohan et al. [59], who investigated the association between p53 protein accumulation and p53 mutations in benign breast tissue and correlates this with increased risk of subsequent breast cancer. They found p53 protein accumulation in 82/104 (79%) cases using in situ hybridization, and they came to the conclusion that p53 protein accumulation was associated with a 2-fold increase in the risk of progression to breast cancer.

Previous findings support the hypothesis that breast cancer evolves by clonal selection of cells that acquire multiple molecular changes through a defined progression of morphologically distinguishable stages, beginning with benign hyperplasia, which progresses to atypical hyperplasia, then to in situ carcinoma, and finally to IBC [60].

Our study showed that, p53 was expressed in 8/11 (72.7%) of DCIS, and it's expression correlated positively with higher tumor grade ($p<0.01$), in agreement with Done et al. [61], who found that the frequency of p53 missense mutations was statistically different among the three overall histological grade categories of DCIS; 0/49 (0%) of low-grade, 1/23 (4.35%) of intermediate-grade, and 9/22 (40.9%) of high-grade DCIS, ($p<0.000$). Our finding indicated that p53 mutations usually occur before invasion during the progression of breast cancer, and that p53 protein expression in DCIS is an important parameter to evaluate the cellular biology and prognosis of DCIS in agreement with Rajan et al. [62].

In this study p53 was positive in 74% (40/54) of IDC. Different ratios for p53 expression were mentioned in the literature; Putti et al. [63], Cho et al. [7], and Lee et al. [55], who found positive p53 in 60% (171/286), 25.3% (25/99), and 37.1% (73/197) respectively. This difference is most likely due to variable proportions of different tumor grades in those studies.

The association between p53 alterations and clinical outcome in breast cancer has been the subject of numerous investigations [64]. The possibility that p53 status influences the biological behavior was raised in an early study in which the presence of p53 mutation in aggressive breast cancer was demonstrated [65]. The majority of studies support an association between worse survival and the presence of p53 mutations [66].

Despite the hypothesis that a reduction in apoptotic response to DNA damage with increasing age may play a significant role in the age-related increase in cancer [67], we found that no significant correlation between p53 expression and age of the

patients ($p<0.5$). This observation concurs with the observations of; Michalides et al. [68] and Zolota et al. [69].

Current study showed significant correlation between p53 expression and larger tumor size ($p<0.05$) consisting with Yamashita et al. [70] and Ferrero et al. [71] who reported a positive correlation between p53 expression and tumor size. Contradictory findings were observed by Noguchi et al. [72] who found no correlation between p53 expression and tumor size. This difference may be due to the presence of other molecules that affect tumor cell apoptosis and proliferation.

This study showed significant positive correlation between p53 expression and higher tumor grade of breast cancer ($p<0.002$) in agreement with Redondo et al. [73], Kourea, et al. [74], Yamashita et al. [70] and Skarlos et al. [75], and indicates that p53 is an indicator of poor prognosis in breast cancer.

The serpin family member "maspin" is an inhibitor of angiogenesis, invasion and metastasis. A step-wise decrease in the expression of maspin in the sequence DCIS > IBC > lymph node metastasis has been described; strongly supporting an important role in breast cancer progression. Maspin is directly transcriptionally induced by wild-type p53, thus providing an interesting connection between p53 and progression in ductal breast carcinoma [76]. Concurring with this notion, we found significant positive correlation between p53 expression and vascular invasion in breast cancer ($p<0.02$).

Axillary lymph node status has repeatedly been shown to be the single most important predictor of disease-free survival and overall survival [77]. Nodal involvement may be an indicator of metastatic disease, the cause of death of patients with breast cancer. Hence, the generally admitted conclusion is that tumor size loses its prognostic role in cases of nodal involvement [78].

In agreement with Arisio et al. [79], and Song et al. [80], we found insignificant correlation between p53 expression and the presence of lymph node metastasis ($p<0.3$). However, Noguchi et al. [77], Gattuso et al. [81] and Amila et al. [82] found significant positive association between p53 expression and lymph node metastasis. This controversy may be attributed to the interplay of other genes which can alter the metastatic potential of breast cancer cells e.g. bcl-xl.

This study revealed insignificant correlation between p53 expression and the presence of desmoplastic stroma ($p<0.1$) in IDC. This is contradictory to Lipponen et al. [83] who found that schirrous carcinoma of the breast expresses p53 oncoprotein more frequently than other carcinomas with less extensive desmoplastic reaction. Our findings can be explained by that; intense stromal reaction in IDC may modulate the expression of p53 [44].

This study revealed positive correlation between p53 expression and lymphocytic infiltration in IDC ($p<0.03$). This is compatible to the findings of Lipponen et al. [83], who stated that lymphocytic infiltration was positively correlated to p53 protein expression, which implies that p53 may have a role in modulation of tumor immunity.

Relation between COX-2 and p53:

P53 gene is inactivated in the majority of human malignancies, representing the most common specific genetic target involved in human malignant transformation. An in vitro study has suggested that wild-type p53 suppresses COX-2 promoter activity by competing against TATA-binding proteins [84]. A link between p53 mutation and COX-2 expression has been demonstrated in various cancer tissues [85].

There have been only a few studies on the correlation between COX-2 and p53 expression in breast carcinomas. Ristimaki et al. [86] and Kim et al. [52] demonstrated a significant correlation between COX-2 expression and p53 expression, while Costa et al. [87] found no correlation. The current study showed a highly significant positive correlation between the expression of COX-2 and p53 in DCIS and in IDC ($p<0.000$).

Conclusions:

Both COX-2 and p53 were increased with poor prognostic parameters; tumor size, tumor grade, lymphovascular invasion, lymph node metastasis and lymphocytic infiltration. P53 is likely to be involved in the regulation of COX-2 expression in ductal breast cancer. These findings might imply for new therapeutic strategies in order to prevent progression of DCIS to IDC and to improve cancer therapy.

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