Cyclooxygenase-2(COX-2) expression in breast carcinomas: Histopathologic and Immunohistochemical study

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Abstract: Background: Breast cancer (BRCA) is the most common cause of cancer related death among women worldwide. Incidence rates are high in more developed countries whereas, in less developed countries and in Japan are low but increasing. Inducible cyclooxgenase-2 (COX-2) is commonly overexpressed in breast tumors and is a target for cancer therapy. Its increased expression occurs early in BRCA and can be detected in ductal carcinoma in situ (DCIS), invasive breast carcinoma and in metastatic lesions. The aim of this study is to analyze COX-2 expression in BRCA and its significant relation to various clinicopathological variables such as age of patient, site, size, type, grade, and stage of tumor. Material and Methods: An archival blocks of formalin fixed paraffin embedded tissue sections of 60 cases of BRCA were collected from the Department of Pathology, Cairo University in the period from February 2011 up to May 2012. All the cases were immersed in monoclonal rabbit primary antibody COX-2 for immunohistochemical staining. Results: COX-2 Immunostaining was observed in almost all cases of BRCA(53out of 60) and lost in only 7 cases. An association was found between COX-2 expression and some clinicopathological features, including tumor size, grade, stage, and lymph node involvement, whereas there was no relationship between COX-2 expression and age of patient and histological type of tumor. Conclusion: COX-2 breast cancer expression was associated with higher stage and worse prognosis and the selective COX-2 inhibitors may be used as a target for cancer therapy.
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Key words: COX-2, breast carcinoma.

1. Introduction

Breast cancer(BRCA) is by far the most common cancer among women both in developed and developing regions, with an estimated 1.38 million new cancer cases diagnosed worldwide in 2008 (23% of all cancers)**(Ferlay *et al.*, 2010).**
In recent years, both incidence of and mortality from BRCA have declined in the United States; between 1999 and 2006, incidence rates decreased by 2.0% per year, and mortality decreased by 1.9% annually between 1998 and 2006 **(Wang *et al.*, 2012).**Theincidence varies widely within regions and countries, likely due to differences in racial and ethnic make-up, health resources, and lifestyle patterns. In Egypt, BRCA rates are intermediate when compared to rates across the world **(Dey, 2010).**

Cyclooxygenease-2 (COX-2) is an inflammation-associated enzyme as it is a key player in the production of prostaglandins and thromboxanes from free arachidonic acid. Increasing evidence suggests that COX-2 plays a role in pathogenesis of many solid tumors. It is over-expressed in non-small cell lung cancer (NSCLC), adenocarcinoma of colon, renal cell carcinoma and others **(Ladetto *et al.*, 2005).**As well as its overexpression is associated with increased angiogenesis, tumor invasion, promotion of tumor cell resistance to apoptosis, and metastasis **(Krysan *et al.*, 2004 and Mitchell *et al.*, 2010).** Additionally it is frequently overexpressed in invasive BRCA and in adjacent ductal carcinoma in situ (DCIS) and, thus, may be an early event in mammary tumorogenesis. These results suggest that non-steroidal anti-inflammatory drugs or aspirin use and selective COX-2 inhibitors may be associated with a survival benefit among women with breast cancer**(Half *et al.*, 2002 and Holmes *et al.*, 2011).** In the same way its intensity and the percentage of positive cells correlated significantly with the size and the histological grade of BRCA, but did not correlate with the outcome (disease-free and overall survival) **(Nassar *et al.*, 2007).**

2.Material and Methods:

Case selection:

Retrospective study including retrieval of formalin fixed paraffin embedded tissue sections from archival blocks of sixty cases of breast carcinoma were collected from the Department of Pathology, Cairo University in the period from February 2011 up to May 2012. The clinical data as regard patient's age, sex and site of the tumor, that could be collected from hospital records and pathology reports. All the cases were modified radical mastectomy that were properly evaluated for tumor type, size, stage ,lymph node status, overlying skin and presence or absence of necrosis, lymphovascular invasion and intraductal component. Serial sections of 5 microns thickness

were prepared from each tissue block, one of them stained by Haematoxylin and Eosin (H&E) for routine histological examination by two consultant pathologists. Breast cancer cases were graded according to Nottingham modification of the Bloom-Richardson grading system**(Elston-Ellis,1991)** and staged according to TNM staging system of American Joint Committee on cancer **(AJCC)**.Then unstained positively charged slides were prepared from each paraffin block for immunostaining using monoclonal rabbit anti-human antibody (anti-Cox 2, Lab vision, USA, Cat#RB-9072), and ultravision detection system (HRP/DAB, Lab vision, USA) in dilution 1/200.

Histological and immunohistochemical interpretation:

Histological interpretation: All cases of BRCA were subcategorized according to WHO classification of the malignant epithelial tumors of the breast **(2003)** into invasive duct carcinoma NOS, invasive lobular carcinoma, medullary carcinoma, metaplastic carcinomas, tubular carcinoma, mucinous carcinoma, mixed carcinoma, invasive papillary carcinoma and invasive cribriform carcinoma. The grade of breast carcinomas was assessed as; I, II or III according to Nottingham modification of the Bloom-Richardson grading system(**Elston-Ellis,1991).** As well as the tumor staging was designated according to TNM staging system of American Joint Committee on cancer **(AJCC).**

Immunohistochemical interpretation: Positive immunoreactivity to COX-2 gives a brown cytoplasmic staining of tumor cells. Semiquantitative assessment of the staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive cells was categorized as 0 = negative, 1 = < 10% positive cells, 2 = 10–50% positive cells, 3 = 51–80% positive cells, and 4 => 80% positive cells. An immunoreactive score of >2 was considered positive **(Shim *et al.*, 2003)**.

Statistical analysis:

Fisher exact and chi square tests were used in the analysis to estimate the correlation between COX-2 immunoreactivity and clinico-pathological data for each case (age, site, size, histological type, grade, stage and lymph nodes involvement). The significance of the results was assessed by determining the probability factor "P" value. A *p*-value of < 0.05 was considered statistically significant.

3.Results

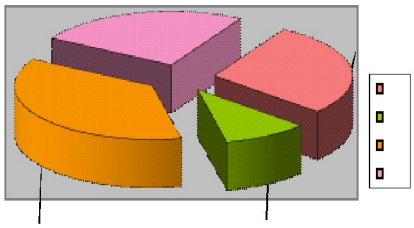
Histopathological findings in all cases studied: A total 60 cases of breast carcinomas (BRCA) removed by modified radical mastectomy were studied. The age ranged from 32 years up to 86 years with mean age 59 years. All the studied cases were females. Regarding to the anatomical location of BRCA, the UOQ was the most common site of BRCA encompassing 29 out of 60 cases (48.3%). Invasive duct carcinoma NOS was the commonest variant in all studied cases that seen in 18 cases (30%) (Fig. 1) followed by invasive lobular carcinoma (16 cases) (Fig. 2). In the same them grade II BRCA represents (65%) as well as most of the studied cases were more than 2 cm in diameter and representing 75% of cases. In concern to lymph nodes status, most of studied cases showed lymph node metastasis that observed in 43 cases (71.7%). As well as most of them were pN2 encompassing 22 cases (36.7%) (Graph 1). Additionally; all these cases were free from distant metastasis (M0).Also, most of the studied cases were stage III which was detected in 37 cases (61.6%) (Table 1).

Table 1: **Histopathological findings in all cases studied:**

Clinicopathological Parameters	Number of cases	Percent
Age:		
≤50	28	46.7
>50	32	53.3
Site of BRCA:		
UOQ	29	48.3
UIQ	8	13.3
LOQ	6	10
LIQ	4	6.7
Retroareolar	13	21.7
Histological Variants:		
Invasive duct carcinoma NOS	18	30
Invasive lobular carcinoma	16	26.7
Medullary carcinoma	5	8.3
Metaplastic carcinoma	5	8.3
Tubular carcinoma	5	8.3
Mucinous carcinoma	3	5
Mixed carcinoma	5	8.3
Invasive papillary carcinoma	2	3.3
Invasive cribriform carcinoma	1	1.7
Nuclear grade:		
1	6	10
11	39	65
111	15	25
Size in centimeters:		
≤ 2cm	15	25
>2cm	45	75
Lymph nodes:		
pN0	17	28.3
pN1	6	10
pN2	22	36.7
pN3	15	25
Stage:		
1	10	16.7
11	13	21.7
111	37	61.6
Total	**60**	**100.0**

COX-2 immunohistochemical findings in all cases studied: in regard to COX-2 immunoreactivity, 53 cases (88.3%) of BRCA were COX-2 positive as well

as most of them revealed strong degree of positivity (51.7%) (Table 2). Strong COX-2 immunostaining was observed in patients more than 50 years which was seen in 19 cases (*P* value=0.173) that was statistically not significant and most of the weak positive cases were ≤50y (8 cases) (table 3, graph 2). Additionally, there was no significant relation between COX-2 immunoreactivity and the tumor sites (*P* value=0.167) and most of the cases that revealed strong COX-2 immunostaining were located in UOQ and detected in 13 cases, whereas most of negative COX-2 cases were also located in UOQ (6 out of 7 cases) (Table 4). As well as the relation between COX-2 immunostaining and the histological types of BRCA was insignificant (*P* value= 0.201). In invasive duct carcinomas (IDCA) 12 out of 18 cases were strongly positive to COX-2 (Fig. 7), one was weak positive and none of them was COX-2 negative so, all cases of IDCA revealed COX-2 immunopositivity. In the same manner 6 cases of invasive lobular carcinoma were strongly positive (Fig. 8), as well as all cases of medullary carcinomaand metaplastic carcinomas were strongly positive to COX-2 (Figs 10 & 11), whereas the only studied case of invasive cribriform carcinoma was weakly positive, one case of invasive papillary carcinoma was moderately positive and the other was negative. Additionally, all cases of tubular carcinomas were negative or weakly positive(3/5,2/5), and 3 cases of mucinous carcinomas showed variable expression ranged from weak to strong positivity (one for each grade) (tables 5-6) (*P* value= 0. 061). In the opposite direction there was a significant relation between COX-2 expression and histological grades of BRCA (P value= 0.052) as all grade III cases were COX-2 strongly positive (15 out of 15),while all grade I cases were either COX-2 negative or weakly positive (3 out of 6 &3 out of 6) (Table 7, & Graph 3). Additionally, there was a significant relation between COX-2 and tumor size (*P* value=0.003) as most of the cases that strongly stained with COX-2 were more than 2 cm in diameter(29 out of 31),while most of negative cases were less than or equal 2 cm in diameter (6 out of 7) (table 8, & graph 4). As well as the relation between COX-2 and lymph nodes status in cases of BRCA was significant (P value=0.035) as most (pN0) tumors were negative or weaklypositivetoCOX-2(7 out of 17 in both scales), while most (pN3) tumors were strongly positive (14 out of 15) (Table 9, & Graph 5). Also there was a significant relation between COX-2 immunostaining and stage of BRCA cases (*P* value=0.003) and most stage III tumors were strongly positive (31 out of 37) while most stage I tumors were negative COX-2 (7 out of 10) (Table 10, & Graph 6). Additionally; 8out of the 60 cases of BRCA showed skin invasion in the form of ulceration and or ipsilateral satellite nodules and or Peaud'orange that all of them were COX-2 strong positive. Six out of the 60 cases revealed tumor necrosis, 5 of them were COX-2 strongly positive and the other one was weak positive. Finally one out of the 60 cases of BRCA exhibited lympho-vascular emboli and it was COX-2 strongly positive**.**



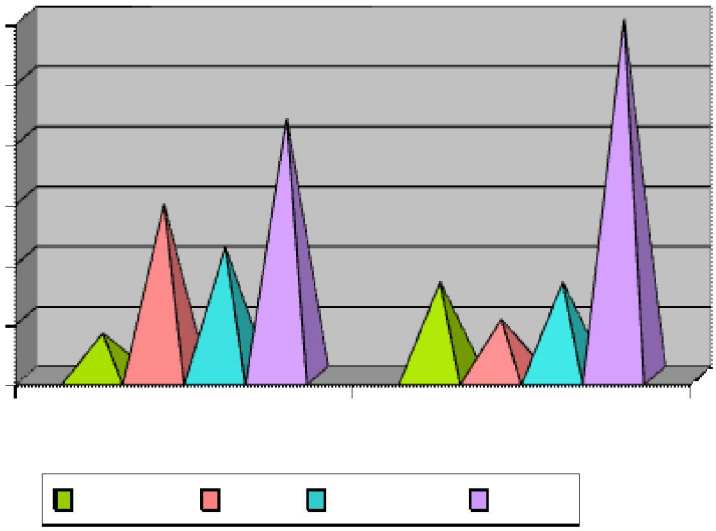
Graph(1): Lymph node status in studied breast carcinoma cases

Table 2:COX-2 immunohistochemical findings in all cases studied:

COX-2 stain findings	Number of cases	Percent
COX-2 immunostain		
Negative	7	11.7
Positive	53	88.3
Intensity of staining:		
Weak + ve	11	18.7
Moderate + ve	11	18.7
Strong + ve	31	51.7
Total	60	100.0

Table (3): Relation between intensity of COX-2 immunostaining and age of cases:

Intensity of COX-2 immunostaining	Age		Total
	≤50 y	>50 y	
Negative	2	5	7
Weak +ve	8	3	11
Moderate +ve	6	5	11
Strong +ve	12	19	31
Total	28	32	60



Graph(2):Relation between intensity of COX-2 immunostaining and age of studied breast carcinoma cases

Table (4): Relation between intensity of COX-2 immunostaining and tumor site:

Degree of staining	UOQ	UIQ	LOQ	LIQ	Retroareolar	Total
Negative	6	0	0	0	1	7
Weak	3	4	0	1	3	11
Moderate	7	1	2	1	0	11
Strong	13	3	4	2	9	31
Total	29	8	6	4	13	60

Table (5): Relation between intensity of COX-2 immunostaining and histological variants of breast carcinomas:

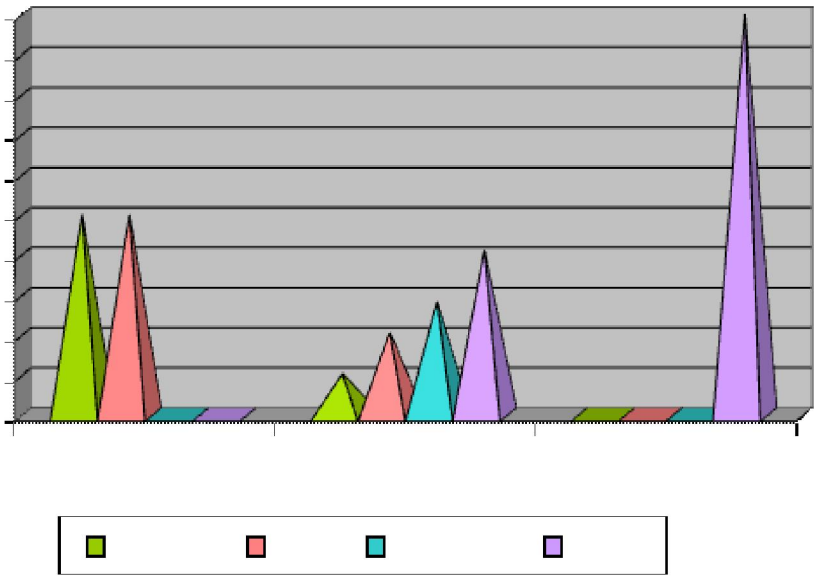
Intensity ofCOX-2 immunostaining	Histological variants									Total
	invasive duct carcinoma (NOS)	invasive lobular carcinoma	medullary carcinoma	metaplastic carcinoma	tubular carcinoma	mucinous carcinoma	mixed carcinoma	invasive papillary carcinoma	invasive cribriform carcinoma	
Negative	**0**	**3**	**0**	**0**	**3**	**0**	**0**	**1**	**0**	**7**
Weak	**1**	**4**	**0**	**0**	**2**	**1**	**2**	**0**	**1**	**11**
Moderate	**5**	**3**	**0**	**0**	**0**	**1**	**1**	**1**	**0**	**11**
Strong	**12**	**6**	**5**	**5**	**0**	**1**	**2**	**0**	**0**	**31**
Total	**18**	**16**	**5**	**5**	**5**	**3**	**5**	**2**	**1**	**60**

Table (6): Relation between intensity of COX-2 immunostaining and histological variants of breast carcinomas:

Intensity ofCOX-2 immunostaining	Histological variants									Total
	invasive duct carcinoma (NOS)	invasive lobular carcinoma	medullary carcinoma	metaplastic carcinoma	tubular carcinoma	mucinous carcinoma	mixed carcinoma	invasive papillary carcinoma	invasive cribriform carcinoma	
Negative	**0**	**3**	**0**	**0**	**3**	**0**	**0**	**1**	**0**	**7**
Positive	**18**	**13**	**5**	**5**	**2**	**3**	**5**	**1**	**1**	**53**
Total	**18**	**16**	**5**	**5**	**5**	**3**	**5**	**2**	**1**	**60**

Table(7): Relation between intensity of COX-2 immunostaining and tumor histological grade:

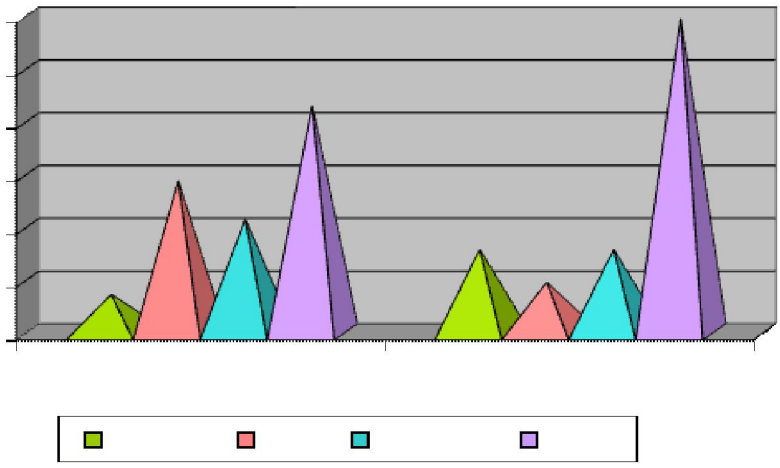
Intensity of COX-2 immunostaining	Tumor grade			
	I	II	III	Total
Negative	3	4	0	7
Weak +ve	3	8	0	11
Moderate +ve	0	11	0	11
Strong +ve	0	16	15	31
Total	6	39	15	60



Graph(3):Relation between intensity of COX-2 immunostaining andhistological grade of tumors

Table (8): Relation between intensity of COX-2 immunostaining and tumor size:

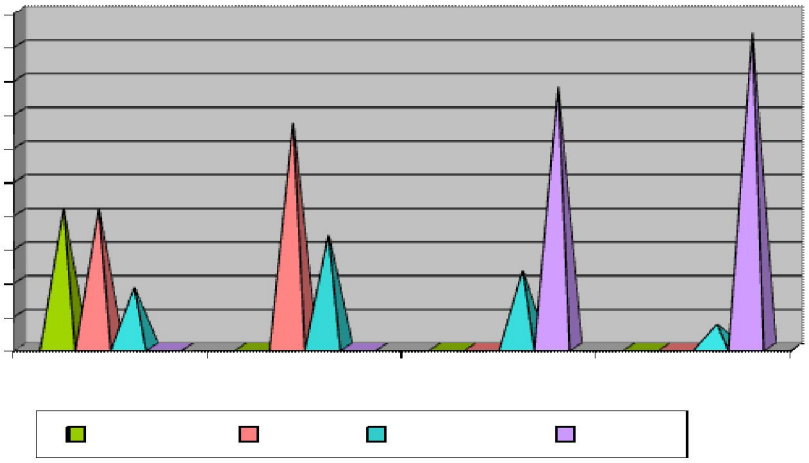
Intensity of COX-2 immunostaining	Size (cm)		Total
	≤2cm	>2cm	
Negative	6	1	7
Weak +ve	5	6	11
Moderate +ve	2	9	11
Strong +ve	2	29	31
Total	15	45	60



Graph(4):Relation between intensity of COX-2 immunostaining and size of tumors

Table (9): Relation between intensity of COX-2 immunostaining and lymph node status in breast carcinoma cases:

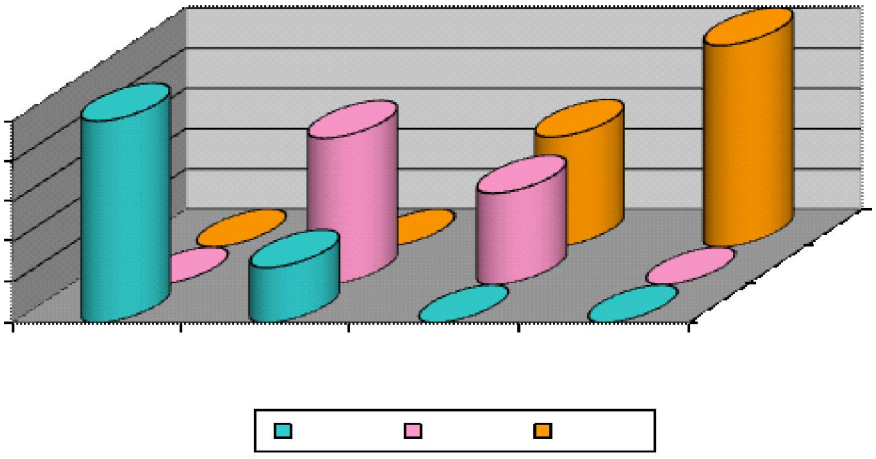
Intensity of COX-2 immunostaining	Lymph Node Status				Total
	pN0	pN1	pN2	pN3	
Negative	7	0	0	0	7
Weak +ve	7	4	0	0	11
Moderate +ve	3	2	5	1	11
Strong +ve	0	0	17	14	31
Total	17	6	22	15	60



Graph(5):Relation between intensity of COX-2 immunostaining and pN

Table (10): Relation between intensity of COX-2 immunostaining and tumor stage:

Intensity of COX-2 immunostaining	Tumor stage			
	Stage I	Stage II	Stage III	Total
Negative	70	0	0	7
Weak +ve	3	8	0	11
Moderate +ve	0	5	6	11
Strong +ve	0	0	31	31
Total	10	13	37	60



Graph(6):Relation between intensity of COX-2 immunostaining and stage of tumor

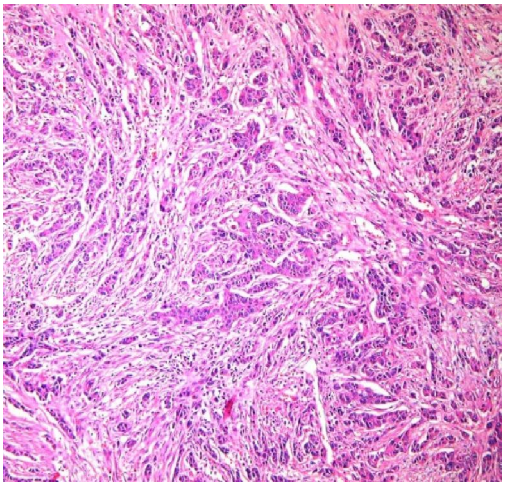


Fig 1: A case of invasive duct carcinoma, not otherwise specified(IDC,NOS), grade II revealed islands of malignant cells surrounded by dense desmoplastic reaction (H&E X 100).

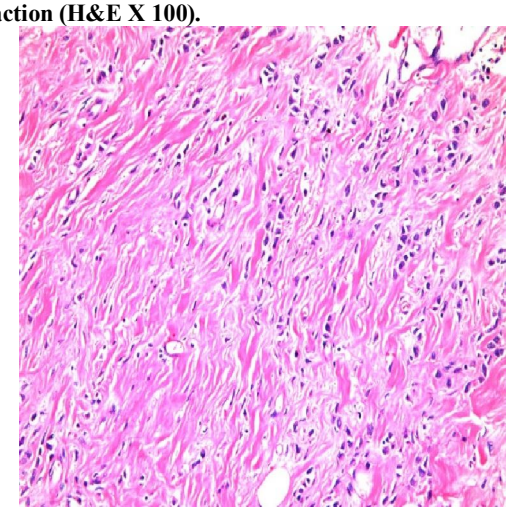


Fig 2: A case of invasive lobular carcinoma (ILC) exhibited monomorphic small malignant cells arranged in Indian file growth pattern surrounded by marked desmoplastic stromal reaction (H&E X200)

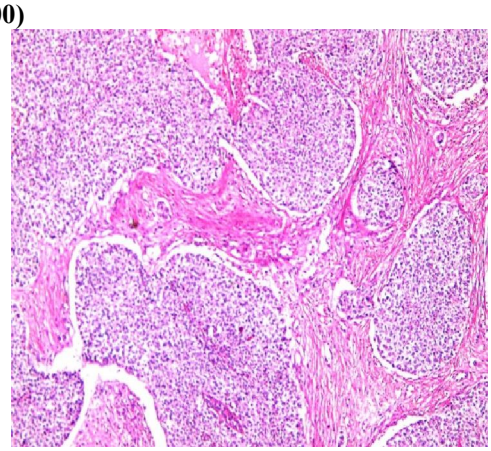


Fig 3: A case of medullary carcinoma showed syncytial growth pattern of tumor cells (H&E X100).

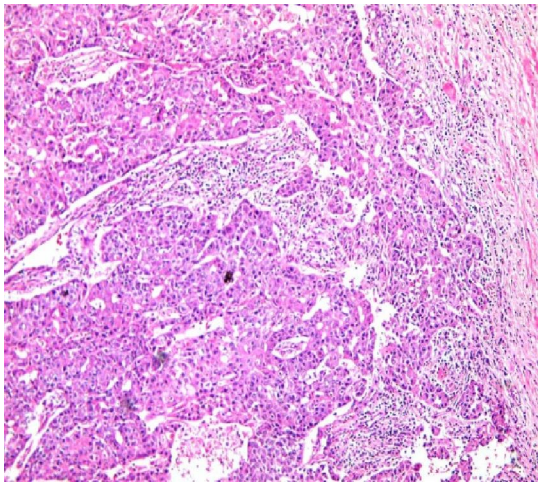


Fig 4: A case of metaplastic carcinoma revealed squamous like features (H&E X100)

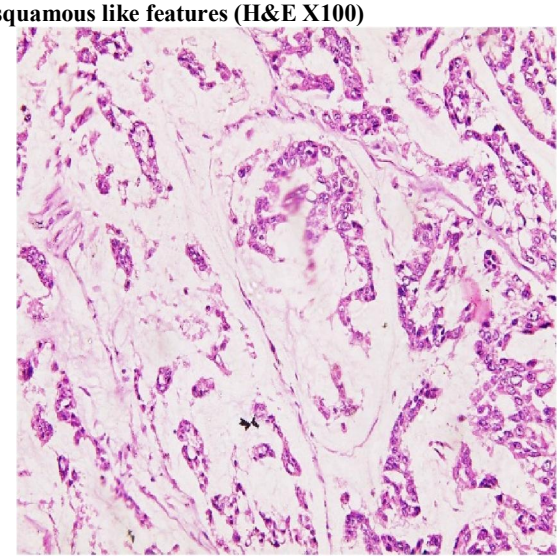


Fig 5: A case of mucinous carcinoma showed malignant cells with lakes of mucin (H&E X200)

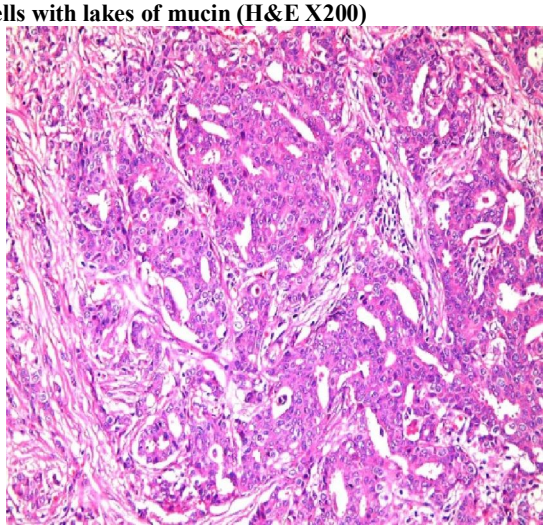


Fig 6: A case of invasive cribriform carcinoma formed of malignant epithelial cells arranged in a cribriform pattern (H&E x200).

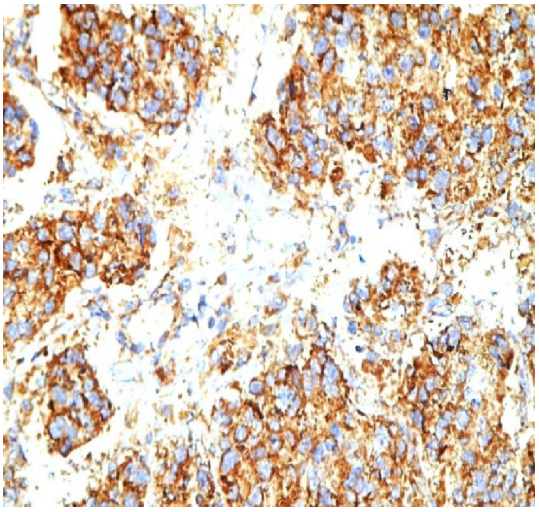


Fig 7: A case of invasive duct carcinoma (IDC) showed strong positive cytoplasmic immunoreactivity for COX-2 (DAB X400).

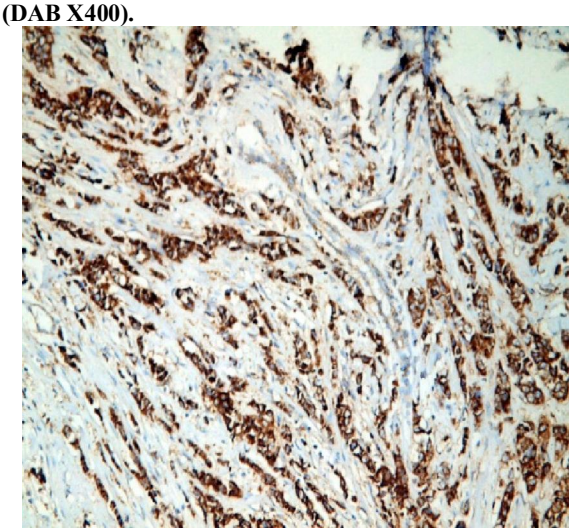


Fig 8: A case of ILC revealed strong positive cytoplasmic immunostaining for COX-2 (DAB X 200)

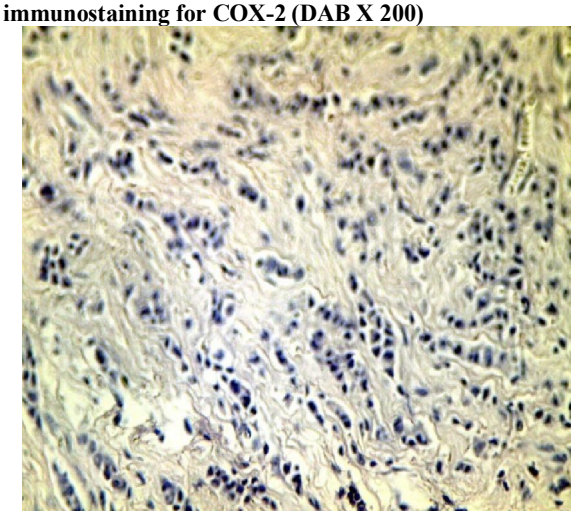


Fig 9: A case of ILC revealed negative COX-2 immunostaining (DAB X 200)

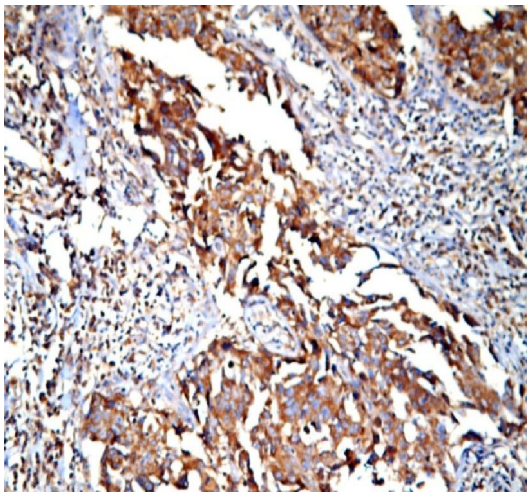


Fig 10: A case of medullary carcinoma revealed strong positive cytoplasmic immunoreactivity for COX-2 (DAB X200)

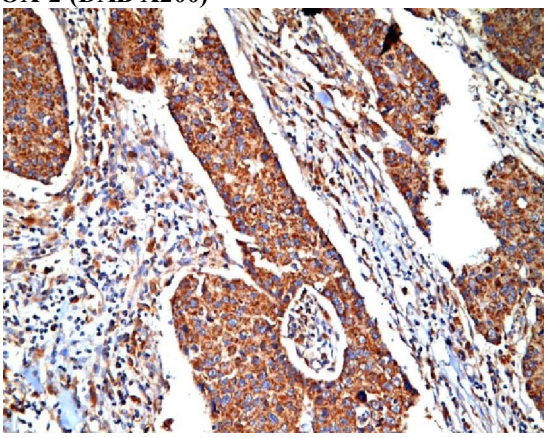


Fig 11: A case of metaplastic carcinoma exhibited strong positive cytoplasmic immunoreactivity for COX-2 (DAB X200)

4. Discussion

Breast cancer (BRCA) is an urgent public health problem in high-resource regions and is becoming an increasingly urgent problem in low resource regions, where incidence rates have been increasing by up to 5% per year (**Aboserea *et al.*, 2011).**

In the present study, 60 cases of BRCA were studied. Theirages ranged from 32 to 86 years with mean age 59 years, and all cases were females, as men are generally at low risk for developing BRCA**(Alteri *et al.*, 2012)**. Histologically invasive duct carcinoma (IDC) was representing 18 out of 60 cases (30%). This result was different from result of **Ristimaki *et al.* (2002)** who found that 1155 out of 1576 studied cases were IDC that representing 73.3%. Additionally, invasive lobular carcinoma (ILC) was found in 16 out of 60 cases studied (26.7%), that was not parallel with

a study performed by **Nam *et al.* (2005)** who reported that ILC seen in 3 out of 112 cases (2.7%).

Immunohistochemically COX-2 cytoplasmic immunopositivity was seen in the majority of cases that 53 out of the 60 cases (88.3%) were positive, while the remaining 7 cases (11.7%) were negative. This was agreed with **Davies *et al.* (2003)** who evaluated COX-2 expression in 80 cases of BRCA and found that immunoreactivity for COX-2 protein was observed in 63 cases (79%). As well as, similar results were given by **Shim *et al.* (2003)** who performed immunohistochemical staining for COX-2 expression in 64 cases of BRCA and reported that 46 cases (72%) were positive.

Concerning the intensity of COX-2 immunostaining, strong positivity was detected in 31 out of the 60 cases (51.7%) whereas, weak cytoplasmic reactivity seen in 11 of them (18.3%). This result was different from results of **Ristimaki *et al.* (2002)** who evaluated COX-2 expression in BRCA cases and found that immunoreactivity for COX-2 was strongly positive in only 5% of studied cases and weakly positive in 54.2%.

In the current study, there was an insignificant relationship between COX-2 immunostaining and age, histological types, and anatomical location of BRCA (P=0.173, P=0.201, and P=0.167). Similar results were reported by **An *et al.* (2009)** after working on 353 patients with BRCA and reported that COX-2 positivity appeared in 41.9% of examined cases without significant relationship between COX-2 expression and age or histological types. Also these results were consistent with **Shim *et al.* (2003)** who reported similar results in which 72% of BRCA cases were COX-2 positive without association with histological types. Additionally, this result disagreed with **Ristimaki *et al.* (2002)** who detected COX-2 positivity in 91.6% of cases, with significant relationship between COX-2 expression and histological type. As well as similar results were reported by **Thorat *et al.* (2009)** who evaluated COX-2 expression in 89 cases and found that immunoreactivity for COX-2 protein was observed in 79% with a significant relationship between COX-2 expression and tumor histological type.

Regarding the tumor size, there was a significant relationship between COX-2 positivity and larger tumor size (>2 cm) (P=0.003) as most of cases that strongly stained with COX-2 (29 out of 31) were more than 2cm,while most of negative COX-2 cases (6 out of 7) were less than or equal to 2 cm. These results agreed with **Shim *et al.* (2003)** who found a significant relationship between COX-2 expression and large tumor size (>2cm). Also similar results were given by **Nassar *et al.* (2007)** who worked on 43 cases of breast carcinomas, 95% of them were COX-2 positive. They reported that there is significant relationship between COX-2 expression and large tumor size (>2 cm). The results in this study are in harmony also with the study done by **Denkert *et al.* (2003)** which reported similar results after working on 221 cases of BRCA with, 36% of them were COX-2 positive with a significant relationship with large tumor size. In the other way this result disagreed with **Davies *et al.* (2003)** who evaluated COX-2 expression in 80 cases and found that immunoreactivity for COX-2 protein was observed in 63 cases (79%) with no relationship was found between COX-2 expression and tumor size. The discrepancy of immunohistochemical results can be explained by the differences in antibody reactivity selection criteria, in this study the primary antibody type is monoclonal rabbit antibody, while in **Davies's** study it was a polyclonal rabbit IgG antibody. Also, **Thorat *et al.* (2009)** found an insignificant relation between COX-2 expression and tumor size.

In this study metastatic deposits in lymph nodes were detected in 43 cases (71.7%). This was consistent with **Costa *et al.* (2002)** who reported similar results after working on 46 cases of BRCA and found 54.5% of them were lymph node positive for malignant involvement. Additionally, the relationship between COX-2 positivity and lymph node metastasis was statistically significant (P=0.035). This finding was consistent with **Costa *et al.* (2002), Denkert *et al.* (2003), and Nam *et al.* (2005)** they reported a significant relation between lymph nodes metastasis and COX-2 positive immunoreactivity. In the opposite waythis result does not in parallel with **Davies *et al.* (2003), Kelly *et al.* (2003), and Thorat *et al.* (2009)** they reported that there was no relationship between COX-2 expression and lymph node metastasis. The first two studies worked on 80, and 106 cases of breast carcinoma. The discrepancy of immunohistochemical results can be explained by the differences in antibody reactivity selection criteria as in this study the primary antibody type is monoclonal rabbit antibody, while in **Davies 's and Kelly's** studies it was a polyclonal rabbit IgG antibody.

Regarding the histological grading, grade II was the commonest grade in the studied BRCA cases (65%), and these results were consistent with **Nam *et al.* (2005)** who found that 54.6% of studied cases were grade II. Additionally, the relationship between COX-2 positivity and histological grade was statistically significant (P=0.052). In this study all cases in grade III revealed strong cytoplasmic COX-2 immunostaining (15/15), while all cases in grade I were either COX-2 negative or weak positive (3/6 for each). This observation agreed with **Takeshita *et al.* (2005)** who evaluated COX-2 expression in 30 cases, 57% of them were COX-2 positive with significant

relation between COX-2 immunoreactivity and histological grade of BRCA. Also similar results were given by **Surowiak *et al.* (2005)** who worked on 104 cases of BRCA and found 44% of them were COX-2 positive. They reported that there is a significant relationship between COX-2 expression and higher tumor grade. This was consistent also with **Nassar *et al.* (2007) and Thorat *et al.* (2009)** who reported similar results in which COX-2 expression was significantly related to the tumor grade. This result disagreed with **Costa *et al.* (2002)** who reported that there was no relationship between COX-2 expression and tumor grade as 17.4% of their cases were COX-2 positive. Also **Davies *et al.* (2003)** reported similar results. As well as **Shim *et al.* (2003)** found an insignificant relation between COX-2 expression and tumor grade. The disagreement of immunohistochemical results can be explained by the differences in primary antibody type and concentration, in the current study the primary antibody was monoclonal rabbit antibody (diluted 1/200), while in **Shim's** study had dilution 1/50.

In the current study, stage III was the commonest stage in the studied breast carcinoma cases (61.6%), and these results disagreed with **Nam *et al.* (2005)** who found that 61% of studied cases were stage II. There was statistical significance relating to COX-2 expression and tumor stage (P=0.003). This was consistent with **Shim *et al.* (2003), Wulfing *et al.* (2003), and Chuah *et al.* (2010)** they found a significant relation between COX-2 expression and tumor stage. The last two studies worked on 192 and 100 cases of breast carcinomas. In the other direction this result disagreed with **Nam *et al.* (2005)** where they found an insignificant relationship between COX-2 expression and tumor stage. The reason for this disagreement of results may be due to the increased number of cases with high stage III(37out of 60 cases) (61.6%) in this study, while in **Nam's** study they were only 14 out of 112(12.5%) cases. Also **Davies *et al.* (2003) and Nassar *et al.* (2007)** reported that COX-2 expression was insignificantly related to tumor stage. The wide discrepancy of immunohistochemical results may be also explained by the presence of strong relation between COX-2 overexpression within breast carcinoma tissue and p53 and HER2-Neu overexpression ***(Cho et al.,2006)***, Proliferating Cell Nuclear Antigen overexpression (PCNA)***(Kirkpatrick et al.,2001)*** and CD31 and VEGF overexpression ***(Ranger et al., 2004)*** within breast carcinoma tissue and the percentage of these factors show wide variations among different patients.

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References:

1. **Ferlay J, Shin HR, Bray F, Forman D, Mathers C, and Parkin DM (2010).** Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International Journal Cancer 127: 2893–2917
2. **Wang Q,Li J, Zheng S, Li JY, Pang Y, Huang R, Zhang BN, Zhang B, Yang HJ, Xie XM, Tang ZH, Li H, He JJ, Fan JH and Qiao YL (2012):** Breast cancer stage at diagnosis and area-based socioeconomic status: a multicenter 10-year retrospective clinical epidemiological study in China,BMC Cancer 12: 122.
3. **Dey S, Soliman AS, Hablas A, Seifeldin IA, Ismail K, Ramadan M, El-Hamzawy H, Wilson ML, Banerjee M, Boffetta P, Harford J, and Merajver SD (2010):** Urban-rural differences in breast cancer incidence by hormone receptor status across 6 years in Egypt. Breast Cancer Research Treatment 120(1):149-60.
4. **Ladetto M, Vallet S, Trojan A, Dell'Aquila M, Monitillo L, Rosato R(2005):** Cyclooxygenase-2 (COX-2) is frequently expressed in multiple myeloma and is an independent predictor of poor outcome**. Blood Journal 12(105):4784-91.**
5. **Krysan K, Merchant F, Zhu L, Dohadwala M, Luo J, Lin Y, Nathalie Heuze-Vourc'h, Põld M, Seligson D and Chia D(2004):** COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB Journal 206-8.
6. **Mitchell K, Svenson KB, Longmate WM, Gkirtzimanaki K, Sadej R, Wang X, Zhao J, Eliopoulos AG, Berditchevski F, and DiPersio CM (2010):** Suppression of integrin α3β1 in breast cancer cells reduces COX-2 gene expression and inhibits tumorogenesis, invasion, and crosstalk to endothelial cells. Cancer Research 70(15): 6359–67.
7. **Half E, Tang XM, Gwyn K, Sahin A, Wathen K and Sinicrope FA (2002).** Cyclooxygenase-2 expression in human breast cancers and adjacent ductal carcinoma in situ. American Association for Cancer Research62:1676-81.
8. **Holmes MD, Wendy Y. Chen WY, Schnitt SJ, Collins L, Colditz GA, Hankinson SE, and Tamimi1 RM (2011):** COX-2 expression predicts worse breast cancer prognosis and does not modify the association with aspirin. Breast Cancer Research Treatment 130(2): 657–62.
9. **Nassar A, Radhakrishnan A, Cabrero IA, Cotsonis G, Cohen C(2007)**, COX-2 expression in invasive breast cancer: correlation with prognostic parameters and outcome. Applied

Immunohistochemistry and Molecular Morphology 15(3):255-9.

10. **Elston, C.W. and Ellis I.O. (1991).**Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer, experience from a large study with long-term follow-up. Histopathology 19: 403-10.
11. **Shim JY, An HJ, Lee YH, Kim SK, Lee KP, Lee KS (2003):** Overexpression of cyclooxygenase-2 is associated with breast carcinoma and Its poor prognostic factors. Modern Pathology 16(12):1199–1204.
12. **Aboserea M, Abdelgawad M and wafik W (2011):**: Early Detection of Breast Cancer among Females at Fakous District, Sharqia Governorate, Egypt. Life Science Journal 8 (1):196-203.
13. **Alteri R, Bandi P, Brinton L, Casares C, Cokkinides V, Gansler T, Gapstur S, Graves K, Kramer J, McNeal B, Magro A, Naishadham D, Newman L, Niemeyer D, Richards C, Runowicz C, Saslow D, Simpson S,Smith R, Sullivan K, Wagner D, Xu J(2012):** American Cancer Society. Breast cancer facts &figures. Atlanta: American Cancer Society, Inc. Pages 2.
14. **Ristimaki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, and Isola J (2002):** Prognostic significance of elevated Cyclooxygenase-2 expression in breast cancer. Cancer Research 62: 632–5.
15. **Nam E, Lee SN, Im SA , Kim DY, Lee KE, and Sung SH (2005):** Expression of cyclooxygenase-2 in human breast cancer:Relationship with HER-2/neu and other clinicopathologicalprognostic factors. Cancer Research Treatment 37(3):165.
16. **Davies G, Salter J, Hills M, Martin LA, Sacks N, and Dowsett M (2003):** Correlation between Cyclooxygenase-2 Expression and Angiogenesis in Human Breast Cancer. Clinical Cancer Research9: 2651–56.
17. **An MS, Kim SH, Yoon HK, Kim WW(2009**): COX-2 Expression in Malignant Breast Tumors. *Journal Korean Surgical Society*; 77 (6): 371-7.
18. **Thorat M.A.·Mehrotra S.·Morimiya A.·Badve S. (2009)**. COX-2 Expression does not correlate with microvessel density in breast cancer. Pathobiology 76:39–44
19. Denkert C, Winzer KJ, Müller BM, Weichert W, Pest S, Köbel M, Kristiansen G, Reles A, Siegert A, Guski H, Hauptmann S (2003). Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer15;97(12):2978-87.
20. **Costa C, Soares R, Reis-Filho JS, Leita˜o D, Amendoeira I, Schmitt FC (2002):** Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. Journal Clinical Pathology 55:429–34.
21. **Kelly LM, Hill AD, Kennedy S, Connolly EM, Ramanath R, Teh S, Dijkstra B, Purcell R, McDermott EW, O'Higgins N (2003):**Lack of prognostic effect of Cox-2 expression in primary breast cancer on short-term follow-up. European Journal Surgical Oncology 29(9):707-10.
22. **Takeshita E,Osanai T, Higuchi T,Saumaoro LT,Sugihara K(2005):** Elevated COX-2 expression is associated with histological grade in invasive ductal carcinoma. Journal Medical Dentistry Science 52:189-193.
23. **Surowiak P**, **Materna V**, **Matkowski R**, **Szczuraszek K**, **Kornafel J**, **Wojnar A**, **Pudelko M**, **Dietel M**, **Denkert C**, **Zabel Mand Lage H (2005):** Relationship between the expression of cyclooxygenase 2 and MDR1/P-glycoprotein in invasive breast cancers and their prognostic significance, Breast Cancer Research, 7: 862-70.
24. **Wülfing P, Diallo R, Müller C, Wülfing C, Poremba C, AchimHeinecke, Rody A, Greb RR, Böcker W and Kiesel L (2003):** Analysis of cyclooxygenase-2 expression in human breast cancer: high throughput tissue microarray analysis. Journal of Cancer Research and Clinical Oncology 129(7):375-382.
25. **Chuba PJ, Hamre MR, Yap J, Severson RK, Lucas D, Shamsa F, and Aref A (2005):** Bilateral risk for subsequent breast cancer after lobular carcinoma-in-situ: analysis of surveillance, epidemiology, and end results data. Journal Clinical Oncology 23(24): 5534-41.
26. **Cho E, Chen WY, Hunter DJ, Stampfer MJ, Colditz GA, Hankinson SE, and Willett WC (2006):** Red meat intake and risk of breast cancer among premenopausal women. Arch Internal Medicine166(20):2253-9**.**
27. **Kirkpatrick KL, Ogunkolade W, Jenkins P, Bustin S, Ghilchick M, Carpenter R, Mokbel K (2001).** Cyclo-Oxygenase-2 and Proliferating Cell Nuclear Antigen in Breast Carcinoma. Proc Am Soc Clin Oncol 20 (abstract 3090).
28. **Ranger GS, THOMAS V, JEWELL A and MOKBEL K(2004).** Elevated Cyclooxygenase-2 Expression Correlates with Distant Metastases in Breast Cancer. Anticancer Research 24: 2349-52.