Impact of COX- 2 “Cyclooxygenase 2” in Breast Cancer

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Abstract

Background: Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma deaths in women. Its etiology is multifactorial, including reproductive factors, hormonal imbalances and genetic predispositions. According to many studies Cycloxygenase-2 (COX-2) plays an important role in the carcinogenesis and increased expression has been regarded as a poor prognostic factor.
Objective: The objective of our study is to evaluate COX-2 expression in breast cancer as a prognostic factor.
Methods: Formalin-fixed and paraffin-embedded tissue blocks were studied for COX-2 expression by immunohistochemistry in 100 patients diagnosed as breast carcinoma. The relationship between COX-2 expression and various clinico-pathological parameters was studied.
Results: The results of our study suggest an association of the expression of COX-2 to the poor prognostic factors in breast cancer, hormonal status and HER2/NEU status. Studying the association between COX-2 protein expression using H-score and clinico-pathological characteristics revealed that the median H-score of COX-2 protein expression was higher in her2/neu positive cases compared to her2/neu negative cases and that was statistically significant with a p value (p = 0.023). Also, statistical significant association was found between hormonal receptor negative and median H-score of COX-2 protein expression (p = 0.029).
Conclusion: Our study showed the association of COX-2 and the adverse prognostic factors in breast cancer.

Keywords: COX-2, Breast cancer, Immunohistochemistry.

Introduction:

Breast cancer is the most frequently diagnosed cancer globally and is the leading cause of cancer-related death in women [1]**.** In 2018, the predicted number of new breast cancers in 28 European Union (EU) countries was 404 920, with estimated age-adjusted annual incidence of breast cancer of 144.9/100 000 and mortality of 32.9/100 000, with 98 755 predicted deaths. In Egypt, it is the most common cancer in females, in 2018 the incidence of breast cancer was 23081 new cases about 35.1% of the incidence of all cancer cases according to Globocan 2018. A female breast cancer is a challenging health problem coming on top of all malignancies [2] with a poor outcome compared to international figures [3] Many studies showed that age at diagnosis of breast cancer in Arab countries is a decade younger than that in Western countries [4].

In breast cancer the molecular characteristics play an important role in tumor prognosis and aggressiveness and may contribute to routine clinical decision making. Additionally, identifying specific molecular patterns help to introduce targeted therapies for cancer treatment. The classical molecular prognostic parameters of breast cancer are estrogen receptor (ER), progesterone receptor (PR) expression and Her-2-neu receptor expression [5],[6].Studies have shown that Cycloxygenase-2 (COX-2) plays an important role in the development of some human cancers, specifically pulmonary, colon and breast cancers. Cyclooxygenase enhances catalyzing the conversion of arachidonic acid to prostaglandin endoperoxide, which is the rate limiting step in prostaglandin and thromboxane biosynthesis. COX-1 and COX-2 are the two isoforms of prostaglandin synthase [7].

COX-1 is characterized as a housekeeping enzyme required for the maintenance of basal level prostaglandins and is expressed constitutively in most tissues. COX-2 is highly inducible and can be rapidly up regulated in response to various proinflammatory agents, including cytokines, mitogens, and tumor promoters, especially in cells involved in inflammation, pain, fever, Alzheimer's disease, osteoarthritis, or tumor formation [8] [9].

Under normal conditions, acute inflammation is a tightly controlled self-limiting response, specific cytokines, including interleukin-1 (IL-1) and IL-6, exert feedback inhibition causing COX-2 expression and

PGE2 production to cease and the inflammatory response to subside. However, with sustained exposure to pro inflammatory stimuli, continued expression of COX-2 leads to the transition from acute to chronic inflammation. Moreover, COX-2 plays a role in the regulation of estrogen by producing prostaglandin E2, which increases the expression of the cytochrome P450 enzyme complex (also known as aromatase) that catalyzes androgen to produce estrogen [10],[11],[12]. During progression of cancer, prostaglandins mediate several mechanisms, including cell proliferation, apoptosis, and angiogenesis. Therefore, the aim of our study is to evaluate the COX-2 protein expression in breast cancer and its relation with clinical and histological prognostic parameters.

Patients and Methods:

A total number of one hundred formalin-fixed and paraffin-embedded tissue blocks were collected from the archived materials of pathology department in the South Egypt Cancer Institute. There were taken either by True cut biopsy, breast conservative surgery or modified radical mastectomy. Clinicopathological parameters such as patient age, sex, tumor size (T), lymph node metastasis (LN)hormonal status (ER& PR), HER2/NEU and stage, all were obtained from the available histopathological reports, and the overall survival was obtained from the patient medical record files of SECI.

Immunohistochemistry:

Three µm thick formalin-fixed paraffin-embedded tissue sections were cut and Sections were dewaxed in Xylene (for half an hour) and rehydrated through graded alcohols from 100%-70% then washed in Distilled water. Pre-treatment with heat-induced epitope retrieval (HIER) was done using citrate buffer pH 9 for 20 minutes at 97 c. Slides were then washed 2-3 times with phosphate buffer solution (PBS). Blocking of endogenous peroxidase activity was performed using peroxidase blocking reagent (Genemed, Sakura, USA) and incubated 5 minutes a Polyclonal Anti-PTGS2/ COX2 antibody with Catalog no. #YPA1044 primary antibody (Chongqing Biospes Co., Ltd, China) diluted by 1:150 was applied to the sections and incubated for 30 minutes at room temperature. Then the slides were washed 2-3 times using PBS. After washing, immunostaining was performed using a universal staining kit, (Poly HRP/DAB (Ready-To-Use), Genemed, Sakura, USA) following the manufacturer's instructions. The secondary antibody was applied to the slides and incubated for 20 minutes at room temperature, then rinsed and washed with PBS twice, the detection was done by DAB chromogen and substrate for 5 min using the same kit. Sections were then counterstained using Mayer's hematoxylin (Dako, Denmark) for 5 minutes then washed in distilled water, dehydrated in ascending alcohols from 70%-100% then cleared in Xylene and left to dry in air room temperature in a humidity chamber to prevent unnecessary background staining.

Evaluation of COX-2 protein expression

COX-2 positivity was indicated by the presence of brown cytoplasmic staining as shown at **figure 1**. Staining was assessed using H-score, which is a semi-quantitative approach. In this approach, staining intensity was first determined for all cells (0,1,2,3,for negative, weak, moderate and strong intensity respectively), then the percentage of cells at each staining intensity was calculated and finally H-score is calculated using the following formula: (3× percentage of strongly staining malignant cells) + (2×percentage of moderately staining malignant cells) + (1× percentage of weakly staining malignant cells) which give a range from 0 to 300[13].

Statistical Analysis:

All statistical calculations were done using SPSS (statistical package for the social science; SPSS Inc., Chicago, IL, USA) version 22. Data which are normally distributed were statistically described in terms of mean ± standard deviation (±SD), frequencies (number of cases) and percentages were used for qualitative data. For comparing quantitative data, Mann Whitney U test was performed because the data were not normally distributed. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Kaplan-Meier test was performed to compare overall survival between both study groups. P-value is always 2 tailed set significant at 0.05 level.

Results:

Table 1 summarize the association between COX-2 protein expression and different clinico-pathologic characteristics of patients.

The mean age of our patients was 50 (50.82 ± 12.69) years. According to the stage 5% of cases were of stage I, 42% were of stage II, 46% were stage III, and 8% were of stage IV. Regarding the tumor size, T2 was the commonest tumor size representing (50%) of cases followed by T3 (32%), T1 (13%) and T4 (5%) of cases The majority of cases presented by invasive ductal carcinoma by 95 %, only 5% were other histo-pathological types Regarding the hormonal profile; 69 cases were estrogen receptor positive. Also 63 cases were progesterone receptor positive, and 12 cases were Her2/ NEU positive. All clinico-pathologic features are summarized in Table 1

Table 2 demonstrates association between cox2 protein expression and different clinico-pathologic features using H-score.

Studying the association between COX-2 protein expression using H-score and clinico-pathological characteristics revealed that the median H-score of cox2 protein expression was higher in her2/neu positive cases compared to her2/neu negative cases and that was statistically significant with a p value ($p = 0.023$). Also,

statistical significant association was found between hormonal receptor status and median H-score of COX-2 protein expression (p = 0.029).

No statistical significant association was found between median H-score of COX-2 protein expression and age (p = 0.19), site of tumor (p = 0.466), stage (p = 0.236), tumor size (0.331), and lymph node metastasis (p = 0.871).

Table 3 & table 4: Survival analysis: The disease free survival according to the H- score were shown using Kaplan-Meier survival curves (figure 2) wasn't show any significance (p = 0.412), also the overall survival show no significance with (p = 0.975) (figure 3).

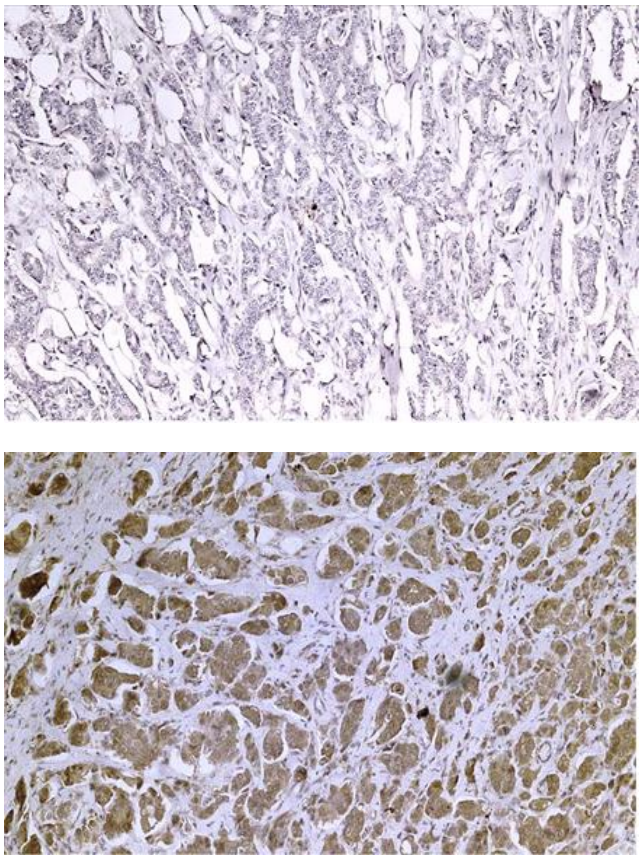

Figure (1): showed cox-2 protein expression in breast carcinoma.
(A) A case of breast carcinoma showed negative immunoreactivity of Cox-2 protein expression.
(B) A case of breast carcinoma showed brown cytoplasmic staining in tumor cells.

Table (1): Clinico-pathological features of the studied Participants:

Variable name		N = 100 N (%)	
Age (years), mean ± SD		50.82 ± 12.69	
Sex	Male	1	(1.0)
	Female	99	(99.0)
Site of tumor	Right	58	(58.0)
	Left	42	(42.0)
Stage	Stage 1	5	(5.0)
	Stage 2	41	(41.0)
	Stage 3	46	(46.0)
	Stage 4	8	(8.0)
Tumor size	T1	13	(13.0)
	T2	50	(50.0)
	T3	32	(32.0)
	T4	5	(5.0)
Lymph node metastasis	N0 (no node)	25	(25.0)
	N1 (1-3 Node)	26	(26.0)
	N2 (4-9 Node)	19	(19.0)
	N3 (10 or more Node)	30	(30.0)
ER	Negative	31	(31.0)
	Positive	69	(69.0)
PR	Negative	37	(37.0)
	Positive	63	(63.0)
HER2/neu	Negative	88	(88.0)
	Positive	12	(12.0)
Pathology	IDC	95	(95.0)
	Other Pathology	5	(5.0)

Table (2): Association of COX-2 protein expression using H-score and different clinico-pathologic features

Variable name		H score Median (range)	p-value
Age	≤ 50	200 (30 – 300)	0.191
	> 50	200 (10 – 300)	
Site of tumor	Right	200 (10 – 300)	0.466
	Left	200 (60 – 300)	
Stage	Early	200 (30 – 300)	0.236
	Advanced	200 (10 – 300)	
Tumor size	< 5	200 (20 – 300)	0.331
	≥ 5	200 (10 – 300)	
Lymph node metastasis	No node	200 (60 – 300)	0.871
	Node positive	200 (10 – 300)	
Hormonal receptors	Negative	200 (120 – 300)	0.029*
	Positive	200 (10 – 300)	
HER2/neu	Negative	200 (20 – 300)	0.023*
	Positive	300 (10 – 300)	

Table (3) Disease free survival according to the H-score result

Survival	Estimate ± SE		P-value
	≤ 200	> 200	
At 1 year	91.1±4.3%	81.8±8.2%	0.412
At 2 year	88.4±4.9%	81.8±8.2%	
At 3 year	77.3±7.4%	65.5±16.0%	
At 4 year	77.3±7.4%	65.5±16.0%	

Table (4): Overall survival according to the H-score result

Survival	Estimate ± SE		P-value
	≤ 200	> 200	
At 1 year	80.7±5.5%	86.4±7.3%	0.975
At 2 year	80.7±5.5%	75.3±9.8%	
At 3 year	77.8±6.0%	75.3±9.8%	
At 4 year	73.5±7.1%	75.3±9.8%	

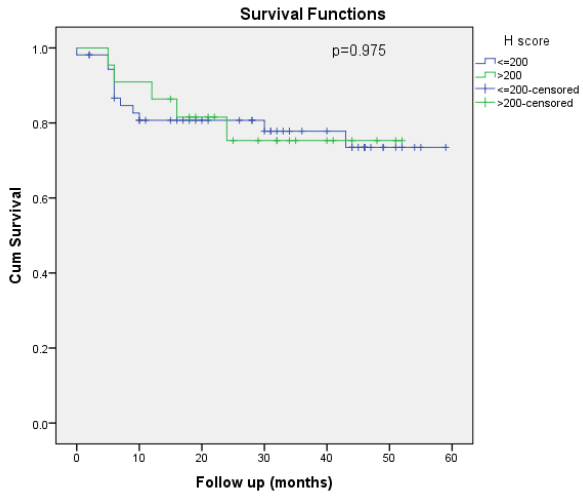

Fig. (2): disease free survival

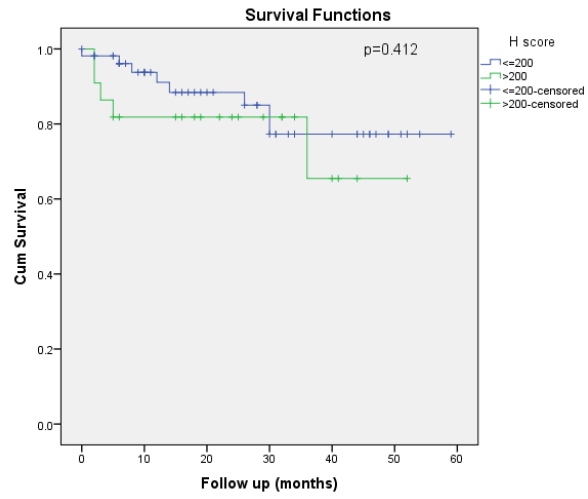

Fig. (3): overall survival

Discussion:

Breast cancer is the most frequently diagnosed cancer globally and is the leading cause of cancer-related death in women [14], female breast cancer is a challenging health problem coming on top of all malignancies with a poor outcome compared to international figures [3].

Therefore, there is substantial interest in identification of novel markers that could be used as prognostic or predictive markers and therapeutic targets. Expression of estrogen receptor (ER), progesterone receptor (PR) and Human Epithelial Growth Factor Receptor 2 (Her2) as predictive and/or prognostic markers has been well established in multiple studies and has led to a major shift in treatment approach.

Cyclooxygenase (*COX*) is a group of enzymes are important for the conversion of arachidonic acid to prostaglandins.

COX-1 is characterized as a housekeeping enzyme required for the maintenance of basal level prostaglandins [15] and is expressed constitutively in most tissues.

In adults, COX2 is found only in the central nervous system, kidneys, vesicles, and placenta, whereas in the fetus, it occurs in the heart, kidneys, lungs, and skin [16].

COX-2 regulates tumor growth, invasion and metastasis in breast cancer. Various research articles suggest that *COX-2*-derived metabolites may contribute to maintenance of tumor viability, premalignant hyper proliferation, tumor growth, transformation, invasion and metastatic spread [17]

Regarding calculating cox2 by H-score in our study, it showed significance association with, negative hormonal status, and positive HER2/NEU which is similar to various studies reported that COX-2 expression was correlated with ER negative[18], PR negative and HER-2/neu positive status [19], which may be explained as 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 [20]. Both PKC [21] and mutated RAS [22] have been associated with an increased metastatic potential in cell lines.

HER-2/neu was over expressed in approximately 20–30 % of invasive breast cancers and was an independent marker of poor prognosis [23]. We found that high levels of COX-2 expression correlated with HER-2⁄neu overexpression which show highly significant, which explained by COX-2 can stimulate HER-2/neu expression via EGFR through PGE2. So COX-2 mediates variety of cellular processes including tumor growth, apoptosis, differentiation, cell cycle, lymph node metastasis and angiogenesis, however no significant correlation was found betwteen COX-2 status and estrogen receptor status, progesterone receptor status or HER-2-neu expression , in the study done by many researches [24], [25].

However, there was no significance was detected as regard other prognostic factors like tumor size, lymph nodes metastasis , and advanced stage , which was different to many studies [24]& [17].

Also the present study failed to find a statistically significant relationship between the COX-2 immunoexpression and the histological subtype, in contrary to our results, many studies found significant correlation between COX-2 immunoexpression and the histological subtypes of breast carcinoma may be due to the majority of our cases were invasive ductal carcinoma with few numbers with other histological subtypes [26] & [27]

Regarding the survival, our study showed no statistically difference in disease free survival or overall survival between COX2 by using H-score, on the other hand there was various studies showed elevated COX-2 expression was significantly associated with decreased 5-year OS and DFS rates of patients with breast cancer [17].

Accordingly, another larger multicenter study is recommended to evaluate COX-2 protein expression as a prognostic factors in breast cancer.

Conclusion:

COX-2 was calculated in breast cancer by using H-score showed that statistically significant association with negative hormonal profile and HER2/Neu but there was no significance with other prognostic factors and survival analysis so that another study with large numbers of patients is recommended to confirm these results.

List of abbreviations:

COX-2= Cycloxygenase-2
PR= Progesterone receptor
ER= Estrogen receptor
PGE2= Prostaglandin E2
SECI = South Egypt Cancer Institute

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