COX-2 "Cyclooxygenase 2"as a Prognostic marker in Breast Cancer

Fatma Z. Abdel-rahman[a], Amen H. Zaky[a], Ashraf Zedan[a], Tarek M. Elsaba[b], Adel Gabr[a]

[a]Department of Medical Oncology and Hematology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
[b]Department of Pathology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.

Abstract

Background: Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among women in both developed and developing countries. Globally, the incidence rate of breast cancer has been rising rapidly over the past few decades.Cycloxygenase-2 (COX-2) regulates tumor growth, invasion and metastasis in breast cancer.

Objective: The objective of our study is to evaluate COX-2 expression in breast cancer as a poor 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, such as larger tumor size, positive lymph node status, higher T stage and N stage as studying the association between Cox-2 protein expression and different clinco-pathologic features revealed that larger tumor size (> 5) and lymph node metastasis showed statistical significant association with Cox2 protein expression ($p = 0.014$ and $p = 0.031$, respectively). While rest of clinico-pathologic features such as age, stage, hormonal receptor status, HER2/NEU and histopathological features showed no statistical significant association.

Conclusion: Our study established the role of COX-2 in carcinogenesis and its association with adverse prognostic factors.

Keywords Breast cancer. COX-2.Prognostic marker. Immunohistochemistry (IHC).

Introduction

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death among women in both developed and developing countries. Globally, the incidence rate of breast cancer has been rising rapidly over the past few decades **(Jemal et al., 2010)**. Worldwide, there were about 2.1 million newly diagnosed female breast cancer cases in 2018, accounting for almost 1 in 4 cancer cases among women. Breast cancer is the most frequently diagnosed cancer in the vast majority of countries (154 of 185) and is also the leading cause of cancer death in over 100 countries **(Bray et al., 2018)**.

Today Breast cancer is considered to be a heterogeneous disease, the term is used as an umbrella for a multitude of molecularly defined tumor types. In addition, there is our knowledge of the intratumoural heterogeneity, since a tumor contains various molecular subpopulations including also cells with stem cell properties **(Zardavas et al., 2013)**.

Although a greater proportion of women are diagnosed in early disease stages because of national screening programs and increasing awareness, 3% to 5% of patients still present with metastatic disease at diagnosis **(Siegel RL et al., 2015)** In addition, 20% to 85% of patients who undergo complete resection develop distant metastasis (DM) **(Desantis et al., 2014).**

The molecular characterization of this malignancy is an indicator for tumor prognosis and aggressiveness and may contribute to clinical decision making. Additionally, identifying specific molecular patterns helps to introduce specifically 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 **(Pakkiri et al., 2009; Ross et al., 2009)**.

Studies have shown that Cycloxygenase-2 (COX-2) plays an important role in the development of some human cancers, particularly pulmonary, colon and breast carcinoma as well as their pre-invasive lesions. Cyclooxygenase (also known as Prostaglandin. 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 **(Williams and Dubois, 1996)**.

COX-1 is constitutively produced by most of the body tissues, while COX-2 is an inducible enzyme and is produced under certain specific conditions like inflammation and tumor microenvironment. 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 **(Richards et al., 2002; Brueggemeier et al., 2003; DíAz-Cruz et al., 2005)**.

During progression of cancer, prostaglandins mediate several mechanisms, including cell proliferation, apoptosis, and angiogenesis.

Therefore, the aim of our present study is to determine the COX-2 expression in as a prognostic marker in breast cancer.

Materials 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 unnecessarybackground staining.

Evaluation of Cox-2 protein expression

COX-2 positivity was indicated by the presence of brown cytoplasmic staining as shown at **figure 1**. We applied for evaluation of Cox-2 protein expression by using scoring system was categorizing Cox-2 protein expression into negative (no stained cells) and positive **(Hwang et al., 1998; Leo et al., 2006)**.

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

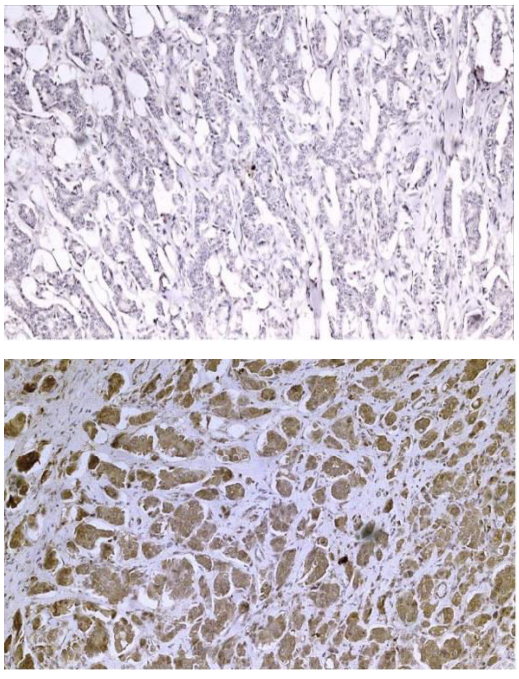

Figure (1): Showed cox-2 protein expression in breast carcinoma.

(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.

Clinic-pathological features

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 histopathological 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. Clinic-pathological features of the 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)

Association of Cox2 protein expression (positive versus negative) and different clinic-pathologic features

Evaluation of Cox-2 protein expression as positive versus negative expression revealed that 76% of cases were positive for Cox-2 protein expression.

Studying the association between Cox-2 protein expression and different clinco-pathologic features revealed that larger tumor size (> 5) and lymph node metastasis showed statistical significant association with Cox2 protein expression (p = 0.014 and p = 0.031, respectively). While rest of clinico-pathologic features such as age, stage, hormonal receptor status and histopathologic features showed no statistical significant association.

Table 2. Association of Cox2 protein expression (positive versus negative) and different clinic-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)	

Expression of cox2 protein in the studied samples

COX-2 scoring system (positive versus negative expression)76% were positive cases showing positive cytoplasmic expression in tumor cells while 24 % of cases were negative (pie chart) **Figure2.**

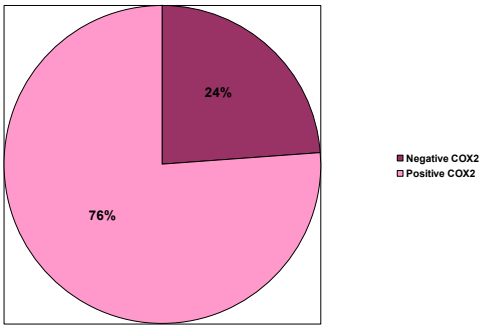

Fig 2. Expression of cox2 protein in the studied sample.s

Survival analysis (DFS and overall survival) according to the COX-2:

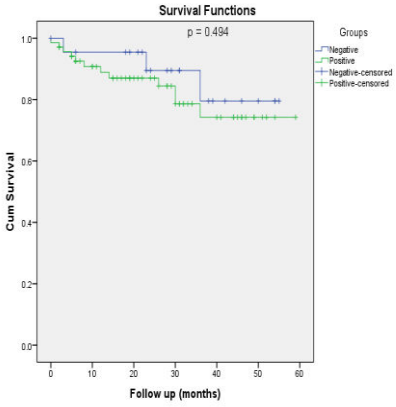

Fig. (3): disease free survival

Survival analysis regarding COX-2 (positive versus negative)

For the disease free survival analysis and overall survival were shown using Kaplan-Meier survival curves **(Figure 3)** and **(Figure 4)**, that wasn't show any significance between cox-2 positive or negative **(p = 0.494)** and **(p = 0.996)**, respectively.

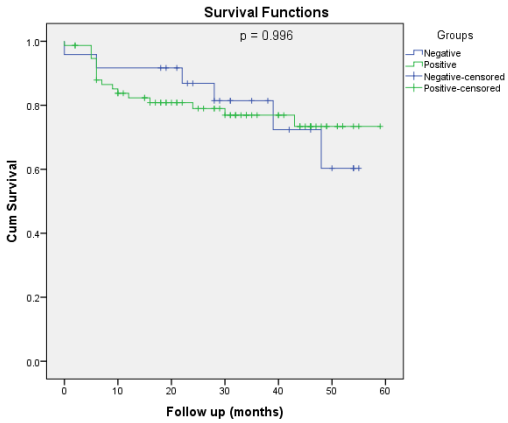

Fig. (4): overall survival

Discussion

Regarding age, median age in our study was 50.84 years we found no significant correlation between COX-2 expression and the patient's age at presentation. This finding was in accordance with the studies done by**Solanki et al. (2018)**and **(Singh et al., 2004)** which showed no association between the mean age of presentation and the expression of COX-2. Similar results were obtained in various other studies performed by **Dannenberg and Howe (2003)**, **Lee et al. (2010)**.

The present study failed to find a statistically significant relationship

between the COX-2 immunoexpression and the histological subtype, in contrary to our results**Ristimäki et al. (2002) and Misron et al. (2015)**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.

No correlation was found between tumor stage and the COX-2 immunoexpression in this study, although COX-2 expression was increasing with increase in tumor stage but did not reach a statistically significant level, the same results were reported by **(Solanki et al., 2018)(Ameen et al., 2018)**, all this studies are not matched with the results which show that COX2 was expressed in advanced stage compared to early stage this may be need increase our cases , in contrast **(Jana et al., 2014)** which showed a significant correlation between early and advanced stages as COX2 protein expression was increased with the advanced stages of breast cancer.

A study done by**(Solanki et al., 2018)** showed a positive correlation between COX-2 expression and larger tumor size with a significant P-value (<0.001)., also There was a significantly positive correlation between positive COX-2 expression and lymph node involvement, another many studies have demonstrated that COX-2 expression was significantly correlated with large tumor size and advanced stage of disease**(Shim et al., 2003)** findings were observed by **Dannenberg and Howe (2003)**, **Ristimäki et al. (2002)** and **Arun and Goss (2004)**. It has been reported that elevated COX-2 expression was more common in tumors with axillary lymph node metastasis and a larger size **(Costa et al., 2002, Ristimäki et al., 2002, Denkert et al., 2003).**

These results correlated to our study that show COX-2 protein expression was statistically significantly correlated with large size tumors which was similar also to**(Jana et al., 2014)** However, in the studies performed by **Lee et al. (2010)** and **Misron et al. (2015)**, no significant correlation was found with the tumoursize.This might be because of the low sensitivity of the immunohistochemical analysis performed.

COX- 2 expression to be more frequent in patients with lymph node

metastasis, these findings were in concordance with the studies done by**Rozenowicz et al. (2012)**; **Lee et al. (2010)**;**Dannenberg and Howe (2003)**;**Jana et al. (2014)**. However, **Costa et al. (2002)**;**Misron et al. (2015)** showed that there was no significant correlation between COX-2 positivity and node status. Correlation between lymph node positivity and higher COX-2 expression is associated with tumor spread and a poor prognosis.

Various studies reported that COX-2 expression was correlated with ER negative **(Denkert et al., 2003)**, PR negative and HER-2/neu positive status **(Zeeneldin et al., 2009**). HER-2/neu is over expressed in approximately 20–30 % of invasive breast cancers and is an independent marker of poor prognosis. **(Tsutsui et al., 2002)** these results were different from our results that didn't show any significance with hormonal profile or HER-2/neu.

Regarding the survival, our study show no statistically difference in disease free survival or overall survival between COX2 negative and positive cases, in contrast to various studies showed that elevated COX-2 expression was significantly associated with decreased 5-year OS and DFS rates of patients with breast cancer **Jana et al. (2014)**.

Logistic regression was done on tumor size , lymph node metastasis , hormonal profile , and HER2/neu for predicting COX-2 positive tumor biomarker but it showed no significance with the previous prognostic factors , in contrast to a study done by **Jana et al. (2014)** which showed a highly significance with those clinico-pathological factors , maybe we need to increase our study participants to reach this results

References

Ameen MaM, Jalal JA,Alnuaimy WMT. (2018). Cyclooxygenase-2 Immunoexpression in Invasive Breast Carcinoma: A Possible Prognostic Factor. J. Journal of Kurdistan Board of Medical Specialties, 4(1):15-24.

Arun B, Goss P. (2004). The role of COX-2 inhibition in breast cancer treatment and prevention. Seminars in oncology, 31(3):22-29.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA,Jemal A. (2018). Global cancer statistics 2018, GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a

cancer journal for clinicians, 68(6):394-424.

Brueggemeier RW, Richards JA,Petrel TA. (2003). Aromatase and cyclooxygenases: enzymes in breast cancer. The Journal of steroid biochemistry and molecular biology, 86(3-5):501-507.

Costa C, Soares R, Reis-Filho J, Leitao D, Amendoeira I, Schmitt F. (2002). Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. Journal of clinical pathology, 55(6):429-434.

Dannenberg A, Howe L. (2003). The role of cox-2 in breast and cervical cancer. ProgExp Tum Res, 37(6):90-106.

Denkert C, Winzer KJ, Müller BM, Weichert W, Pest S, Köbel M, Kristiansen G, Reles A, Siegert A,Guski H. (2003). Elevated expression of cyclooxygenase-2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer, 97(12):2978-2987.

Desantis C, Ma J, Bryan L,Jemal A. (2014). Breast cancer statistics, 2013. CA: a cancer journal for clinicians, 64(1):52-62.

DíAz-Cruz ES, Shapiro CL,Brueggemeier RW. (2005). Cyclooxygenase inhibitors suppress aromatase expression and activity in breast cancer cells. The Journal of Clinical Endocrinology, Metabolism, 90(5):2563-2570.

Hwang D, Scollard D, ByrneJ. et al.(1998). Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. J Natl Cancer Inst, 90:455–60.

Jana D, Sarkar DK, Ganguly S, Saha S, Sa G, Manna AK, Banerjee A, Mandal S. (2014). Role of Cyclooxygenase 2 (COX-2) in Prognosis of Breast Cancer. Indian Journal of Surgical Oncology, 5(6):59-65.

Jemal A, Siegel R, Xu J, Ward E. (2010). Cancer statistics, 2010. CA: a cancer journal for clinicians, 60(5):277-300.

Lee JA, Bae JW, Woo SU, Kim H. Kim CH (2010). Correlation between COX-2 expression and hormone receptors in invasive ductal breast cancer. Journal of the Korean Surgical Society, 78(3):140-148.

Leo C, Faber S, Hentschel B, Hockel MHL.(2006). The status of cyclooxygenase-2 expression in

ductal carcinoma in situ lesions and invasive breast cancer correlates to cyclooxygenase-2 expression in normal breast tissue. Ann DiagnPathol, 10:327–32.

Misron NA, Looi L-M & Nik Mustapha N. (2015). Cyclooxygenase-2 expression in invasive breast carcinomas of no special type and correlation with pathological profiles suggest a role in tumorigenesis rather than cancer progression. Asian Pac J Cancer Prev, 16(4):1553-1558.

Pakkiri P, Lakhani SR, Smart CE. (2009). Current and future approach to the pathologist's assessment for targeted therapy in breast cancer. Pathology, 41(1):89-99.

Richards JA, Petrel TA, Brueggemeier RW. (2002). Signaling pathways regulating aromatase and cyclooxygenases in normal and malignant breast cells. The Journal of steroid biochemistry and molecular biology, 80(2):203-212.

Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H, Isola J. (2002). Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer research, 62(3):632-635.

Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM,Hortobagyi GN. (2009). The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. Oncologist, 14(4):320-321.

Rozenowicz R, Santos R, Rodrigues F, Oliveira A, Oliveira V, Aldrighi J, Silva MG (2012). 213. Cox-2 and its association with prognostic factors and response to neoadjuvant chemotherapy in patients with breast cancer. European Journal of Surgical Oncology, 38(9):803-810.

Siegel RL, Miller KD,Jemal A, (2015). Cancer statistics, 2015. CA: a cancer journal for clinicians, 65(1):5-11.

Singh SK, Saibaba V, Ravikumar V, Rudrawar SV, Daga P, Rao CS, Akhila V, Hegde P, Rao YK. (2004). Synthesis and biological evaluation of 2, 3-diarylpyrazines and quinoxalines as selective COX-2 inhibitors. J Bioorganic medicinal chemistry, 12(8):1881-1893.

Solanki R, Agrawal N, Ansari M, Jain S. Jindal A. (2018). COX-2 expression in breast carcinoma with correlation to clinicopathological

parameters. Asian Pacific Journal of Cancer Prevention, 19(2):1971-1975.

Tsutsui S, Ohno S, Murakami S, Hachitanda Y. Oda S. (2002). Prognostic value of c-erbB2 expression in breast cancer. Journal of surgical oncology, 79(4):216-223.

Williams CS, Dubois RN. (1996). Prostaglandin endoperoxidesynthase: why two isoforms? American Journal of Physiology-Gastrointestinal and Liver Physiology, 270(3):G393-G400.

Zardavas D, Baselga J,Piccart M. (2013). Emerging targeted agents in metastatic breast cancer. J Nature reviews Clinical oncology, 10(4):191-198.

Zeeneldin A, Mohamed A, Abdel H, Taha F, Goda I,Abodeef W. (2009). Survival effects of cyclooxygenase-2 and 12-lipooxygenase in Egyptian women with operable breast cancer. Indian journal of cancer, 46(1):54-62.