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Cyclooxygenase-2 Immunoexpression in Invasive Breast Carcinoma: A Possible Prognostic Factor

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Abstract

Background and objectives: cyclooxygenase-2 is well known to be overexpressed in breast cancer and it plays negative effects on tumor growth and progression. The aims of this study were to determine the frequency of Cyclooxygenase-2 immunoexpression in invasive breast carcinoma and to evaluate its relationship with some clinicopathological parameters. **Methods:** During the period January 2015 to July 2016, 100 formalin-fixed, paraffin-embedded tissue specimens of invasive breast carcinoma were collected from pathology departments of Rizgary teaching hospital and private labs in Erbil city. Using labeled polymer and enhanced polymer system (DakoEnVisionTMFlex) Dakoprotocol, Cyclooxygenase-2 expression was assessed immunohistochemically. **Results:** The Cyclooxygenase-2 immunoexpression was detected in 48% of invasive breast carcinoma. A statistically significant association was found between Cyclooxygenase-2 immunoexpression and lymphovascular invasion. Also a rising trend of Cyclooxygenase-2 immunoexpression was observed with increasing age, tumor size and tumor stage but didn't reach a statistically significant level. No any statistical significant association was found between Cyclooxygenase-2 immunoexpression and tumor grade. **Conclusions:** cyclooxygenase-2 overexpression is observed in 48% of cases of invasive breast carcinoma and was significantly correlated to lymphovascular invasion,

Keywords: Cylooxygenase-2; Invasive breast carcinoma; Immunohistochemistry.

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide[1]. It is the most common type of malignancy among Iraqi population in general. It accounts for approximately one third of the registered female cancers according to the Iraqi Cancer Registry publication[2]. Molecular characterization of this cancer is an indicator for tumor prognosis and aggressiveness and may contribute to the routine clinical decision-making[3,4]. Many studies have defined prostaglandin E2 (PGE2) and associated proteins in the cyclooxygenase (COX)-system as poor prognostic biomarkers[4-8]. The cyclooxygenases (COXs) are a family of enzymes, which catalyze the rate-limiting step of prostaglandin biosynthesis[9]. Three isomers has been identified, cyclooxygenase-1 which play role in tissue homoeostasis[10]. Cyclooxygenase-2 (COX-2) is the inducible isoform, which is over expressed during both inflammation and cancer[11]. COX-3 has been identified as a splice variant of COX-1, and it is present mainly in brain and spinal cord[12]. COX-2 play role as a Tumor Promoter as its overexpression has been detected in a number of tumors, such as colorectal and breast cancers[13,14]. Also it plays role as a regulator of cell proliferation as it has been found that prostaglandins were able to increase cell proliferation of hormonal-dependent breast cancer by increasing transcription of CYP19 aromatase implicated in estrogen biosynthesis. It's also implicated in extrinsic apoptotic cell signaling mechanisms[14].

Materials and methods

This cross-sectional study was conducted after permission approval granted by Kurdistan Board of Medical Specialties. Samples of primary breast carcinoma were obtained from 100 female patients who underwent radical mastectomy or wide excision with or without axillary clearance for breast cancer. They were collected from Rizgary teaching hospital and some private labs in Erbil city where tissue blocks were collected during a period spanning from January 2015 to July 2016. For each case, all Hematoxyline and Eosin stained sections were reviewed, concerning the type and grade of tumor, lymphovascular invasion, axillary lymph node status and tumor stage. Information was gained from the patient's case sheaths from the histopathology department archive. Tumors were typed according to the WHO classification system and graded according to the Nottingham modification of the Bloom - Richardson grading scheme[15].

A full clinicopathological staging were evaluated, depending on American Joint Committee on Cancer (AJCC) criteria[16].

Labeled polymer and enhanced polymer systems (Dako EnVision™ Flex) method according to Dako recommendation was used to stain the tissue by COX-2 antibody. The COX-2 expression was observed in the cytoplasm of the cancer cell, but when the staining was strong it also stained the cell membrane. According to Spizzo et al [17]; the immunohistochemical score (IHS) was calculated by

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multiplying an estimate of the percentage of immunoreactive cells (proportional score) with an estimate of the staining intensity (intensity score), as follows:
No staining was scored as 0, 1–10% of cells stained scored as 1, 11–50% as 2, 51–80% as 3 and 81–100% as 4. Staining intensity was rated on a scale of 0 to 3; 0 = negative; 1 = weak; 2 = moderate, and 3 = strong. When there was multifocal immunoreactivity in staining intensities between foci, the average of the staining intensity was recorded. An IHS score of 9 –12 was considered strong immunoreactivity, 5 –8 was considered moderate, 1 –4 was considered weak, and 0 was scored as negative. Positive and negative control slides were involved in each run of staining. Positive controls for COX-2 include sections of COX-2 positive colonic adenocarcinoma. While negative controls were prepared from the same tissue block, but incubated with TBS instead of the primary antibody.
Only samples with a final expression score >4 were defined as 'overexpressing'. Sections in which the staining could not be well characterized considered as equivocal. The collected data was analyzed using computerized statistical software; Statistical Package for Social Sciences (SPSS) version[18]. Association between COX2 expression and some clinicopathologic factors were evaluated using the Chi square test and a P value equal or less than 0.05 was considered significant.

Results

Ages of patients ranged from 23-86 years with a mean age ±SD of 50.20±13.324 years, regarding size of the tumors, it ranged from 1-10 cm with a mean size±SD of 3.94±1.945cm. The other pathological data of the patients are summarized in Table 1.

Table (1): Pathological data of the sampled cases

Variables	Categories	No.	%
Tumor subtype	Invasive ductal carcinoma, NST	89	89%
	Invasive lobular carcinoma	4	4%
	Others *	7	7%
Tumor grade	Grade I	3	3%
	Grade II	62	62%
	Grade III	35	35%
Lymphovascular invasion	Present	18	18%
	Absent:	82	82%
Lymph node involvement	Present	50	50%
	Absent	23	23%
	No data available	27	27%
Tumor stage	Stage I	16	16%
	Stage II	58	58%
	Stage III	20	20%
	Stage IV	5	5%
	No data available:	1	1%

*Including combined ductal and lobular carcinoma 3 (3%), papillary carcinoma 2(2%), medullary carcinoma 1(1%) and mucinous carcinoma 1(1%).
COX-2 was found to be overexpressed in 48 % (48 cases), 18 % showed a strong expression (Figure 1), 30% moderate expression (Figure 2). It was found to be negative in 52 % (52 cases) including 37% weak expression and 15% completely negative.

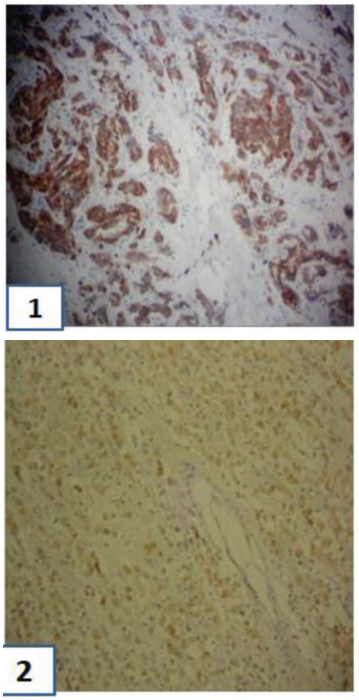

Figure (2): Invasive ductal carcinoma, Strong COX-2 (1) and Moderate (2) immunoreactivity (IHC, x100).

The associations between COX-2 expression and clinicopathological characteristics of patients are illustrated in Table 2.

Table (2): Correlation between COX-2 over expression and clinicopathological parameters

Variables	Categories	No.	COX-2 overexpression		P value
			Negative	Overexpression	
Age groups	< 50	64	35	29	0.4
	> 50	36	17	19	
Tumor size	< 5 cm	77	42	35	0.2
	> 5 cm	23	10	13	
Histological type	Ductal	89	46	43	0.3
	Lobular	4	1	3	
	Others	7	5	2	
Tumor grade	Grade I	3	1	2	0.7
	Grade II	62	32	30	
	Grade III	35	19	16	
Lymphovascular invasion	Present	18	4	14	0.005
	Absent	82	48	34	
Lymph node status	Present	50	24	26	0.71
	Absent	23	10	13	
	No data available	27	18	9	
Tumor stage	Stage I	16	9	7	0.5
	Stage II	58	32	26	
	Stage III	20	9	11	
	Stage IV	5	1	4	
	No data available:	1	1	0	

Discussion

As breast cancer forms a major medical problem globally, it has been shown to have an interesting subject for researchers to understand the molecular bases of its tumorogenesis, tumor progression and to find superior ways for its management. Many researches have been done on the COX-2 and linked it's overexpression with poor prognostic biomarkers. The current study was a trial to follow these researches and link the COX-2 over expression with the clinicopathological data in Erbil city.

In the present study COX-2 overexpression was seen in 48% of the cases. This result fell within rate ranges (21.2% to 79.6%) reported in different studies.

This wide range of COX-2 immunoexpression in different studies may be attributed to the number of studied cases, immunohistochemical methodology used including tissue fixation, the choice of the antibody, sensitivity of the detection system and the determination of the criteria used for the positive result. Also differences in population group, diversity of risk habits and variation of genetic predisposition may contribute to that wide range of COX-2 immunoexpression that reported in different countries[32-35].

COX-2 immunoexpression was noted to be more with increase in the tumor size but didn't reach a statistically significant level just similar to what have been reported by other studies done in different countries[17,36, 34, 37-41], in contrast, Ristimäki et al.[5] found a significant correlation with tumor size. Actually our study also found a statistically significant correlation when the COX-2 immunoexpression was taken into consideration as four categories i.e. (negative, weak, moderate, strong) instead of two categories i.e. (negative and positive) with a p value of less than 0.0[5]. The present study failed to find any statistical significant relationship between the COX-2 immunoexpression and the histological subtypes although it was found to be more in the lobular type invasive breast carcinoma. In contrary to our results, Ristimäkiet al.,[5] Spizzo et al.,[17] and Misron et al.,[36] found significant correlation between COX-2 immunoexpression and the histological subtypes of breast carcinoma.

Most of our cases were grade II (65%), the immunoexpression of COX-2 was more in grade I followed by grade II and grade III respectively, but no statistically significant relationship was noted to be present between the COX-2 immunoexpression and tumor grade. This is in agreement with the findings of other studies done in different countries [17, 18, 31, 34, 37- 41]. In contrary, Ristimäki et al., [5] Misron et al., [36] found positive correlation between COX-2 expression and tumor grade.

A positive correlation was detected between COX-2 immunoexpression and Lymphovascular invasion. This is consistent with findings of Ristimäki et al.,[5] Zhang et al.,[37] who proposed that COX-2 reduces the adherence of tumor cells to the extracellular matrix promoting angiogenesis and development of metastasis.[42] These findings suggest that elevated expression of COX-2 in breast carcinoma may reflect a more aggressive biological behavior. In contrary, Cho et al.,[39] Leo et al.,[40] Misron et al.,[36] Davies et al.,[34] and Serra et al.,[18] didn't found a significant correlation between COX-2 expression and lymphovascular invasion.

Lymph node involvement by metastasis was detected in 50% of the current cases, but statistically no significant relationship was detected with the COX-2 immunoexpression and this is consistent with the findings of other studies done in different countries[17,34-36,38-41] where a significant correlation between COX-2 expression and lymph

node involvement was demonstrated.

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 Aggarwal et al.,[43] and Surowiak et al.[24].

Conclusions

COX-2 immunoexpression was seen in 48% of cases of invasive breast carcinoma with a statistically significant association with lymphovascular invasion. Further studies are required to verify COX-2 as independent prognostic factor.

References

1. Pakkiri P, Lakhani SR, Smart CE. Current and future approach to the pathologist's assessment for targeted therapy in breast cancer. Pathology. 2009; 41: 89-99.
2. Iraqi Cancer Registry 2012. Iraqi Cancer Board. Ministry of Health, Republic of Iraq, 2015.
3. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti–HER-2 therapy and personalized medicine. Oncologist. 2009; 14: 320-68.
4. Rozic JG, Chakraborty C, Lala PK. Cyclooxygenase inhibitors retard murine mammary tumor progression by reducing tumor cell migration, invasiveness and angiogenesis. IJC. 2001; 93: 497-506.
5. Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res. 2002; 62: 632-5.
6. Subbaramaiah K, Dannenberg AJ. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. Trends Pharmacol Sci. 2003; 24: 96-102.
7. Ali-Fehmi R, Morris RT, Bandyopadhyay S, Che M, Schimp V, Malone JM, et al. Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma:correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival. AJOG. 2005; 192: 819-25.
8. Ranger GS, Thomas V, Jewell A, Mokbel K. Elevated cyclooxygenase-2 expression correlates with distant metastases in breast cancer. Anticancer rese. 2004; 24:2349-52.
9. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M. The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol. 2010; 2010:17
10. Chandrasekharan NV, Simmons DL. The cyclooxygenases. Genome biology. 2004; 5: 241.
11. Ramsay RG, Ciznadija D, Vanevski M, Mantamadiotis T. Transcriptional regulation of cyclo-oxygenase expression: three pillars of control. Int J Immunopathol Pharmacol. 2003; 16: 59-67.
12. Kis B, Snipes JA, Isse T, Nagy K, Busija DW. Putative cyclooxygenase-3 expression in rat brain cells. J Cereb Blood Flow Metab. 2003; 23: 1287-92.
13. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al . Cyclooxygenase in biology and disease. FASEB J. 1998; 12: 1063-73.
14. Falandry C, Canney PA, Freyer G, Dirix LY. Role of combination therapy with aromatase and cyclooxygenase-2 inhibitors in patients with metastatic breast cancer. Ann Oncol. 2009:mdn693.
15. Rakha EA. , El-Sayed ME., Lee AH, Elston CW, Grainge MJ, Hodi Z, et al. Prognostic significance of Nottingham histological grade in invasive breast carcinoma. J Clin Oncol 2008; 26:3153-8.
16. Singletary SE, Allred C, Ashley P, Bassett LW, Berry D, Bland KI, et al.Staging system for breast cancer: revisions for the 6th edition of the AJCC Cancer Staging Manual. Surg Clin North Am 2003; 83: 803-19.
17. Spizzo G, Gastl G, Wolf D, Gunsilius E, Steurer M, Fong D, et al. Correlation of COX-2 and Ep-CAM overexpression in human invasive breast cancer and its impact on survival.. B J Cancer 2003; 88, 574 – 578
18. Serra KP, Peres RM, Sarian LO, Vassallo J, Pinto GA, de Paiva Silva GR, et al. Cyclooxygenase-2 (COX2) and p53 protein expression are interdependent in breast cancer but not associated with clinico-pathological surrogate subtypes, tumor aggressiveness and patient survival. Acta histochem. 2016; 118: 176 -82.
19. Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, et al. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res. 2002; 62: 632 -5.
20. Denkert C, Winzer KJ, Müller BM, Weichert W, Pest S, Köbel M, et al. Elevated expression of cyclooxygenase 2 is a negative prognostic factor for disease free survival and overall survival in patients with breast carcinoma. Cancer. 2003; 97: 2978-87.
21. Wülfing P, Diallo R, Müller C, Wülfing C, Poremba C, Heinecke A, et al. Analysis of cyclooxygenase-2 expression in human breast cancer: high throughput tissue microarray analysis. J Cancer Res Clin Oncol . 2003; 129: 375-82.
22. Witton CJ, Hawe SJ, Cooke TG, Bartlett JM. Cyclooxygenase 2 (COX2) expression is associated with poor outcome in ER negative, but not ER positive, breast cancer. Histopathology. 2004; 45: 47-54.
23. Sivula A, Talvensaari-Mattila A, Lundin J, Joensuu H, Haglund C, Ristimäki A, et al. Association of cyclooxygenase-2 and matrix metalloproteinase-2 expression in human breast cancer Breast Cancer Res Treat. 2005; 89: 215-20.
24. Surowiak P, Materna V, Matkowski R, Szczuraszek K, Kornafel J, Wojnar A, et al. Relationship between the expression of cyclooxygenase 2 and MDR1/P-glycoprotein in invasive breast cancers and their prognostic significance. Breast Cancer Res. 2005; 7: R862- R870.
25. Gunnarsson C, Jansson A, Holmlund B, Ferraud L, Nordenskjold B, Rutqvist LE, et al. Expression of COX-2 and steroid converting enzymes in breast cancer. Oncol Rep. 2006; 16: 219-24.
26. Schmitz KJ, Callies R, Wohlschläger J, Kimmig R, Otterbach F, Bohr J, et al. Overexpression of cyclo-oxygenase-2 is an independent predictor of unfavourable outcome in node-negative breast cancer, but is not associated with protein kinase B (Akt) and mitogen-activated protein kinase (ERK1/2, p38) activation or with Her-2/neu signalling pathways. J Clin Pathol. 2006; 59: 685-91.
27. Yamamoto Y, Ibusuki M, Okumura Y, Kawasoe T, Kai K, Iyama K, et al. Hypoxia-inducible factor 1Ś is closely linked to an aggressive phenotype in breast cancer. Breast Cancer Res Treat. 2008; 110: 465-75.
28. Zhao X, Goswami M, Pokhriyal N, Ma H, Du H, Yao J, et al. Cyclooxygenase-2 expression during immortalization and breast cancer progression. Cancer Res. 2008; 68: 467-75
29. Darb-Esfahani S, Loibl S, Müller BM, Roller M, Denkert C, Komor M, et al. Identification of biology-based breast cancer types with distinct predictive and prognostic features: role of steroid hormone and HER2 receptor expression in patients treated with neoadjuvant anthracycline/taxane-based chemotherapy. Breast cancer Res. 2009; 11: R69.

30. Muhammad EM, Edin HS, Guirguis MN, Osman SM. Immunohistochemical Cyclooxygenase-2 (COX-2) and P53 Expression in Breast Carcinoma with Correlation to Clinico-Pathological Parameters. Med. J. Cairo Uni. 2013; 81(2).

31. Kargi A, Uysal M, Bozcuk H, Coskun HS, Savas B, Ozdogan M. The importance of COX-2 expression as prognostic factor in early breast cancer. J BUON. 2013; 18: 579-84.

32. Lee JA, Bae JW, Woo SU, Kim H, Kim CH. Correlation between COX-2 expression and hormone receptors in invasive ductal breast cancer. J Korean Surg Soc. 2010; 78: 140-8.

33. Glover JA, Hughes CM, Cantwell MM, Murray LJ. A systematic review to establish the frequency of cyclooxygenase-2 expression in normal breast epithelium, ductal carcinoma in situ, microinvasive carcinoma of the breast and invasive breast cancer. Br J Cancer. 2011; 105: 13-7.

34. Davies G, Salter J, Hills M, Martin LA, Sacks N, Dowsett M. Correlation between cyclooxygenase-2 expression and angiogenesis in human breast cancer. Clin Cancer Res. 2003; 9: 2651-6.

35. Takeshita E, Osanai T, Higuchi T, Soumaoro LT, Sugihara K. Elevated cyclooxygenase-2 expression is associated with histological grade in invasive ductal breast carcinoma. J Med Dent Sci. 2005; 52: 189-93.

36. Misron NA, Looi LM, Nik Mustapha NR. 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. 2015; 16: 1553-8.

37. Zhang XH, Huang DP, Guo GL, Chen GR, Zhang HX, Wan L, et al. Coexpression of VEGF-C and COX-2 and its association with lymphangiogenesis in human breast cancer. BMC cancer. 2008; 8: 4.

38. Nam E, Lee SN, Im SA, Kim DY, Lee KE, Sung SH. Expression of cyclooxygenase-2 in human breast cancer: relationship with HER-2/neu and other clinicopathological prognostic factors. Cancer Res Treat. 2005; 37: 165-70.

39. Cho MH, Yoon JH, Jaegal YJ, Choi YD, Lee JS, Lee JH, et al. Expression of cyclooxygenase-2 in breast carcinogenesis and its relation to HER-2/neu and p53 protein expression in invasive ductal carcinoma. Breast. 2006; 15: 390-8.

40. Leo C., Faber S., Hentschel B., Hfckel M. and Horn L.C.: 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 Diagn Pathol. 2006; 10: 327-32.

41. Dillon M., Stafford A., kelly G., Redmond A., Mcilroy M., Crotty T.,et al.: Cyclooxygenase-2 predicts adverse effects of tamoxifen: A possible mechanism of role for nuclear HER2 in breast cancer patients. Endocr Relat Cancer. 2008; 15:745-53.

42. Costa C, Soares R, Reis-Filho JS, Leitao D, Amendoeira I, Schmitt FC. Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. J Clin Pathol. 2002; 55: 429-34.

43. Aggarwal A, Al-Rohil RN, Batra A, Feustel PJ, Jones DM, DiPersio CM. Expression of integrin $\alpha 3\beta 1$ and cyclooxygenase-2 (COX2) are positively correlated in human breast cancer. BMC cancer. 2014; 14: 459.