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Original Article

Role of Cyclooxygenese-2 (COX-2) Expression in Breast Cancer Differentiation and Its Relationship with Hormone Receptors Status

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ABSTRACT

Background and Objectives: **Cyclooxygenese-2 (COX-2) expression in breast cancer and its correlation with tumor prognosis is unclear. We investigated the incidence of COX-2 expression in patients and assessed interactions between COX-2 and clinical features of cancer and expression of HER2/neu, estrogen receptor (ER), and progesterone receptor (PR).**

Methods: **COX-2 expression was investigated by immunohistochemistry in 29 patients' specimens diagnosed as primary breast cancer between 2006 and 2008 at the Fatemieh Hospital, Semnan, Iran. Relationship between COX-2 expression and age, histological grade, histological type, nodal status, and hormone receptor status were evaluated.**

Results: **We used IHC method although it was not a quantitative study. Its expression depends on quality of antibody, staining and selection of analyzed region. COX-2, HER-2, ER, and PR were detected in 89.7%, 51.7%, 82.8%, and 79.3% of samples, respectively. Elevated COX-2 expression was not associated with size and grade of tumor, while mean numbers of involved lymph nodes was significantly higher in those with elevated expression of COX-2 (P = 0.001). There were no significant correlations between COX-2 expression and HER-2, ER, and PR receptors.**

Conclusion: **Only tumor tissue was analyzed and did not compare to normal tissue. Elevated COX-2 expression can be found in most patients with breast cancer and has a crucial role in tumor differentiation regarding degree of lymph node involvement. It seems that correlation between COX-2 and other oncogens and hormonal receptors might be influenced by geographical and racial factors, so, assessment of these relationships in each patient's population may be necessary.**

Keywords: **Cyclooxygenase-2, Breast Cancer, Estrogen Receptors, Progesterone Receptor , HER-2 Proto-Oncogene Protein**

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Introduction

Cyclooxygenese (COX-1 and COX-2) are prostaglandin (PG) synthases which catalyse sequential synthesis of prostaglandin G2 (PGG2) and PGH2 from arachidonic acid. During tumorigenesis, COX-2 is upregulated in response to growth factors, tumor promoters, cytokines and several oncogenes, including v-src, v-Ha-ras, HER-2/neu and Wnt genes. In vitro overexpressing COX-2 demonstrate several altered characteristics, including increased adhesion to extracellular matrix, resistance to butyrate-induced apoptosis, a delayed transit through the G1 phase of the cell cycle and increases expression or activity of enzymes capable of digesting the basement membrane, most probably contributing to the observed increase in ability to invade through a layer of Matrigel (1-9). Therefore, COX-2 is as a mediator of tumor epithelial–stromal cell interactions in breast cancer. (10)

Recent immunochemical analysis of breast cancers in human has revealed a significant COX-2 expression in different types of this cancer so that the degree of COX-2 expression is positively correlated with poor prognosis of tumor (11-13). The elevated level of COX-2 mRNA are present in the tissue adjacent to cancerous lesions (14). Hence, abnormal COX-2 expression seems to have a pivotal beginning pathogenetic role in mammary carcinogenesis and has positive implications for COX-2 inhibition. In this context, inhibition of COX-2 not only may prevent onset of the disease in women, but also treatment of established breast cancer with COX-2 inhibitors can reduce cancer aggressiveness and induce its remission (15, 16). Some studies on animal models demonstrated that prophylaxis approaches with selective COX-2 inhibitors reduced tumor multiplicity and also treatment of established breast cancer with these inhibitors led to a reduction in tumor volume (17, 18). Moreover, the disappointing effect of COX-2 inhibitors in some recent clinical studies indicated that COX-2 might not be as crucial for the progression of human breast cancer as previously hypothesized (19). On the contrary, COX-2 over expression in breast cancer cells enhances cell motility and invasiveness thus suggesting a mechanism of COX-2 mediated metastasis (20, 21).

Furthermore, these recent observations did not prove correlation of COX-2 mRNA expression in the tumor tissues with the mRNA expression of HER2/neu, estrogen receptor (ER), or the progesterone receptor (PR) (20). However, other studies showed clear and or positive relationship between HER-2/neu status and COX-2 expression in human breast tumors and these two enzymes did not show expression in normal epithelium (22, 23). Thus, correlation between COX-2 expression and clinical features of breast cancer as well as its association with hormonal receptors has been already unclear.

We first investigated the overall incidence of COX-2 expression in our breast cancer patients› population and then assessed interactions between COX2 and breast cancer clinical features as well as with expression of HER-2 and other hormonal receptors.

Material and Methods

Surgical specimens from 29 consecutive patients diagnosed as having primary invasive breast cancer and operated on between 2006 and 2008 at the Fatemieh Hospital, Semnan City, Semnan, Iran were prospectively studied.

The ethics and research committees of the Semnan University of Medical Sciences approved the research protocols and all patients gave written consent to participate in the study.

For immunohistochemical examination of COX-2, a universal immunoenzyme polymer method was used. Paraffin-embedded tumor tissue was stained for COX-2 using a monoclonal antibody, for estrogen receptor, for progesterone receptor using a mouse monoclonal antibody, and for HER-2/neu, using a mouse monoclonal

antibody. Immunohistochemistry was performed on formalin-fixed paraffin embedded tissue sections using peroxidase with counter stain using hematoxylin. Pretreatment consisted of microwave heating for 5 min in 0.01 M citrate buffer.

Expression of COX-2, estrogen and progesterone receptors were scored according to the proportion of positive-staining cells: 1+, <10%; 2+, 10–50%; and 3+, >50%. A score ≥2+ was considered positive; while cases scored as 0 were considered negative (24, 25).

IHC scoring system according to the guidelines given by ASCO/CAP:

Score 0: No staining is observed or cell membrane staining is observed in less than 10% of the tumor cells.

Score 1+: A faint perceptible membrane staining can be detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

Score 2+: A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+: A strong complete membrane staining is observed in more than 30% of the tumor cells.

Continuous data were shown as mean and standard deviation (SD) and categorical variables were presented as percentages. Patients' characteristics were compared by means of the *t* test for continuous variables and the chi-square test or the Fisher's exact test for categorical variables. Comparative analysis was performed using SPSS (version 13.0, SPSS Inc., Chicago, IL, USA). All *P*-values were two-sided, with statistical significance defined by $P \leq 0.05$.

Results

Mean age of patients was 55 years (ranged 31 to 75 years). Baseline characteristics including histological type, histological grade, nodal status, and hormone receptor status were presented in Table 1.

Table 1- Baseline characteristics and histological features in patients with breast cancer

Histological type	
DCI	28/29 (96.6)
LCI	1/29 (3.4)
Histological grade	
Poor differentiated	11/29 (37.9)
Moderate differentiated	14/29 (48.3)
Well differentiated	4/29 (13.8)
Tumor size (cm^3)	4.57 ± 2.03
Number of involved lymph node	4.92 ± 3.30
Positive estrogen receptor	24/28 (82.8)
Positive progesterone receptor	23/29 (79.3)
Positive HER-2	15/29 (51.7)

In the present study, 29 samples of breast cancer tissues (28 samples of invasive ductal carcinoma and 1 sample of invasive lobular carcinoma obtained from pathology ward archive) were studied. Elevated COX-2 expression was found in 89.7% of breast cancer samples (26 out of 29 samples).

Tumor markers of HER-2, estrogen receptor (ER), and progesterone receptor (PR) were also detected in 51.7%, 82.8%, and 79.3% of samples, respectively. Although patients› age was numerically higher in the group with elevated COX-2 expression (56.04 ± 13.24 versus 48.67 ± 11.37), but this difference was not statistically significant. Elevated COX-2 expression was not associated with size and grade of tumor (Table

2), but mean numbers of involved lymph nodes was significantly higher in those with elevated expression of COX-2 ($P = 0.001$). No significant correlations were observed between COX-2 expression and expression of HER-2, ER, and PR receptors.

Table 2- Baseline characteristics and histological features in patients with breast cancer

Tumor characteristics	With elevated COX-2 expression (n=26)	Without elevated COX-2 expression (n=3)	*P* -Value
Tumor size (cm^3)	4.65 ± 2.07	3.80 ± 1.84	0.516
Tumor grading	2.31 ± 0.68	1.67 ± 0.58	0.185
Number of involved lymph node	5.32 ± 4.36	0.50 ± 0.31	< 0.001
Positive estrogen receptor	21/26 (80.8)	3/3 (100)	0.404
Positive progesterone receptor	20/26 (76.9)	3/3 (100)	0.350
Positive HER-2	13/26 (50.0)	2/3 (66.7)	0.584

Discussion

In breast cancer, the prognostic impact of COX-2 expression varies widely between studies. In the current study, we examined the correlation between COX-2 expression and features of breast cancer as well as its relationship with hormonal receptors in a cohort of breast cancer patients among Iranian patients. Based on our findings, overexpression of COX-2 was detected in 89.7% of breast cancer samples that was notably higher than that was reported previously that occurred in 43% of human invasive breast cancers and 63% of ductal carcinomas in situ (26). At least 8 different immunohistochemical studies have investigated expression of COX-2 in a total of 2392 primary breast carcinomas, of which 40% were found to be COX-2 positive (27).

In addition, in our study and among different features of tumor progression, COX-2 expression was positively associated with the severity of lymph node involvement, but not with tumor size or tumor grading. Our study suggested the probable role of COX-2 expression in invasion of breast tumor and its metastasis. Co-expression of COX-2 and *c-erb*-B2 may be a useful prognostic marker in patients with operable breast cancer (28).

Treatment with COX-2 inhibitors reduces incidence and growth of breast carcinomas (29). Possible mechanisms include regulation of invasion, increased proliferation, and suppression of apoptosis by COX-2. Moreover, there may be an indirect effect of prostaglandins, for example in tumor host interactions such as induction of stromal aromatase activity or enhancement of angiogenesis in tumor tissue (27). Prostoglandin E2 (PGE2) is a major downstream mediator of COX-2 that promotes cellular proliferation and angiogenesis, makes cells resistant to apoptosis, enhances invasiveness, and modulates immunosuppression. Compelling evidence gained from mechanistic studies with cancer cell lines, mouse models of intestinal tumorigenesis and a number of clinical supports an important role for the COX–prostaglandin path- way in tumourogenesis. Evidence suggests that without COX–prostaglandin path- way tumours cannot sustain their growth and development (30).

We did not show significant correlations between COX-2 expression and expression of HER2, ER, and PR receptors. Similarly, elevated COX-2 expression was not associated with size, grade, and high Nottingham prognostic index (NPI) or estrogen receptor (ER) negativity. Besides, no association was observed between COX-2 and HER1-4 expression (31). It may be due to the analysis of COX-2 expression by immunohistochemistry method that is not quantitative and

would strongly depend on the quality of the antibody and the staining protocol and also on the selection of the analyzed region. Contrary to our study, in some previous studies, some connections were demonstrated between COX- 2 and a few oncogenes including v-src, v-Ha-ras, and HER-2\neu. But similarly, they did not also confirm correlation of COX-2 expression with hormonal receptors (31).

It seems that the correlation between COX-2 expression and other oncogens and hormonal receptors might be influenced by the geographical and racial factors and therefore, assessment of these relationships in each patient's population may be necessary.

Our study was the first study on this hypothesis in our population and thus further assessment among our breast cancer patients is recommended.

Conclusion

The over-expression of COX-2 is positively associated with the severity of lymph nodes involvement, but is not correlated with the expression of HER2, ER, and PR receptors.

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