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Immunohistochemical Expression of Cyclooxygenase-2 and Its Relationship With Prognostic Parameters in Breast Cancer

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BACKGROUND/AIMS
Cyclooxygenase-2 (COX2) plays an important role in the development of some human cancers, especially in the development of pulmonary, colon, and breast carcinomas. Overexpression of COX2 has been involved in the pathogenesis of a wide range of malignancies, such as colon, breast, and lung cancer, and has been associated with carcinogenesis and tumor progression. The COX2 pathway has been involved in several processes associated with tumor progressions, such as angiogenesis, proliferation, and invasion. This study aimed to determine whether COX2 can be used as a prognostic marker in breast cancer.

MATERIAL and METHODS
We evaluated immunohistochemical expressions of COX2 in 100 patients with invasive ductal breast carcinoma and compared its utility as a prognostic parameter. In the evaluation of the data, chi-square test was used to assess the relationship between mean, standard deviation, and COX2 and the relationship between other independent variables in the Statistical Package for the Social Sciences version 24 software package.

RESULTS
A positive correlation was found between COX2 expression and estrogen receptor positivity, tumor grade, Ki67 proliferation index, tumor size, advanced age, and triple-negative subtypes (P<.005). However, there was no association with HER2 positivity, progesterone receptor positivity, and nonluminal type.

CONCLUSION
In breast cancer, COX2 expression has a positive correlation with some prognostic parameters; however, it has an inverse correlation with some others.

Keywords: Breast cancer, COX2, immunohistochemistry

INTRODUCTION

Breast cancer is the most common malignancy in women, and its malignancy is one of the most common causes of death (1). Molecular characterization of this malignancy is an indicator for tumor prognosis and aggression. The classical molecular parameters of breast cancer are estrogen receptor (ER), progesterone receptor (PR), and cerbB2 expressions, and Ki67 proliferation index (2, 3).

Previous studies have shown that cyclooxygenase (COX)-2 plays an important role in the development of some human cancers, especially pulmonary, colon, and breast carcinomas, as well as preinvasive lesions. COX catalyzes the synthesis of prostaglandin endoperoxidase from arachidonic acid, which means that it is the first step in the biosynthesis of prostaglandins and thromboxane and is also known as prostaglandin endoperoxide synthase.

Of note, 2 prostaglandin synthase isoforms have been identified, which are often referred to as COX1 and COX2 (4). Although COX1 is structurally produced by most body tissues, COX2 is an inducible enzyme and is produced under certain conditions, such as inflammation and tumor microenvironment. COX2 plays a role in estrogen regulation by producing prostaglandin E2 that increases the expression of cytochrome P450 enzyme complex (also known as aromatase) that

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catalyzes estrogen production, which is mediated through androgen (5).

COX2 is the enzyme that regulates the inflammatory process and the first step of prostaglandin synthesis. Tissue expression of COX2 is regulated by cytokines, endotoxins, and growth factors (6). Overexpression of COX2 has been defined in the pathogenesis of a wide range of malignancies, such as colon, breast, and lung cancer, and has been associated with carcinogenesis and tumor progression, such as angiogenesis, proliferation, and invasion (7-9). COX2 plays a role in the induction of apoptosis (10). High COX2 expression is more common in poorly differentiated tumors than in well- and moderately differentiated tumors. It has been shown that COX2 expression also correlates with poor prognostic factors, such as high Ki-67 proliferative rate and low differentiation (1).

We aimed to evaluate COX2 expressions in invasive ductal breast cancer and adjacent benign breast tissue and to correlate them with clinical and histological prognostic parameters, including hormone receptor status.

MATERIAL and METHODS

This study was based on the retrospective analysis of tumors diagnosed as invasive breast carcinoma (without special type) from patients who underwent mastectomy between 2014 and 2019. A total of 100 patients were included in the study. A total of 4-µm-thick sections were prepared from the paraffin block of tumors, and ER (clone: 6F11, 1:50, Leica Biosystems, Thermo Fisher Scientific, IBM SPSS Corp, Germany), PR (clone: Pgr16, 1:100, Leica Biosystems), Ki67 (clone: MM1 optimized for use, Leica Biosystems), cerbB2 (clone: 10A7, 1:40, Leica Biosystems), and COX2 (clone: SP21, 1:50, Thermo Fisher Scientific, Waltham, MA, USA) antibodies were applied by Leica Bond-Max brand fully automatic immunohistochemistry device. For each slide, hematoxylin was used as the counterstain. Immunohistochemical staining was evaluated with a light microscope (BX46 Clinical Microscope, Olympus, Tokyo, Japan). A minimum of 500 tumor cells was counted for the immunohistochemical evaluations per antibody.

Immunohistochemical staining percentages for ER and PR were made according to Allred's criteria, and the staining intensity of positive tumor cells was also categorized into 4 groups (0, no staining; 1, weak staining; 2, medium staining; and 3, strong staining). CerbB2 receptor status was considered negative for 0 and 1+ test results and positive for 3+ test results; however, tumors with the 2+ test results were retested by FISH.

Main Points:

- It is important to monitor the progression of breast cancer, which is the most common type of cancer in women, and to direct the treatment. Therefore, the searches for various prognostic markers of this cancer continue.
- It is still early for COX2 to be an immunohistochemical prognostic marker for breast cancer because different results have been found in different studies.
- Further standardized studies may be needed before it can be considered a prognostic marker.

Medium to strong nuclear staining of >1% of tumor cells for ER and >20% of tumor cells for PR was considered positive. For Ki67, cases showing >14% medium/strong nuclear tumor staining were considered positive. Cytoplasmic immunoreactivity of COX2 was graded according to the German Immuno Reactive Score (12). The staining intensity was graded from 0 to 3 (0=no staining, 1=weak staining, 2=moderate staining, 3=strong staining). For statistical calculations, the COX2 status was evaluated by establishing 2 thresholds: positive and negative (negative and weak staining were grouped as negative, whereas medium and strong staining were grouped as positive [Figure 1]).

Breast Cancer Classification

Breast cancer was classified as follows:

1. Luminal A: when estrogen/progesterone are positive, cerbB2 is negative, and Ki67 is low (<14%).
2. Luminal B negative, cerbB2 negative: when estrogen is positive, cerbB2 is negative, and ki67 is high (≥14%) and/or progesterone is positive (<20%).
3. Luminal B negative, cerbB2 positive: when estrogen is positive, cerbB2 is positive, there is any ki67 and any PR.
4. CerbB2 positive (nonluminal): when cerbB2 is positive, and estrogen/progesterone is negative.
5. Triple negative: estrogen/progesterone and cerbB2 are negative.

Statistical Analysis

In the evaluation of the data in this study, chi-square test was used for the assessment of the relationships between the mean, standard deviation, and COX2 and other independent variables by the Statistical Package for the Social Sciences 24 package program (IBM SPSS Corp.; Armonk, NY, USA). The relationships between COX2 and triple negative and those between COX2 and ER-positive, PR-positive, and cerbB2-positive cases were assessed. In the analyses, $P<.05$ values were accepted as statistically significant.

RESULTS

The distribution of the surrogate subtypes of breast cancer in 100 samples was 56 (56%) luminal A; 9 (9%) luminal B, cerbB2

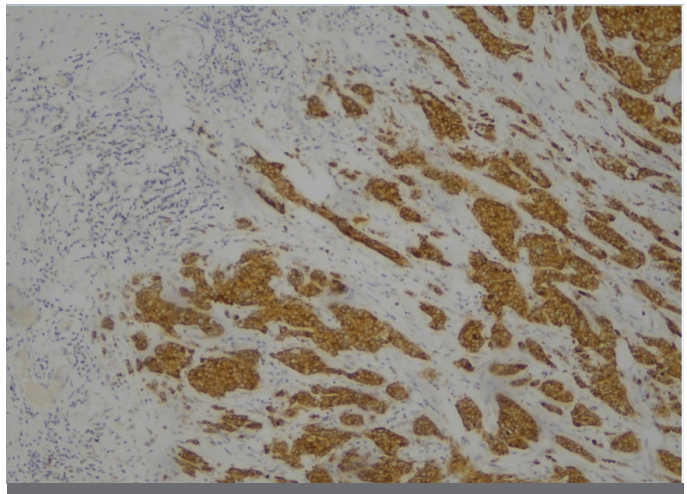

FIGURE 1. Cytoplasmic staining of the tumor cells with COX2 antibody (×100 magnification)

TABLE 1. The demographic characteristics of the cases

Demographic Parameters		n (100)	%
Tumor grade	Grade 1	20	20
	Grade 2	62	62
	Grade 3	18	18
Tumor size	2cm<	27	27
	2cm≥	73	73
Age	30-48	40	40
	49≥	60	60
Lymph node metastasis	(+)	34	34
	(-)	66	66

TABLE 2. COX2 and Independent Variables

Prognostic parameters	COX2 X±SD	Level of significance
Grade	.66±.76	
Low (G1)	.40±.50	
Medium (G2)	.66±.47	X2=0.01
High (G3)	.94±.23	p<0.05
ER+	.77±42	X2=0.04
		p<0.05
PR+	.58±.50	X2=0.094
		p>0.05
cerbB2+	.67±.49	X2=0.095
		p>0.05
Ki67(above 14%)	.82±.38	X2=0.09
		p<0.05
Tumor size >2cm	.80±.40	X2=0.00
		p<0.05
Age 49≥	.81±.39	X2=0.00
		p<0.05
Lymph node metastasis (+)	.85±.34	X2=0.01
		p<0.05
Triple negativity	.94±.25	X2=0.011
		p<0.05

negative; 12 (12%) luminal B, cerbB2 positive; 7 (7%) cerbB2 positive (nonluminal); and 16 (16%) triple negatives. The demographic characteristics of the cases are shown in Table 1. For threshold definition, negative and poor staining results were grouped as negative, whereas medium and strong staining results were grouped as positive. When the statistical results were examined, the correlations between advanced/intermediate grades and COX2 expression (χ^2=0.01, P<.05) and that between ER positivity and COX2 expression (χ^2=0.004, P<.05) were statistically significant. It was determined that there was no statistical relationship between CerbB2 positivity and COX2 expression (χ^2=0.095, P>.05) and between PR positivity and COX2 expression (χ^2=0.094, P>.05). In addition, the relationships between Ki67 and COX2 expression (χ^2=0.09, P<.05), between tumor size >2 cm and COX2 expression (χ^2=0.00, P>.05), between age >49 years and COX2 expression (χ^2=0.00, P>.05), and between lymph node metastasis and COX2 expression (χ^2=0.01, P>.05) were found statistically significant. The relationship between COX2 expression and triple-negative subtype was found to be statistically significant (χ^2=0.00, P<.05) (Table 2).

Pathological parameters, such as peritumoral angiolymphatic invasion, perineural invasion, dermal invasion, in situ carcinoma component, luminal A, luminal B, and nonluminal subtypes were not associated with COX2 expression.

DISCUSSION

COX2 expression has been widely described in breast cancers. It shows high expression in tumor breast tissue compared with that in benign breast tissue and seems to have a clinical potential use in predicting the prognosis (13). In an analysis of 12 studies, COX2 positivity was found in 42% of the tumors (14). In our study, 67% of tumors showed positivity for COX2. COX2 was found to be associated with increased tumor grade and poor prognosis among patients with estrogen-independent breast cancer because it is a main agent in the inflammation-cancer signal axis (15). Although COX2 expression was presented as a prognostic parameter in basal carcinomas, it showed no prognostic significance in luminal A cancers (16). However, Serra et al. (17) declared that COX2 expression was not related to clinical and pathological subtypes, tumor characteristics, and prognosis. COX2 positivity was found in the invasive and in situ carcinomas and also around the tumor (14, 17, 18).

In a retrospective study of 303 high-grade breast cancers, COX2 was evaluated immunohistochemically, and a positive correlation was observed between COX2 overexpression and high tumor grade; however, no correlation was found with ER positivity (15).

There are some other studies that found a reverse correlation between ER and COX2 expressions (19), and some others showed a positive correlation (20) such as in our study. Ristimaki et al. (20) suggested that elevated COX2 expression in ER-positive cancers could be because of the enhancement of the microenvironment for cancer cells to grow by inducing estrogen production. In addition, some studies have found a strong association of COX2 overexpression with ER negativity and the worse prognosis (21).

Triple-negative breast cancers do not have ER, PR, and HER2 expressions, and they constitute 15% of all breast cancers and are associated with aggressive progress, high metastasis, and poor prognosis (22). It has been reported that COX2 expression is increased in triple-negative and HER2+ (nonluminal) tumors (23, 24). In our study, COX2 expression seemed to be increased in triple-negative tumors, but no statistically significant relationship was observed with the HER2+ group. There are variations in the literature regarding COX2 positivity and negativity; therefore, it is difficult to compare between studies. Different results may occur in different immunohistochemical analyses owing to different antibodies, nonstandardized staining methods and differentiation differences of the tumors, and the differences in COX2 expression analysis.

A recent meta-analysis of 21 studies and 6739 patients with breast cancer showed that the presence of high COX2 levels predicted a larger tumor size and lymph node metastasis, similar to our finding (25). When Ki67 is highly expressed as a nuclear cell proliferation marker in breast cancer, it is associated with a poor prognosis (26). There are studies emphasizing that there is a correct relationship between the number of cells expressing Ki67 and proliferation (27) or that there are no significant statistical results with Ki67 expression (28). In this study, a positive correlation was found between the high value of Ki67 (>14%) and COX2 expression.

In carcinogenesis, inflammatory cytokines, growth factors, endotoxins, and oncogenes trigger the induction of COX2 and cause tumor progression by participating in COX2 tumor proliferation, invasion, angiogenesis, apoptosis resistance, and metastasis (29, 30). Therefore, it is associated with poor prognosis in patients with cancer (20).

We have found a correlation of COX2 expression with some of the prognostic parameters in breast cancer. However, it appears to be early for COX2 immunoexpression to be used daily as a prognostic marker in breast cancer. It may be a good prognostic marker if it gains support from further studies with large series.

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