Association between Cyclooxygenase-2 and Indoleamine 2,3-Dioxygenase Expression in Breast Cancer Patients from Pakistan

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Abstract

**Background:** Tumors use several immunosuppressive mechanisms to evade immune destruction. Cyclooxygenase-2 (COX-2) expression may be a driver of immunosuppression in breast cancer, but the mechanisms involved remain elusive. COX-2 expression induces the expression of indoleamine 2,3 dioxygenase (IDO) in tumor cells. IDO is an immunosuppressive enzyme which is involved in tumor immune escape mechanisms in breast cancer. Our aim was to evaluate the association between COX-2 and IDO expression to find evidence of immunosuppression in Pakistani breast cancer patients. **Methods:** Immunohistochemical analysis was performed to evaluate the expression of COX-2, IDO, estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) on formalin-fixed paraffin-embedded breast cancer tissues of 100 patients. Univariable and multivariable logistic regression model was used to identify the independent risk factors of COX-2. **Results:** A total of 100 patients were included with a mean age and standard deviation of 48.28 ± 11.83. A significant association was observed among COX-2, IDO, ER, PR and tumor grade. In multivariable analysis, three variables were identified as significant independent risk factors for high COX-2: IDO expression high; [adjusted odds ratio (AOR) 6.51; 95% confidence interval (CI) (2.00-21.20), p=0.001], ER; [AOR 5.62; 95% CI (1.80-17.84), p=0.002] and age [AOR 1.04; 95% CI (1.00-1.10), p=0.05] respectively. **Conclusion:** Our data showed that high IDO expression is associated with high COX-2 expression in Pakistani breast cancer patients. The co-expression of both enzymes may suggest their role in disease pathogenesis. Hence the concurrent targeting of COX-2 and IDO may be a promising therapy for breast cancer.

**Keywords:** Cyclooxygenase-2- indoleamine 2-3-dioxygenase- breast cancer- Pakistan

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Introduction

The cyclooxygenase (COX) family of enzymes contains two members (COX-1 and COX-2) (Chen et al., 2014). COX-1 is expressed ubiquitously (Williams and DuBois, 1996). COX-2 is expressed in distinct tissues and is involved in inflammatory processes (Herschman, 1996). Chronic inflammation is well known to be linked with cancer progression (Howe, 2007). Recent emerging epidemiologic, preclinical, and clinical data suggest that COX-2 up-regulation is a fundamental step in carcinogenesis (Cao and Prescott, 2002) and tumor angiogenesis (Davies et al., 2003). Elevated COX-2 expression has been detected in human breast tumor tissues (Ristimaki et al., 2002).

The age-associated increase in COX-2 activity has been noticed in animal models (Claycombe et al., 2002). Elevated level of COX-derived products has been identified in platelets and peripheral blood mononuclear cells (PBMCs) in elderly humans (Vericel et al., 1988; Meydani et al., 1990). Furthermore, COX-2 up regulation is associated with distinct pathological features such as large tumor size, high tumor grade and metastasis (Ristimaki et al., 2002; Wulfing et al., 2003). COX-2 induces aromatase in breast tissue (Salhab et al., 2007). Aromatase activity increases estrogen levels (Vienonen et al., 2002). Consequently, COX-2 expression increases the estrogen levels and subsequently tumor progression in hormone receptor positive breast cancer (Hoellen et al., 2011).

Tryptophan catabolism is linked with immunosuppression in the tumor microenvironment. Indoleamine 2,3-dioxygenase (IDO) is a heme containing tryptophan-degrading enzyme (McGaha et al., 2012). IDO overexpression in tumors results in prompt conversion of tryptophan into kynurenine. Tryptophan depletion...
and enhanced levels of kynurenine play pivotal role in immunosuppression (McGaha et al., 2012; van Baren and Van den Eynde, 2015). IDO expression is involved in breast tumor growth and pulmonary metastasis (Levina et al., 2012). Furthermore, high IDO expression is significantly linked with overall decreased patient survival (Asghar et al., 2019). COX-2 and IDO promote breast cancer progression (Chen et al., 2014). The current study has been conducted in Pakistan, a country with one of the high incidence of breast cancer in its region, to investigate the association between COX-2 and IDO expression in breast cancer patients.

Materials and Methods

Tumor tissue specimens

A retrospective study was performed on formalin-fixed paraffin-embedded (FFPE) tumor specimens of breast cancer patients (n=100). These specimens were retrieved from pathology department, at Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH and RC) Lahore, Pakistan. The patients selected for the current study were diagnosed with breast cancer between 2007 and 2009. All the patients were treatment naïve. Tumor grade was allocated using the Nottingham Histologic Score. IHC analysis of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) expressions were conducted and interpreted using standard methods (Chen et al., 2010). The comprehensive information about the clinico-pathological characteristics was retrieved from medical records and pathology reports.

Evaluation of COX-2 and IDO scoring

It was a blind histopathologic assessment. The slides were evaluated by pathologists. The COX-2 and IDO immunostaining were examined by using the H-score, well-defined by the following equation: $H$-score = $\Sigma Pi (i + 1)$, as described previously (Chan et al., 2012), $Pi$ is the percentage of stained tumor cells and $i$ is the intensity of cytoplasmic staining of tumor cells (0 to 3+).

Statistical analysis

Statistical analysis was carried out using SPSS software (version 20.0; SPSS, Chicago, IL, USA). Percentages (proportions) were used for categorical variables while mean and standard deviation were used for continuous variables. Bivariate analysis was done using chi-square or fisher exact test (when necessary). For continuous explanatory variables such as age, independent t-test was performed. Univariable and multivariable logistic regression model was used to identify the risk factors.

Results

Demographic characteristics versus low and high COX-2 scores

Demographic characteristics of 100 breast cancer patients are summarized in Table 1. Overall the mean age at breast cancer diagnosis was 48.28 ± 11.83, and there was a mean difference of age in low and high COX-2
COX-2 and IDO

In order to examine the association between COX-2 and IDO, we categorized the patients into COX-2 low versus high. There was a statistically significant association of COX-2 with IDO expression (p = 0.004), ER (p = 0.003), PR (p = 0.002) and tumor grade (p= 0.02) respectively (Table 2).

Table 1. Demographic Characteristics versus Low and High COX-2 Scores

| Variables          | Characteristics | Total 100 (100.0%) | Low COX-2 41 (41.0%) | High COX-2 59 (59.0%) | p-value |
|--------------------|-----------------|---------------------|-----------------------|-----------------------|---------|
| Age                | Mean ± SD       | 48.28 ± 11.83       | 45.02 ± 11.20         | 50.54 ± 11.82         | 0.02*   |
| Region             | Punjab          | 88 (88.0)           | 35 (39.8)             | 53 (60.2)             | 0.5     |
|                    | Khyber Pakhtunkhwa | 7.0 (7.0)        | 3 (42.9)              | 4 (57.1)              |         |
|                    | Kashmir         | 3.0 (3.0)           | 1 (33.3)              | 2 (66.7)              |         |
|                    | Sindh           | 2.0 (2.0)           | 2 (100.0)             | 0 (0.0)               |         |

Majority of patients belonged to the Punjab region (88%).

Clinicopathological characteristics versus low and high COX-2 scores

Table 2. Clinicopathological Characteristics versus Low and High COX-2 Scores

| Variables          | Characteristics | Total 100 (100.0%) | Low COX-2 41 (41.0%) | High COX-2 59 (59.0%) | p-value |
|--------------------|-----------------|---------------------|-----------------------|-----------------------|---------|
| IDO Score          | Low             | 24 (24.0)           | 16 (66.7)             | 8 (33.3)              | 0.004*  |
|                    | High            | 76 (76.0)           | 25 (32.9)             | 51 (67.1)             |         |
|                    | Total           | 100 (100.0)         | 41 (41.0)             | 59 (59.0)             |         |
| Estrogen receptor  | Negative        | 69 (69.0)           | 35 (50.7)             | 34 (49.3)             | 0.003*  |
|                    | Positive        | 31 (31.0)           | 6 (19.4)              | 25 (80.6)             |         |
|                    | Total           | 100 (100.0)         | 41 (41.0)             | 59 (59.0)             |         |
| Progesterone receptor | Negative  | 74 (74.0)           | 37 (50.0)             | 37 (50.0)             | 0.002*  |
|                    | Positive        | 26 (26.0)           | 8 (30.8)              | 18 (69.2)             |         |
|                    | Total           | 100 (100.0)         | 41 (41.0)             | 59 (59.0)             |         |
| HER2–neu receptor  | Negative        | 74 (74.0)           | 33 (44.6)             | 41 (55.4)             | 0.22    |
|                    | Positive        | 26 (26.0)           | 8 (30.8)              | 18 (69.2)             |         |
|                    | Total           | 100 (100.0)         | 41 (41.0)             | 59 (59.0)             |         |
| Metastasis         | Negative        | 38 (38.0)           | 19 (50.0)             | 19 (50.0)             | 0.21    |
|                    | Positive        | 49 (49.0)           | 18 (36.7)             | 31 (63.3)             |         |
|                    | Total           | 87 (87.0)           | 37 (42.5)             | 50 (57.5)             |         |
| Grade              | II              | 36 (36.0)           | 10 (27.8)             | 26 (72.2)             | 0.02*   |
|                    | III             | 56 (56.0)           | 29 (51.8)             | 27 (48.2)             |         |
|                    | Total           | 92 (92.0)           | 39 (42.4)             | 53 (57.6)             |         |
| Nodes              | N0              | 37 (37.0)           | 18 (48.6)             | 19 (51.4)             | 0.17    |
|                    | N1              | 24 (24.0)           | 7 (29.2)              | 17 (70.8)             |         |
|                    | N2              | 13 (13.0)           | 4 (30.8)              | 9 (69.2)              |         |
|                    | N3              | 13 (13.0)           | 8 (61.5)              | 5 (38.5)              |         |
|                    | Total           | 87 (87.0)           | 37 (42.5)             | 50 (57.5)             |         |
| Histology          | Ductal          | 91 (91.0)           | 38 (41.8)             | 53 (58.2)             | 0.82    |
|                    | Mammary         | 6 (6.0)             | 2 (33.3)              | 4 (66.7)              |         |
|                    | Lobular         | 2 (2.0)             | 1 (50.0)              | 1 (50.0)              |         |
|                    | Metaplastic     | 1 (1.0)             | 0 (0.0)               | 1 (100.0)             |         |
|                    | Total           | 100 (100.0)         | 41 (41.0)             | 59 (59.0)             |         |
| Tumor Size         | T1              | 7 (7.0)             | 2 (28.6)              | 5 (71.4)              | 0.34    |
|                    | T2              | 49 (49.0)           | 18 (36.7)             | 31 (63.3)             |         |
|                    | T3              | 8 (8.0)             | 5 (62.5)              | 3 (37.5)              |         |
|                    | Total           | 64 (64.0)           | 25 (39.1)             | 39 (60.9)             |         |

*values indicate statistical significance p< 0.05; IDO, Indoleamine 2,3-dioxygenase.

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COX-2 and IDO immunostaining
To investigate the expression of COX-2 and IDO, FFPE tumor specimens (n=100) of same patients were selected. Out of 100 tumor specimen, COX-2 high, and low scores were 59%, and 41%, respectively (Figure 1). IDO positivity was observed in all breast tumor specimens. COX-2 and IDO co-expression is shown in Figure 2.

Risk factors of COX-2 high expression
Table 3 summarizes various clinical and pathological factors that were included in the univariable and multivariable analyses to identify the COX-2 association with IDO expression. In multivariable analysis, three variables were identified as significant independent risk factors for high COX-2: IDO expression high; [adjusted odds ratio (AOR) 6.51; 95% confidence interval (CI) (2.00-21.20), p=0.001], ER, [AOR 5.62; 95% CI (1.80-17.84), 0.002] and age [AOR 1.04; 95% CI (1.00-1.10), 0.05].

Discussion
Previous studies established that IDO overexpression is involved in tumor immune escape in various cancers (Muller et al., 2005; Mansfield et al., 2009). It has been documented that IDO and COX-2 promote breast cancer progression (Chen et al., 2014). To the best of our knowledge, this is first study which revealed that high IDO expression is associated with high COX-2 expression in Pakistani breast cancer patients; coherent with the findings demonstrated by Mei et al., (2012). It is well established that COX-2 expression induces constitutive expression of IDO in human tumor cells (Hennequart et al., 2017). But interestingly Mei et al., (2012) demonstrated that IDO inhibitors suppressed the COX-2 expression and IDO may be involved in endometriosis pathogenesis via promoting COX-2. Our data showed as well that high IDO expression is associated with COX-2 expression which might play a role in breast cancer pathogenesis.

COX-2 upregulation is involved in age-related dysregulation of the immune responses (Wu and Meydani, 2004). Siironen et al., (2004) demonstrated that an increase in the COX-2 expression is associated with age in papillary thyroid cancer patients. Our data revealed that increase in the COX-2 expression is associated with age in Pakistani breast cancer patients. In addition, it has been established that ER-positive breast cancer may evolve from low to high grade (Lopez-Garcia et al., 2010; Natrajan et al., 2010). COX-2 expression upregulates transcription of aromatase and consequently stimulates tumor cell progression in ER-positive breast cancer (Diaz-Cruz et al., 2005). Our results are in agreement that high COX-2 expression is associated with ER-positive breast cancer.

As we aimed to investigate the association between COX-2 and IDO expression in breast cancer patients from Pakistan, a country with high incidence of breast cancer cases, our data showed the high IDO expression is associated with high COX-2 expression.

Figure 2. COX-2 and IDO co-Expression. (A) Strong COX-2 expression was detected in invasive ductal carcinoma. Sections from the same breast cancer patients were stained for IDO (B) Strong and diffuse IDO staining in invasive ductal tumor cells. Both staining were cytoplasmic. Images were captured at x 40 magnification.

Table 3. Risk Factors of COX-2 High Expression

| Variables          | Characteristics | Univariable analysis odds ratio (95% CI), p-value | Multivariable analysis odds ratio (95% CI), p-value |
|--------------------|-----------------|--------------------------------------------------|--------------------------------------------------|
| Age (years)        | Mean ± SD       | 1.04 (1.00-1.10), 0.02                           | 1.04 (1.00-1.10), 0.05*                           |
| Estrogen receptor  | Negative        | Ref                                              | Ref                                              |
|                   | Positive        | 4.30 (1.60-11.76), 0.01                          | 5.62 (1.80-17.84), 0.002*                         |
| IDO Score          | Low             | Ref                                              | Ref                                              |
|                   | High            | 4.10 (1.54-10.81), 0.01                          | 6.51 (2.00-21.20), 0.001*                         |

*values indicate statistical significance p< 0.05; IDO, Indoleamine 2,3-dioxygenase
present between IDO and COX-2 expression in Pakistani breast cancer patients. The data presented in this study will certainly serve as a useful addition to the already available knowledge, while the molecular mechanisms underlying IDO mediated COX-2 expression need further investigation. Hence the simultaneous targeting of IDO and COX-2 may be a promising therapy for breast cancer.

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Conflict-of-interest statement

Authors declare no conflict of interests for this manuscript.

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