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Relationship Between COX-2 Expression with ER, PR, HER-2, and KI-67 Expression in Breasr Cancer Patients at Dr Saiful Anwar Malang Hospital

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ABSTRACT

Breast cancer is a malignancy of the breast which is one of the leading causes of death in women. COX-2 plays a role in increasing cell proliferation, delaying apoptosis, increasing angiogenesis, and increasing invasiveness of surrounding cells. Non-steroidal anti-inflammatory drug (NSAID) or anti-COX-2 therapy has the potential to reduce COX-2 overexpression and decrease breast cancer progression. COX-2 expression is thought to be related to hormone receptor expression of ER, PR HER-2, and Ki-67. The purpose of this study was to determine the relationship between COX-2 expression and ER, PR, HER-2, and KI-67 expression in breast cancer patients at DR Saiful Anwar Hospital, Malang so that it can be used as a basis for NSAID therapy. This study using cross-sectional study and carried out with a time span of July-November 2021 at the Anatomical Pathology Installation of Saiful Anwar Hospital, Malang. Sampling using consecutive sampling method with a total sample of 30 samples of breast cancer tissue. COX-2 expression with ER, PR, HER-2, and KI-67 was analyzed using Kendall's tau B with a significance level of $p<0.05$. There is a correlation between COX-2 expression with PR ($r=-0.382$; $p=0.037$) and Ki-67 ($r=0.532$;$p=0.004$). There was no correlation between COX-2 expression with ER and HER-2 receptors ($p>0.05$). COX-2 expression was negatively correlated with PR and positively correlated with Ki-67.

1. INTRODUCTION

Breast cancer is an abnormal growth in the epithelial cells of the lobules and ducts of the breast. Based on the WHO report, the prevalence of breast cancer worldwide in women is 2.1 million cases per year and the mortality rate is 627,000 cases per year, which is the highest cause of death in women. Based on the 2011 Ministry of Health report, the prevalence of breast cancer in Indonesia is 12/100,000 population and 80% is found at an advanced stage [1], [2].

The exact cause of breast cancer is currently still unclear, some researchers state that the causes of breast cancer include (1) molecular changes in the TP-53, PIK3CA, and MYC proteins; (2) changes in the hormones estrogen and progesterone; (3) genetic alteration of the Ki-67 gene and human epithelial growth factor receptor-2 (HER2); (4) changes in the immune system of macrophages, CD4 and CD8; (5) increase in inflammatory cells of COX-2 [3- 6].

Several examinations currently used to diagnose breast cancer include clinical examination, supporting examinations including ultrasonography (USG), mammography, digital mammary tomosyntegesis (DMS), magnetic resonance imaging (MRI), histopathological examination, and molecular examination including examination of the estrogen receptor hormone. and progesterone, HER2, and Ki-67 [7- 13].

Molecular examination is currently used because it has a significant relationship in breast cancer and can determine future prognosis [14- 16]. In recent years, many researchers have studied the expression of COX-2 to increase the incidence of breast cancer, increased COX-2 was associated with the progression of breast cancer [17]. COX-2 plays a role in increasing cell proliferation, delaying apoptosis, increasing angiogenesis, and increasing angiogenesis and increasing invasiveness of surrounding cells [18- 20]. Therefore, non-steroidal anti-inflammatory drug (NSAID) or anti-COX-2 therapy can reduce COX-2 overexpression and decrease breast cancer progression.

Several studies showed that NSAID or anti-COX-2 therapy had an effect on decreasing breast cancer progression [21], [22]. Another study conducted by [23] in a retrospective and prospective study in 8233 patients taking aspirin showed a reduced risk incidence of breast cancer by 37%-39%.

Several studies have shown that COX-2 expression was associated with the expression of hormone receptors of estrogen and progesterone. These receptors become protective agents inhibiting chronic inflammatory pathways. This process showed that COX-2 expression has the potential to become a standard test that can be done for the administration of NSAID therapy and can be considered to reduce breast cancer progression [24- 27].

From the above explanation, futher study is needed to evaluate the relationship between COX-2 expression and the expression of estrogen receptors, progesterone receptors, HER-2, and Ki67 on immunohistochemical examination of breast cancer patients at Dr. Saiful Anwar Malang Hospital.

2. Methods

This research is a cross-sectional observational study. A cross sectional study is a research design in which the measurement of the variables is carried out only once at a time to determine the relationship between COX-2 and ER, PR, HER-2, and Ki-67 in breast cancer patients. This research was conducted at the Anatomical Pathology Installation of Saiful Anwar Hospital Malang with from July – November 2021.

2.1 Population and sample

The population in this study were all patients with a diagnosis of breast cancer. The sample in this study was Paraffin Block of breast cancer patients who were inpatient at RSSA and outpatient at RSSA from January to November 2021. The sampling technique used was consecutive sampling, where all research subjects were used from January to November 2021. The inclusion criteria of this research were paraffin block of mammary carcinoma patients with histopathological staining results with ER, PR, HER-2 and Ki-67 profiles. The exclusion criteria of this study was paraffin block of mammary carcinoma patients with damaged histopathological staining data

2.2 Research procedure

2.2.1 Tools and materials

Poly L-Lysin, Xylol, Ethanol 95%, Alcohol 90%, 80%, H2O2, Diva Solution, Paraffin, phosphate buffered saline (PBS), Sniper background solution, Trecavidin HRP, DAB Larutan solution, Haematoxylin Solution, Antigen retrieval, ER, PR, HER-2, Ki-67, and COX-2 (CPI) antibodies. Microtome, Decloaking Chamber, Moisture chamber, Glass object.

2.3 Measurement Procedure on ER, PR, HER-2, Ki-67, and COX-2

First, preparations are cut with a microtome 4 to 5 microns thick, Attach to the glass object that has been coated with poly L-Lysin, Store in an incubator at 40ºC overnight for stronger adhesion, Deparaffinization with Xylol I, II, III 3 minutes each, Put in ethanol, 90% alcohol, 80% each 2-3 minutes, Put in H2O2 in 100ml 0.5% methanol + 1.6ml H2O2 for 20 minutes, Antigen retrieval soak in DIVA solution, heat in Decloaking Chamber, Cool at room temperature 20 to 30 minutes, Soak with PBS for 2 to 5 minutes, Place the slide in the moisture chamber and place a barrier around the pap pen preparation. Drop sniper background 10-15 minutes, Drop the primary antibody (universal link), incubate overnight, Wash with PBS 2 to 5 minutes, Drop secondary antibody (universal link) incubation for 10 minutes, Wash with PBS for 2 to 5 minutes, Drop trackavidin Hrp label, incubate for 10 minutes, Wash with PBS for 2 to 5 minutes, Drop DAB incubation for 2 to 4 minutes (1 ml Betazoid DAB Substrate Buffer plus 1-2 drops of DAB chomogen), Wash with running water 5-7 minutes, Counterstain with mayers haematoxilin 2 to 3 minutes. Soak in saturated lithium carbonate 2 to 3 minutes, Wash with running water 5 to 7 minutes, Dehydrated with alcohol 80%, 95%, absolute alcohol up to xylol I, II, III 3 minutes each, Mounting with entelan.

2.4 Data analysis

The relationship between COX-2 expression and the expression of estrogen receptors, progesterone receptors, HER-2, and Ki-67 was carried out using Kendall's tau B test with signification level of p-value <0.05.

3. Results

3.1 Correlation Analysis of COX-2 and ER Expression in Breast Cancer Patients

Table 1 Correlation Test of COX-2 and ER Expression in Breast Cancer Patients

COX-2 Expression	ER Expression		Correlation	
	Positive	Negative	*r*	*p-value*
Strong	16 (53,3)	6(20)	0,011	0,951
Medium	6(20)	1 (3,3)		
Weak	0	1 (3,3)		

3.2 Correlation test using Kedall's tau B; r<0.4, weak; r 0.4-0.6, moderate; r > 0.6 strong.

Based on table 1 shows the correlation of COX-2 expression with ER in breast cancer patients. Most of the research subjects had strong COX-2 expression with positive ER expression (53.3%). The results of the correlation test analysis using Kendall's tau B showed that there was no significant correlation between COX-2 expression and ER expression (p>0.05)

3.3 Correlation Analysis of COX-2 and PR Expression in Breast Cancer Patients

Table 2 Correlation Test of COX-2 and PR Expression in Breast Cancer Patients

COX-2 Expression	PR Expression		Correlation	
	Positive	Negative	*r*	*p-value*
Strong	17(56,7)	5(16,7)		
Medium	3(10)	4(13,3)	-0,382	0,037
Weak	0	1 (3,3)		

Correlation test using Kedall's tau B; r<0.4, weak; r 0.4-0.6, moderate; r > 0.6 strong.

Based on table 2 shows that the correlation of COX-2 and PR expression in breast cancer patients. Most of the research subjects had strong COX-2 expression with positive PR expression (56.7%). The results of the correlation test analysis using Kendall's tau B showed that there was a significant negative correlation between COX-2 expression and PR expression, meaning that the higher PR expression was accompanied by a decrease in COX-2 (p<0.05).

3.4 Correlation Analysis of COX-2 and HER-2 Expression in Breast Cancer Patients

Table 3 Correlation Test of COX-2 and HER-2 Expression in Breast Cancer Patients

COX-2 Expression	HER-2 Expression		Correlation	
	Positive	Negative	*r*	*p-value*
Strong	10 (33,3)	12 (40)	0,045	0,807
Medium	2 (6,7)	5(16,7)		
Weak	1 (3,3)	0		

Correlation test using Kedall's tau B; r<0.4, weak; r 0.4-0.6, moderate; r > 0.6 strong.

Based on table3 shows that the correlation of COX-2 and HER-2 expression with in breast cancer patients. Most of the research subjects had strong COX-2 expression with negative HER-2 expression (40%). The results of the correlation test analysis using Kendall's tau B showed that there was no significant correlation between COX-2 expression and HER-2 expression (p>0.05).

3.5 Correlation Analysis of COX-2 and Ki-67 Expression in Breast Cancer Patients

Table 4 Correlation Test of COX-2 and Ki-67 Expression in Breast Cancer Patients

COX-2 Expression	Ki-67 index		Correlation	
	High	Low	r	p-value
Strong	6 (20)	16 (53,3)		
Medium	6 (20)	1(3,3)	0,532	0,004
Weak	1 (3,3)	0		

Correlation test using Kedall's tau B; r<0.4, weak; r 0.4-0.6, moderate; r > 0.6 strong.

Based on table 4 shows the correlation of COX-2 expression with Ki-67 in breast cancer patients. Most of the research subjects had strong COX-2 expression with a low Ki-67 index (53.3%). The results of the correlation test analysis using Kendall's tau B showed that there was a significant positive correlation between COX-2 expression and the Ki-67 index (p<0.05). This means that the higher COX-2 expression is accompanied by an increase in the Ki-67 degree index.

Histological Overview	Intensity

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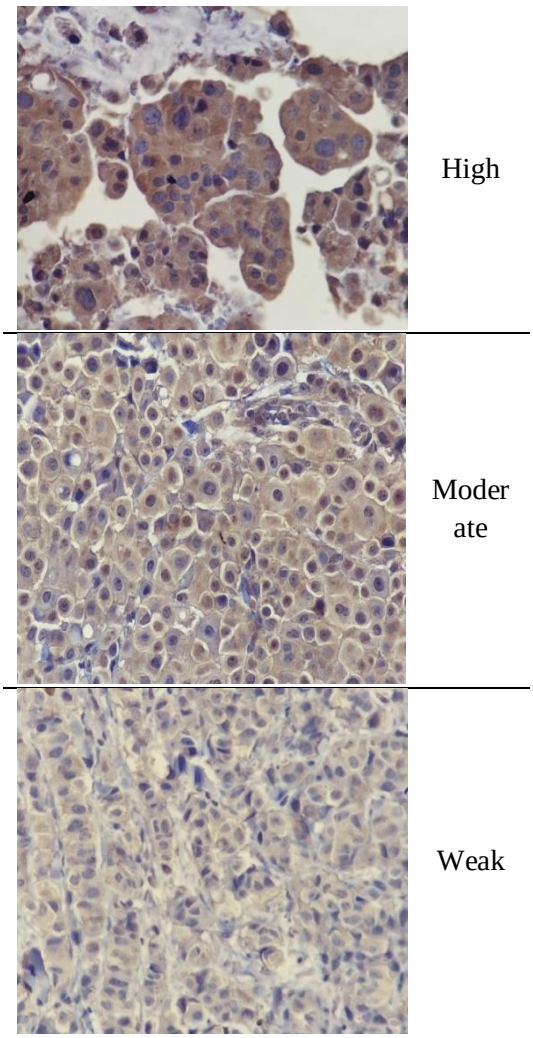

Figure 1. Histology Expression of COX-2

Figure 1 showed that histology of breast cancer tissue with COX-2 expression with 400x magnification. It can be seen that the strong intensity has a more dominant brown color than the other intensities.

4. Discussion

In this study, it was found that there was a relationship between COX-2 expression and Ki-67 and there was no relationship with ER, PR, and HER-2. In this study, it showed that COX-2 expression was strong in about 63.3% of all samples. In a previous study, COX-2 expression in breast cancer was around 76.9% [28]. Positive ER expression in breast cancer has a percentage of 73.3%. These results are similar to previous studies that approximately 80% of breast cancer patients are estrogen receptor positive [29]. The positive PR expression in this study was around 66.67% while the positive HER-2 expression was around 43.3%. This study is supported by previous studies that positive PR expression in breast cancer is around 50-70% [30]. The Ki-67 index is divided into two types, namely high and low with a percentage of 43.3% and 66.6%, respectively. These results are in line with previous studies that high Ki-67 in luminal B is around 69% and HER-2 is around 34% [31]. Chronic inflammation promotes cancer progression by increasing cellular proliferation. The COX-2 gene is known to be associated with inflammation and cancer. The COX-2 gene can be induced by various factors that are the main triggers that activate the inflammatory response. COX-2 induction can be carried out by various inflammatory stimuli e.g. tobacco, alcohol, ischemia, trauma, pressure, foreign bodies, toxins, bacteria, viruses, lipopolysaccharides, etc. This induction results in the biosynthesis of prostaglandins

that regulate the inflammatory response. The cyclooxygenase pathway produces a variety of prostaglandins, prostacyclin and thromboxane from arachidonic acid and other fatty acids. Initially COX catalyzes the oxidation of arachidonic acid to prostaglandin H-2 (PGH-2) which is rapidly converted to biologically active prostaglandins by specific enzymes [32].

Downregulation of COX-2 decreases the extracellular matrix in tumors. Decreased COX-2 is also known to decrease VEGF thereby decreasing vascular permeability. Decreased cellular permeability reduces the survival and metastasis of cancer cells [33]. COX-2 expresses 40% of invasive breast cancer. Bone is the dominant site of breast cancer metastases. The presence of COX-2 is important for the process of breast cancer metastasis in bone. Studies in mice have shown that COX-2 enhances the osteolytic process in bone metastases from breast cancer patients [34].

The results of a meta-analysis consisting of 21 clinical studies showed that increased expression of COX-2 was associated with decreased overall survival and disease free survival for 5 years. In addition, an increase in COX-2 is also associated with an increase in tumor size and the incidence of lymph metastases in breast cancer [35]. Increased COX-2 expression in lobular and ductal breast cancer correlates with clinical parameters so that it can be a marker of poor prognosis in patients [36].

In this study there was a negative correlation between PR and COX-2. These results are in line with previous research. Increased PR led to decreased aromatase expression, COX-2, and HER-2/neu expression and decreased PR led to induction of aromatase and HER-2/neu mRNA. Based on these results indicate that PR plays an important role in anti-inflammatory breast cancer in cultured cells [37]. Another study showed that COX-2 expression was also correlated with HER-2/neu expression which was expressed in >30% of breast tumors. Induction of COX-2 by inflammatory cytokines acting through NF-kappaB contributes to increased expression of CYP19 and breast cancer progression, and that PR plays a role in preventing breast cancer cell development by antagonizing NF-kappaB activation of COX-2 [38]. This difference in results could be due to other diseases that can induce an increase in COX-2.

The results of previous studies are consistent with this study, namely that there is no correlation between COX-2 and ER in breast cancer patients [39]. COX-2 levels can be induced by ER negative and ER positive through different pathways. In cancer cells with negative ER induce COX-2 through the RAS/MAPK/PKC pathway, whereas in cancer cells with positive ER it induces through the Activated Protein-1 (AP-1) pathway [40]. This is thought to cause insignificant results because both types of estrogen receptors both positive and negative can induce COX-2.

The results of this study indicate that there is no correlation between COX-2 and HER-2. The results of this study are supported by previous studies which showed that there was no correlation between COX-2 and HER-2 [36]. Generally, there is an increase in COX-2 in HER-2 positive patients but this depends on the expression of the epidermal growth factor receptor (EGFR) induced by other inflammatory mediators such as prostaglandins [36]. This research was conducted in Indonesia so that it is possible to have different signaling from other studies. Research by [41] showed that ethnic differences cause gene mutations in EGFR so that they have different signaling. These signaling differences may provide different responses to tumor development.

In this study, there was a positive correlation between Ki-67 and COX-2. Other studies have also shown that COX-2 is positively correlated with size, grade, and vascular invasion but not with ER, PR, and HER-2 [36]. In addition, COX-2 is positively correlated with Ki-67 in patients with poor prognosis (Park et al., 2012). The

results of this study are supported by previous studies showing that COX-2 expression is positively correlated with Ki-67 levels in breast cancer patients so that the presence of COX-2 and Ki-67 can be a prognostic indicator in breast cancer [42]. The results of this study could be clinically useful as a basis for the administration of COX-2 anti-inflammatory drugs. Anti-inflammatory COX-2 is recommended to be given to patients with negative PR and high Ki-67 index.

COX-2 has the potential as a prognostic indicator in breast cancer, but in this study, no measurements were made on the prognosis of breast cancer. In this study, the clinical characteristics of breast cancer patients were also not measured. Therefore, the next study needs to measure the correlation between COX-2 and Ki-67 on the characteristics and prognosis of breast cancer patients.

5. Conclusions

According to this research result, it could be concluced that:

1. There is no correlation between COX-2 expression and ER in breast cancer patients
2. There is a negative correlation between COX-2 expression and PR in breast cancer patients
3. There is no correlation between COX-2 expression and HER-2 in breast cancer patients
4. There is a positive correlation between COX-2 expression and Ki-67 in breast cancer patients
5. High COX-2 expression in breast cancer patients with high Ki-67 hormone receptors and negative PR

Author Contribution

All authors are contributed to conceived and design the analysis, cellected the data; contributed data and analysis tools; performed analysis and wrote the paper.

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6. References

[1] Singh, B., Berry, J. A., Shoher, A., Ayers, G. D., Wei, C., & Lucci, A. COX-2 involvement in breast cancer metastasis to bone. 2007. Oncogene, 26(26),3789–3796. https://doi.org/10.1038/sj.onc.1210154

[2] Kemenkes RI. InfoDATIN Bulan Peduli Kanker Payudara. InfoDatin Bulan Peduli Kanker Payudara. Jakarta: Kementerian Kesehatan RI Pusat Data dan Informasi. 2016; p. 2–6.

[3] Wu, F., Wang, Z. B., Cao, Y. D., Zhu, X. Q., Zhu, H., Chen, W. Z., Zou, J. Z. “Wide local ablation” of localized breast cancer using high intensity focused ultrasound. Journal of surgical oncology, 96(2), 2007; 130-136.

[4] Santen, R. J. (2011). Clinical review: effect of endocrine therapies on bone in breast cancer patients. J. Clin. Endocrinol. Metab. 96, 308–319.

[5] Yates, LR., Desmedt, C. Translational genomics: practical applications of the genomic revolution in breast cancer. Clin. Cancer Res. 2017;23, 2630–2639.

[6] Dieci, MV., Robin NR., Fineberg S., Eynden G., Ternes N., Llorca FP., et al. Update on tumor-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease

after neoadjuvant therapy and in carcinoma in situ: a report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer. Semin. Cancer Biol. 52, 16–25 (2018).

[7] Manuaba TW. Panduan Penatalaksanaan Kanker Solid PERABOI. Jakarta: Sagung Seto. 2010.

[8] Sardanelli, F. Boetes, C., Borisch, B., Decker, T., Federico, M., Gilbert, FJ., et al. Magnetic resonance imaging of the breast: recommendations from the EUSOMA working group. Eur. J. Cancer. 2010; 46, 1296–1316.

[9] Lauby-Secretan, B., Scoccianti, C., Loomis, D., Talla, LB., Bouvard, V., Bianchini, F. Breast-cancer screening — viewpoint of the IARC Working Group. N. Engl. J. Med. 2015; 372, 2353–2358.

[10] Phi, X.-A., Houssami, N., Obdeijn, IM., Warner, E., Sardanelli, F., Leach, M.O., et al. Magnetic resonance imaging improves breast screening sensitivity in BRCA mutation carriers age ≥ 50 years: evidence from an individual patient data meta-analysis. J. Clin. Oncol. 2015;33, 349–356.

[11] Sun Y., Zhao, Z., Yang, Z., Xu, F., Lu, HJ., Zhu, ZY., et al. Risk Factors and Preventions of Breast Cancer. 2017; 13.

[12] Weaver O., Leung JWT. Biomarkers and Imaging of Breast Cancer. Am J Roentgenol. 2018;210(2):271–8.

[13] Pierce, D. W., Zavodovskaya, M., & Fan, J. (2018). U.S. Patent Application No. 15/555,310.

[14] Anderson, H., Hills, M., Zabaglo, L., A'hern, R., Leary, A. F., Haynes, B. P., ... & Dowsett, M. Relationship between estrogen receptor, progesterone receptor, HER-2 and Ki67 expression and efficacy of aromatase inhibitors in advanced breast cancer. Annals of oncology. 2011;22(8), 1770-1776.

[15] Allison, K. H. Molecular pathology of breast cancer: what a pathologist needs to know. American journal of clinical pathology. 2012;138(6), 770-780.

[16] Parise, C. A., & Caggiano, V. Breast cancer survival defined by the ER/PR/HER2 subtypes and a surrogate classification according to tumor grade and immunohistochemical biomarkers. Journal of cancer epidemiology. (2014).

[17] Gupta, R. A., and DuBois, R. N. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat. Reviews/ Cancer. 2001;1: 11.

[18] Zhao, Y., Agarwal, V. R., Mendelson, C. R., and Simpson, E. R. Estrogen biosynthesis proximal to a breast tumor is stimulated by PGE2 via cyclic AMP, leading to activation of promoter II of the CYP19 (aromatase) gene. Endocrinology. 1996; 137: 5739.

[19] Gately, S. The contribution of cyclolxygenase-2 to tumor angiogenesis. Cancer Metastasis Rev. 2000;19: 19.

[20] Guyton, D. P., Evans, D. M., and Sloan-Stakleff, K. D. Urokinase plasminogen activator receptor (uPAR): A potential indicator of invasion for in situ breast cancer. Breast J. 2000; 6:130.

[21] Hoellen, F., Kelling, K., Dittmer, C., Diedrich, K., Friedrich, M., & Thill, M. Impact of cyclooxygenase-2 in breast cancer. Anticancer research. 2011; 31(12), 4359-4367.

[22] Moris, D., Kontos, M., Spartalis, E., & Fentiman, I. S. The role of NSAIDs in breast cancer prevention and relapse: current evidence and future perspectives. Breast Care. 2016 ;11(5), 339-344.

[23] Kehm, R. D., Hopper, J. L., John, E. M., Phillips, K. A., MacInnis, R. J., Dite, G. S. Southey, M. C. Regular use of aspirin and other non-steroidal anti-inflammatory drugs and breast cancer risk for women at familial or genetic risk: a cohort study. Breast Cancer Research. 2019; 21(1), 52.

[24] Glynn, S. A., Prueitt, R. L., Ridnour, L. A., Boersma, B. J., Dorsey, T. M., Wink, D. A., Ambs, S. COX-2 activation is associated with Akt phosphorylation and poor survival in ER-negative, HER2-positive breast cancer. BMC cancer. 2010; 10(1), 626.

[25] Sicking, I., Rommens, K., Battista, M. J., Böhm, D., Gebhard, S., Lebrecht, A., ... & Schmidt, M. Prognostic influence of cyclooxygenase-2 protein and mRNA expression in node-negative breast cancer patients. BMC cancer. 2014; 14(1), 952.

[26] Güler, S. A., Uğurlu, M. Ü., Kaya, H., Şen, S., Nazlı, Y., & Güllüoğlu, B. M. Impact of cyclooxygenase-2 over-expression on the prognosis of breast cancer patients. Turkish Journal of Surgery/Ulusal cerrahi dergisi. 2016; 32(2), 81.

[27] Basudhar, D., Glynn, S. A., Greer, M., Somasundaram, V., No, J. H., Scheiblin, D. A., ... & Cheng, R. Y. Coexpression of NOS2 and COX2 accelerates tumor growth and reduces survival in estrogen receptor-negative breast cancer. Proceedings of the National Academy of Sciences. 2017; 114(49), 13030-13035.

[28] Jana, D., Sarkar, D. K., Ganguly, S., Saha, S., Sa, G., Manna, A. K., Banerjee, A., & Mandal, S. Role of Cyclooxygenase 2 (COX-2) in Prognosis of Breast Cancer. Indian journal of surgical oncology. 2014; 5(1), 59–65. https://doi.org/10.1007/s13193-014-0290-y

[29] Sleightholm, R., Neilsen, B. K., Elkhatib, S., Flores, L., Dukkipati, S., Zhao, R., Choudhury, S., Gardner, B., Carmichael, J., Smith, L., Bennion, N., Wahl, A., & Baine, M. Percentage of Hormone Receptor Positivity in Breast Cancer Provides Prognostic Value: A Single-Institute Study. Journal of clinical medicine research, 2021; 13(1), 9–19. https://doi.org/10.14740/jocmr4398

[30] Nordenskjöld, A., Fohlin, H., Fornander, T., Löfdahl, B., Skoog, L., & Stål, O. Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast cancer research and treatment. 2016; 160(2), 313–322. https://doi.org/10.1007/s10549-016-4007-5

[31] Soliman, N. A., & Yussif, S. M. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer biology & medicine. 2016; 13(4), 496–504. https://doi.org/10.20892/j.issn.2095-3941.2016.0066

[32] Harris, R. E., Beebe-Donk, J., & Alshafie, G. A. Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC cancer. 2006; 6(1), 27.

[33] Krishnamachary, B., Stasinopoulos, I., Kakkad, S., Penet, M. F., Jacob, D., Wildes, F., Mironchik, Y., Pathak, A. P., Solaiyappan, M., & Bhujwalla, Z. M. Breast cancer cell cyclooxygenase-2 expression alters extracellular matrix structure and function and numbers of cancer associated fibroblasts. Oncotarget. 2017; 8(11), 17981–17994. https://doi.org/10.18632/oncotarget.14912

[34] Singh, B., Berry, J. A., Shoher, A., Ayers, G. D., Wei, C., & Lucci, A. COX-2 involvement in breast cancer metastasis to bone. Oncogene. 2007; 26(26), 3789–3796. https://doi.org/10.1038/sj.onc.1210154

[35] Xu, F., Li, M., Zhang, C., Cui, J., Liu, J., Li, J., & Jiang, H. Clinicopathological and prognostic significance of COX-2 immunohistochemical expression in breast cancer: a meta-analysis. Oncotarget. 2017; 8(4), 6003–6012. https://doi.org/10.18632/oncotarget.13990

[36] Miglietta, A., Toselli, M., Ravarino, N., Vencia, W., Chiecchio, A., Bozzo, F., Motta, M., Torchio, B., & Bocca, C. COX-2 expression in human breast carcinomas: correlation with clinicopathological features and prognostic molecular markers. Expert opinion on therapeutic targets. 2010; 14(7), 655–664. https://doi.org/10.1517/14728222.2010.486792

[37] Hardy, D. B., Janowski, B. A., Chen, C. C., & Mendelson, C. R. Progesterone receptor inhibits aromatase and inflammatory response pathways in breast cancer cells via ligand-dependent and ligand-independent mechanisms. Molecular endocrinology (Baltimore, Md.). 2008; 22(8), 1812–1824. https://doi.org/10.1210/me.2007-0443

[38] Mendelson, C. R., & Hardy, D. B. Role of the progesterone receptor (PR) in the regulation of inflammatory response pathways and aromatase in the breast. The Journal of steroid biochemistry and molecular biology. 2006;102(1-5), 241–249. https://doi.org/10.1016/j.jsbmb.2006.09.029

[39] Nassar, A., Radhakrishnan, A., Cabrero, I. A., Cotsonis, G., & Cohen, C. COX-2 expression in invasive breast cancer: correlation with prognostic parameters and outcome. Applied immunohistochemistry & molecular morphology: AIMM. 2007; 15(3), 255–259. https://doi.org/10.1097/01.pai.0000213130.63417.b3

[40] Jana, D., Sarkar, D. K., Ganguly, S., Saha, S., Sa, G., Manna, A. K., Banerjee, A., & Mandal, S. Role of Cyclooxygenase 2 (COX-2) in Prognosis of Breast Cancer. Indian journal of surgical oncology. 2014; 5(1), 59–65. https://doi.org/10.1007/s13193-014-0290-y

[41] Shen, Y., Wang, J., Han, X., Yang, H., Wang, S., Lin, D., & Shi, Y. Effectors of epidermal growth factor receptor pathway: the genetic profiling ofKRAS, BRAF, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. PloS one. 2013; 8(12), e81628. https://doi.org/10.1371/journal.pone.0081628

[42] Qiu, X., Wang, H., Wang, Z., Fu, Y., & Yin, J. Expression of PCNA, Ki-67 and COX-2 in breast cancer based on DCE-MRI image information. Journal of infection and public health. 2020; 13(12), 2032–2037. https://doi.org/10.1016/j.jiph.2019.06.024