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Cyclooxigenase-2 (COX-2) expression in colorectal cancer and the correlation with histopathological grading, depth of tumor invasion and regional lymph node metastasis

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Abstract

Colorectal cancer is malignancy that originates from epithelium lining of colon and rectum. GLOBOCAN reported that the incident in 2018 were 18 million new cases and 9.6 million deaths in global world. The Indonesian cases reached 34,189 in 2020. COX-2 suspected to play role in colorectal cancer carcinogenesis. Several studies have stated that any COX-2 expression in colorectal cancer, but research that shows correlation with prognostic factors is still controversial. This study aims to determine COX-2 expression in colorectal cancer using immunohistochemistry examination and the correlation with histopathological grading, depth of tumor invasion and regional lymph node metastasis. This research used analytical observational design, sample consisted of 30 paraffin blocks of colectomy patients diagnosed with colorectal cancer in 2022-2023 as well as medical record data from histopathological results. The analysis used univariate and bivariate analysis. Chi Square test results of COX-2 expression showed 100% positive staining with categories strong 93.4%, and moderate 6.7%. Results Spearman correlation test of COX-2 expression with histopathological grading obtained p value 0.856 (correlation not significant), correlation with the depth of tumor invasion obtained p value 0.763 (correlation not significant), and correlation with regional lymph node metastasis obtained p value of 0.023 (negative correlation). Overexpression of COX-2 was found in colorectal cancer and no correlation was found with the prognosis markers studied. Further research on COX-2 is needed by comparing other molecular markers such as KRAS, TP53, TILs.

Keywords: Colorectal Adenocarcinoma; COX-2; Histolpathological Grading; Depth of Tumor Invasion; Regional Lymph Node Metastasis

1. Introduction

Colorectal cancer is a malignancy that originates from epithelium of the colon and rectum, is the third most common disease in the world, the second most common cause of death due to cancer, and the main cause of death from gastrointestinal cancer in western countries (1). The Global Burden of Cancer Study (GLOBOCAN) said that in 2019 there were 18 million new cases of colorectal cancer and 9.6 million deaths due to colorectal cancer in the world (2). Colorectal cancer is also the second most common malignancy in women (9.2% of all cancer cases) and the third most common malignancy in men (10% of all cancer cases) worldwide (3). American Cancer Society states that there will be around 152,810 new cases of colorectal cancer (81,540 in men and 71,270 in women) in the United States in 2024 (4).

The incidence of colorectal cancer 2020 in Indonesia reached 34,189 (8.6%) of the total cancer cases in Indonesia. Based on the results of previous studies at the Sanglah Central General Hospital, the number of colorectal cancer sufferers in

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2010-2014 was 435 people and increased every year. Research conducted at Saiful Anwar Hospital Malang in 2016 - 2018 included 40 cases of colorectal adenoma and 300 cases of colorectal adenocarcinoma. These cases came from biopsy preparations with colonoscopy or colectomy operations (1,5).

Colorectal cancer starts from precursor lesions which generally progress slowly until they become malignant. The journey from normal colonic epithelium to precursor lesions and ultimately to cancer involves two main pathways (6). The first is the classical pathway, involving the change of normal epithelium into an adenoma and later becoming carcinoma, and the alternative pathway involves the change in normal epithelium into serrated polyps before becoming carcinoma (7). Arachidonic acid metabolism is thought to play a very important role in carcinogenesis. Cyclooxygenase (COX) is a key enzyme in the conversion arachidonic acid into prostaglandins Cyclooxygenase has two forms namely COX-1 and COX-2, these two enzymes are located intracellular in the endoplasmic reticulum and nucleus (8). COX-2 expression in colorectal cancer occurs through a variety of different mechanisms and involves several signaling pathways. Several cellular markers, oncogenes, growth factors, and cytokines are thought to play a role in COX-2 expression in colorectal cancer. COX-2 expression is associated with inhibition of apoptosis, increased metastatic potential, and increased neoangiogenesis (neovascularization), or in other words its expression is associated with the aggressiveness and poor clinical outcome of tumor (9).

Based on previous research data that cyclooxygenase-2 (COX-2) has a role in the carcinogenesis of colorectal cancer and states that there is an overexpression of COX-2, this study wants to know the expression of COX-2 in colorectal cancer in relation to histopathological grading, depth of tumor invasion and regional lymph node metastasis. This is related to the patient's prognosis. Looking at COX-2 expression in colorectal cancer, it can also be used to consider the use of combination COX-2 inhibitor therapy in colorectal cancer as a companion therapy to chemotherapy.

2. Materials and Methods

This study aims to determine COX-2 expression in colorectal cancer using immunohistochemistry examination and the correlation with histopathological grading, depth of tumor invasion and regional lymph node metastasis. This research used analytical observational design, using univariate and bivariate analysis. Sample consisted of 30 paraffin blocks of colectomy patients diagnosed with colorectal cancer in 2022-2023 as well as medical record data from histopathological results. The research was conducted from October 2023 to February 2024. The research location was at the Anatomical Pathology Installation at General Hospital dr Saiful Anwar Malang.

2.1. Histopathological Examination

Tissue processing begins with macroscopic cutting and fixation using 10% formalin for a minimum of 4-6 hours. The process continues by inserting the tissue into a tissue processor which continues with making paraffin blocks. Paraffin blocks from tissue that meet the criteria as samples are cut using a microtome, soaked in a tissue floating bath, then continued with deparaffinization for 2 hours in a microwave oven. Processing is then continued with routine staining using hematoxylin-eosin.

2.2. Immunohistochemistry Procedure

The immunohistochemistry examination stage starts with thinly cut tissue placed on a glass slide, then deparaffinized by placing it in a solution of xylol and alcohol with decreasing concentrations. Next, it was soaked in peroxide block solution for 25 minutes followed by DIVA solution before being put into a decloacking chamber at a temperature of 900 for 45 minutes. The daubing was continued by soaking in PBS solution for 5 minutes, then incubating the primary antibody for 60 minutes, then applying polymer. The next step is to give DAB chromogen and do a counter staining using hematoxylin for 2 minutes and lithium carbonate. The next process is clearing into xylol solution which is then continued with mounting. The process was carried out using manual techniques with a dilution of 1:100.

COX-2 expression was measured using a light microscope, counting neoplastic columnar epithelial cells in glands with a brown stain on the cell nucleus, as many as 1000 cells from 5 large fields of view (x40) in the largest area. Each large field of view counted 200 cells stained brown in the cytoplasm. The percentage of cells stained is expressed as a score of 0 = 0 - 5%, 1 = 6 – 25%, 2 = 26 – 50%, 3 = 51 – 75%, 4 = 76 – 100%. The intensity of the appearance is expressed as a score of 1 = weak, 2 = medium, 3 = strong. The score of the percentage of cells stained and the intensity of the smear combined and summed is expressed in a combination score of negative results (score 0-1), weak positive (score 2-3), moderate positive (score 4-5), strong positive (score 6-7) (10).

3. Results and Discussion

Based on data demographic characteristics of the research, From 30 research samples, the average age of patients was 62.7 years, with the youngest being 23 years and the oldest being 83 years. There was 1 person (3.3%) aged between 17.25 years (late teens), 6.7% aged between 36-45 years (late adulthood), 20.0% aged between 46-55 years (early elderly), 26.7% aged between 56- 65 years (late elderly), and 43.3% aged more than 65 years (seniors). Based on gender, there were 17 male (56.7%) and 13 female (43.3%). Based on the tumor location, was found 20.0% in ascending colon, 13.3% in descending colon, and 66.7% in rectosigmoid colon. Complete results of research sample characteristics are listed in table 1.

Table 1 Research Sample Characteristics

Parameters	**Frequency (N=30)**	**Percentage (%)**
Age		
17-25 years (late teens)	1	3.3%
36-45 years (late adulthood)	2	6.7%
46-55 years (early elderly)	6	20.0%
56-65 years (late elderly)	8	26.7%
>65 years (seniors)	13	43.3%
Gender		
Male	17	56.7%
Female	13	43.3%
Location		
colon ascendens	6	20.0%
colon descendens	4	13.3%
colon rectosigmoid	20	66.7%
Histopathological Subtype		
Adenocarcinoma	28	93,4%
Mucinous adenocarcinoma	1	3,3%
Signet ring cell carcinoma	1	3,3%
Histopathological Grading		
Low grade	28	93.3%
High grade	2	6.7%
Regional Lymph Node Metastasis		
N0 (No metastasis)	9	30.0%
N1 (Metastasis 1-3)	13	43.3%
N2 (Metastasis >3)	8	26.7%
Depth of Tumor Invasion		
T1 (Infiltration into submukosa)	0	0%
T2 (Infiltration into muscularis propria)	3	10.0%
T3 (Infitration penetrates beyond serosa)	27	90.0%

Based on histopathological subtype, 93.4% were classified as adenocarcinoma, 3.3% were classified as mucinous adenocarcinoma and 3.3% were classified as signet ring cell carcinoma.

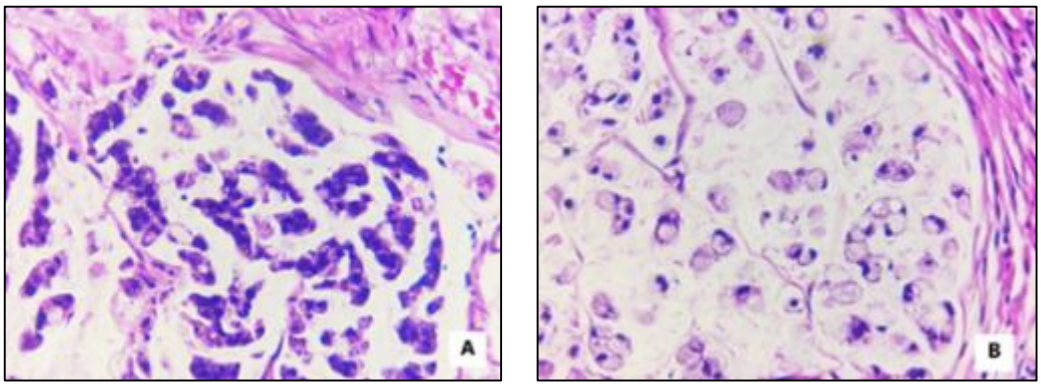

Figure 1 Histopathological subtypes of colorectal adenocarcinoma A) Mucinous adenocarcinoma B) Signet ring cell carcinoma

Histopathological grading of the 30 research samples, it was found that 93.3% of cases were classified as low grade, and the other 6.7% were classified as high grade.

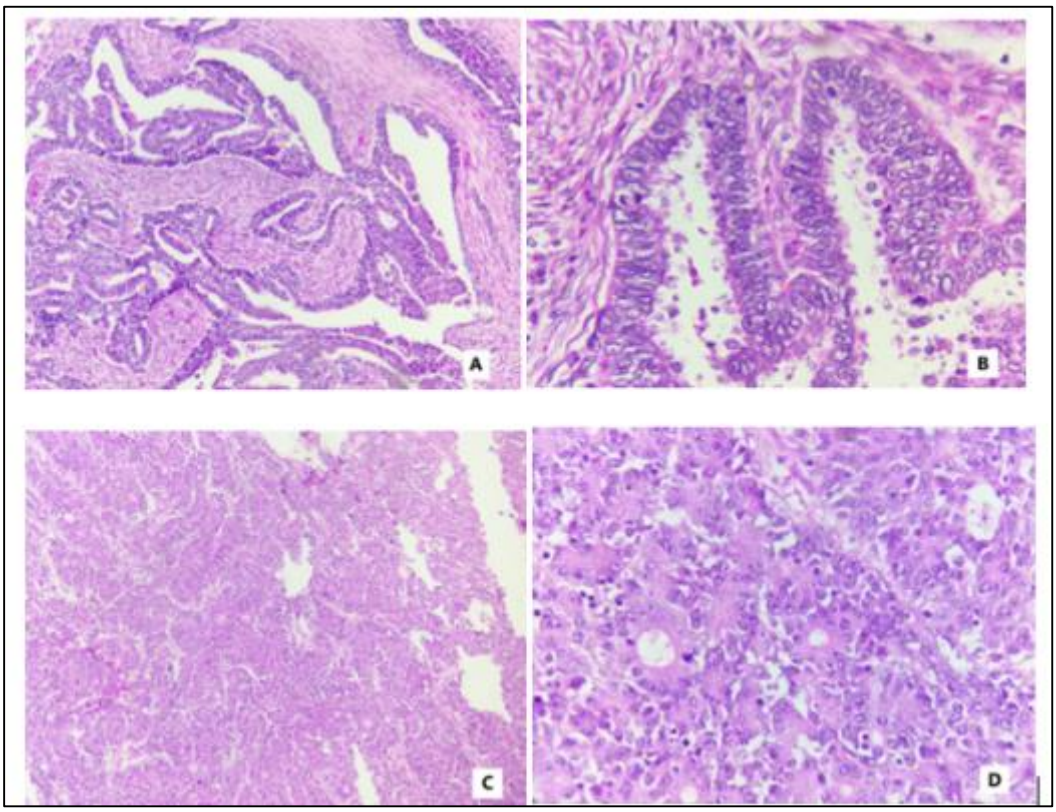

Figure 2 Histopathological grading of colorectal adenocarcinoma. A) low grade adenocarcinoma 100X B) low grade adenocarcinoma 400X, C) high grade adenocarcinoma 100X, D) high grade adenocarcinoma 400X

Based on the depth of tumor invasion from 30 research samples, there are 90% of patients including T3 with tumor invasion that penetrates the serosa, and 10.0% of patients including T2 with infiltration up to the muscularis propria layer and there are no (0%) patients classified as T1 with invasion up to the layer submucosa. Based on regional lymph node metastases from 30 research samples, it was found that 30.0% were classified as N0, 43.3% were classified as N1, and another 26.7% were classified as N2.

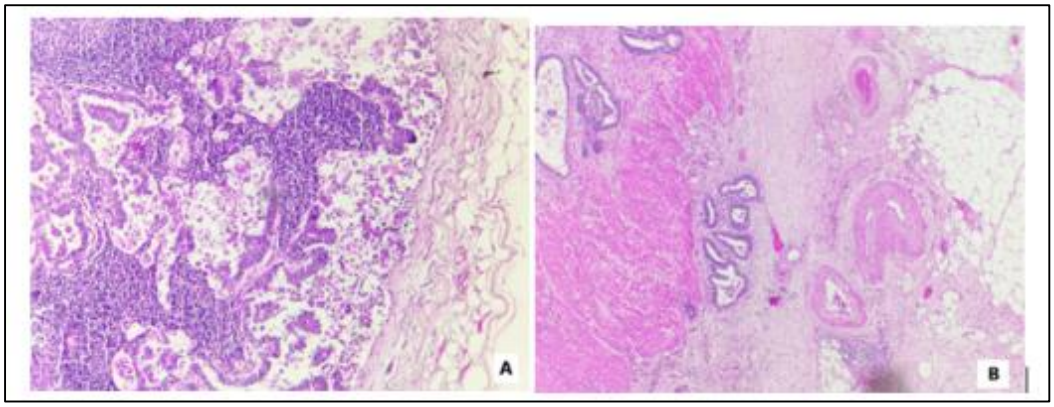

Figure 3 A) Regional lymph node metastasis, B) Tumor invasion penetrates serosa

Results of this study showed that COX-2 expression in 30 colorectal adenocarcinoma samples showed 100% positive staining and no negative staining, with a category of 93.4% strong positive and 6.7% moderate positive and no weak staining. COX-2 expression results based on combined score criteria showed 46.7% score 7 (strong positive), 46.7% score 6 (strong positive) and 6.7% score 5 (moderate positive). This shows that there is overexpression of COX-2 in colorectal adenocarcinoma.

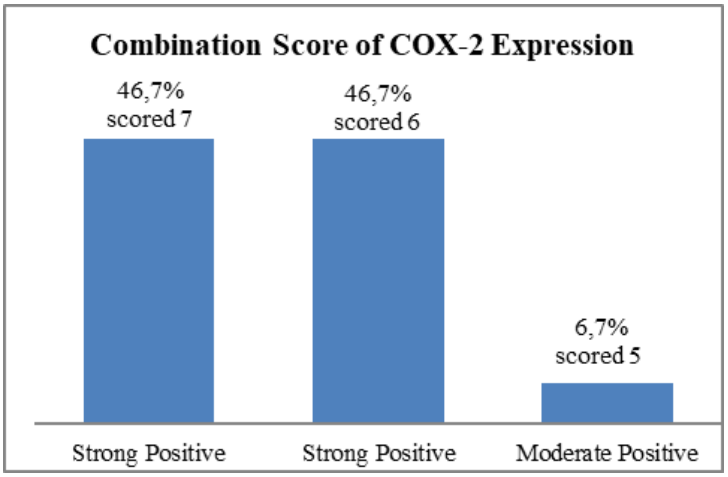

Figure 4 COX-2 Expression in Colorectal Adenocarcinoma

This is in line with other research conducted by *Negi et al* which stated that 70% of colorectal carcinoma cases expressed strong positive COX-2 (overexpression) compared to normal colon tissue (11). Other research bt Mahmoud *et al* also states that COX-2 is expressed positively in 77% of colorectal carcinoma cases and this makes it possible for COX-2 to be an additional therapy to accompany chemotherapy in colorectal cancer cases (12). Another study by Setiawan *et al* carried out at Sanglah Hospital Indonesia involving 71 samples of colorectal carcinoma surgery cases, from this research it was found that 91.3% were positive for expressing COX-2 and this was related to the patient's clinical stage (13). Another study in China with a larger sample size of 1056 cases of colorectal carcinoma diagnosed in 2002-2007 conducted by Wu et al showed 77.9% of cases with COX-2 expression (10).

Table 2 COX-2 Expression Results using Chi Square Test

Variable	Frequency (N=30)	Percentage (%)
COX-2 Expression (percentage)		
Mean±standard deviation	84.81±9.60	
Min-max	57.3 – 97.8	
COX-2 Expression (intensity)		

Moderate	23	76.7%
Strong	7	23.3%
Combination Score		
5	2	6.7%
6	14	46.7%
7	14	46.7%
Combination Score (category)		
positif sedang	2	6.7%
positif kuat	28	93.3%

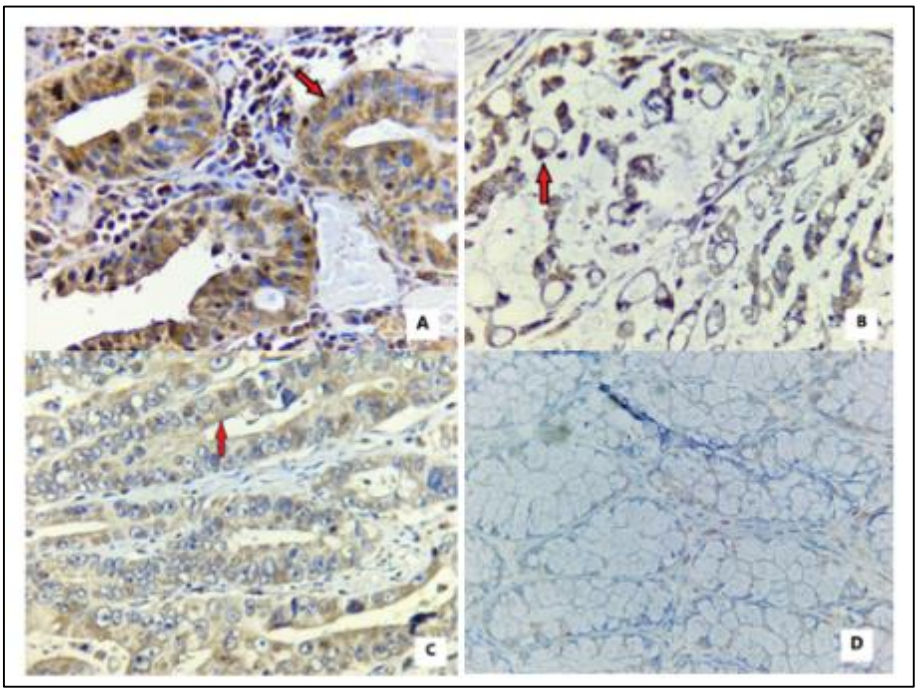

Figure 5 A) Strong intensity COX-2 expression in adenocarcinoma B) Strong intensity COX-2 expression in signet ring cell carcinoma C) Moderate intensity COX-2 expression in adenocarcinoma D) Negative control of COX-2 in normal colonic mucosal crypts

Overexpression of COX-2 in colorectal carcinoma is associated with tumor aggressiveness. COX-2 expression in colorectal cancer occurs through a variety of different mechanisms and involves different signaling pathways. COX-2 expression is associated with inhibition of apoptosis, increased metastatic potential, and increased neoangiogenesis (14).

The results of the Spearman correlation test for COX-2 expression with histopathological grading, depth of tumor invasion and regional lymphnode metastasis are completely explained in table 3.

Correlation between COX-2 expression combination score (scored 5,6,7) and histopathological grading obtained a spearman correlation coefficient of 0.035 with a p value of 0.856 ($p>0.05$) that meaning no significant correlation. These results are in line with research conducted by Negi *et al* and Mahmoud *et al* which stated that COX-2 overexpression was found in colorectal cancer but there was no significant correlation between COX-2 overexpression and histopathological grading or Duke's staging (11,12). This result is in contrast to previous research conducted by Setiawan et al in Bali, Indonesia which stated that there was a correlation between COX-2 overexpression and histopathological grading (13).

The correlation between COX-2 expression and various prognostic markers is still debated. The insignificant correlation between COX-2 expression and histopathological grading could be caused by the influence of other molecular pathways that can also influence prognostic markers. Research conducted by Berbecka *et al* stated that there was no correlation between COX-2 expression and histopathological grade and other prognostic markers, which could be due to the influence of other molecular markers such as KRAS, TP53, BCL2, VEGFR2 gene mutations (15). Another study by Lee *et al* said that there was a strong correlation between KRAS gene mutations and solid areas in the tumor which affected histopathological grading (16).

Table 3 COX-2 Expression and correlation with histopathological grading, depth of tumor invasion and regional lymphnode metastasis

	Spearman correlation coefficient	**P value**
Correlation between combined score COX-2 expression and histolopathologycal grading	0.035	0.856
Correlation between combined score COX-2 expression and depth of tumor invasion	0.058	0.763
Correlation between combined score COX-2 expression and regional lymph node metastasis	-0.415	0.023

The results of spearman correlation between COX-2 expression (scored 5,6,7) and depth of tumor invasion (T2 and T3) above obtained a spearman correlation coefficient value of 0.058 with a p value of 0.763 ($p>0.05$) that meaning no significant correlation. This is in contrast by Setiawan *et al* and Shechan *et al* to previous research which stated that there was a correlation between COX-2 overexpression and a worse prognosis at the TNM stage, one of the components of which was the depth of tumor invasion (13,17).

Berbecka *et al* and Lee *et al* stated that the correlation between COX-2 expression and depth of tumor invasion which was not significant could be caused by the influence of other molecular pathways such as mutations in the KRAS, TP53, BCL2, VEGFR2 genes (13,17). Research by Tamari *et al* stated that there was a significant relationship between Tumor Infiltrating Lymphocytes (TILs) and the depth of tumor invasion which was studied using immunofluorescence examination with CD4 and CD8 antibodies in 84 cases of stage T1b colorectal cancer (18).

Correlation between COX-2 expression combination score (scored 5,6,7) and regional lymph node metastasis (N0, N1, N2) obtained a spearman correlation coefficient value of -0.415 with a p value of 0.023 ($p <0.05$) so it can be concluded that there are negative correlation meaning higher COX-2 expression is closely related to lower regional lymph node metastasis. The results of this study are in line with research conducted by Negi *et al* and Mahmoud *et al* which stated that COX-2 overexpression was found and there was no significant correlation between COX-2 overexpression and the number of regional lymph node metastases at Duke's staging (11,12). This is in contrast by Setiawan *et al* and Shechan *et al* to previous research which stated that there was a correlation between COX-2 overexpression and regional lymph node metastasis (13,17).

Berbecka *et al* and Lee *et al* stated that the correlation between COX-2 expression and regional lymph node metastasis was not significant could be caused by the influence of other molecular pathways such as mutations in the KRAS, TP53, BCL2, VEGFR2 genes (13,17). Research by Tamari *et al* stated that there was a significant correlation between Tumor Infiltrating Lymphocytes (TILs) and the depth of tumor invasion which was studied using immunofluorescence examination with CD4 and CD8 antibodies in 84 cases of stage T1b colorectal cancer who underwent early resection surgery to remove the lymph node (18).

4. Conclusion

There are strong expression (overexpression) of COX-2 in colorectal cancer and there are no significant correlation between COX-2 expression with histopathological grading, depth of tumor invasion and regional lymph node metastasis.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest in constructing this manuscript.

Statement of Ethical Approval

Ethical approval was obtained from Health Research Ethics Commision General Hospital Dr. Saiful Anwar Malang number 400/262/K.3/302/2023

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