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The Relationship of Cyclooxygenase -2 (COX-2) Expression with Clinical Presentation, Staging, and Degree of Differentiation in Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is a common malignancy worldwide. Although the recent development of adjuvant treatment has developed rapidly, it has only slightly Increased the survival rate of CRC Patients in an advanced stage. The prognosis of CRC patient strongly influenced by Several factors, such as tumor stage, clinical manifestations, histopathology and molecular oncogenicity of the tumor. COX-2 is an enzyme that plays a role in converting arachidonic acid into prostaglandins. The end product of COX-2 contributes to various biological factors in triggering tumor growth. The purpose of this research is to know the relationship of COX-2 expression with the clinical presentation such as patient age, location, and size of the tumor and histopathology in CRC patients. Fifty-eight CRC Patients included in the study; the research ranges from December 2016 to February 2017.

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The assessment of the COX-2 protein using the Immunohistochemistry methods, Dako polyclonal antibody, a semi-quantitatively. We Consider positive COX-2 when the score 2-3, negative if the score is 0-1. The subjects of this study consisted of 55.2% men and 44.8% women with a mean age of 57 years. There was no significant difference between COX-2 expression and age group <65 vs.> 65 years, and tumor location with p = 0.437 and p = 0.719. There was a significant relationship between COX-2 expression and tumor size (p <0.01) in, wherein the percentage of positive COX-2 expression on tumor size> 3 cm is higher than the negative expression (100% vs. 75%, p = 0.007). There was a significant relationship between COX-2 expression and degree of differentiation, in the which, the percentage of positive COX-2 expression was Significantly higher in moderate differentiation and well differentiation (78.9% and 10.9%) Compared with negative expression (50.0% and 8, 3%), with p = 0.042. The relationship between CRC staging and histopathology type with COX-2 expression was not found statistically significant with p = 0.588 and p = 0.100.

This study reported a significant relationship between COX-2 expression and patient's tumor size and the degree of differentiation of CRC Patients, but not with age, tumor location and TNM stage.

Keywords: Colorectal Cancer; COX-2; Immunohistochemistry; Expression; Differentiation; Stage.

1. Introduction

Mortality due to Colorectal cancer (CRC) estimated 655,000 per year worldwide and is the third leading cause of death from cancer in Western countries [1]. In Indonesia, there are no exact figures by CRC incident because of they no population-based data (population-based registry). In Makassar, based on hospital-based data in Sub-section Digestive Surgery / Department of Surgery Hasanuddin University Makassar, every year increase in CRC cases [2]. Identification of risk factors CRC is essential to the success of screening and surveillance program. Several environmental factors considered as a risk factor for CRC include lifestyle, a diet high in fat and low in fiber, alcohol, smoking, obesity and physical activity as well as infection. Some of the genetic factors that considered as the risk factors include a history CRC had suffered from benign tumors or other cancers in addition to CRC or CRC have a family history, or other cancers experienced non-*CRC* (Familial Cancer Syndrome) [3]. CRC patient prognosis influenced by several factors, such as tumor stage, clinical, histopathological and molecular oncogenic of the tumor [3]. Stadium pathology is an important prognostic indicator to date. System TNM (Tumor-nodule-metastasis) and AJCC VII in 2010 (American Joint Committee on Cancer) is now a system of staging the most widely used on the TRC and used as a standard to predict five-year survival, as it covers the stage is clinically and pathologically [4]. Age is one of the risk factors that play a role in CRC. The risk of CRC begins at age 40 and rose sharply after the age of 50-55 years [5]. Someone who has a family history of suffering from CRC then that person would be an increased risk of 2-4 times to get the TRC [6]. COX-2 is an enzyme that plays a role in converting arachidonic acid to prostaglandins. The end product of COX-2 is which contribute to a variety of biological factors in triggering the growth of tumors. Literature is explaining the relationship of COX-2 and tumor cell growth. The role of the differentiation of cell types (epithelial and interstitial) with the expression of COX-2, the factors which induce COX-2 such as, oncogenes, anti-oncogene inactive, cytokines, factor growth, fatty acids, bile gram, and mucin. Under these conditions, the use of selective COX-2 inhibitors has been reported to prevent and treat adenomas and CRC [7].

The purpose of this study was to determine the relationship of COX-2 expression with a clinical picture of the patient such as age, location, and size of the tumor and histopathology in patients with CRC.

2. Materials and Method

2.1. Collection of Samples

This is an observational study with longitudinal research model. The study subjects were patients based on clinical and endoscopic CRC were treated in Wahidin Sudirohusodo Hospital Makassar. The study conducted in December 2016 to February 2017. Exclusion criteria were patients with CRC receiving chemotherapy/radiotherapy, and immunohistochemical examination results are not perfect. Sampling for histopathology taken from cancer tissue of patients then conducted histopathology and IHC COX-2 by Pathology Department of Wahidin Sudirohusodo Hospital Makassar.

2.2. Expression COX-2 by Immunohistochemistry

Assessment of COX-2 protein by IHC using polyclonal antibody Dako. Observed with a light microscope binocular brands Olympus Type CX 21, ranging from enlargement weak (40x) then enlargement was (400x), and expansion of the high (1000x), Calculations performed on whole cell tumor starts from the tumor with the highest expression of COX-2 to the weaker sections. Interpretation of the expression of COX-2 by researchers and a specialize in Pathology. Cells that express COX-2 will appear brown in the cytoplasm. Rating expression of COX-2 based on the analysis of the percentage of positive tumor cells and staining intensity. The expression of COX-2 was given a score of 0 (<6%), +1 (6-25%), +2 (26-50%) and +3 (51-75%), and +4 (76-100%) of whole tumor cells. The intensity of the painting given a score of 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). To summarize the results of IHC staining score Cox-2 is an amount greater than or equal to ≥2 (2-3) considered positive, while a score of 0-1 is considered negative.

2.3. Histopathology and Grade Classification

Histopathology type is determined based on the results of CRC preparations with hematoxylin-eosin staining (HE) were classified into adenocarcinoma, mucinous adenocarcinoma, signet ring cell carcinoma, squamous cell adenocarcinoma, undifferentiated carcinoma, carcinoma Unclassified. Determined also the degree of differentiation by Grade I:

The tumor is well differentiated, contain components glandular> 95%. Grade II tumors moderate differentiated, comprising components glandular 50-95%. Grade III poorly differentiated tumors include components secretory 5-50%. Grade IV: the tumor is undifferentiated, the glandular component content of <5%. CRC Stadium based on AJCC TNM system according to VII in 2010, where the grouping relies on the size of the tumor (T), the location and number of lymph nodes that look (N) and tumor metastasis (M).

2.4. Data Analysis

Data analysis using the SPSS (Statistical Package for Social Science) version 22. Chi Square test and Spearman's correlation test with significance limit of $p < 0.05$ utilized for this study.

2.5. Ethical Clearance

Ethical approval for this study obtained from Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Number; 671/H4.8.4.5.31/PP36-KOMETIK/2016.

3. Results

During the study from November 2016 - February 2017 CRC in patients treated at the hospital Wahidin Sudirohusodo digestive surgery and networks gained 58 research subjects who met the inclusion criteria. In Table 1 shows the distribution of subjects and clinical characteristics of the tumor.

Table 1: Characteristics of Samples (n = 58)

Variables	Category	n	%
	Male	32	55.2
	Female	26	44.8
Age	<65 years	45	77.6
	> = 65 years	13	22.4
tumor location	right colon	6	10.3
	left colon	15	25.9
	Rectum	37	63.8
tumor size	<= 3 cm	3	5.2
	> 3 cm	55	94.8
Stadium	I-II	25	43.1
	III-IV	33	56.9

From the distribution of the data of patients, this study subjects consisted of 55.2% men and 44.8% of women aged 27 years to 86 years with a mean age of 57 years. Comparative study subjects aged <65 years were 45 (77.6%) and> = 65 years (22.4%).

CRC locations most frequently encountered are the rectum 37 patients, the left colon followed by as many as 15 patients and six patients is the right colon.

According to tumor size <= 3 cm by 3 patients (5.2%) and> 3 cm by 55 patients (94.8%). Based on the TNM staging of 58 patients, found patients with stage III were 25 patients (43.1%) and stage III-IV total of 33 patients (56.9%).

Table 2: Distribution of Histopathologic and Expression of COX-2 (n = 58)

Variables	Category	n	%
Histopathology Type	Adenocarcinoma	46	79.3
	Mucinous	8	13.8
	Signet Ring Cell	1	1.7
	other ***	3	5.2
Degrees of Differentiation	Good	6	10.3
	Moderate	42	72.4
	Bad	10	17.2
The expression of COX-2	Negative	12	20.7
	Positive	46	79.3

***Clear cell carcinoma*, Large cell lymphoma, non-Hodgkin's malignant Lymphoma

In the tables two show the results of histopathology with HE staining, gained as much as 79.3% are adenocarcinomas, followed by 13.8% mucinous adenocarcinoma, signet ring Cell 1.7% and other types (Unclassified) as much as 17.2%. Of the 58 patients with CRC, obtained the degree of differentiation histopathologic differentiation well as 6 (10.3%) patients, moderate differentiation were 42 (72.4%) and 10 (17.2%) patients with moderately differentiated.

The expression of COX-2 positive patients as many as 46 subjects (79.3%) and negative expressions as much as 12 (20.7%).

Based on patient age relationship with the expression of COX-2, there were no significant differences according to the age distribution of COX-2 expression (p = 0.437), although seen the percentage of positive expression of COX-2 at age <65 years is higher than negative expression, i.e., 80.4 % to 66.7%. While at age> = 65 years, visible expression of COX-2 positive was lower (19.6%) (Table 3).

Table 3: Age Relationship with Expression of COX-2

	The expression of COX-2		
Age	Negative	Positive	Total
<65 years	8 (66.7%)	37 (80,4%)	45 (77.6%)
> = 65 years	4 (33.3%)	9 (19.6%)	13 (22.4%)
Total	12 (100%)	46 (100%)	58(100%)

p = 0.437

The percentage of COX-2 expression was higher in rectal (65.2%) and the left colon (26.1%), compared with COX-2 expression in the right colonic 8.7%. Compared to the negative expression, the result of the statistical test showed p> 0.05, which means that there is a significant correlation between the expression of COX-2 to the tumor site. (Table 4)

Table 4: Relationship Expression of COX-2 by the tumor site

	The expression of COX-2		
location of the tumor	Negative	Positive	Total
right colon	2 (16.7%)	4 (8.7%)	6 (10.3%)
left colon	3 (25.0%)	12 (26.1%)	15 (25.9%)
rectum	7 (58.3%)	30 (65.2%)	37 (63.8%)
Total	12 (100.0%)	46 (100.0%)	58 (100.0%)

p = 0.007

In Table 5 shows there is a significant correlation between the expression of COX-2 by tumor size (p <0.01), where the percentage of positive expression of COX-2 in tumor size> 3 cm higher than negative expression, i.e., 100% vs. 75%. While tumor size ≤3 cm not found any positive expression

Table 5: Relationship Expression of COX-2 by Tumor Size

	The expression of COX-2		
Tumor size	Negative	Positive	Total
≤3 cm	3 (25.0%)	0 (0.0%)	3 (5.2%)
> 3 cm	9 (75.0%)	46 (100.0%)	55 (94.8%)
Total	46 (100.0%)	58 (100.0%)	12 (100.0%)

Table 6 shows the stage according to the TNM classification and stage III and stage III-IV group. Based on statistical test, not found a significant association between the expression of COX-2 with stage (p> 0.05), but it appears that at stage III-IV percentage of positive expression is higher than the negative, i.e., 58.7% to 50.0%, While on stage I-II, the percentage of positive expression was lower than a negative, namely 41.3% to 50.0%.

Table 6: Relationship stadium with COX-2 expression

	The expression of COX-2		
Stadium	Negative	Positive	Total
I-II	6 (50.0%)	19 (41.3%)	25 (43.1%)
III-IV	6 (50.0%)	27 (58.7%)	33 (56.9%)
Total	12 (100.0%)	46 (100.0%)	58 (100.0%)

p = 0.588

On histopathologic type group, the expression of COX-2 positive adenocarcinoma of 38/46 (78.3) and negative 10/12 (83,%), the percentage of COX-2 expression was higher in mucinous types 8/46 (17.4%) and signet ring cell (2.2%) than negative expression, although the statistical test results show the value of p = 0.100, which means there is no significant correlation between the expression of COX-2 with histopathological types in table 7.

Table 7: Relation Expression of COX-2 with type Histopathology

	THE EXPRESSION OF COX-2		
HISTOPATHOLOGY	Negative	Positive	Total
ADENOCARCINOMA	10 (83.3%)	36 (78.3%)	46 (79.3%)
MUCINOUS	0 (0.0%)	8 (17.4%)	8 (13.8%)
SIGNET RING CELL	0 (0.0%)	1 (2.2%)	1 (1.7%)
MISC	2 (16.7%)	1 (2.2%)	3 (5.2%)
TOTAL	12 (100.0%)	46 (100.0%)	58 (100.0%)

p = 0.100

There is a significant correlation between the expression of COX-2 with the degree of differentiation ($p < 0.05$), where the percentage of positive expression of COX-2 was significantly higher in moderately differentiated as many as 36 patients (78.3%) and a real differentiation that 5 patients (10, 9%) compared with negative expression 6 and 1 patient (50.0% and 8.3%). (table 8)

Table 8: Relationship with the Expression of COX-2 Degrees of Differentiation

	The expression of COX-2		
Levels of Differentiation	Negative	Positive	Total
Good	1 (8.3%)	5 (10.9%)	6 (10.3%)
Moderate	6 (50.0%)	36 (78.3%)	42 (72.4%)
Poor	5 (41.7%)	5 (10.9%)	10 (17.2%)
Total	12 (100.0%)	46 (100.0%)	58 (100.0%)

p = 0.042

4. Discussion

The results of this study reported some CRC in men more than women are 32 (55.2%) vs. 26 (44.8%). These results are no different to those published in national seminar I Makassar Colorectal Cancer in 2011, where data are collected from Medan - Aceh, Padang, Palembang, Samarinda, Banjarmasin, Makassar, Jakarta, Surabaya, Bandung, Semarang, Yogyakarta, Solo, Manado and Bali , which reported the percentage of male: female, 54.57% and 43.45% [8].

Several studies by Yamuchi and his colleagues 2002, Joo and his colleagues 2002, Xiong and his colleagues in 2005 reported that the expression of COX-2 reaches 80% of colorectal cancers and adenomas reach 40%. Another study by Miladi-Abdelkader and his colleagues in 2012, conducted a study on 35 patients CRC expression of COX-2 68.6% [9]. In this research report different results with other studies where we get the expression of COX-2 by 46 patients (79.3%) and a negative result by 12 (20.7%).

Masunaga and his colleagues 2000 reported a study of 100 patients with CRC earned their positive expression by 53% and 46% in patients less than 65 years and> = 65 years, but not statistically significant [10]. Results did not differ reported by Tomozawa and his colleagues (11), In 2000 said that their robust and weak expression of COX-2 in 63 patients with colorectal cancer by age group over 60 years and <= 60 years (p = 0.94). Different reports by Milada-abdennadher and his colleagues in 2012, i.e., no significant correlation between the expression of COX-2 in patients age ≤ 60 years and> 60 years [9]. In our research obtained comparative study subjects aged <65 years were 45 (77.6%) and> = 65 years (22.4%). The results of this research report are no different with the study by Masunaga and his colleagues [10], no significant differences according to the age distribution of COX-2 expression (p = 0.437). Up to now, there is no theory that explains the relationship of COX-2 expression with patient age CRC. Several clinicopathologic studies with COX-2 gets different results.

Comparison of the expression of COX-2 in the right colon left colon and rectum patient CRC widely considered. These comparisons have therapeutic potential in patients with COX-2 CRC active and COX-2 negative. Location TRC can be used as a stratification provision of anti-COX-2. Nasir and his colleagues reported the existence of differences in the expression of COX-2 by 46% the right colon and left colon 70%; they conclude that the discrepancies in clinical expression may be used as a combination therapy with a COX-2 inhibitor. Another study reported that COX-2 mRNA expression in distal adenomas was higher than proximal colorectal [12].

Several studies and theories to explain if there is an increased expression of COX-2 and prostaglandin excessive production of intestinal epithelial cells, the cells are protected from apoptosis and cell stimulating angiogenesis factor production. Peroxisome Proliferator-Activated Receptor (PPARγ) is a transcription factor that has a regulatory function of cell differentiation and found to be highly elevated in colon cancer. Differences in fatty acids and prostaglandins will bind to PPARγ and stimulate transcription of target genes. When excessive PPARγ expression in colon cancer cells, apoptosis does not occur even if given NSAIDs. In colon cancer PPARγ Overexpression very visible. Under normal conditions, PPARγ suppressed by the APC gene. When the APC gene mutation occurs in the case of the TRC, indeed PPARγ levels will increase. One important factor that associated with colon cancer is β catenin proteins form a complex with APC. Mutation of the APC gene and or β catenin, the separation of APC-β catenin complex and β catenin bind to T-Cell Factor / Lymphoid E Factor-1 (TCF / LEF-1) controls the transcription factor gene targets, for example, c-Myc, contribute as a cause of colon tumors. The possibilities can be considered that the β catenin that activates TCF / LEF-1 mutated will regulate the expression of COX-2. This theory which explains the low expression of COX-2 colon cancer than rectal cancer [13].

Dimberg and his colleagues reported that overexpression of COX-2 protein rectal cancer by 90% compared to 20.06% in colon cancer ($p < 0.001$).(13)Different results were reported by Masunaga and his colleagues where the report found no significant association of expression of COX-2 with location CRC [10].

Our research to get results, the percentage of COX-2 expression was higher in the left colon and rectum than the right colon. Although the expression of COX-2 negative small, the test results showed no significant statistically ($p > 0.05$), which means there is no significant correlation between the expression of COX-2 to the tumor site.

The relationship between COX-2 and the CRC is through the role of prostaglandin E2 (PGE2) derived from arachidonic acid metabolism mediated COX-2 increases the proliferation of colorectal cancer cells. There inhibitory effect of NSAIDs against CRC cell proliferation. Miladi-Abdennadher, and his colleagues reported a significant association between overexpression of COX 2 with tumor size greater than 5 cm and depth of tumor invasion [9]. Other studies comparing the expression of COX-2 by tumor size ≤ 3 and> 3, and the presence of lymph node metastasis, the degree of differentiation and stage colorectal cancer patients get statistically significant results [10].

Our study reported a significant correlation between the expression of COX-2 by tumor size ($p < 0.01$), where the percentage of positive expression of COX-2 in tumor size> 3 cm higher than negative expression, i.e., 100%

to 75%. While tumor size ≤ 3 cm did not reveal any positive expression.

Research in humans and experimental animals discovered COX-2 was higher in intestinal type adenocarcinoma than in precancerous lesions such as familial adenomatous polyposis. It is similar to some of the squamous cell carcinoma of the head and neck, the level of COX-2, PG, such as PG2α, PGE2 and its metabolic found to be higher than normal tissue [14]. Peng and his colleagues conducted a meta-analysis of 23 studies that include 4567 patients CRC matches COX-2 expression associated with tumor infiltration depth, differentiation, Duke's stage and distant metastases [15]. Al-Maghrabi, and his colleagues reported a COX-2 expression related to tumor stage, lymph node invasion and distant metastasis [16].

This study obtained different results based on the stage according to the TNM. Where there is no significant correlation between the expression of COX-2 in the group stage III and stage III-IV group, but it appears that at stage III-IV percentage of positive expression is higher than the negative, i.e., 58.7% to 50.0% , While on stage I-II, the percentage of positive expression was lower than a negative, i.e., 41.3% and 50.0%. These significant results may be due to small sample number and uneven distribution by stage. However, when analyzed with the degree of differentiation, there is a significant correlation between the expression of COX-2 with the level of differentiation ($p < 0.05$), where the percentage of positive expression of COX-2 was significantly higher in the differentiation were 36 patients (78.3%) and differentiation of both 5 patients (10.9%) than negative expressions 6 and 1 patient (50.0% and 8.3%).

Several studies by immunohistochemical examination showed almost all types of cells and stroma of colorectal adenocarcinoma could raise levels of COX-2, such as fibroblasts, myofibroblasts, mononuclear inflammatory cells, and endothelial cells [17]. Wu and his colleagues split histopathological types of papillary adenocarcinoma and carcinoid into differentiated, while the mucous-adenocarcinoma, signet ring cell carcinoma, undifferentiated carcinoma, neuroendocrine carcinoma in poorly differentiated [18]. Increased expression of COX-2 more on the type of adenocarcinoma than squamous carcinoma types and is associated with a shorter life expectancy in lung cancer patients with Non-Small Cell Lung Cancer [19]. Sheehan and his colleagues assess the expression of COX-2 of 123 adenomas and malignant transformation obtained relationship and increased levels of COX-2 with histologic type [20]. Nogueira and his colleagues evaluating the degree of expression of COX-2 in patients with non-neoplasia as controls and patients with tubular, tubulovillous, villous adenomas, found no statistically significant relationship [21]. The results of the evaluation group histopathology type of research we obtained the expression of COX-2 positive adenocarcinoma of 38/46 (78.3) and negative 10/12 (83,%), the percentage of COX-2 expression was higher in mucinous types 8/46 (17, 4%) and signet ring cell (2.2%) than negative expression, although the test results showed no significant statistically ($p = 0.100$).

5. Conclusion

In conclusion, this study reported a significant association between the expression of COX-2 by tumor size of patients CRC and CRC patient's degree of differentiation but did not obtain the relationship between COX-2 by age, the location of the tumor and TNM staging.

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6. Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare

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