Correlation Intramural Vascular Invasion and Extramural Vascular Invasion with Histopathological Grading of Adenocarcinoma Colorectal

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Background: Carcinoma colorectal is a malignancy originating from the colorectal epithelium which ranks the third-largest in the world after lung and breast cancer, leading to the highest mortality in the world with new cases of about 1.8 million (10.2%). Ninety percent of colorectal malignancies are a type of adenocarcinomas. Vascular invasion of the adenocarcinoma of the colorectal could be one of the most damaging prognostic indicators and can be helpful for the selection of adjuvant chemotherapy.

Material and methods: This study is a sectional cross-sectional study conducted on 35 patients with adenocarcinoma colorectal taken from their formalin-fixed paraffin-embedded tissue blocks in period 2018-2019, which is colorectal by hematoxylin and eosin to assess intramural vascular invasion and extramural with histopathology grade of adenocarcinomas. Sample characteristic data from a medical file was retrieved. Mann-Whitney test u (p< 0.005) is used to assess the correlation of intramural vascular invasion and extramural vascular invasion with histopathological grade.

Results: Among 35 samples of those with adenocarcinomas of colorectal, the most cases were low grade by 30 cases (85.7%) and vascular invasions have been found in common, both intramural and intramural and extramural as many as 10 cases (26.6%). Mann-Whitney U correlation test and found a significant correlation between intramural and extramural vascular invasion with histopathological grade (p = 0.001)

Conclusion: There is a significant correlation of statistical analysis between intramural and extramural vascular invasion and histopathology grade of adenocarcinomas colorectal.

Keywords: adenocarcinomas colorectal, intramural vascular invasion, extramural vascular invasion, grade.

INTRODUCTION

Colorectal carcinoma is a malignancy originating from the colorectal epithelium which ranks third in the world after lung and breast cancer, it is the most common cause of death in the world with a new case of about 1.8 million (10.2%).[1] In 2017 in the United States, there were some 95,520 new cases of colonic cancer and 39,910 new cases of rectal cancer. This colorectal carcinoma is found at the age of 65-79 years and is mostly found in men, which is about 26,950 cases.[2,3] Sightly different from the United States, colorectal carcinoma cases in Asia rank the second most of all malignancies in men and women, while the death from the colorectal carcinomas came in sixth of all those caused by malignancy. Where rectal carcinomas occupied the seventh and colon carcinomas occupied the eighth-most.[1]

The survey of the incidence of colorectal carcinoma in Indonesia has been found at 30,017 (8.6%) new cases and topped fourth in number after breast, cervical, and lung cancer. Incidence according to gender, colorectal carcinomas ranked second in men after lung cancer, which is 19,113 (11.9%) new cases, and in women ranked fourth after breast cancer, cervix, and ovarium of 10,904 (5.8%).[1] Person reporting the incidence of colorectal carcinoma obtained from the medical records of H. Adam Malik Hospital, Medan, increased in recent years in 2015 (75 cases), 2016 (83 cases), and 2017 (98 cases).[4]

Ninety percent of the malignancy in colorectal is a type of adenocarcinoma. The incidence of colorectal carcinomas began to increase significantly after age 40 years. Colorectal carcinomas aged under 40 years are usually associated with genetic factors that increase the risk of colorectal carcinomas 3 times greater than non-familial ones.[5] Other risk factors that influence the occurrence of colorectal carcinoma are a diet high in calories and animal fat (Western type) and low physical activity. Several studies of epidemiology suggest that aspirin and some non-steroid-anti-inflammatory-drugs (*NSAID*) have protective effects.[6.7]

The pathogenesis of colorectal adenocarcinoma is generally preceded by a history of precursor lesions such as adenoma and dysplasia.[6,7] Most colorectal carcinomas develop through the conventional pathogenesis of classic adenoma-carcinoma, but they can also evolve through hyper mutant pathways or ultramutane pathways. This can lead to a change from normal mucosal epithelial stem cells developing adenoma to carcinoma, with the gradual accumulation of genetic and epigenetic abnormalities in a very diverse pattern of tumor cell development. There are 3 mechanisms of molecular change in the form of genetic instability that underlies colorectal carcinoma, namely: (1) chromosomal instability which results in increased somatic copy number alterations (SCNA) DNA; (2) Microsatellite instability (MSI) which is hypermutant which functions to repair damaged DNA mismatches and (3) Damage to the proofreading ultramutane polymerase. The most common and typical pathways for the genetic alteration of conventional colorectal adenocarcinoma are (84%) APC, KRAS, TP53, SMAD4, or PIK3CA while for MSI are MLH1 and MSH2 (13%) and (3%) ultramutane pathway namely POLE.[8]

Based on the structure of the gland, the histological grading of colorectal adenocarcinoma can be divided into 2 criteria, namely: low grade (well to moderately differentiated) and high grade (poorly differentiated). Vascular invasion can be sub-classified according to location, namely: on the intestinal wall (intramural vascular invasion / IMVI) and outside the intestinal wall (extramural vascular invasion / EMVI). IMVI is reported to have an incidence of 4-40% (12.5% of overall reported incidence) and is associated with a poor prognosis. The incidence of EMVI is higher than IMVI, however, this is still not widely found in the literature8. EMVI has also been reported as an important prognostic indicator of adverse events. EMVI detection can help people with colorectal carcinoma to increase their preference for adjuvant chemotherapy. EMVI is one of the important parameters that must be reported in the minimum data set for reporting the histopathology of colorectal carcinoma as described by the Royal College of Pathologists. [9-11] Detection of EMVI varies greatly from pathologist to other pathologists.[11]

Based on the description above, the researchers wanted to know the role of intramural and extramural vascular invasion with histopathological grading in colorectal adenocarcinoma.

MATERIALS AND METHODS

This study was conducted in an analytic study with a cross-sectional approach looking for a correlation between intramural and extramural vascular invasion with the histopathological grade of colorectal adenocarcinoma. We studied 35 cases taken from 2018-2019 originating from colorectal surgery tissue diagnosed as colorectal adenocarcinoma at the Anatomical Pathology Department, Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of Adam Malik Hospital, Medan. Samples were scored from paraffin blocks that met the inclusion criteria with consecutive sampling technique and obtained clinical data, namely age, gender, depth of tumor invasion (based on the "T" criteria of AJCC 2017), tumor location, followed by histopathological examination of colorectal adenocarcinoma which was evaluated by three Researchers through microscopic examination of the Haematoxylin and Eosin stained slides, assessed grading according to WHO 2019, intramural and extramural vascular invasion, lymphatic invasion and perineural invasion.

In this study, the tools needed include a microtome, water bath, hot plate, coated object-glass, antigen retrieval (PT Link Dako), staining jar, glass shelf, moist chamber, Pap pen, micropipette, white, yellow, blue tip, aliquots, chemical scales, filter paper, stopwatches, beaker glasses, cover glasses, porcelain and light microscopes (Olympus CX23). The materials in this study included sample paraffin blocks, Mayer Hematoxylin Counterstain solution, 1% Eosin Solution, Xylol, 1% Acid Alcohol, Absolute Alcohol, 96%, 80%, and 50% and Ex-Mount xylene base.

Data processing was carried out with the help of SPSS statistical software. All data processing results will be presented in tabular form. Data analysis was performed using the Mann-Whitney U test. The p-value <0.05 was statistically significant.

RESULTS

Based on clinical data from medical records/pathology files, it was found that the most colorectal adenocarcinoma patients were aged ≥40 - <60 years, namely 16 cases (45.8%). There were more males, namely 24 cases (68.6%) than 11 cases of females (31.4%). The highest depth of tumor invasion was T3, namely 13 cases (37.1%), then followed by T2 as many as 10 cases (28.6%), T1 as many as 8 cases (22.4%), and T4 as many as 4 cases (11.4%). The location of the tumor was more frequent in the rectum, namely 23 cases (65.7%) followed by a transverse and descending colon, respectively 5 cases (14.3%), and ascending colon in 2 cases (5.7%). The histopathological grading in this study was mostly found in low grade, which was 30 cases (85.7%) compared to a high grade in 5 cases (14.3%). Vascular invasion in this study resulted in 10 cases of intramural vascular invasion (28.6%), intramural and extramural invasion in 10 cases (28.6%) and no intramural or extramural vascular invasion was found in 15 cases (42.8%). The lymphatic invasion was assessed based on positive and negative, where there were more negative ones, namely 24 cases (68.6%) while positive ones were 11 cases (31.4%). Perineural invasion in this study was found to be more negative with invasion, namely 25 cases (71.5%) than positive ones, namely 10 cases (28.5%) (Table 1).

Table 1. Distribution of sample characteristic adenocarcinomas colorectal.

	Amount (n)	Percentase (%)
Number of samples	35	100
Age:		
- <20 years	0	0
- ≥20 - < 40 years	6	17.1
- ≥40 - <60 years	16	45.8
- ≥60 years	13	37.1
Sex:		
- Male	24	68.6
- Female	11	31.4
Invasion Depth:		
- T1	8	22.4
- T2	10	28.6
- T3	13	37.1
- T4	4	11.4
Location:		
- Colon Asenden	2	5.7
- Colon Tranversum	5	14.3
- Colon Desenden	5	14.3
- Rectum	23	65.7
Grade:		
- Low grade	30	85.7
- High grade	5	14.3
Vascular Invasion:		
- Negatif	15	42.8
- Intramural	10	28.6
- Intramural and Extramural	10	28.6
Lymphatic Invasion:		
- Negative	24	68.6
- Positive	11	31.4
Perineural Invasion		
- Negative	25	71.5
- Positive	10	28.5

The trial results found that there is a significant correlation between intramural and extramural vascular invasion with histopathological grade assessment where the value of p = 0.0016 (p <0.05). Intramural and extramural vascular invasion trials with histopathological grade assessments are presented in the following table (table 2).

Table 2. Correlation test of intramural and extramural vascular invasion with histopathological grading.

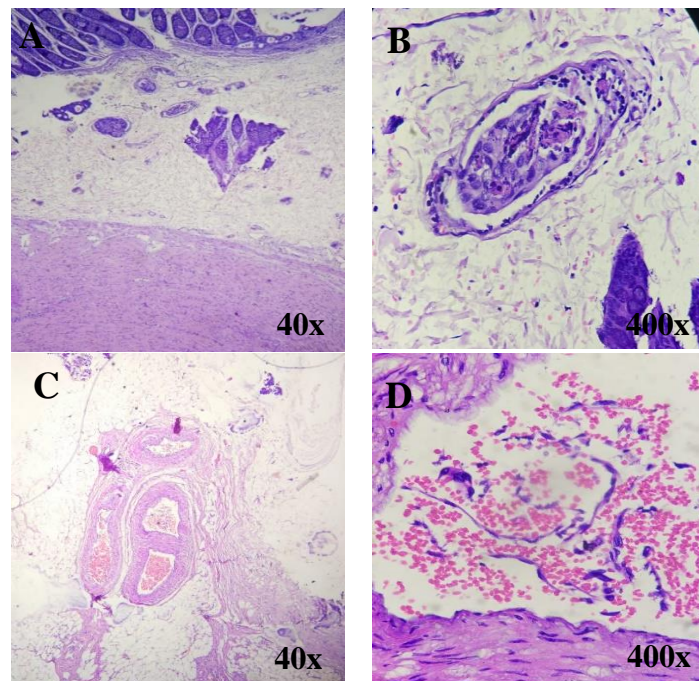| Vascular Invasion	Grade				p-value*
	Low grade		High grade		
	n	%	n	%	
Negative	15	50,0	0	0	
IMVI	10	33,3	0	0	0,0016
IMVI & EMVI	5	16,7	5	100	

Test *Mann-Whitney U*

Figure 1. Microscopic Sample **A&B**. Intramural vascular invasion (IMVI). **C&D.** Extramural vascular invasion (EMVI).

DISCUSSION

One of the assessments to determine the prognosis in colorectal adenocarcinoma is to assess intramural vascular invasion (IMVI) and extramural vascular invasion (EMVI). Patients with vascular invasion are more likely to have a large bowel obstruction.[12] Vascular invasion has been associated with lymph metastases and distant metastases. Betge et al, suggested that vascular (venous) invasion proved to be a strong predictor and was associated with outcome. Patients with vascular invasion were twice as likely to experience carcinoma progression or die as people without vascular invasion.

The assessment of colorectal adenocarcinoma in this study was determined based on the degree of differentiation according to WHO 2019, namely low grade and high grade. In the multivariate analysis conducted by Marzuok and Schofield, it was found that the assessment was

related to a person's prognosis and survival. High-grade tumors (poor differentiation) are associated with distant lymph metastases and metastases and the prognosis is worse.[13]

In this study, it appears that the presence of intramural and extramural vascular invasion is followed by an increase in histopathological grading in colorectal adenocarcinoma. After the statistical correlation test was carried out with the Mann-Whitney U correlation test, there was a significant correlation between intramural vascular invasion alone and both vascular invasion (intramural and extramural) with grading ($p < 0.001$) (table 2). In line with the study conducted by Betge et al, which stated that there was a significant correlation between vascular invasion and histopathological grade ($p < 0.001$).[13]

Extramural vascular invasion was shown to be a strong predictor and was associated with survival, whereas patients with intramural vascular invasion alone had comparable outcomes with no invasion. Patients with positive EMVI will have an increased recurrence rate and even death from colorectal carcinoma compared to patients without invasion. The adverse prognostic impact of patients with EMVI on colorectal carcinoma is comparable to the presence of lymph node metastases, high grade, and perineural invasion.[12,13] Patients with stage II colorectal carcinoma in the presence of EMVI receive adjuvant treatment twice as often as IMVI patients or without invasion. Patients with stage II colorectal carcinoma in the presence of EMVI have worse survival than patients with stage III without EMVI, regardless of adjuvant therapy.[12]

There is a need for more aggressive follow-up that benefits people with EMVI-positive colorectal carcinoma. Investigations MRI has been performed to detect preoperative rectal carcinoma, however, MRI is not useful for colon carcinoma because it cannot explain the location and motion of colonic peristalsis. Computed tomography can be used as an alternative examination but is less sensitive in identifying vascular invasion with certainty. The hypothesis of Leijssen et al. vascular invasion can be detected by histopathological examination and is very important for predicting recurrence and death from colorectal carcinomas. [12]

CONCLUSION

Colorectal adenocarcinomas in this study was more common at age ≥ 40 - < 60 years and was more common in men than in women. The depth of tumor invasion was more common in T3 and the location was more common in the rectum. Grading colorectal adenocarcinomas was more common at low grade than high grade and intramural vascular invasion as well as intramural and extramural vascular invasion was found equally. Lymphatic invasion and perineural invasion in this study were more negative than positive. There was a significant correlation between intramural and extramural vascular invasion with the histopathological grade of coloretal adenocarcinoma.

COMPETING INTERESTS

The author has no financial interests that are relevant to the products or company described in this article.

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ETHICAL APROVAL

The Health Research Ethical Committee, University of North Sumatra , Medan, Indonesia approved this study.

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