

Histomorphology of Colorectal Adenocarcinoma at Anatomic Pathology Unit of the H. Adam Malik Hospital Medan in 2019-2020

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Background: Colorectal carcinoma is a malignant epithelial tumor originating from the large intestine (colon and rectum) which shows differentiation of glands and mucin, accompanied by invasion between the stroma of the mucous muscularis layer to the submucosa layer. World Health Organisation (WHO) recommends reporting several histopathological features in colorectal carcinoma related to the prognosis of the patient.

Methods: This study is a descriptive study with a cross sectional approach. There were 60 samples diagnosed histopathologically as adenocarcinoma colorectal from paraffin block at RSUP H. Adam Malik Medan in 2019-2020. Data regarding age, sex, tumor location, and clinical stage were obtained from medical records. Slaid reviews were performed, histopathologic subtypes, grade, Lymphatic invasion, Intra Mural Vascular Invasion (IMVI), Extra Mural Vascular Invasion (EMVI), Perineural Invasion (PNI), tumor budding, TILs, and depth of invasion (T) were determined.

Results: The mean age of the patients was 51.9 years with a minimum age of 22 years and a maximum of 83 years, the most common age group was 50-59 years, men were found more than women, and the location of the tumor was most found in the rectum. The most histopathologic subtypes were adenocarcinoma NOS, with common grade was low grade (73.3%). Lymphatic invasion was found in 22 cases (36.7%), most vascular invasion found was EMVI (35%), PNI was found in 16 cases (26.7%), most tumor budding were low budding, most frequent grade of TILs was moderate, and most clinical stage is stage II.

Keyword: histomorphology, adenocarcinoma, colorectal

INTRODUCTION

Colorectal carcinoma is a malignant epithelial tumor originating from the large intestine (colon and rectum) which shows gland and mucin differentiation, accompanied by invasion between the stroma of the mucous muscularis layer to the submucosa layer.¹ This cancer is the third most common malignancy in the world, and the second most common cause of death in the world.² The incidence of colorectal carcinoma in Indonesia is reported at 12.8 per 100.000 adult population, and accounts for 9.5% of all cancer mortality.³ Data from GLOBOCAN shows that there are 30.017 (8.6%) new cases of colorectal carcinoma. in Indonesia at 2018. Based on gender, colorectal carcinoma in men ranks second most frequently after lung cancer with 19.113(11.9%) new cases in 2018, and the fourth most common malignancy after breast, cervical and ovarian carcinoma with 10,904 (5.8%) new cases in women.² Colon and rectal carcinoma often grouped together because they share many features.^{1,4} The risk of developing this malignancy tends to be higher in men than women for reasons not fully known. It is suspected related with exposure to risk factor such as smoking and hormones.¹ In addition, the risk of developing this disease also increases with age.^{1,5}

World Health Organisation (WHO) recommends reporting additional histological features that need to be assessed in the reporting of colorectal carcinoma patients, including lymphatic invasion, Intra Mural Vascular Invasion (IMVI), Extra Mural Vascular Invasion (EMVI), Perineural Invasion (PNI), tumor budding, as well as a description of the immune respons.¹ These histopathological assessments should be reported routinely in every case of colorectal adenocarcinoma, because this is related to the prognosis that will affect the outcome of the patients.

MATERIALS AND METHODS

In this study, 60 samples of adenocarcinoma colorectal who underwent surgery at H. Adam Malik General Hospital Medan in 2019-2020 were diagnosed histopathologically with Hematoxylin Eosin (H&E) staining. Data regarding age, sex, tumor location, and clinical stage were obtained from medical records. While histopathologic subtypes, grade, Lymphatic invasion, Intra Mural Vascular Invasion (IMVI), Extra Mural Vascular Invasion (EMVI), Perineural Invasion (PNI), tumor budding, TILs, and depth of invasion (T) were determined from slide review.

RESULTS

From this study obtained the mean age of the patient was 51,9 years with a minimum age of 22 years and a maximum of 83 years with the most common age group was 50-59 years. Male was more common than female (56.7% vs 43,3%), and the most colorectal adenocarcinoma locations were found in the rectum for 19 cases (31.7%), followed in the colon for 18 cases (30%), then 15 cases in the left colon (25%), and the least number was found in the right colon by 8 cases (13.3%).

Table 1. Distribution of colorectal adenocarcinoma samples based on clinical data for age,sex, and tumor location.

Clinical data	n	Percentage (%)
Age		
< 30 years	2	3,3
30-39 years	10	16,7
40-49 years	12	20,0
50-59 years	22	36,7
60-69 years	10	16,7
70-79 years	3	5
≥ 80 years	1	1,7
Sex		
Male	34	56,7
Female	26	43,3
Tumor location		
Colon	18	30
Rectum	19	31,7
right kolon	8	13,3
left kolon	15	25

The microscopic examination results showed that the majority of samples were a non-specific histopathological type of colorectal adenocarcinoma as many as 52 cases (86.7%), and 8 other cases had a specific histopathological type of colorectal carcinoma consisting of 6 cases of mucinous adenocarcinoma and 2 cases of serrated adenocarcinoma.

Table 2. Distribution of samples of colorectal adenocarcinoma patients based on histopathological subtypes.

Histopathological subtypes	n	Percentage (%)
Adenocarcinoma NOS	52	86,7
Mucinous adenocarcinoma	6	10
Serrated adenocarcinoma	2	3,3
Amount	60	100

Grading colorectal adenocarcinoma in this study was assessed based on the percentage of the glandular structure according to the World Health Organization (WHO) in 2019. There were 41 cases of low grade adenocarcinoma NOS, and 11 cases of high grade adenocarcinoma NOS. Meanwhile, high grade mucinous adenocarcinoma was found in 6 cases, and low grade and high grade serrated adenocarcinoma each in 1 case.

Table 3. Distribution of colorectal adenocarcinoma samples based on histological grading.

Histopathological subtypes	Grading			
	Low grade		High grade	
	n	%	n	%
Adenocarcinoma NOS	41	68,3	11	18,3
Mucinous adenocarcinoma	-	-	6	10
Serrated adenocarcinoma	1	1,7	1	1,7
Amount	42	70	18	30

Colorectal adenocarcinoma with positive lymphatic invasion was found in 21 cases (35%), IMVI as many as 13 cases (21.7%), EMVI as many as 26 cases (43.3%) and positive perineural invasion (PNI) 16 cases (26.7%).

Table 4. Distribution of lymphatic invasion, IMVI, EMVI and PNI in colorectal adenocarcinoma samples.

Histopathological subtypes	Invasion							
	Lymph		IMVI		EMVI		PNI	
	n	%	n	%	n	%	n	%
Adenocarcinoma NOS	17	28,4	12	20	22	36,6	13	21,7
Mucinous adenocarcinoma	2	3,3	-	-	3	5	2	3,3
Serrated adenocarcinoma	2	3,3	1	1,7	1	1,7	1	1,7
Amount	21	35	13	21,7	26	43,3	16	26,7

Assessment of tumor budding in this study obtained the most were low budding in 35 cases (58%), followed by intermediate budding in 14 cases (23%), and the lowest was high budding in 11 cases (19%). Meanwhile, the stromal distribution of TILs in this study was found to be moderate as many as 34 cases (57%).

Table 5. Distribution of budding tumors and stromal TILs in colorectal adenocarcinoma samples.

Histopathological subtypes	Tumor budding								Stromal TILs			
	Low		Intermediate		High		Minimal		Moderate		High	
	n	%	n	%	n	%	n	%	n	%	n	%
Adenocarcinoma NOS	31	51	13	21	8	13	3	5	29	48,3	20	33
Mucinous adenocarcinoma	3	5	1	2	2	4	-	-	4	7	2	3,3
Serrated adenocarcinoma	1	2	-	-	1	2	-	-	1	1,7	1	1,7
Amount	35	58	14	23	11	19	3	5	34	57	23	38

The distribution of colorectal adenocarcinoma samples based on the depth of tumor invasion (T) was mostly T3 as many as 43 cases (71.7%), followed by T2 in 11 cases (18.3%), then T4 as many as 6 cases (10%), and not found. sample with T1. Meanwhile, based on KGB involvement (N), there were 49 cases (81.7%) without KGB involvement, and as many as 11 cases (18.3%) with KGB involvement. Of the 60 samples of colorectal adenocarcinoma in this study, 51 cases (85%) were found without metastasis and as many as 9 cases (15%) with metastases. Based on the criteria for the TNM system, the most tumor stages were stage II in 37 cases (61.7%), followed by stage I with 10 cases (16.7%), then stage IV in 9 cases (15%), and the least found was stage III in 4 cases (6.7%).

Table 6. Distribution of colorectal adenocarcinoma samples by tumor TNM stage.

Histopathological subtypes	Depth of tumor invasion (T)				Lymph nodes involvement		Meta stasis (M)		Stage			
	T 1	T 2	T 3	T 4	N 0	N 1-2	M0	M 1	I	II	III	IV
Adenocarcinoma NOS	-	9	38	5	43	9	44	8	11	31	3	7
Mucinous adenocarcinoma	-	1	4	1	4	2	5	1	2	2	1	1
Serrated adenocarcinoma	-	1	1	-	2	-	2	-	1	1	-	-
Amount	-	11	43	6	49	11	51	9	14	34	4	8

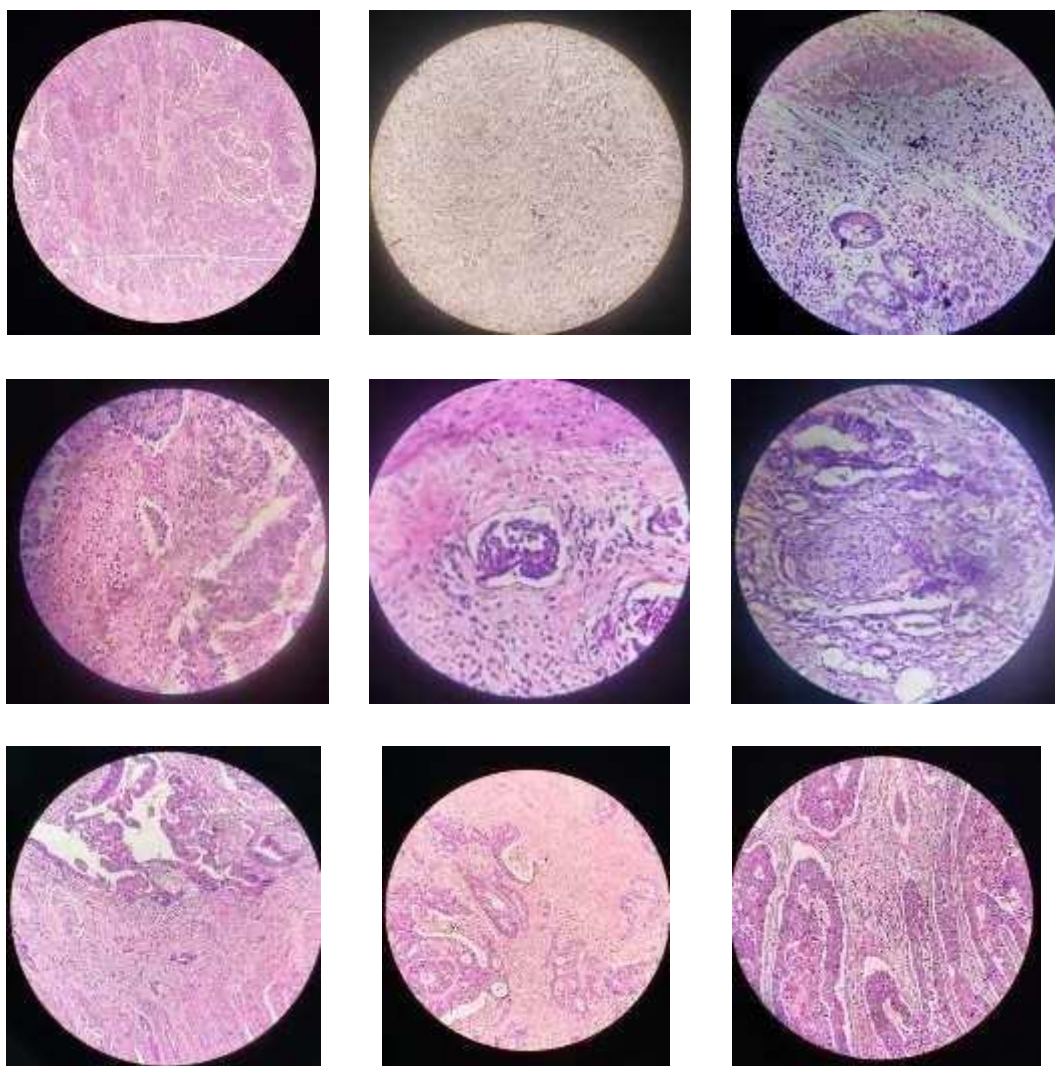


Figure 1. A. Low grade CRC (H&E 100x), B. High grade CRC (H&E 100x), C. Lymphatic invasion (H&E 400x), D. IMVI (H&E 400x), E. EMVI (H&E 400x), F. PNI (H&E 400x), G. Moderately tumor budding (H&E 200x), H. Moderate stromal TILs (H&E 200x), I. High stromal TILs (H&E 200x).

DISCUSSION

The mean age of patients with colorectal carcinoma in this study was 51.9 years, with the most common age group being 50-59 years. Accordance with the theory stated in the WHO book 2019 and various literature which states that the most people with colorectal carcinoma are found in the age group over 50 years, because this cancer is closely related to dietary patterns and lifestyles that accumulate with age. This is also supported by various previous studies which found that the incidence of colorectal carcinoma is more dominated by the older age group, although several recent studies have also shown an increase incidence of patients young adults (under 40 years).⁶ People under 40 years old with colorectal adenocarcinoma generally have genetic disorders such as a history of HNPCC, FAP, chronic intestinal infection, and ulcerative colitis. The age plays more important role in rectal adenocarcinoma than the colon.⁷ In this study, 12 samples (20%) were under 40 years old, with 2 of them even under 30 years (22 years and 28 years). However, the researchers did not get more information whether the young patients in this study were accompanied by other genetic disorders or not.

As many as 56.7% cases of colorectal carcinoma in this study were male. This is in accordance with the literature which explains that this malignancy affects men than women because of hormonal differences between men and women.¹ Experimental research conducted by Li et al found that sex hormones in women, especially estrogen, have a protective role against colorectal carcinogenesis, indirectly by reducing secondary bile acids and insulin-like growth factor (IGF-1), or directly by regulating colonic epithelial cell growth or by inhibiting colorectal tumor proliferation via estrogen receptors.⁸ 26 female patients in this study were more than 50 years old. This also indicates that most of these female patients have reached menopause, that decrease endogenous estrogen levels which leads to decreased antineoplastic estrogen activity against colorectal carcinoma. In addition, unhealthy lifestyle in men such as smoking and alcoholic beverages also trigger the vulnerability of men to suffer this malignancy. The most tumor location in this study was in the rectum as many as 19 cases (31.7%), and the rarest location was in the right colon as many as 8 cases (13.3%). Based on the literature, most cases of colorectal carcinoma appear in the left colon and rectum,^{1,9} in line with that Wang et al reported that the right colon is a rare location.¹⁰ Because in the right colon this cancer more likely cause hidden bleeding that causes atypical clinical symptoms, in contrast with tumor in the left colon or rectum that blockage and bloody the feses. One of the weaknesses of this study is still found medical records that do not include specific location of the tumor, because of that in this study researchers divided tumor location into four parts, namely the colon, rectum, right colon, and left colon.

Based on the WHO classification in 2019, colorectal adenocarcinoma consists of eleven histopathological subtypes that can be distinguished and have specific clinical and molecular characteristics. However, of all these subtypes this study only found three subtypes, were dominated by adenocarcinoma NOS (86.7%), and there were two specific histopathological subtypes of colorectal carcinoma, namely mucinous adenocarcinoma and serrated adenocarcinoma. The grade in this study was assessed based on the percentage of gland differentiation components according to WHO 2019 which divides the grade of colorectal carcinoma into low grade and high grade. This study found that cases of low grade adenocarcinoma NOS were more common than high grade. This may be due to the increasing knowledge and information about this malignancy, as well as the increasing use of colonoscopy or other imaging support tools, which causes this malignancy to be detected more quickly. Although dominated by low grade adenocarcinoma NOS, this study also found 6 cases of high grade mucinous adenocarcinoma with a worse prognosis.

Lymphatic invasion positive was found in 35% of cases, slightly more than the study conducted by Betge et al that found lymphatic invasion positive in 30% of the study cases.¹¹ Lymphatic invasion was associated with metastasis to lymph nodes, as well as in several other studies proved to be an independent prognostic indicator patients adenocarcinoma colorectal.^{1,12,13} Vascular invasion in this study is divided into Intramural Vascular Invasion (IMVI) and Extramural Vascular Invasion (EMVI). It was found that EMVI was more common than IMVI. This is in accordance with the literature and research conducted by Gibson et al which reported that the EMVI was more dominant than IMVI (15.1% vs 3.3%).^{1,14} In line with that Betge et al also reported that EMVI more dominant than IMVI.¹¹ The relationship of EMVI as an independent prognostic factor in the survival of colorectal carcinoma patients has been widely studied, while the role of IMVI is still not fully understood. This may be due anatomically and histologically EMVI is outside of the muscularis externa (serosa and subserosa) that it closer to the main

branches of the blood vessels that supply the large intestine, whereas IMVI represents an end vascular with smaller diameter. Therefore, when there is invasion of the tumor into the blood vessels (vascular) it will be easier to see it in larger blood vessels.

Of the 60 samples studied, only 16 samples (36.7%) were found to have positive perineural invasion (PNI). In accordance with the literature which states that the incidence of PNI in colorectal cancer ranges from 9% -30%^{1,15,16} Zhou et al reported that PNI status can be used as a complementary factor for TNM staging in colorectal carcinoma patients. His study found that there was a significant influence between PNI status on life expectancy in patients adenocarcinoma colorectal with stage II and III. Stage II patients are the most important group to benefit from PNI identification, because by determining their PNI status, they can be considered for adjuvant chemotherapy. In addition, Liebig et al also found that the reporting of PNI status was still low in routine daily reporting, even though in particular it was found that PNI was associated with histopathological indicators that showed a tumor aggressiveness and tumor metastatic behavior such as the presence of LVI, high tumor budding and the presence of metastasis to the KGB.¹⁶

Until now there is no standard method used to assess the infiltration of lymphocyte in adenocarcinoma colorectal. Therefore, in this study, the assessment of TILs was carried out based on the recommendations of the International Immuno-Onocology Biomarker Working Group in 2017 about how to assess TILs in solid tumors adopted from the method of assessing TILs in breast cancer according to the International TILs Working Group in 2014.¹⁷ Initially, there were hypothesis that lymphocyte cells that interact directly with carcinoma cells (intratumoral TILs) are more relevant than stromal TILs, but currently several studies have found stromal TILs to be a superior parameter.¹⁸⁻¹⁹ This may be due to intratumoral TILs are generally present in smaller numbers, more heterogeneous, and difficult to observe in H&E staining.²⁰ In addition, limiting the evaluation of TILs to the stromal compartment means that the number of TILs assessed will not be affected by the density and growth pattern of tumor cells. Klintrup et al reported that the number of inflammatory cells correlated with a good prognosis in colorectal carcinoma,¹⁸ in line with that Canna et al reported that the absence of CD4 + T lymphocytes in a study using CD4 + immunohistochemical staining correlated with poor survival.¹⁹

In this study the assessment of tumor budding was carried out in the invasive front tumor area after selecting the hotspot with the highest number of buds. The examination of tumor budding found that the highest proportion was low budding in 30 cases (60%), followed by intermediate budding in 13 cases (21.7%), and high budding in 11 cases (18.3%). This is consistent with van Wyk et al study that found low budding was higher than high budding.²⁰ However there were differences in the both about reporting system where in this study the researcher used the three tier scoring system recommended by the International Tumor Budding Consensus Conference (ITBCC) 2016, whereas van Wyk et al used the two tier scoring system. The three tier scoring system is recommended by the ITBCC 2016 because it can stratification the risk of pT1 and stage II colorectal cancer. Whereas in pT1, the intermediate and high budding was associated with an increased risk metastasis to lymph nodes, whereas in colorectal cancer stage II high budding was associated with a reduction survival life and increased recurrence.²¹ The most clinical stage in this study was stage II (61.7%). This is accordance with the research conducted by Gunasekaran in Bali in 2019 which found that the highest stage was stage II (53.7%), as well as several other studies in various regions in Indonesia which found the same results.²²⁻²⁴ It may be caused by due to the increasing use of colonoscopy as a screening in patients carcinoma colorectal that can be found at a lower stage than before.

CONCLUSION

After conducting research on 60 samples of adenocarcinoma colorectal it found that the average age of patients was 51.9 years with a minimum age of 22 years and a maximum of 83 years, the most common age group was 50-59 years, men was found more than women, and the most tumor location was found in the rectum. The most histopathologic subtypes were adenocarcinoma NOS, with common grade was low grade (73.3%). Lymphatic invasion was found in 22 cases (36.7%), most vascular invasion found was EMVI (35%), PNI was found in 16 cases (26.7%), most tumor budding were low budding, most frequent grade of TILs was moderate, and most clinical stage is stage II.

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ETHICAL APPROVAL

Health Research Ethical Committee. Universitas Sumatera Utara, Medan, Indonesia approved this study.

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