CORRELATION OF INTRATUMORAL TUMOR-INFILTRATING LYMPHOCYTES (TILs) WITH HISTOLOGICAL GRADING OF ADENOCARCINOMA COLON

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ABSTRACT

Background : Most malignant epithelial tumors originating from the colon are adenocarcinomas, which account for >90% of all carcinoma colon. Tumor-Infiltrating Lymphocytes (TILs) have been extensively studied and are associated with prognostic factors for several solid tumors. Research on TILs with Hematoxylin & Eosin staining for colon adenocarcinoma is rarely done until now.

Objective : To know the correlation between Intratumoral histological grading of adenocarcinoma colon.

Material and Methods : This is analytical research with a cross-sectional approach using the slide and the paraffin block from the patients diagnosed as adenocarcinoma colon stained with Hematoxylin&Eosin. The statistical analysis with the Spearman method is used to analyze the correlation of intratumoral TILs with histological grading of adenocarcinoma colon.

Results : Based on the statistical analysis with the Spearman method for 32 samples of the patients with adenocarcinoma colon to analyze the correlation of intratumoral TILs TILs with the histological grading show the p-value of p=0,006 (if p-value <0.05, the correlation was significant).

Conclusion : There is a significant relationship between Intratumoral Tumor-Infiltrating Lymphocytes (Intratumoral TILs) and grading histology of adenocarcinoma colon.

Keywords : Adenocarcinoma colon, TILs, grading

1. Introduction

Most malignant epithelial tumors originating from the colon are adenocarcinomas, which account for> 90% of all colon carcinomas. [1] Dietary factors that are closely associated with an increase in carcinoma colon numbers are low intake of vegetable fiber and high intake of processed carbohydrates and fats. Colon carcinoma occurs presumably because it is mediated by the inhibition of the enzyme cyclooxygenase-2 (COX-2), which is highly expressed in 90% of colorectal carcinoma and 40% to 90% of adenomas and is known to accelerate epithelial proliferation, especially in response to lesions. [2,3]

According to data from Globocan 2012, the incidence of carcinoma colon in Indonesia is 12.8 per 100,000 adult population, with a mortality rate of 9.5% of all cancer cases. [4] Indonesia ranks third in colorectal cancer, this is caused by changes in the diet of Indonesian people who follow the Western diet (westernization) which consumes foods that are higher in fat and low in fiber. Generally, cases of colon carcinoma are only known when cancer has entered a more advanced stage. This is due to a lack of public knowledge about cancer and only to consult a health care provider if there are symptoms that greatly interfere with activity. [2,4] According to the American Cancer Society, carcinoma colon is the third most cancer and the second most leading cause of death in cancer male and female population in the United States. [4] The risk of carcinoma colon begins to increase at the age of 60-70 years and less than 20% of cases occur before the age of 50 years. Now an age shift has begun. Many rectal colon cancers are found at a younger age, this is due to an unhealthy lifestyle. [1,2,6]

Tumor-infiltrating lymphocytes (TILs) is one of the important immune cells and can be found in primary and metastatic tumors. [7,8] TILs infiltrate into tumors which are referred to as intratumoral TILs around the tumor dwelling place called stromal TILs. Increased intratumoral and stromal TIL are associated with a better prognosis than some studies suggest that stromal TIL has a significant effect.[9]

2. Material and Method

We examined 32 cases of adenocarcinoma colon histopathology slides in the Anatomic Pathology Laboratory of the Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of H. Adam Malik Hospital using an analytical research design with a cross-sectional approach to assess the relationship between the degree of infiltrating lymphocytes (TILs)

intratumoral tumors with histopathological grading in sufferers adenocarcinoma colon. evaluated by three researchers through microscopic examination of Hematoxylin and Eosin staining slides. Histopathological specimens stained with H&E after fixation with 95% ethanol and with dry drying fixation were retrospectively reviewed by the authors. Histopathological slides were examined for the presence or absence of intratumoral tumor-infiltrating lymphocytes (TILs) by looking at the total intratumoral lymphocyte cells in each of the 5 fields of view. The measurement result is the total number of intratumoral lymphocyte cells from the 5 high power fields (HPF).

3 Results

The research sample was a histopathological slide diagnosed as adenocarcinoma of the large intestine in the Anatomic Pathology Laboratory of the Faculty of Medicine, University of North Sumatra and the Anatomic Pathology Unit of H. Adam Malik General Hospital. The total sample is 32 slides that meet the inclusion criteria. The following are the results of the research obtained.

Table 1. Distribution of characteristics of the Colon Adenocarcinoma study sample based on clinical data.

.	Characteristics	Score (n(%))
	Average ± SD	48.7 ± 12,50
	Age (years):	
	• < 20	4(12,5)
	• 20-30	3(9,4)
	• 30-40	10(31,1)
	• 40-50	9(28,1)
	• 50-60	3(9,4)
	• 60-70	3(9,4)
	Gender:	
	• Man	15(46,9)
	• Woman	17(53,1)
	Grading histopatology	
	• Well differentiated	15 (46,9)
	• Moderatly differentiated	13(40,6)
	• Poorly differentiated	4(12,5)

Based on age, it was found more at the age of 30-40 years in 10 cases (31.3%), followed by 40-50 years in 9 cases (28.1%), starting at age <20 years in 4 cases (12.5%).)%) at least 20-30 years old, 50-60 years old, and 60-70 years old who both have 3 cases (9.4%). Obtained an average value of 48.7 years and SD ± 12.50. Distribution of samples of large intestinal adenocarcinoma in this study, based on sex more in women by 17 cases (53.1%) compared to men by 15 cases (46.9%).

Colon adenocarcinoma samples based on histopathological assessment in this study were the most well-differentiated adenocarcinomas of 15 cases (46.9%), which were needed by moderately differentiated adenocarcinoma in 13 cases (40.6%), and adenocarcinoma of poorly differentiated cases in 4 cases (12.5%). (Table 1)

Table 2. Age Correlation Test with Intratumoral Tumor Infiltrating Lymphocytes (TILs).

Variable	**Age**		
	n	**r**	**p**
Intratumoral Tumor-Infiltrating Lymphoytes (TILs)	32	-0,050	0,785

***** *Spearman*

The correlation test results showed that the correlation between age and tumor infiltrating lymphocytes (TILs) had a significant correlation with the value of the correlation coefficient between age and TILs classified as "very weak" r = -0.050 in the negative direction. However, the analysis showed that the relationship between age and TILs did not have a significant correlation p = 0.785 (if the of p value <0.05, the correlation was significant).

Table 3. Gender Correlation Test with Intratumoral Lymphocyte Infiltration Tumors (TILs).

Variabel	Gender		
	n	r	p
Intratumoral Tumor-Infiltrating Lymphoytes (TILs)	32	0,248	<0,05

* *Eta*

The correlation test results showed that the correlation between sex and tumor infiltrating lymphocytes (TILs) was 0.248 (classified as a weak correlation). **(Table 3)**

Table 4. Grading histopalogy Correlation Test with Intratumoral Tumor Infiltrating Lymphocytes (TILs).

Variabel	*Grading Adenocarcinoma Colon*		
	n	r	p
Intratumoral Tumor-Infiltrating Lymphoytes (TILs)	32	0,477	0,006

**Spearman*

The correlation test results showed that the correlation between age and tumor infiltrating lymphocytes (TILs) had a significant correlation with the value of the correlation coefficient between age and TILs classified as "very weak" r = -0.050 in the negative direction. However, the analysis showed that the relationship between age and TILs did not have a significant correlation p = 0.785 (if the of p value <0.05, the correlation was significant). **(Table 4)**

MICROSCOPIC SAMPLES IMAGES

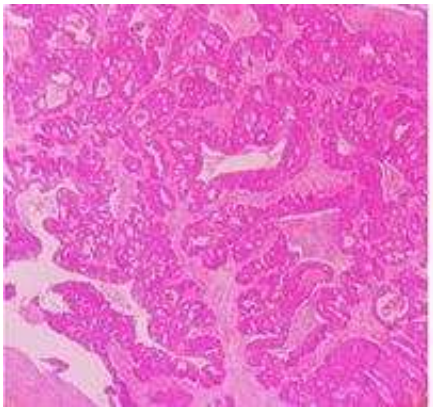

Fig 1. *Well differentiated adenocarcinoma colon*

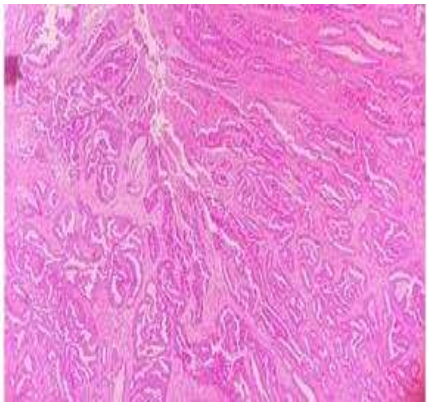

Fig 2. *Moderrately adenocarcinoma colon*

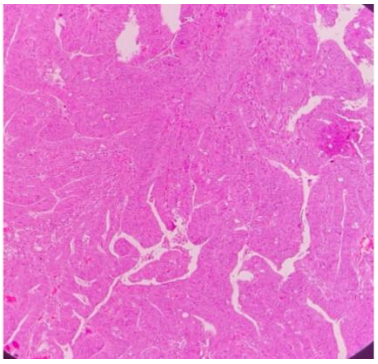

Fig 3. *Poorly adenocarcinoma colon*

4. Discussion

In this study, adenocarcinoma colon pendendita recorded in the data storage software at the Department of Anatomic Pathology, Faculty of Medicine, University of North Sumatra and the medical records of the Anatomic Pathology Unit of the H. Adam Malik Hospital in Medan who met the inclusion and exclusion criteria were 32 samples. In this study it is known that the distribution of adenocarcinoma colon samples by age is more common in the age of 30-40 years in 10 cases (31.3%), followed by the age of 40-50 years in 9 cases (28.1%) and then age <20 years of 4 cases (12.5%) and at least at the age of 20-30 years, ages 50-60 years, and ages 60-70 years which both had 3 cases (9.4%). Obtained a mean value of 48.7 years and SD ± 12.50. According to the literature, the risk of colon adenocarcinoma increases significantly with increasing age, where at the age of 40 years in men and women, the risk will double in every decade until the age of 75 years. [1] Adenocarcinoma colon, found at a younger age, is more associated with an unhealthy lifestyle. The results of this study are almost the same as the research by PR Monitor, et al which states that the most age of adenocarcinoma colon is age 46-60 as many as 16 cases and at least age 30-45 years which is as many as 5 cases studied during the 2016-2017 period. [3] Research conducted by Jakubowska K, et al. showed the same results as this study, the most obtained age was> 60 years with more samples. [7] Increased incidence in old age can occur due to accumulation of somatic mutations caused by the development of neoplasms and also a factor in decreasing immunity with increasing age. [1,2] The incidence of adenocarcinoma colon, peaks in the age of 60 years to 70 years, and less than 20% of cases occur before 50 years old. [2]

The incidence of colon adenocarcinoma sufferers in this study was more in women than in men which is different from the literature that discusses how to increase colon adenocarcinoma in men and women alike. [1] The results of this study are also different from those of Jakubowska K, et al. which states the male group more than the female group of 160 cases. [7] The differences that occur because of this study have a very different sample size and length of study. However, the reasons why men or women are more questionable for colon adenocarcinoma are not recognized for certain. [10]

Histological grading for colon adenocarcinoma is one of the prognostic factors for this malignancy. Based on World Health Organization classification, adenocarcinoma colon is divided into four levels of differentiation based on glandular structure. [1,2] In table 1, it can be seen that from 32 samples studied, as many as 15 cases (46.9%) were well differentiated adenocarcinoma colon, 13 cases (40.6%) were moderately differentiated adenocarcinoma colon, and 4 cases (12.5%) were poorly differentiated adenocarcinoma colon. This is different from the Pantau RP research, et al. which states the most grading obtained is moderately adenocarcinoma colon in 13 cases and the lowest is poorly differentiated adenocarcinoma colon in 3 cases.[3]

In this study, researchers observed intratumoral infiltration of TILs. This may be due to the observation that intratumoral TILs are easier to do in hematoxylin & eosin staining because these inflammatory cells show direct interaction with tumor cells. [23] The study also showed that from 32 samples TILs were found in every five fields of view of the three grading adenocarcinoma colon. In well differentiated adenocarcinoma colon, TILs were found in 15 cases (46.9%), moderately differentiated adenocarcinoma colon, TILs were found in 13 cases (40.6%), and poorly differentiated adenocarcinoma colon, TILS were found in 4 cases (12.5%)). This is different from the research Jakubowska, et al. who examined intratumoral TILs on 160 samples with a degree of assessment found or not found TILs and in his study found that there were 32 cases without TILs and 128 cases found TILs. Research Jakubowska, et al. showing a weak inflammatory response in the invasive part of the tumor, a low proportion of TILs at the center of the tumor is associated with increased severity of colon adenocarcinoma. [7]

The relationship between intratumoral infiltrating lymphocytes (TILs) tumor infiltration with age in patients with adenocarcinoma colon which was also tested by Spearman statistics. In this study, the correlation test results showed that the correlation between age and tumor infiltrating lymphocytes (TILs) had a significant correlation with the value of the correlation coefficient between age and TILs classified as "very weak" p = -0.050 in the negative direction. However, the analysis showed that the relationship between age and TILs did not have a significant correlation p = 0.785 ((if the of p value <0.05, the correlation was significant). This is in accordance with research Jakubowska, et al. for intratumoral or intraepithelial TILs which say that TILs are not age-related. [7]

The relationship between intratumoral infiltrating lymphocyte (TIL) with sex in patients with large intestinal adenocarcinoma which was also statistically tested. In this study, the results of

a debate trial about the differences between sex and TIL have a very large number. This study is the same as research Jakubowska, et al. for intratumoral or intraepithelial TIL that says lymphocyte infiltration (TIL) tumors are sex-related. [7]

The relationship between infiltrating tumor infiltrating lymphocytes (TILs) intratumoral with intratumoral adenocarcinoma colon grading which was also tested using the Spearman test. In this study, the results of the correlation test showed that the correlation between grading Adenocarcinoma Colon with the number of intratrating lymphocytes (TILs) histology was also tested using the Spearman test. p value = 0.006 (if the of p value <0.05, the correlation was significant). Spearman's correlation coefficient shows a sufficient correlation category) and in a positive direction (improvement in grading adenocarcinoma colon will be followed by an increase in the number of intratumoral infiltrating lymphocytes (TILs) tumor cells, and vice versa). This is in accordance with research Jakubowska, et al. for intratumoral or intraepithelial TILs which say increased grading is followed by a decrease in the number of TILs. [7]

5. Conclusion

In this study conducted on 32 samples there was an association of intratumoral infiltrating lymphocytes (TILs) with histopathological grading of adenocarcinoma colon and there was also a relationship that had a weak correlation level between intratrating lymphocytes (TILs) intratumoral tumors with age and gender.

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REFERENCES

1. Hamilton RS, Bosman TF, Boffetta P, Iiyas M, Morreau H. Carcinoma of the colon and rectum. 2010. In: Bosman TF, Carneiro F, Hruban HR, Theise DN, eds. WHO Classification of tumours of the digestive system, 4th. IARC. Lyon; pp132-46
2. Kumar V, Abbas AK, Fausto N, Aster JC. 2013. Oral Cavity and Gastrointestinal tract. Robbins Basic Pathology. 9th ed. Philladelpia; Elsevier Saunders. pp598-602
3. Pantow PR, Waleleng JB, Sedli PB. 2017. Profil Adenocarcinoma Kolon di RSUP Prof Dr. R. D. Kandou dan Siloam Hospitals Periode Januari 2016-Juni 2017. Jurnal e-Clinic (eCl), Volume 5, Nomor 2; pp. 326-31
4. American Cancer Society. Key Statistics for Colorectal Cancer. [cited 2019 feb 9] Available from: https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html
5. Benson BA, et al. 2017.Colon cancer, Version 1. J Natl Compr Canc Netw 2017;15(3);pp370–98
6. Komite Penanggulangan Kanker Nasional. Pedoman Nasional Pelayanan Kedok-teran Kanker Kolorektal. Jakarta, 2014
7. Jakubowska K, Kisielewski W, Kańczuga-KL, Koda M, Famulski W. 2017. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. Oncol Lett. Dec; 14(6): pp6421–32
8. Perez EA, Ballman KV, Tenner KS, Thompson EA, Badve SS, Bailey H,Baehner FL. 2016. Association of Stromal Tumor-Infiltrating Lymphocytes With Recurrence-Free Survival in the N9831 Adjuvant Trial in Patients With Early-Stage HER2-Positive Breast Cancer. Jama Oncol. Jan 1; 2(1): pp56–64
9. Matsutani S, Shibutani M, Maeda K, Nagahara H, Fukuola T, Iseki Y, et al. Verification of the methodology for evaluating tumor-infiltrating lymphocytes in colorectal cancer. Oncotarget. 2018 Mar 16; 9(20): pp15180–197
10. Lee CT, Huang YC, Hung LY, Chow NH, Su Pei-fang, Ho Chung- Liang et al. Serrated adenocarcinoma morphology in colorectal mucinous adenocarcinoma is associated with improved patient survival. Oncotarget, 2017, Vol. 8, (No. 21), pp35165-75