Lower Number and Percentage of Activated Natural Killer Cells in Colorectal Cancer Patients

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ABSTRACT

Background: *Colorectal cancer is a type of cancer that begins in the colon and/or rectum tissue. Natural killer (NK) cells play a critical role in the first line of defense against infection and tumors, as well as in autoimmunity and hypersensitivity reactions. NK cells also play a role in regulating tumor cell growth and metastasis. The number and percentage of activated natural killer cells have been determined in patients with colorectal cancer and benign lesion.* ***Methods:*** *This was a cross-sectional observational analytic study. The number and percentage of activated NK cells in peripheral blood were determined using the flow cytometry method in 50 samples from patients who underwent colonoscopy and obtained a mass as evidenced by histopathological examination.* ***Results:*** *Among the 50 samples, 24 samples included in the colorectal cancer group and 26 samples from benign lesion group. The mean number of NK cells in colorectal cancer was 161.71 ± 62.666 cells/µL, benign lesion was 553.92 ± 269.173 cells/µL. The mean percentage of activated NK cells in colorectal cancer was 2.82 ± 1.19%, benign lesion was 5.10 ± 2.48%. There was a significant difference in the number of NK cells and the percentage of activated NK cells between colorectal cancer and benign lesion patients (p = 0.000).* ***Conclusion:*** *The number and activity of NK cells decreases in patients with colorectal cancer.*

Keywords: *The number of NK cells, percentage of activated NK cells, colorectal cancer, cancer.*

INTRODUCTION

Colorectal cancer is a type of cancer that begins in the colon and/or rectum tissue. Recently the incidence of colorectal cancer has been increasing in both Western and developing countries. Colorectal cancer is the third most common type of cancer worldwide, the second leading cause of death from cancer, and the leading cause of death from gastrointestinal cancer.[1] Colorectal cancer is the third most common type of cancer and the third leading cause of death in men and women in the United States, according to the American Cancer Society. In 2018, 19,113 men (11.9% of new cancer cases) and 10904 women (5.8% of new cancer cases) were diagnosed with colorectal cancer in the world.[2] Data in Dr. Soetomo Surabaya Hospital, Indonesia, recorded as many as 852 patients diagnosed with colorectal cancer from 1 January 2012 to 31 December 2017. Other data obtained were 201 patients were recorded as having colorectal tumors at the Gastroentero-

hepatology Center Dr. Soetomo Hospital from June 2013 to May 2015. The risk of developing colorectal cancer can be associated with aging, poor dietary habits, lack of exercise, smoking, and obesity.[3] In general, it is stated that the development of colorectal cancer is an interaction of various factors, namely environment and genetics. Natural killer (NK) cells are a type of large granular lymphocyte with a distinctive morphology that participates in innate immunity. NK cells play a role in the early stages of infection and tumor defense and may also play a role in autoimmunity and hypersensitivity reactions. NK cells protect the body by killing specific cells and secreting chemokines and cytokines (innate immune system), as well as assisting other immune cells in eliminating the targeted cells (adaptive immune system). NK cell functions are regulated by two types of receptors, namely receptor activation, and inhibition.[4]

NK cells are the primary cells in cancer immune surveillance. The role of NK cells in colorectal cancer can predict the occurrence of postoperative recurrence and metastases. A previous study shows that the number of NK cells is related to the life expectancy of people with colorectal cancer.[5] The decrease in NK cell activity was not associated with the cancer staging, is associated with a lower life expectancy.[4] Colorectal cancer was tenfold more likely to develop in patients with low NK cell activity.[6]

The incidence of colorectal cancer is increasing in developing countries such as Indonesia. A recent study compared patients at high risk of colorectal cancer, specifically those over the age of 40 who had colonoscopy.[6] This study compares the number and percentage of activated natural killer cells in colorectal cancer patients and benign lesion patients based on this description.

METHODS

The method used was observational analytic with a cross-sectional design. The population of this study was all patients who underwent colonoscopy at Dr. Soetomo Hospital, Surabaya, East Java, Indonesia. The study sample was patients who met the inclusion and exclusion criteria, and had a mass taken in colonoscopy which was proven by histopathological examination. The inclusion criteria were consenting to participate in the study with informed consent, aged 20-60 years and not undergoing anticancer treatment, including surgery and chemotherapy. While the exclusion criteria were patients who are not pregnant and not currently using contraception, currently has no active bacterial or viral infection, had hepatitis B or C infection, had a history of using corticosteroids and immunosuppressants for the past six months, had autoimmune diseases (rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, kidney disease), intestinal inflammation including Crohn's disease, type 1 diabetes, Guillain Barre syndrome), currently has no active TB or a history of HIV infection. The number of subjects was obtained after calculating the minimum sample size using the formula unpaired numerical analytic. The number of samples was obtained with 24 colorectal cancer samples and 26 benign lesion samples.

Colorectal cancer patients are patients whose masses are suspected of malignancy originating from the colon and/or rectum on colonoscopy examination, as well as evidenced by histopathological examination, and benign lesion patients are patients who do not show a mass suspected of malignancy (polyps, internal and external hemorrhoids, normal) as evidenced by histopathological examination. This study has approved by the Ethics Committee of dr. Soetomo Hospital (Ref. No. 2028/KEPK/VII/2020).

The research location was the Gastroentero-Hepatology Center, Internal Medicine Department/SMF at Dr. Soetomo Hospital, Surabaya, East Java, Indonesia. Samples were taken in the period July 2020 to February 2021. Sampling was done by consecutive sampling until the sample size was met. This research was conducted by taking the peripheral blood of the research subject to check the number and percentage of activated NK cells using the flow cytometry method. After conducting research and obtaining the desired data, the data were collected and analyzed using SPSS version 25 software.

RESULTS

Based on the 50 subjects obtained, 26 subjects had colorectal cancer and 24 subjects had benign lesion. Based on the age profile, the mean age of the subjects for colorectal cancer was 44 years while for benign lesion it was 49 years as shown in **Table 1**. Most of the subjects (57.69%) were female in the benign lesion group (15 people), and most 75% were male in the colorectal cancer group (18 people).

Characteristics of the Research Subject's Laboratory Examination

From a total of 50 research subjects, 24 subjects had colorectal cancer, and 26 subjects had benign lesion. As can be seen in **Table 2** shows that the average Hb value in the benign lesion was higher than the colorectal cancer group. The average value is not more significant. Leukocyte levels in benign lesion subjects were higher (10353.46 cells/µL) than in the colorectal cancer group. At the same time, the platelet level was higher (371037.50 cells/µL) in the colorectal cancer group. Lymphocyte levels were greater (2110.77 cells/µL) in the benign lesion group. The mean *Serum Glutamic Oxaloacetic Transaminase* (SGOT) was 30.09 mg/dL for the colorectal cancer group. Mean *Serum Glutamic Pyruvic Transaminase* (SGPT) level 34.50 mg/dL for benign lesion.

The average Blood Urea Nitrogen (BUN) level was 7.07 mg/dL for the colorectal cancer group. The average albumin level in the colorectal cancer group was 3.55 g/dL. Examination of the Serum Creatinine (SK) test showed the mean SK level for the benign lesion group was 3.32 mg/dL. The average Na level for the colorectal cancer group was 135.79, while the average K level in the benign lesion group was 4.05.

Distribution of Colorectal Cancer and Benign Lesion Based on Histopathological Type

The results of this study found that the most frequent histopathological types found in the colorectal cancer group was adenocarcinoma (36%), followed by carcinoma well differentiated (12%). While for the benign lesion group, non-specific chronic colitis was most frequent (28%),

Table 1. Demographic characteristics.

Characteristics	Colorectal cancer n = 24	Benign lesion n = 26	p-value
Age (years)			
Mean ± SD	44.83 ± 12.11	49.35 ± 10.94	0.540
Median	46.0	54.0	
Gender			
Women (%)	25	57.69	0.019
Men (%)	75	42.30	
Body Massa Index (kg/m^2)			
Mean ± SD	25.754 ± 0.84	22.096 ± 3.07	0.034
Median	25.60	21.75	
Ethnicity			
Javanese (%)	79.17	76.92	0.957
Madura (%)	12.5	15.38	
Tionghoa (%)	8.33	7.7	
Symptoms			
Hematochezia (%)	29.17	38.46	
Diarrhea (%)	29.17	23.07	
Constipation (%)	8.33	3.85	0.821
Abdominal pain (%)	33.33	34.62	

Table 2. Characteristics of the research subject's laboratory examination.

Laboratory parameters	Colorectal cancer n=24	Benign lesion n=26	p-value
Hemoglobin (g/dL)			
Mean ± SD	12.27 ± 2.10	12.85 ± 2.21	0.565
Median	12.65	12.85	
Leukocytes (cell/µL)			
Mean ± SD	9618.67 ± 5994.57	10353.46 ±12677.35	0.432
Median	8385.00	6755.00	
Platelets (cell/µL)			
Mean ± SD	371037.50 ± 193426.06	326938.46 ± 135021.05	0.352
Median	4.20	4.20	
Lymphocytes (cell/µL)			
Mean ± SD	1147.50 ± 449.44	2110.77 ± 635.13	0.069
Median	1090.00	2300.00	
SGOT			
Mean ± SD	30.09 ± 27.26	27.46 ± 17.75	0.191
Median	20.500	24.500	
SGP-T			
Mean ± SD	31.15 ± 17.71	34.50 ± 21.11	0.651
Median	28.00	28.00	
BUN			
Mean ± SD	7.07 ± 3.41	6.19 ± 2.35	0.652
Median	6.00	6.00	
Albumin			
Mean ± SD	3.55 ± 0.69	2.90 ± 0.94	0.232
Median	3.70	2.80	
SC			
Mean ± SD	2.47 ± 2.12	3.32 ± 2.67	0.321
Median	1.3200	1.3200	
Na			
Mean ± SD	135.79 ± 6.70	131.08 ± 24.60	0.363
Median	135.00	134.50	
K			
Mean ± SD	3.99 ± 0.49	4.05 ± 0.35	0.390

*SGOT : Serum Glutamic Oxaloacetic Transaminase
SGP-T : Serum Glutamic Pyruvic Transaminase
SC : Serum Creatinine
Na : Natrium
K : Kalium

followed by non-specific chronic colitis with mild dysplasia (12%), and active chronic colitis (12%).

Number of NK Cells in Colorectal Cancer and Benign Lesion Group

The mean number of NK cells in the colorectal cancer group was 161.71 ± 62.66 cells/µL, which was greater than the average number of NK cells in the Benign lesion group, which was 553.92 ± 269.17 cells/µL. The lowest number of NK cells in the colorectal cancer group was 25, while the highest number was

Table 3. Histopathological classification.

Variables	n (%)
Colorectal cancer	
Adenocarcinoma	18 (36)
Carcinoma well differentiated	6 (12)
Benign lesion	
Non-specific chronic colitis	14 (28)
Non-specific chronic colitis with mild	6 (12)
dysplasia	6 (12)
Active chronic colitis	
Total	(100)

244. The lowest number of NK cells in the benign lesion group was 272, while the highest number was 1095. The range of minimum and maximum values in both groups was also quite large, as indicated by the very high standard deviation of 62.66 in the colorectal cancer group and 269.17 in the benign lesion group. Comparative analysis of the number of NK cells in patients with colorectal cancer and benign lesion patients were performed using the Mann Whitney's test. The analysis results show a significant difference between the number of NK cells in patients with colorectal cancer and benign lesion (p-value 0.000).

Percentage of Activated NK Cells in Colorectal Cancer and Benign Lesion Group

The average percentage of activated NK cells in the colorectal cancer group is 2.82 ± 1.19%, which is smaller than the benign lesion group with 5.10 ± 2.48%. The lowest percentage of activated NK cells was 0.92, and the highest was 4.67 in the colorectal cancer group, while the lowest percentage of NK cells in the benign lesion group was 1.23, and the highest was 11.44. The range of minimum and maximum values in both groups was also quite large, as indicated by the high standard deviation values, namely 1.19 in patients with colorectal cancer and 2.48 in patients without colorectal cancer. The analysis results show a significant difference between the percentage of activated NK cells in patients with colorectal cancer and benign lesion with a p-value of 0.000.

DISCUSSION

Colorectal cancer group is a patient whose mass is suspected of malignancy originating from the colon and/or rectum on colonoscopy examination, as well as evidenced by histopathological examination.[1] The number and activity of natural killer cells (NK cells) decreased in patients with colorectal cancer in this study. NK cells are effector lymphocytes of the innate immune system that regulate the growth and spread of several types of tumors. NK cells are a promising cell type for adoptive immunotherapy. Transplantation of tumor-infiltrating lymphocytes has demonstrated some remarkable responses in patients with metastatic melanoma.[7] Although NK cells have a limited ability to infiltrate the colorectal cancer microenvironment, a subpopulation of colorectal cancer patients had lesions that are sufficiently infiltrated with NK cells for statistical analysis. NK cell infiltration was previously detected in approximately 30% of colorectal tumor specimens.

Notably, NK cell infiltration was not found to be associated with disease progression.[8] Following a 5-year follow-up, the beneficial effect of NK cell-T cell cooperation on the clinical course of colorectal cancer is diminished. The mechanism underlying this phenomenon is obscure. One could hypothesize that tumor-infiltrating NK cells lose their helper function over time as a result of altered activities induced by cancer cells via a variety of mechanisms. These include indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) production by malignant cells, metalloproteinases (MMPs), transforming growth factor b1 (TGFb1), and integrin b2 production by malignant cells (ITGB2, also known as LFA1).[9]

Another study found that the mean age of patients with colorectal cancer was 42 years.[10] Most colorectal cancer sufferers are at the age of 40-49 years. Other studies mostly found colorectal cancer at the age of over 40 years. The age of 40 years is when the diagnosis of colorectal cancer begins to increase sharply.[11] In this study the colorectal cancer group was also found to be at an average age of 44 years.

This situation may occur due to the slow metabolic process, inactivity, and more frequent food consumption. As many as 70% of colorectal cancer cases are sporadically caused by poor lifestyle, such as a diet low in fiber and fruits, excessive red meat and saturated fat consumption, lack of physical activity, alcohol consumption, and smoking.[12] Based on gender, the proportion of female subjects in the benign lesion group significantlt larger than that the colorectal cancer group. As for the colorectal cancer group, there was a significantly higher proportion of males. In most studies, colorectal cancer were mostly found in male patients. The incidence of colorectal cancer in men is related

to the level of the hormone estradiol, which in normal amounts, functions in spermatogenesis and fertility. However, excessive amounts of estradiol will inhibit the secretion of gonadotropin proteins such as luteinizing hormone (LH), further reducing testosterone secretion. A high amount of testosterone is associated with a reduced risk of colorectal cancer.[13]

Another study discovered that the majority of colorectal cancer patients had an overweight BMI. Obesity causes hormone accumulation, increased insulin levels, and insulin-like growth factor-1 (IGF-1), triggering tumor growth regulators, impaired immune response, and oxidative stress, thus triggering colorectal carcinoma.[14] Based on ethnicity, the proportion of subjects with Javanese ethnicity in the colorectal cancer group was more significant than the benign lesion group. Another study found colorectal cancer patients of Javanese ethnicity. The ethnic groups chosen for this study are critical because they will influence cancer treatment strategies, cancer prevention strategies, early detection, and appropriate treatment.[15] Ethnic variations in the risk of colorectal cancer should affect results if adjusted for known or suspected risk factors and environmental exposures.[16] Although there was no statistically significant difference in the number of NK cells in the peripheral blood of healthy donors and colorectal cancer patients, the colorectal cancer group had 10.10 cells/L in the CD45+ CD56+ cell population. Whereas in healthy donors, the results were 12.3 cells/μL.[17] Increased loss of CD16 expression via release could explain the increase in the frequency of the CD56-dimmed CD16 population. CD16 release can also be induced by activating NK cells with cytokines such as IL-2, IL-15, and IL-18, TNF, or target cells (such as tumor cells). Many of these cytokines are increased in the blood of patients with colorectal cancer. When NK cells are activated via CD16 or NKG2D signaling, the ADAM17 metalloprotease, which also cleaves CD16 in NK cells, is increased.[18] The increase in CD56+CD16 + NK cell counts in colorectal cancer patients is statistically significant. CD16 is a low-affinity FcRIII that recognizes antibody-coated targets and signals antibody-dependent cytotoxicity (ADCC). CD16 binds specifically to the Fc moiety of IgG antibodies on the surface of coated cells and induces degranulation of intracellular granules, resulting in the death of infected or tumor cells.[19] NKG2D expression was significantly downregulated in NK cells isolated from patients with colorectal cancer. The decreased expression of NKG2D may be associated with the suppression of NK cell activity in colorectal cancer.[20] NKG2D is required for NK cell activation, and decreased NKG2D expression may result in decreased NK cell activity in patients with colorectal cancer. In colorectal cancer patients, the NKG2 pathway can be used to inhibit NK cell-mediated antitumor immune responses. The imbalanced expression of NKG2A and NKG2D may contribute to the suppression of NK cell activity in colorectal cancer patients, thereby allowing tumor cells to escape NK-mediated lysis. Numerous cytokines, including IL-2, IL-12, IL-15, and IFN-, can increase NKG2 expression and NK cell-mediated cytotoxicity. Due to the decreased level of NKG2D expression in colorectal cancer patients, which may be related to NK cell suppression, tumor cells can evade NK cell control via the NKG2 pathway.[21]

CONCLUSION

A significant difference was found between the number and percentage of activated NK cells in colorectal and benign lesion patients.

CONFLICT OF INTEREST

The authors declare that they have no conflicting interests.

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