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Tumour-Infiltrating Lymphocytes in Colorectal Adenocarcinoma

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Abstract

Several studies have indicated that lymphocytes found in tumour area, known as TILs (tumour-infiltrating lymphocytes), can provide prognostic information in colorectal cancer (CRC). The purpose of this study was to evaluate degree of stromal TILs according to histological grading of CRC. This study used a cross sectional design method by taking random samples of data and formalin-fixed paraffin-embedded of colorectal adenocarcinoma patients treated in Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar during the period of 2014-2016. The histological grading of each sample was reviewed and TILs were assessed according to recommendation of the International TILs Working Group, 2014 on hematoxylin-eosin (H-E)-stained sections. Based on histological grading it was found that from 98 samples, 23 samples (23.5%) were well-differentiated, 66 samples (67.3%) were moderately differentiated, and 6 samples were (9.2%) poorly differentiated. While based on degree of TILs found that low- and moderate-score were 38 (38.8%) and 42 (42.9%) samples respectively, and high-score were 18 (18.4%) samples. Data were analysed using the Chi-square test and a p value of 0.865 ($p > 0.05$) was obtained and therefore it was concluded that there was no significant difference of degree of TILs according to histological grading of colorectal adenocarcinoma in this study.

Keywords: TILs; colorectal cancer; adenocarcinoma; histological grading.

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer found and is the fourth most common cause of cancer death in the world, with approximately 1,4 million new cases recorded and nearly 700.000 deaths in 2012 [1,2]. By 2030, it is estimated that the incidence of CRC would be increased by 60% to more than 2,2 million new cases and 1,1 million deaths [2]. Worldwide deaths attributable to CRC is reported higher in less-developed regions which were recorded 52% in 2012 that presumed a worse survival in the regions [1]. In Indonesia, the incidence of CRC in men is the second most common after lung cancer and in women is the third most common after breast cancer and cervical cancer. While the number of deaths from CRC ranks the third most common cause of cancer death in men, and the fourth most common cause of cancer death in women [3]. Lymphocytes found in tumour area known as tumour-infiltrating lymphocytes (TILs) [4,5]. TILs considered as primary immune response of host against the tumour, which may suggest that TILs are a part of tumour microenvironment that play a role in suppressing tumour growth [6,7,8]. One of the subpopulations of TILs which holds an important role in immunity against tumour is CD8+ cytotoxic T cells (CTLs), is a form of adaptive immune response, which can perform the function of surveillance by recognizing and directly killing tumour cells that express peptides derived from tumour antigens and are presented on major histocompatibility complex (MHC) class I [5,9]. Previous studies reported that lymphocytes infiltration is associated with better survival in several types of cancer [6]. Large scale study in Israel which analyzed 2369 CRC patient samples shows that TILs are a prognostic indicator for CRC after adjusting for age, sex, ethnicity, stage, grade, and status of microsatellite instability (MSI) [10]. Hyuk and his colleagues in their study found a significant correlation between degree of TILs and degree of tumour differentiation of CRC, which low-degree of TILs is correlated with poorly differentiated of tumour [11]. Some studies also suggest that the infiltration of TILs, both inside of tumour nest (intratumour) and stroma of tumour, contribute to a better prognosis in CRC [12]. However, some current studies conclude that stromal TILs are better used as a parameter for the diagnostic purposes compared intratumour TILs on H-E-stained sections. Assessment of stromal TILs will not be influenced by the density and the pattern of tumour growth because TILs are assessed on the area between nests of the tumour [13]. The purpose of this study was to evaluate degree of stromal TILs according to histological grading (tumour differentiation) on a sample of colorectal adenocarcinoma patients, which is important as both predictive and prognostic factor in patients with CRC.

2. Materials and Methods

2.1. Collection of Samples

There were a total of 98 formalin-fixed paraffin-embedded of samples of colorectal adenocarcinoma patients treated in Dr. Wahidin Sudirohusodo Hospital and Hasanuddin University Hospital in Makassar during the period of 2014-2016, were collected retrospectively.

2.2. Histological Evaluation and TILs Scoring

Each formalin-fixed paraffin-embedded of the samples was cut with microtome to the thickness of 3-μm and

then through hematoxylin-eosin (H-E) staining procedure and then reviewed its histological grading by two experts of pathological anatomy, and also TILs were assessed as recommended by the International TILs Working Group, 2014. The histological grading of colorectal adenocarcinoma is assessed based on the percentage of glandular formation of the tumour according to the World Health Organization (WHO) criteria. In well-differentiated adenocarcinoma, >95% of the tumour shows glandular formation, moderately differentiated adenocarcinoma shows 50-95% glandular formation, while in poorly differentiated adenocarcinoma which is mostly solid, shows <50% glandular formation [14].TILs were assessed in the stromal compartment (reported as a percentage of stromal TILs), which was the area infiltrated by mononuclear inflammatory cells on stromal tumour sites; the area between the nests of tumour and included those distributed along the invasive margin of tumour; the area occupied by tumour cells has not been investigated. Areas with necrosis, artefacts, and regressive hyalinises around the area of the tumour have not been investigated [13]. The percentage reported was the average value of some representative areas of any different density of TILs to assess stromal TILs (not focusing on the area of the hot spot). This study investigated 5 different areas per section (with microscopic magnification of objective (obj.) x10) to determine stromal areas of each tumour and then to assess TILs (with higher microscopic magnification). The samples were grouped based on the same value of scoring applied by Jakubowska and his colleagues i.e. score 1 = low (0-10% TILs), 2 = moderate (20-40% TILs), and 3 = high (50-90% TILs) [4].

2.3. Data Analysis

All data obtained from this study were recorded and grouped according to their purpose and type, to be then analyzed by the Chi-square test to evaluate degree of TILs according to histological grading of colorectal adenocarcinoma.

3. Results

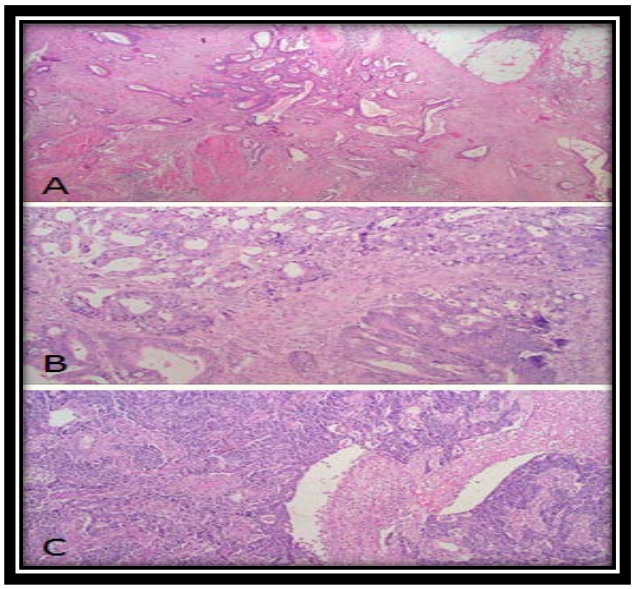

Figure 1: Representative samples of colorectal adenocarcinoma based on histological grading : well-differentiated, obj.x4(A), moderately differentiated, obj.x10(B), and poorly differentiated, obj.x10(C).

A total of 98 samples (n = 98) from colorectal adenocarcinoma patients were obtained in this study. Based on histological grading, the well-differentiated were count for 23,5% samples, the moderately differentiated were 67.3% samples, and the poorly differentiated were 9.2% samples.

The scores of TILs are shown in Figure 2, and the characteristics of samples are outlined in Table 1.

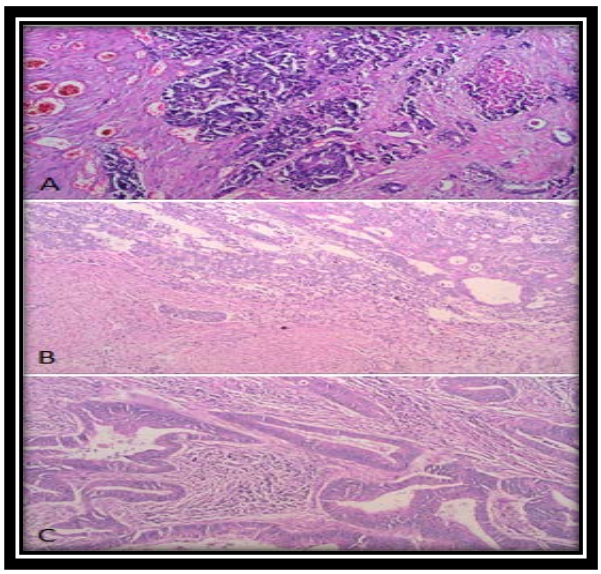

Figure 2: Stromal TILs : low-score TILs with stromal area showing 5% of TILs, obj.x10 (A); moderate-score TILs with stromal area showing 20% of TILs, obj.x10 (B). Note the area included on the stroma of invasive margine of tumour. High-score TILs with stromal area showing 50% of TILs, obj.x10 (C).

Table 1: Characteristics of 98 samples of colorectal adenocarcinoma patients

Characteristic	**n (%)**
Age	
< 40 years	6 (6.1)
≥ 40 years	92 (93.9)
Sex	
Male	46 (46.,9)
Female	52 (53.1)
Histological grading	
Well-differentiated	23 (23.5)
Moderately differentiated	66 (67.3)
Poorly differentiated	9 (9.2)
Degree of TILs	
Low-score	38 (38.8)
Moderate-score	42 (42.9)
High-score	18 (18.4)

Based on Table 1, from a total of 98 samples, 6 (6.1%) samples were in the category of < 40 years of age and ≥ 40 years of age were 92 (93.9%) samples with the mean age was 57.33 years. Males were count for 46 (46.9%) samples and females were 52 (53.1%) samples. Based on histological grading, the number of samples classified as well-differentiated were 23 (23.5%), moderately differentiated were 66 (67.3 %) samples, and 9 (9.2%) samples were poorly differentiated. As for degree of TILs, score 1 (low) was 38 (38.8%) samples, score 2 (moderate) was 42 (42.9%) samples, and score 3 (high) was 18 (18.4%) samples.

Table 2: Degree of TILs and histological grading of samples

Histological grading	Degree of TILs			Total	
	Low (%)	Moderate (%)	High (%)	(%)	
Well-differentiated	9	10	4	23	
	(39.1)	(43.5)	(17.4)	(100)	
Moderately differentiated	24	29	13	66	
	(36.4)	(43.9)	(19.7)	(100)	p = 0,865
Poorly differentiated	5	3	1	9	
	(55.6)	(33.3)	(11.1)	(100)	
Total	38	42	18	98	
	(38.8)	(42.9)	(18.4)	(100)	

Chi-Square test

As seen on Table 2, on the well differentiated group of samples, out of 23 total samples, 9 (39.1%) samples were low-score TILs, 10 (43.5%) samples were moderate-score TILs, and 4 (17.4%) samples were high-score TILs. On the moderately differentiated group, out of 66 total samples, 24 (36.4%) samples were low-score TILs, 29 (43.9%) samples were moderate-score TILs, and 13 (19.7%) samples were high-score TILs. As for the poorly differentiated group, 5 (55.6%) samples were low-score TILs, 3 (33.3%) samples were moderate-score TILs, and only 1 (11.1%) sample was high-score TILs out of 9 total samples. Data were analyzed by the Chi-square test and resulted the p value of 0.865 ($p > 0.05$), therefore it was concluded that there was no significant difference of the degree of TILs according to histological grading of colorectal adenocarcinoma in this study.

4. Discussion

Predictive and prognostic factors are important in the selection of cancers therapy. Infiltration of lymphocytes is a part of the primary tumour characteristics and is considered as one of the important prognostic factors in CRC, and some studies have shown that the infiltration of lymphocytes is related to a better prognosis [15]. This study

assessed stromal TILs. Currently, stromal TILs is considered as a better diagnostic parameter compared to intratumour TILs based on H-E-stained sections. This is because it is still difficult to assess intratumour TILs in minuscule amounts and only detected at a fraction of the cases [13]. Previous studies assess stromal TILs on the area of invasive front of tumour, where the area is considered as an optimal area to assess TILs [16]. Therefore, the assessment of stromal TILs in this study also covered the stromal area of the invasive margin of tumour. TILs reportage in the form of percentage by most pathologist closer to 5-10% of the score seemed to be more appropriate, for example the percentage TILs score of 13.5% is rarely reported, and most of this would be reported as a score of 15% [13]. As in this study, TILs were classified into three groups, 1 = low (0-10% TILs), 2 = moderate (20-40% TILs), and 3 = high (50-90% TILs). Some scores between 10-20% or in between 40-50%, will be determined by the observer to be included in the group which has the nearest results. For example, the score of 13.5% reported as 15%, but classified as score 1 = low (0-10% TILs). Table 1 describes the characteristics of samples, it is shown that in the age category, corresponding samples aged < 40 years was fewer compared to the ≥ 40-years-old (6 vs 92 samples) with the mean age was 57.33 years. Similar result is also found in the study of Shi and his colleagues which reports higher numbers of CRC patients of age > 40 years, and especially of age >60 years [17]. This is in accordance with the trend of an increased risk of CRC in older age[18]. Transformation of colon mucosa from normal into invasive cancer could arise through a stage where genetic and epigenetic modifications were accumulated. Most of CRC were told to be developed from preceded adenoma that had malignant genetic lesions, and this transformation could take about 10-15 years[19]. Such length of time might be one of the factors we found many cases of CRC in older ages. While the correlation of gender to the development of CRC is still unclear [18]. This study found that the number of cases in male and female were almost the same, although female was slightly more than male (52 vs 46 samples). Some previous studies also obtained similar results, such as study by Valentini and his colleagues found the frequency of CRC patients of the subject study between male and female is almost the same [20]. While Rosenbaum and his colleagues also reported from 181 samples used, 53% of all the samples of CRC patients are female [21]. This study found that there was no significant difference of the degree of TILs according to histological grading of colorectal adenocarcinoma. It may be because of the differences in the number of samples between the well-differentiated, the moderately differentiated, and the poorly differentiated were very large. A more uniform distribution of the number of samples in the histology grade group is needed in the subsequent study that assess the degree of TILs based on histological grade of colorectal adenocarcinoma. Study by Hyuk and his colleagues found a significant correlation between degree of TILs and degree of tumour differentiation of CRC, where low-degree of TILs is correlated with poorly differentiated tumour [11]. Whereas based on the study of breast cancer patients by Huszno and his colleagues found that higher degree of TILs appear to be associated with higher histological grading [22]. In this study, as shown in Table 2, the poorly differentiated tumours had been found to be of more low-degree of TILs. Programmed Death-1 (PD-1) expression can experience upregulation on exhausted T cells because of constant exposure of the tumour antigens [23]. PD-1 is a co-receptor inhibitor that can be expressed on some types of TILs, including CTLs [23,24]. PD-1 expressed on T cells that is bind to the Programmed Death-Ligand 1 (PD-L1) expressed by tumour cells would inhibit T-cell receptor (TCR) signal transduction, resulting in inhibition of the activity of CTLs and can ultimately increase apoptosis in T cells [25,26]. Some studies have found the correlation of PD-1 overexpression and tumour grade of several types of cancer [27,28]. This may be associated with increasing T

cells that are exhausted in line with the increase in histological grading of tumor. Some studies suggest that high lymphocytes infiltration in colorectal cancer associated with a specific molecular profile of this cancer, in this case MSI-high (MSI-h) [29]. MSI-h is more immunogenic than microsatellite stable tumors due to the presence of large numbers of abnormal peptides as a result of frameshift mutations [12]. In addition, large amounts of regulatory T cells (Treg) are known also to be present in various types of cancer, including CRC. Believed, Treg can suppress anti-tumour immune responses in the tumour microinvironment. The increasing of Treg in the tumour area, will affect the ratio of Treg with CTLs, and are reported to be associated with the poor prognosis in a number of malignancies [30]. This may be associated with the result of this study, in which the samples with the worse of differentiation grade, still had a high-degree of TILs.

5. Conclusion

This study concluded that there was no significant difference of the degree of stromal TILs according to histological grading of colorectal adenocarcinoma.

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