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C-KIT EXPRESSION IN VARIOUS DEGREES OF HISTOPATHOLOGY DIFFERENTIATION OF RETINOBLASTOMA IN MOHAMMAD HOESIN HOSPITAL PALEMBANG

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ABSTRACT

Retinoblastoma is a malignant intraocular neoplasm in pediatric which composed of embyonic tumour cells from retinoblasts of neuroepithelial origin with a relative incidence of 3% of all pediatric tumours. Histopathology examination can confirmed the diagnosis of retinoblastoma. Immunohistochemical staining with C-kit antibody can be used to identify the expression of C-kit in paraffin block of retinoblastoma sample.This study was conducted to identify C-kit expresion in various degrees of histopathology differentiation's retinoblastoma in Mohammad Hoesin Hospital Palembang.. A descriptive study used on the paraffin blocks of 19 cases retinoblastoma in RSUP dr. Mohammad Hoesin from January 2012- July 2017. The degree of histopathologic differentiation and invasion of retinoblastoma was investigated by Hematoksilin-Eosin stain, and the C-kit expression stained by immunohistochemistry using antibody C-kit *ready to use*. Data was being presented in tabular form to identify C-kit expression. Retinoblastoma which expressed C-kit in this study are 63,16% among all sample. C-kit expressions were detected in 25% *well differentiated*, 50 % *moderately differentiated*, and 25% *poorly differentiated* of histopathological differentiation degree, also in 50% sample with optic nerve invasion and 58,33% in scleral invasion. C-kit expression was negative in 36,84% sample. In this study, C-kit expression was found more than half in retinoblastoma sample.

Keyword: C-kit, degree of histopathology differentiation, retinoblastoma

1. INTRODUCTION

Retinoblastoma is a primary intraocular malignant tumor in children whose tumor cells derived from embryonic retinoblast of neuroepitel structure are biologically similar to neuroblastoma and medulloblastoma. [1-4] The incidence of retinoblastoma is 3% of all childhood tumors.[1-3] Based on data obtained from the pedoatric hemato-oncology at RSCM 2010-2016, retinoblastoma incidence was 156 cases and was the second most common malignancy after acute lymphocytic leukemia.[7] Data from the pediatric hemato-oncology Dr. Hospital Mohammad Hoesin Palembang reported retinoblastoma cases as many as 37 cases calculated from the year 2008-2014.[8] Precise diagnosis of

retinoblastoma confirmed by histopathology examination. [2,3,9] Youssef *et al* (2014) classified three degrees of differentiation of retinoblastoma, consist of well differentiated, moderately differentiated and poorly differentiated.[1]

Retinoblastoma's pattern of growth was studied in many research centers and found involvement of molecular processes in the case. Some specific proteins are known to be involved in the developmental process of retinoblastoma, and one of them is the CD117 protein otherwise known as C-kit. This protein is encoded by the Kit gene, a human proto-oncogen located on the long arm of chromosome 4 q11-21. This gene encodes a 145 kDa transmembrane protein of tyrosine kinase, a protein that acts as a type III tyrosine kinase receptor. These receptors form complexes with SCF ligands (stem cell factors) and induce cellular processes such as cell proliferation, migration and differentiation.[1,10-14]

The role of C-kit has been found in more than 120 types of tumors by using immunohistochemical methods on TMA (tissue microarray). C-kit is generally found to be positive in GIST (Gastrointestinal Stromal Tumor), but in some other tumors also found positive C-kit expression. [1,10,11,13-15]

Bosch *et al* (2005) conducted a study of immunostaining with C-kit antibodies in 73 retinoblastoma cases. The results found positive expression in 14 cases of 19.2%.[11] The interesting thing about this protein is the potential for the development of cancer therapy targeting the C-kit protein. [1,11,14,15]

A study conducted by Barry *et al* (2007) showed that 52.38% of cases of retinoblastoma express C-kit on immunohistochemical examination.10 Youssef *et al* (2014) conducted a C-kit expression study on retinoblastoma and obtained positive C-kit results as much as 48.2%. Immunoreactivity is associated with invasion of the sclera and optic nerve and may represent more invasive retinoblastoma.[1,10]

The prognosis is influenced by various factors including the extent of tumor invasion into the structure of the globe and optic nerve.1,2,16,17 Looking at the magnitude of retinoblastoma morbidity and mortality rates and taking into account the absence of data on C-kit expression in retinoblastoma cases in Indonesia , this study was conducted to identify C-kit expression in different degrees of histopathologic differentiation and invasion of retinoblastoma at Dr. Mohammad Hoesin General Hospital Palembang.

2. METHODS

This study is a descriptive study to identify C-kit expression in various degrees of retinoblastoma histopathologic differentiation by immunohistochemical examination of retinoblastoma tissue samples at Dr. Mohammad Hoesin General Hospital Palembang. This research was conducted at Eye Polyclinic and Anatomical Pathology Laboratory of Dr. Mohammad Hoesin General Hospital Palembang from January 2017 until July 2017. The target population was all retinoblastoma patients who went to the Dr. Mohammad Hoesin Palembang who had and will do surgical removal of the tumor. The research sample is determined by time-based method. The subjects of the study were retinoblastoma patients who came to the Eye Polyclinic of Dr. Mohammad Hoesin General Hospital Palembang which has been and will be carried out surgical removal of the tumor and has been given informed consent and signed the agreement in the period January 2017 to July 2017 and preparations

histopathology retinoblastoma from January 2012 to December 2016.

Sample recruitment criteria: The sample in this study is the population that meets the acceptance criteria for the research sample. The Inclusion criteria were (a) All retinoblastoma patients who seek treatment at Eye Polyclinic of Dr. Mohammad Hoesin General Hospital Palembang. (b)The preparation is derived from surgical measures containing retinoblastoma tumor tissue. (c) Histopathological preparations are in good condition or eligible for immunohistochemical examination. (d) Willing to follow the research stated by signing informed consent (for new cases / primary data). The Exclusion criteria were: (a) Postoperative, after histopathological examination did not show Retinoblastoma (b) Suspected Retinoblastoma patients (patients died or delayed surgery) during the period from January 2017 to July 2017. Sampling is done by total sampling technique. Patients suspected retinoblastoma that meet the criteria of inclusion were informed consent, ophthalmological and auxiliary examination. Histopathological preparations of retinoblastoma that meet the inclusion criteria are taken as samples to meet the specified sample size. The degree of histopathologic differentiation of retinoblastoma is classified as follows:

a. Well differentiated: Flexner-Wintersteiner rosettes or fleurettes are found.
b. Moderately differentiated: The tumor consists of isolated Homer-Wright rosettes.
c. Poorly differentiated: Flexner-Wintersteiner rosettes and Homer-Wright rosettes are not found.

Expression C-kit is an expression of receptor tyrosine kinase that is inserted on immunohistochemical examination by using C-kit primary antibody. The cells are rated in 10 large fields. The coloring results are categorized into four groups. Tumors in the absence of staining are called negatives. Tumors with 1+ staining on <60% cells and 2+ staining at <30% intensity are relatively weak. Tumors with 1+ staining at >60% cell intensity, 2+ staining at 30-79%, or 3+ on <30% cells are considered moderate. Positively strong if tumor with intensity 2+ in> 80% cells or 3+ at> 30% cells.[15]

3. RESULTS

This study is a descriptive study to identify C-kit expression in various degrees of histopathologic differentiation and invasion of retinoblastoma. This research was conducted at Eye Polyclinic and Department of Anatomy of Dr. Mohammad Hoesin General Hospital Palembang. Samples in this study were paraffin block preparations taken from retinoblastoma patients who were referred and went to Eye Polyclinic of RSUP Mohammad Hoesin Palembang which had been and will be carried out surgery action in the research period and confirmed histopathologically then done immunohistochemical staining.

There were 24 retinoblastoma patients recorded at RSMH Palembang during the period of January 2012-July 2017. Of these 24 patients, 4 of which paraffin blocks were not available for immunohistochemical staining and 1 paraffin block did not have sufficient tissue count for immunohistochemical readings, the number of samples meeting the inclusion criteria in this study were 19 samples. General characteristics of the subjects in this study include the sex, age and laterality of the tumor.

Table 1. General Characteristics of Research Subjects

Characteristics	n	%
Sex		
Boys	11	57,89
Girls	8	42,11
Age		
Youngest	0 year	
Oldest	8 years	
Mean (SD)	3 (2,4) years	
Median (Min-Max)	3 (0 – 8 years)	
Tumor Lateralitity		
Unilateral :		
Right eye	10	52,63
Left eye	7	36,84
Bilateral	2	10,53
Total	19	100,00

Based on table 1, samples suffering from retinoblastoma were mostly male (57,89%), and samples suffering from female retinoblastoma were 8 samples (42.11%). In terms of age, the average sample suffering from retinoblastoma is 3 years with the youngest age suffering from retinoblastoma is 2 months but categorized in 0 years and the oldest age of samples suffering from retinoblastoma is 8 years. When studied further, as many as 68.42% of the total sample suffered from retinoblastoma at <3 years old. Retinoblastoma can occur unilaterally or bilaterally. Table 1 also presents data on retinoblastoma laterality. Of the total 19 samples, 17 samples suffered from unilateral retinoblastoma (89.47%) and 2 samples (10.53%) suffered from bilateral retinoblastoma.

Table 2. Histopathological Sample Features

Degree of differentiation	**n**	**%**
Well	3	15.79
Moderate	13	68..42
Poor	3	15..79
Optic nerve invasion	**n**	**%**
Positive	12	63.16
Negative	4	21.05
Difficult to determined	3	15.79
Sclera invasion	**n**	**%**
Positive	11	57.89

Negative	8	42.11

In this study, we assessed a histopathologic feature of a sample consisting of degrees of differentiation and invasion. Most samples were categorized as moderately differentiated, as many as 13 samples (68.42%) and there were 3 well differentiated samples (15.79%) and 3 other samples categorized as poorly differentiated (15.79%). In this study, 12 samples of retinoblastoma were invasive to the optic nerve (63.16%). A total of 21.05% or 4 samples showed no invasion to the optic nerve and 3 samples (15.79%) of the invasion were difficult to determined. And 11 samples of retinoblastoma were invasive to sclera (57.89%) and no invasion in 8 samples was 42.11%. In this study showed positive C-kit expression on 12 samples (63,16%) and 7 samples with negative C-kit expression (36,84%). Based on table 3, the results of positive C-kit expression were classified based on differentiation degree of well-differentiated by 3 samples from total of 12 samples (25.0%), moderately differentiated as many as 6 cases (50.0%) and poorly differentiated as many as 3 cases (25.0%). For negative C-kit expression results, the overall sample was classified as moderate differentiation (100%). In the sample with positive C-kit expression, the result of positive weak coloring category was 41.67% and moderate was 58.33%.

Table 3. Expression of C-kit on Differentiation Degree of Retinoblastoma Histopathology and various intensities and cell proportions

C-kit Expression	**Degree of Differentiation**					
	Well		***Moderate***		***Poor***	
	n	**%**	**n**	**%**	**n**	**%**
Positive	3	25%	6	50%	3	25%
Negative	0	0%	7	100%	0	0%

	Intensities and cell proportions					
	Negative		**Weak Positivity**		**Moderate Positivity**	
	n	**%**	**n**	**%**	**n**	**%**
Postive	0	0	5	41.67	7	58.33
Negative	7	100	0	0	0	0

This picture below shows us the results of histopathology and immunohistochemical examination :

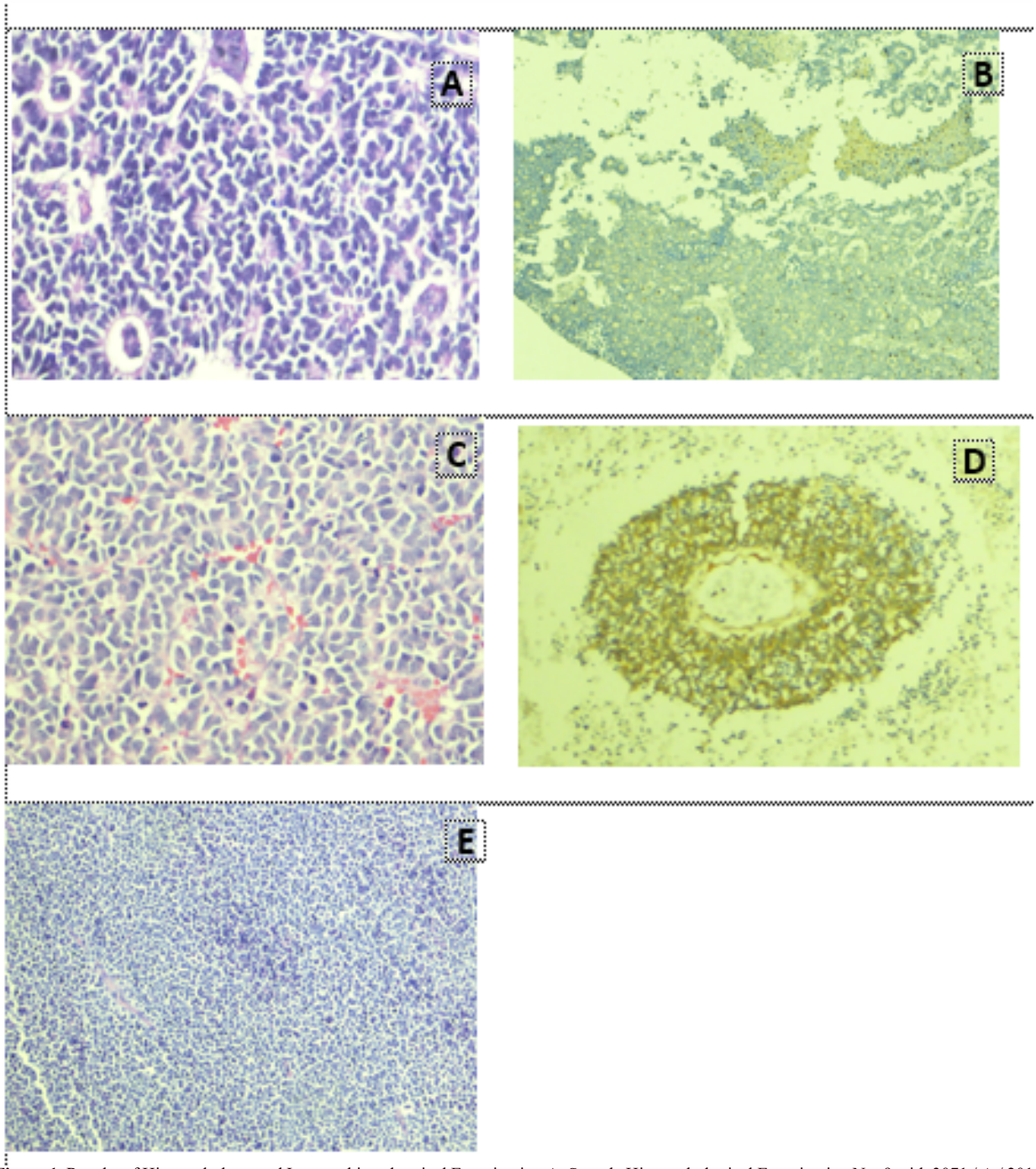

Figure 1. Results of Histopathology and Immunohistochemical Examination A. Sample Histopathological Examination No. 9 with 3071 / A / 2015 (Well-differentiated) availability. Hematoxylin-Eosin staining, 400x magnification. B. Examination of Immunohistochemical samples No.9 Expression of C-kit (+) with weak positive intensity. 100x magnification. C. Histopathological Examination of sample No.12 with no.supplies 3771 / A / 2014 (Moderately-differentiated). Hematoxylin-Eosin staining, 400x magnification. D. Immunohistochemical examination of sample No.12

Expression C-kit (+) with moderate positive intensity 100x magnification. E. Sample Histopathological Examination No. 2 with no.supply 506 / A / 2017 (Poorly-differentiated). Hematoxylin-Eosin staining, 400x magnification.

Here we present a histopathological picture of retinoblastoma with invasion to the optic nerve and sclera in the image below:

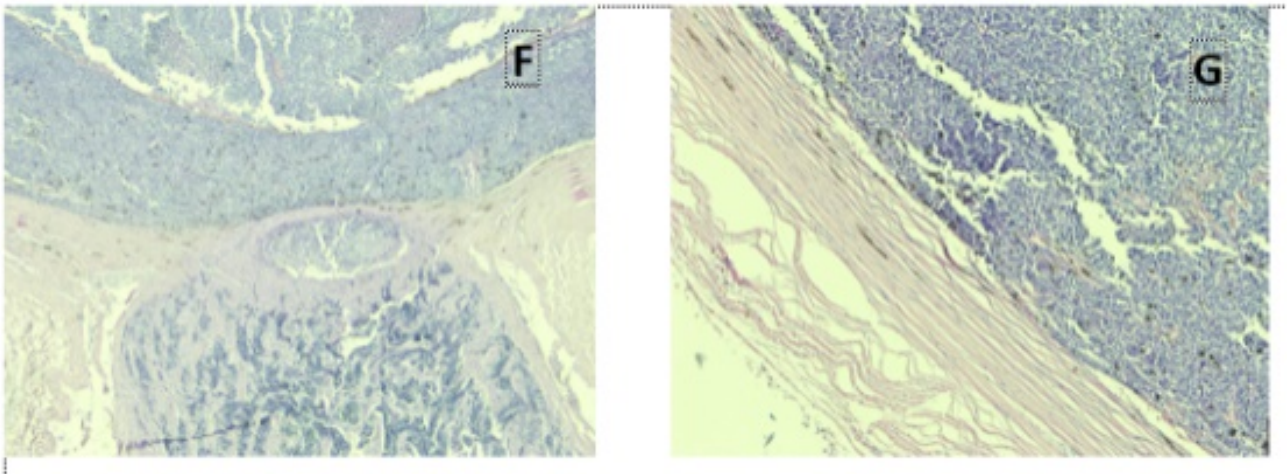

Figure 2. Histopathological Examination Result
F. Invasion of optic nerve
G. Sclera invasion on sample no.4 (1086/A/2016). Hematoxylin-Eosin staining, 40x magnification

4. DISCUSSION

Based on sex, the incidence of retinoblastoma in this study was 57,89% sample of male and female sex of 42,11%. The American Academy of Ophthalmology reports that there is no sex predilection for retinoblastoma. The incidence of retinoblastoma is reported almost the same among men and women. Research conducted by Youssef *et al* reported the incidence of retinoblastoma occurred in 57.1% of women and 42.9% occurred in men. This study was slightly different from that of Bosch *et al* reported in 95 immunohistochemical retinoblastoma samples, a sample of 29 males, 56 females and 9 samples of unrecognized gender. When viewed from the data above, the incidence of occurrence in women more.[1,2,11]

Most of the subjects of this study were 0-3 years old as much as 68.42% and over 3 years age of 31.58%. The mean age of the samples in this study who suffered from retinoblastoma was 2.47 years with the youngest age exposed to retinoblastoma was 2 months and the oldest age was 8 years. The results of this study are not much different from the research Youssef *et al*, that the occurrence of retinoblastoma most in the age range 3-48 months. Skuta *et al* states that about 90% of retinoblastoma cases are diagnosed in patients younger than 3 years. Shields *et al* describes retinoblastoma generally manifests at the age of the first 3 years of life. The average age at diagnosis is related to family history and laterality of the disease. Patients with a family history of retinoblastoma are commonly diagnosed at 4 months of age, patients with bilateral retinoblastoma are diagnosed at 12 months of

age and patients with unilateral retinoblastoma are diagnosed at 24 months of age.[1,2,9,12]

Assessed in terms of tumor laterality, retinoblastoma generally occurs unilaterally. In this study, 88.47% of samples were classified as unilateral retinoblastoma and 10.53% were bilateral retinoblastoma. This is supported by Youssef *et al* reporting as many as 87.5% of unilateral cases.[1,2,12] In addition, the age of patients with bilateral retinoblastoma in this study was 0 and 1 years (samples 3 and 9), so the data this is supported by a report from Skuta *et al* as mentioned in the previous discussion.2 When we examine theoretically, in 60-70% of cases are sporadic and non-hereditary due to somatic mutations clinically representing unilateral retinoblastoma. The rest (30-40%) are hereditary due to germinal mutation rates that cause bilateral retinoblastoma. In the results of this study, there were 2 bilateral retinoblastoma patients shown in samples no.3 and 9 with younger ages (0 and 1 years). [2,6,9,12]

Sample Histopathological Feature

In this study, the paraffin preparation of the retinoblastoma tissue block subjects stored in the Pattern Anatomy Pathology Department of RSMH Palembang was made in 4 micrometer thick pieces then painted with C-kit primary antibody ready to use by immunohistochemical technique. The degree of differentiation is then read under a microscope to observe various histopathologic features in the sample tissue. Assessment of degree of differentiation is done by observing the criteria for well-differentiated focus of Flexner-Wintersteiner rosettes or fleurettes, moderately differentiated in isolated Homer-Wright rosettes, whereas for the category of poorly differentiated in the absence of the above-mentioned structures. On examination of electron microscopy, Flexner-Wintersteiner rosettes describes primitive photoreceptor cells. Furthermore, the lumen rosette describes the same staining pattern as stem cells and cones, indicating that Flexner-Wintersteiner rosettes represents a specific form of retinal differentiation.[39] In this study, results were similar to those of Youssef *et al* (2014) reported that 48.2% of the samples were stratified in moderate differentiation, followed by 35.7% with poorly differentiated and 16.1% of well differentiated.[1] Retinoblastoma samples at RSMH Palembang showed differentiated histopathology differiated by 68.42%, followed by both differentiated and poorly differentiated which have the same percentage, that is 15.79% respectively. Differences were reported by Barry *et al* in 2007 of 63 cases, 7.94% belonging to well differentiated categories, 31.75% were moderately differentiated, and 60.32% or 38 out of 63 cases were poorly differentiated. 10 Several factors may be the cause of this difference, including sample age. Younger age is usually the degree of differentiation is still good or medium, while at an older age the degree of differentiation tends to be worse. Eagle *et al* explained that retinoblastoma with older age tends to be poorly differentiated, while more rosettes are clearly visible in younger children.[25,37] In a study conducted at RSMH Palembang, there were three samples with degrees differentiated poorly differentiated with older age (samples no.2, no.5 and no.16). In this study, also reported the results of invasion of retinoblastoma to optic nerve and sclera, respectively as much as 63.16% and 57.89%. This is almost similar to that of Youssef *et al* who reported that 46.4% of the sample had an invasion to the optic nerve and 62.5% of the samples had an invasion of the

sclera. In this study there are also 3 cases reported difficult to determine the level of invasion (samples no.8, no.12 and no.18). This may be due to the difficult optical nerve structure identified in histopathologic preparations. Laurie *et al* reported that invasion of retinoblastoma into the optic nerve may be caused by several changes related to cadherin- mediated adhesion of cells.[38,39]

Expression of C-kit on Different Degrees of Histopathology Differentiation and Retinoblastoma Invasion

In this study, we used positive C-kit controls on GIST (Gastrointestinal Stromal Tumors) with very high positivity (90-95%) and even other literature mentioned 100%. The role of C-kit has been found in more than 120 types of tumors by using immunohistochemical methods on TMA (tissue microarray). C-kit is generally found to be positive in GIST (100%), seminomas (84%), and adenoid-cystic carcinomas (65%) and in some other tumors also found positive C-kit expression, as well as retinoblastoma but in percentages smaller. [1,10,11,13-15] Sengupta *et al* reported that immunohistochemistry can confirm retinoblastoma by identifying photoreceptors and glial cells, but the exact mechanism remains unclear.[37]

Bosch *et al* (2005) conducted a study of immunostaining with C-kit antibodies in 73 retinoblastoma cases and was found to be positive in 14 cases of 19.2%, then re-classified as positive weak, moderate and strongly positive.[11] In this study C-kit expression was positive as much as 63,16%. This percentage is slightly larger than previous research conducted by Barry *et al* with a 52.38% positive C-kit expression on retinoblastoma and followed by Youssef *et al* of 48.2%. Bosch *et al* reported 19.2% of positive C-kit expression in retinoblastoma. This may occur due to Bosch *et al*'s research using TMA (tissue microarray analysis) which includes 3500 paraffin-embedded tumor samples for 120 tumor types so that only a small fraction (0.6 mm diameter) in each specimen compared to standard preparations. It may be possible that some areas of tumor belonging to the TMA can be negative while other areas may be positive.[1,11,15]

In this study, the results of positive C-kit expression were classified based on differentiation degrees of well-differentiated (25.0%), moderately differentiated (50.0%) and poorly differentiated (25.0%). This is supported by Youssef *et al* research obtained positive C-kit expression results in 20 of 36 degrees of differentiation of well and moderate as much as 55.6%. In the case of poorly differentiated obtained 35.0%. This is almost similar to Barry *et al* described positive C-kit expression in 14 of 25 moderate and well differentiated samples (56%) and 19 of 38 poorly differentiated (50%).[11]

The factors that may affect the positivity of C-kit expression results are tissue handling or whether there is a history of preoperative chemotherapy may affect the reading outcome. In this study, it can not be known the overall history of patient's chemotherapy whether pre-operative or not, this is due to incomplete medical record data. However, in 1 patient recorded the sample # 15 had a history of preoperative chemotherapy and obtained negative C-kit expression results. This is supported by a study by Youssef *et al* (2014) describing that patients undergoing preoperative chemotherapy may influence the interpretation of immunohistochemical results. Barry *et al* (2007) explains that immunohistochemical results are highly

dependent on specific techniques in tissue fixation used by each laboratory.[1,10,40]

Invasion is one of the things that need to be observed in cases of retinoblastoma. The histopathologically observed invasion features are represented by the criterion that there is an invasion of the optic nerve if tumor cells can be identified behind the cribrosa lamina and sclera invasion when tumor cells have infiltrated Bruch's membrane. Research conducted by Youssef *et al* (2014) describes tumor growth patterns in the presence of invasion into the optic nerve showing significant positive C-kit expression. It is concluded that C-kit represents a more aggressive retinoblastoma of growth with extensive invasion.[1]

Barry *et al* reported that of 33 samples with positive C-kit expression there were 31 samples showing an invasion image to the sclera and / or optic nerve. In this study, samples with positive C-kit showed 6 of 12 samples (50%) experienced invasion of the optic nerve and 7 of 12 samples (58.33%) experienced invasion of the sclera. Both of these studies provide a similar picture, ie most of the positive C-kit samples have a good invasion of the optic nerve and the sclera. These two networks are structurally very close to the retina so invasion of this tissue can help assess the severity of retinoblastoma and invasion of tumor cells into other tissues. The increased expression of this protein is due to uncontrolled spread of tumor cells to surrounding tissue.[10] This study has some limitations such as the limited number of samples contained in Mohammad Hoesin Palembang General Hospital, incomplete medical record data so as not to connect clinical complaints with histopathologic picture in more detail. Because of the limited number of retinoblastoma cases results in uneven distribution of proportionally each degree of histopathologic differentiation and invasion of retinoblastoma.

5. CONCLUSION

The degree of differentiation of retinoblastoma in this study is mostly in moderately differentiated category. The invasion of retinoblastoma to the optic nerve and sclera based on observations of histopathologic preparations. C-kit was expressed in most of retinoblastoma samples. It is necessary to do further research with larger number of samples with different degrees of retinoblastoma histopathologic differentiation to determine the association of C-kit expression in retinoblastoma cases.

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