Biomedical & Pharmacology Journal, December 2018. Vol. **11**(4), p. 2031-2036

Pathological Risk Factor Profile for Enucleated Retinoblastoma at Sanglah General Hospital

Putu Yuliawati[1]* and Ni Putu Ekawati[2]

[1]Department of Ophthalmology, Faculty of Medicine Udayana University, Sanglah General Hospital, Denpasar, Indonesia.
[2]Department of Pathology Anatomy, Faculty of Medicine Udayana University, Sanglah General Hospital, Denpasar, Indonesia.
*Corresponding author E-mail: putu.yulia@gmail.com

http://dx.doi.org/10.13005/bpj/1579

(Received: 25 June 2018; accepted: 27 September 2018)

Retinoblastoma is a primary intraocular tumor on childhood. It has a good prognosis with early detection. Aims: **Aim of this study is to know Pathological Risk Factors (PRF) profile of enucleated retinoblastoma at Sanglah General Hospital.** Methods: **This study is an observational retrospective study based on patient's medical record and pathological examination. Twenty patients were included in this study, 50% were female and 50% male within age ranging from 7 month – 12 years old. Unilateral retinoblastoma was found in 18 patients (90.5%), bilateral retinoblastoma was found in 2 patients (9.5%). Majority PRF profile found that cancer cell invasions was 60% choroid infiltration and 45% of anterior segment invasion, 60% of optic nerve infiltration, and 50.1% scleral invasion. Tumor cell aggregation showed 61.9% poorly differentiated malignant cells. None of the patients had family history of retinoblastoma. This study revealed optic nerve and choroid invasion were the major PRF found in retinoblastoma, but there is a significant association between scleral infiltration and tumor cell differentiation (p = 0.025). There was no statistical difference of PRF based on age, gender, and laterality.**

Keywords: Retinoblastoma, enucleated retinoblastoma, pathological risk factors, tumor differentiation.

Retinoblastoma is a primary intraocular malignancy that occurs most often in children with incidence of 1:20.000. Developed under 5 years old children, it mostly occurs in the first two years of life.[1,2,3] The incidence on both sexes are almost the same. Retinoblastoma patient life expectancy exceeds 95% in western countries and decreased to less than 50% in case of the extraocular spreading.[2,4]

Pathological Risk Factors (PRF) of the tumor is the result of eye enucleation pathology and associated with a higher risk of extraocular relapse.[5,6] For a hundred years, we have been observing two typical true rosettes in retinoblastoma in the form of Flexner- Wintersteiner (FW) and Homer Wright (HW) rosettes.[7] Pathological Risk Factors include the invasion of the anterior segment, minor choroid invasion, invasion of major choroid, pre lamina optic nerve invasion, post lamina optic nerve invasion, intra sclera invasion and trans sclera invasion. High-risk Pathological Risk Factor (HRF) includes major invasion of the choroid, pre and post lamina optic nerve invasion, and intra-trans sclera invasion.[7,8]

In Indonesia and other developing countries, diagnosis of retinoblastoma had only done based on clinical examination and general investigations such as ultrasound and CT Scan, instead of tumor pathology examination. Therefore it is necessary to confirm the results of anatomic pathology as gold standard in retinoblastoma cases for further management, determining the adjuvant therapy, prognostic factor, and determine the survival rate of retinoblastoma patients. [9,10] Patients with HRF must receive an adjuvant chemotherapy.[11]

This study purposeis to collect an overview of PRF and HRF in retinoblastoma patients in Sanglah General Hospital.

METHODS

This study is a retrospective descriptive study involving 20 cases of retinoblastoma that enucleated at Sanglah General Hospital and the laboratory results from Pathological Department Sanglah General Hospital. This study used consecutive sampling from 1st January 2014 until 31st December 2017. Qualified samples which fitted the inclusion criteria were collected. Incomplete data was excluded.

RESULT AND DISCUSSION

The study plan was approved from ethical committee of Udayana University/Sanglah General Hospital. There were 20 cases, retrieved from the previous medical record (2014-2017) and patient characteristics presented in Table 1.

The minimum age was seven months, while maximum age was 12 years (Age Range 7 months - 12 years) while the mean age was 3.03 ± 2.69 years. The higher frequency of retinoblastoma cases founded inaged 2 – 5 years old 13/20 (65.0%), while 4cases (20.0%) were found less than 2 years old. Most of the patients came from Bali (80.95%), only 3 patients (14.29%) were coming from West South East Nusa andone patient (4.76%) was coming from Timor Leste.

Sex and Family History

There were 10 female patients (50.0%) and 10 male patients (50.0%) suffering from Retinoblastoma involving in this study. The frequency on both sexes is almost the same with

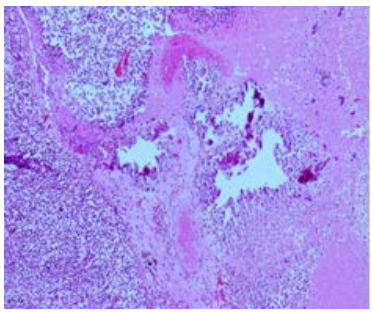

Fig. 1. The pathological risk of intratumoral necrosis and calcification

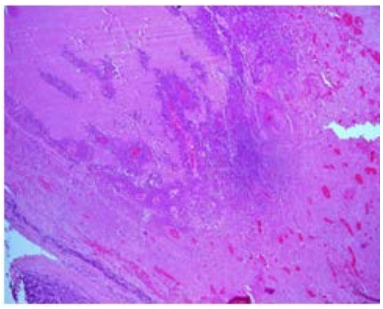

Fig. 2. Peritheliomatous pattern of tumor cells and neural invasion to the optic nerve

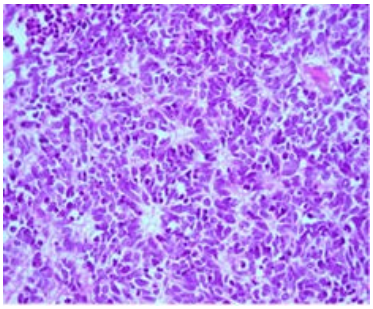

Fig. 3. Retinoblastoma with typical rosettes. Histomorphology was consistent with retinoblastoma

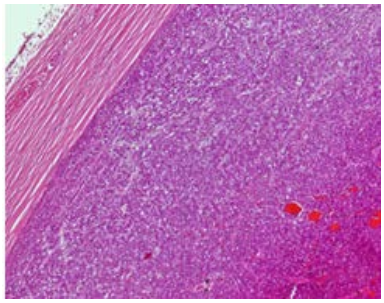

Fig. 4. Low power of dense masses of small round cells tumor in poorly differentiated retinoblastoma

male to female ratio was 1:1 (Table 1). There is no sex preference regarding retinoblastoma. From all patients, no one has family history of retinoblastoma.

Laterality

There were only 2 cases of bilateral retinoblastoma and 18 cases with unilateral neoplasms. The bilateral to unilateral neoplasm ratio was 1: 9. The age range of bilateral retinoblastoma was 1 - 2 years while the range in unilateral tumor was 7 month - 12 years old. In Unilateral cases, 6 patients (28.6%) were from right eye and 12 cases (57.1%) were from left eye. The percentage of bilateral retinoblastoma was 9.5%, and unilateral retinoblastoma was 90.5%. Extraocular invasion founded in 8 patients (35%)(Table 1).

Table 1. Characteristic of Enucleated Retinoblastoma Patient

Subject Characteristic	N (%)	Mean ±SD
Sex		
Female	10	-50.00%
Male	10	-50.00%
Age (years)		
< 2 years old	4	-20.00%
2 – 5 years old	13	-65.00%
> 5 years old	3	-15.00%
Age (years)		3.03 ± 2.69
Domicile		
Bali	16	-80.95%
West South East Nusa	3	-14.29%
Timor Leste	1	-4.76%
Laterality		
Unilateral	18	-90.50%
Bilateral	2	-9.50%
Stage		
Intraocular	12	-65%
Extraocular	8	-35%
Family history		
Yes	0	0%
None	20	-100%

Table 2. PRF Percentage and Tumor Differentiation

Pathology item	N (%)	
PRF		
Anterior segment invasion	9	-45.00%
Choroid invasion	12	-60.00%
Optic nerve invasion	12	-60.00%
Sclera invasion	10	-50%
Differentiation		
Well differentiated	3	-14.30%
Flexner-Wintersteiner rosettes	3	-14.30%
Homer – Wright rosettes	2	-9.50%
Undifferentiated	11	-61.90%

Differentiation

There was cell differentiation suspected as active malignant cells in microscopic readings for 17 patients and only 3 patients with well-differentiated cell. On review of pathological slides, 3 eyes (4.3%) had well-differentiated tumor, 3eyes (4.3%) had Flexner-Wintersteiner rosettes differentiation which is related to retinoblastoma, 2 eyes (9.5%) had Homer-Wright rosettes differentiation, 12 eyes (61.9%) had undifferentiated tumor.

Pathological Risk Factor

Pathological risk factor of retinoblastoma present in all enucleated eyes, including 9 cases (45.0%) of anterior chamber invasion, vitreous body involvement in 6 eyes (28.6%), 12 cases (60.0%) of massive choroid infiltration, 12 cases (60.0%) of optic nerve invasion, 10 cases (50.0%) of scleral invasion, and extraocular extension in 7 cases (33.3%). Majority of the samples had more than one PRF at the same time, which are 2 PRF present in 1 eye (5.3%), 3 PRF present in 3 eyes

Table 3. High-Risk PRF Based on Tumor Differentiation

High-risk PRF (HRF)	Tumor Differentiation	
	Well Differentiated	Malignant cells
Optic Nerve Invasion		
No	1	8
Yes	3	8
Total	4	16
Statistical Analysis	(p = 0.375)	
Major Scleral invasion		
No	0	10
Yes	4	6
Total	4	16
Statistical Analysis	(p = 0.025)	
Extraocular Invasion		
No	1	12
Yes	3	4
Total	4	16
Statistical Analysis	(p = 0.061)	

*(± = 0.05)

Table 4. Pathological Risk Factor Profile of Retinoblastoma Based on Age, Gender, and Lateralization

High-Risk PRF (HRF)	Range of Age (years)			Gender		Laterality	
	< 2	2 – 5	>5	Female	Male	Unilateral	Bilateral
Optic Nerve Invasion							
No	1	6	2	2	6	1	1
Yes	3	7	1	7	5	10	8
Total	4	13	3	9	11	11	9
Statistical Analysis	(p = 0.096)			(p = 0.535)		(p = 0.711)	
Major Scleral invasion							
No	2	6	2	3	6	8	2
Yes	2	7	1	7	4	10	0
Total	4	13	3	10	10	18	2
Statistical Analysis	(p = 0.815)			(p = 0.301)		(p = 0.237)	
Extraocular Invasion							
No	3	7	3	3	5	12	1
Yes	1	6	0	7	6	6	1
Total	4	13	3	9	11	18	2
Statistical Analysis	(p = 0.286)			(p = 0.271)		(P = 0.589)	

*(± = 0.05)

(15.8%), 4 PRF present in 4 eyes (2.05%), and more than five PRF present in 6 eyes (31.6%).

Raju *et al*. reported 21% of 76 enucleated eye in India has spread to the anterior segment, 54% with major choroid invasion, 46% with optic nerve invasion and 7% with sclera invasion and extra sclera extension.[12] Kashyap *et al*. found only 3% has anterior segment invasion, and the remaining cases (97%) had more than one PRF.[15]

This study found high-risk PRF in 20 patients (100%), all of them have optic nerve invasion. However, the optic nerve invasion did not divide into pre and post lamina because not listed in the pathological report. Inferential analysis showed that major scleral invasion has significant association with tumor cell malignancy (p = 0.025). The result of current study is similar with study conducted by Suryawanshi *et al*. which found the incidence of trans-scleral invasion was 23.4%, higher than other studies which are about 15%.[14] It can be caused by high stage retinoblastoma when enucleation performed.

Cuencha *et al*. divides the sclera invasion into two, namely the intra-sclera invasion if tumor cells are still confined within the sclera, not passing episclera, and trans-scleral invasion if tumor cells have penetrated the layers of the sclera and began to invade the periorbital tissue.[18]Pathology reports did not describe in detail whether the invasion was found intra-sclera or trans-sclera. Invasion of the sclera especially trans-sclera is an important risk factor as predictors of recurrence, metastasis, and death, so it is necessary to give more intensive adjuvant chemotherapy to improve survival for patient with trans-sclera invasion.[13,14]

Previous research also found many cases of extraocular relapse after enucleation in patients with scleral invasion as well as the pre and post lamina optic nerve invasion, so that both are approved as a high-risk PRF.[15] It suggests that the invasion of tumor cells in the post lamina optic nerve has the higher risk of metastases to the central nervous system through the subarachnoid space, it can even recur retinoblastoma in orbital.

Invasion of the choroid as predictors of extraocular relapse is still controversial. Font suggests the spread of tumor cells through the blood vessels of the choroid and ciliary can cause metastasis to the lungs, bones, and other tissues through the lymphatic system and blood-borne.[16] Khelfaoui *et al*. found four cases of extraocular relapse in 20 patients with major choroid invasion.[17] The incidence of choroid invasion on research by Suryawanshi *et al*. was 35.1%, higher than the research in Western countries, but research in developing countries showed that generally

around 23% - 32 %.[14] Suryawanshi *et al*. found no significant extra-ocular relapse in patients with major choroid invasion.[14]

The parameters of the pathology specimen examination play an important role to determine the stage of tumor growth and metastasis. It is useful in determining the prognosis and plan of adjuvant chemotherapy; it can also be used to evaluate the enucleation technique and examine whether the distance of the optic nerve resection far enough. Chantada, *et al*., (2004) found the identification of PRF is helpful in selecting patients who underwent adjuvant chemotherapy, so it can avoid not necessary adjuvant therapy that can cause complications such as myelosuppression, the risk of sepsis during chemotherapy, severe contractures of the orbital due to radiotherapy, and the risk of secondary malignancies due to chemotherapy and radiotherapy.[11]

Tumor cell differentiation varies among reports from various countries. Differentiation is divided into well-differentiated and poorly differentiatedt umors. Well-differentiated tumors give an image of rosettes and fleurettes. Filho *et al*. reported a high incidence of poorly differentiated tumors (80%) because of the age of the patient is older.[19] Yousef *et al*. found 74% of patients with well-differentiated tumors at a median age of 26 months.[20]Kashyap *et al*. also concluded that well-differentiated tumors are more common in younger patients.[21]Suryawanshi *et al*. reveal that poorly differentiated tumor is a useful predictor for prognosis, but the results were not statistically significant because of the small number of patients, so it is necessary to conduct studies with a considerable number of patients.[14]

All patients in recent study had adjuvant chemotherapy after enucleation, but the data recorded in the medical record was not completed makes it difficult to further follow up. Future research with long-term follow-up should be carried out to obtain useful information.

CONCLUSION

High-PRF of retinoblastoma present in all enucleated eyes (20 cases) in this study. There was cell differentiation suspected as active malignant cells in microscopic readings for 17 patients and only 3 patients with well-differentiated cell. More than one PRF were found in one sample at the same time. It revealed optic nerve and choroid invasion were the major PRF found in retinoblastoma, but, there is a significant association between scleral infiltration and tumor cell differentiation ($p = 0.025$). There was no statistical difference of PRF based on age, gender, and laterality. Further study with larger sample size is needed to investigate the PRF of retinoblastoma.

REFERENCES

1. Arif, Muhammad, Nasser, Farida, Khan,Mumtaz. Histopathological Features of Retinoblastoma. *KUST Med J*; **2**(1):19-23 (2010).
2. Marichelle, AI. 2010. Retinoblastoma. Cited atJune, 11, 2012. Available at: http://www.emedicine.medscape.com/article/1222849-overview.
3. Balmer, A., Zografos, L., and Munier, F. *A Review Diagnosis and CurrentManagement of Retinoblastoma*. *Oncogene*; **25**: 5341-5349 (2006).
4. American Academy of Ophthalmology staff (2010-2011a).*Ocular and PeriocularTumors in Childhood*. In: basic and clinical science course: Pediatric Ophthalmologyand Strabismus. Section 6. San Fransisco: *American Academy of Ophthalmology,* 390-399.
5. Mudita, I.B. Pola Penyakitdan Karakteristik Pasien Hemato-Onkologi BagianIlmu Kesehatan Anak Fakultas Kedokteran Universitas Udayana/ RSUPSanglah Denpasar Periode 2000-2005. *Sari Pediatri*; **9**(1):13-16 (2007).
6. Setiowati, S. 2006. Diagnosis and Management Retinoblastoma in Ciptomangunkusumo Hospital.*Update in Retinoblastoma and Pediatric Ophthalmology*. 9th Continuing Ophthalmology Education.March 4–5, *Jakarta:* 22–27 (2006).
7. Sedani, Ni Wayan. Karakteristikdan Gambaran Klinis Pasien Retinoblastoma di RumahSakitUmumPusatSanglah Denpasar. Tesis Program Pendidikan Dokter Spesialis Mata pada BagianIlmu Kesehatanmata Fakultas Kedokteran Universitas Udayana: Perpustakaan Prodi Kesehatan Mata (2014).
8. Chantada, G., Fineman, S.L., Sitorus, R.S., Kruger, M., Israels, T., Bakhshi, S., and Abramson, H. Clinical Practice Guidelines Siop-PodcRecommendations for Graduated-Intensity Treatment of Retinoblastoma in Developing Countries.*Pediatr Blood Cancer*; **60**: 719–727 (2013).
9. Dunkel, I.J., Khakoo, Y., Kernan, N.A., Gershon, T., and Gilheeney, S. Intensive multimodality

therapy for patients with stage 4a metastatic retinoblastoma. *Pediatr Blood Cancer*; **55**: 55-9 (2010).

10. Eagle, Ralph C. High-Risk Features and Tumor Differentiation in Retinoblastoma: A Retrospective Histopathologic Study. *Arch Pathol Lab Med*: **133**: 1203-1209 (2009).
11. Chantada, G.L., Dunkel, I.J., Davila, M.T.G., and Abrasam, D.H. Retinoblastoma patients with high risk ocular pathological features: who needs adjuvant therapy? *British Journal Ophthalmology*; **88**:1069-1073 (2004).
12. Raju, NSD., Panda, Anita., Maskati, Quresh., Verma, Lalit., Rao, Sambasiva., *et al.,*. Retinoblastoma: They Live and See!*AIOS CME Series*. India: All India Opthalmological Society (2012).
13. Soebagjo, Hendrian.,Husein, Farouk., Notobroto, HariBasuki., Sumitro, Sutiman B. Histopathologic Profile Grading of Haematoxylene Eosin on Retinoblastoma Stadium.*JurnalOftalmologi Indonesia*. **7**(5): 2011 (2011).
14. Suryawanshi, Pallavi.,Ramadwar, Mukta., Dikshit, Rajesh., Kane, Shubhada., Kurkure, Puna., A Study of Pathologic Risk Factors in Postchemoreduced, Enucleated Specimens of Advanced Reetinoblastomas in a Developing Country. *Arch PatholLab Med*; **135**: 1017-1023 (2011).
15. Kashyap, Seema.,Meel, Rachna., Pushker, Neelam. Clinical Predictors of High Risk Histopathology in Retinoblastoma. *Pediatr Blood Cancer* (2011) DOI 10.1002/pbc.
16. Font, Ramon.,Croxatto, Oscar., Rao, Narsing. *AFIP Atlas of Tumor Pathology. In Tumors of the Retina*.Series 4. Washington: American Registry of Pathology incollaboration with the Armed Force Institute of Pathology, 85-100 (2006).
17. Khelfaoui F, Validire P, Auperin A, *et al*. Histopathologic risk factors in retinoblastoma: a retrospective study of 172 patients treated in a single institution. *Cancer;* **77**(6):1206-13 (2016).
18. Cuencha, Adriana.,Giron, Fior., Castro, Daisy., Fandino, Adriana., Chantada, Guilermo. Microscopic Scleral Invasion in Retinoblastoma: Clinicopathological Features and Outcome. *Arch Opthalmol*. **127**(8):1006-1010 (2009).
19. Filho, JP., Correa, ZM., Odashiro, AN., Coutinho, AB., Martins.MC, Histopathological Features and Pglycoprotein Expression in Retinoblastoma: A Retrospective Histopathologic Study. *Arch Pathol Lab Med*; **133**: 1203-9 (2005).
20. Yousef, Yacoub.,Hajja, Yasmin., Nawaiseh, Ibrahim., Mehyar, Mustafa., Sultan, Iyad., Deebajah, Rasha., Rawashdeh, Khalil. A Histopathologic Analysis of 50 Eyes Primarily Enucleated for Retinoblastoma in a Tertiary Cancer Center in Jordan. *Turk Patologi Derg*. **30**: 171-177 (2014).
21. Kashyap, Seema.,Meel, Rachna., Pushker, Neelam., Bajaj, MS. A Histopathologic Analysis of 232 Eyes with Retinoblastoma Conducted in an Indian Tertiary Care Ophthalmic Center. *J PediatrOphthalmol Strabismus*. **40**: 265-7 (2012).