Original Article

Pathologic Risk Factor in Retinoblastoma: An Institutional Experience Based on Analysis of Enucleated Eyes

Anita Shah[1], Manisha Shrestha[2], Saurav Man Shrestha[3], Anadi Khatri[4], Prateek Krishna Shrestha[5]

[1]Universal College of Medical Sciences- Teaching Hospital, Bhairahawa, Nepal
[2]Patan Academy of Health Sciences, Lagankhel, Nepal
[3]B.P. Koirala Lions Centre for Ophthalmic Studies, Institute of Medicine, Tribhuvan University Teaching Hospital
[4]Birat Eye Hospital, Birat Medical College and Teaching Hospital, Biratnagar, Nepal.
[5]Nidan Hospital, Lalitpur, Nepal

Abstract

Background: Mortality resulting from the metastasis of retinoblastoma is uncommon in the developed world, however it still constitutes a major problem in developing countries like Nepal. The cases of retinoblastoma with increased risk of metastasis even after enucleation can be predicted from the histopathological examination of the enucleated specimen. We conducted this study aiming to assess the frequency and spectrum of high-risk histological features in enucleated specimens of retinoblastoma.
Materials and methods: Forty-two specimens of primary enucleation done for treatment of retinoblastoma received in the Department of Pathology at UCMS from 1st January 2016 to 31st December 2018 were included in the study. All slides were reviewed for high-risk histological features along with tumor differentiation, tumor extension, necrosis, and staging. Correlation of high-risk histological features with age and tumor size was calculated using unpaired t-test and correlation with tumor differentiation, necrosis and staging was done using Pearson Chi square test.
Results: The median age at enucleation was 24 months. All patients had endophytic lesion with a mean tumor size of 1.8cm. One or more high-risk histological features were identified in 30.9% (13/42). The most common high-risk histological feature was retrolaminar optic nerve invasion (10/12, 71.4%). Statistically significant correlation of high risk histological features was noted with tumor size (p=0.011) and AJCC stage of tumor (p=0.0001).

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Corresponding author
Dr. Manisha Shrestha
Patan Academy of Health Sciences, Lalitpur, Nepal.
E-mail: manishashrestha@pahs.edu.np

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Conclusion: Complete histopathological evaluation of retinoblastoma requires searching for high-risk histological features, the presence of which will guide the clinician in timely planning for subsequent neoadjuvant therapy.

Key words: Retinoblastoma; High risk histological features; Optic nerve invasion; Choroidal invasion

Introduction

Retinoblastoma is the most common intraocular tumor of childhood and represents 4% of all childhood malignancies. (Pam 2001) Retinoblastoma results from the mutation, inactivation or deletion of both copies of the retinoblastoma gene at the 13q14 locus. Knudson in 1971 proposed the "two hit hypothesis" for the development of heritable and non heritable forms of retinoblastoma. In the heritable form of retinoblastoma, inherited germline mutation occurs in the first copy of the retinoblastoma gene and the second somatic mutation occurs during development. In the non heritable or sporadic form, both the mutations are somatic and occur during development. (Knudson et al. 1976) Approximately 4% of newly diagnosed cases of retinoblastoma are inherited and 96% are sporadic. (Shields & Shields 2004)

The major cause of mortality in both hereditary and non- hereditary forms of retinoblastoma is metastasis. Although mortality resulting from the metastasis of retinoblastoma is uncommon in the developed world with the survival rate of 95%, it still constitutes a major problem in the developing nations with only 50% survival rate.(Cozza et al. 2009) The patterns of metastasis in retinoblastoma include trilateral retinoblastoma, regional metastasis, extension to the central nervous system and distant metastasis.(Jubran et al. 2004) For vision salvageable cases, enucleation is the preferred method of treatment and adjunctive post-enucleation chemotherapy is given in cases with metastatic disease. A careful histopathological examination of these enucleated specimens can predict the increased risk of metastasis in cases of retinoblastoma even after enucleation and can help in planning of treatment. The high-risk histological features which are known to predict increased risks of metastasis and mortality in retinoblastoma include tumor invasion of the optic nerve (Shields et al. 1994), choroid (Shields et al. 1993), and anterior chamber. (Haik BG et al.1987)

As with any histopathological specimen, pathologists are the very first to identify any of these high-risk histological features on enucleated specimens. Thus, communication between the pathologist and the clinician/ oncologist who are treating the patients is of utmost importance as it aids in the timely planning of post enucleation adjuvant therapy, preventing metastasis and saving lives. (Kaliki S et al. 2011) This approach has led to the fall in rates of metastasis and mortality in retinoblastoma patients even after enucleation of the affected eye, leading to better outcomes in these patients. (Honavar et al. 2002)

In developing countries like Nepal, many patients do not have access or affordability to routinely evaluate for metastasis. In this condition, the role of identifying high-risk histological features in enucleated specimens of retinoblastoma is even more important. We conducted this retrospective study aiming to evaluate the incidence and the spectrum of the high-risk histological features in enucleated specimens of retinoblastoma and its correlation with age, tumor size, tumor differentiation, necrosis and American Joint Committee on Cancer (AJCC) staging.

Materials and methods

All histopathological specimens of primary enucleation done for the treatment of

retinoblastoma, received in the Department of Pathology at Universal College of Medical Sciences from 1st January 2016 to 31st December 2018 were included in the study. The cases receiving any chemotherapy, laser therapy or radiation therapy were excluded. Records of the patients including age, gender, laterality of the tumor, gross features of the tumor which included tumor size and growth pattern (exophytic, endophytic, or combined) were analyzed from the archives of the Pathology Department. Hematoxylin and eosin–stained slides prepared from formalin-fixed, paraffin-embedded sections were re-evaluated. All slides were reviewed for tumor differentiation (moderately differentiated, poorly differentiated, retinocytoma), tumor extension (limited to posterior chamber, involving anterior, posterior chamber and uveal tract, involving posterior chamber and uveal tract, involving extraocular muscles), necrosis (mild, extensive, no necrosis), pathologic staging as per the AJCC staging criteria, 8th edition (Anon 2018), choroidal invasion (focal choroidal invasion, massive choroidal invasion, absent), optic nerve invasion (prelaminar, laminar, retrolaminar), and optic nerve resection margin status by a single pathologist to maintain uniformity and consistency. The criteria for high-risk histological features and definitions for "optic nerve invasion" and "choroidal invasion" were adopted from the International Retinoblastoma Staging Working Group (IRSWG) consensus guidelines. (Sastre et al. 2009) The high-risk histological features are defined as the presence of one or more of the following:

- retrolaminar optic nerve invasion,
- massive choroidal invasion,
- combination of pre- laminar or laminar optic nerve invasion and focal choroidal invasion or tumor invasion into the anterior chamber.

Permission was obtained from the ethical committee and the study adheres to the tenets of Helsinki. Data entry and statistical analysis was done using SPSS version 24.0 statistical software. Correlation of high-risk histological features with age and tumor size were calculated using unpaired t- test for equality of means. Pearson Chi square test was used to evaluate the relationship of high-risk histological features with tumor differentiation, necrosis and AJCC stage. A p value less than 0.05 was considered statistically significant.

Results

A total of 42 enucleated eyes with retinoblastoma were included in the study. The median age at presentation was 24 months with age range from 4 months to 9 years and mean age of 31.2 (+/- 19.08) months. There was male preponderance (n= 25, 59.5%). The right eye was slightly more involved (n= 22, 52.3%) and grossly all cases had an endophytic lesion. The size of the tumor ranged from 0.6 cm to 3cm with a mean tumor size of 1.8cm and 83.3% of the cases with tumor size more than 1.5cm.

The histopathological findings are illustrated in Table 1. The presence of one or more high-risk histological features was identified in 30.9% cases (13/42). The most common high-risk histological feature was retrolaminar optic nerve invasion. Solely retrolaminar optic nerve invasion was noted in 9 cases (21.4%) and solely massive choroidal invasion was noted in 1 case (2.3%). One case (2.3%) had both retrolaminar optic nerve invasion and massive choroidal invasion. One case (2.3%) had both laminar optic nerve invasion and focal choroidal invasion, and one case (2.3%) had both focal choroidal invasion and tumor invasion of tumor into the anterior chamber. All the cases with the presence of at least one high-risk histological feature were congregated into a single group and correlated with the other variables.

Correlation of high-risk histological features showed statistically significant positive correlation with tumor size (p=0.011) but not with the age of the patients (p=0.067).

The high-risk histological features showed a statistically significant correlation with the AJCC stage (p=0.0001). However, this was not noted with tumor differentiation and tumor necrosis (p=0.078, 0.716 respectively). Age at enucleation did not significantly correlate with tumor differentiation. Additional finding of intra-tumoral suppuration was noted in 3 cases (7.1%) and other findings of pigmentation, calcification, fibrosis and lymphoid aggregates were noted in 1 case (2.4%) each respectively.

Table 1: Histopathological details of enucleated eyes with retinoblastoma (N=42)

S.N.	Histopathological findings	n (%)
1.	**Tumor extent**	
	Limited to posterior chamber	34 (80.9)
	Involving anterior, posterior chamber and uveal tract	4 (9.5)
	Involving posterior chamber and uveal tract	3 (7.1)
	Involving extraocular muscles	1 (2.3)
2.	**Tumor differentiation**	
	Moderate differentiation	36 (85.7)
	Poor differentiation	6 (14.2)
3.	**Necrosis**	
	Mild	18 (42.8)
	Extensive	14 (33.3)
	No necrosis	10 (23.8)
4.	**AJCC stage**	
	pT1	32 (76.1)
	pT2	1 (2.3)
	pT4	9 (21.4)
5.	**Choroidal invasion**	
	Present • Focal choroidal invasion • Massive choroidal invasion	14 (33.3) • 12 (85.7) • 2 (14.2)
	Absent	28 (66.6)
6.	**Optic nerve invasion**	
	Present • Retrolaminar invasion • Laminar invasion	14 (33.3) • 10 (71.4) • 4 (28.5)
	Absent	28 (66.6)
7.	**Optic nerve resection margin**	
	Free of tumor	33 (78.5)
	Positive for tumor	9 (21.4)

Discussion

Retinoblastoma has a worldwide annual incidence of one case per 15,000 to 20,000 live births. (Parkin et al. 1988) Many decades ago, it was considered to be fatal but now with advances in the field of medicine and early detection, the survival approaches up to 95% at major centers in developed countries. (Shields et al. 2006) The primary goal in the management of retinoblastoma is the survival of the patient and preservation of the globe. The treatment options include intravenous chemoreduction, transpupillary thermotherapy, chemotherapy, laser photocoagulation, plaque radiotherapy, external beam radiotherapy, enucleation, exenteration, and systemic chemotherapy for metastatic cases. (Shields & Shields 2004)

In the present study, one or more high-risk histological features were present in 30.9% of the enucleated specimens for retinoblastoma. Another study from Nepal showed massive choroidal invasion in 20% and post- laminar optic nerve invasion in 8% cases. (Karki et al. 2015) Similar studies by Gupta et al (Gupta et al. 2009) and Biswas et al (Biswas et al. 2003) from India found high-risk histological features in 54.2% cases and 29% cases respectively. In contrast, Eagle et al (Eagle 2009) from USA reported 18.5% cases to have high risk features. Another study from the USA (Uusitalo et al. 2001) reported an incidence of 9.3% for massive uveal invasion and 11.6% for retrolaminar optic nerve invasion. In a developing country like Nepal, much of the population is still uneducated and live below the poverty line. They present to the hospital at an advanced stage of disease which is supported by this study where the age at enucleation is higher compared to the western world. This could also be an important factor responsible for relatively higher incidence of high-risk histological features in enucleated specimens for retinoblastoma in developing countries like ours. The mean age of the patients at the time of enucleation in this study was 31.2 months, which is similar to another study done in Nepal where the mean age at enucleation was 34.32 months. (Karki et al. 2015) Gupta et al. (Gupta et al. 2009) from India reported a mean age of 29.74 months at the time of enucleation. Studies done by Eagle et al (Eagle 2009) and Shields et al (Shields et al. 1994) from USA reported mean ages of 29.5 months and 23 months at time of enucleation. The mean age of patients with high-risk histological features in our study was 38.4 months. In comparison, Kashyap et al (Kashyap et al. 2012) reported that age more than 24 months at presentation was significant for predicting the presence of high-risk histological features.

Our study showed a statistically significant association of tumor size with the presence of high-risk histological features. However, Shields et al (Shields et al. 1994; Shields et al. 1993) did not find tumor size to be a predictor of high-risk histological features. This could be because 83.3% of cases in our study had tumor size more than 1.5cm, whereas in the study by Shields et al (Shields et al. 1994; Shields et al. 1993) only 41.4% cases have tumor size greater than 1.5cm. Our patients presented at a later stage of disease with a larger tumor size which had already extended beyond the retina.

Studies have shown an inverse relation between tumor differentiation and age at enucleation. (Eagle 2009; Kashyap et al. 2012) Tumors enucleated at an earlier age showed numerous rosettes (which is a feature of tumor differentiation) whereas progressive loss of rosettes (feature of poor differentiation) accompanied increasing age of enucleation. Our study did not find a statistically significant correlation between age at enucleation and degree of tumor differentiation. Kashyap et al (Kashyap et al. 2012) reported poorly differentiated tumors to be associated with simultaneous presence of three of more high-risk histological features. They also reported

massive necrosis to be associated with two or more high-risk histological features. An increased association of massive necrosis with high-risk histological features was also noted in our cases, however it was statistically not significant.

This study also showed retinoblastoma with a higher AJCC stage to be associated with the presence of high-risk histological features, supporting the theory that tumors at a higher stage have greater chances of metastasis. However, an important finding in this study was that 25% of cases with high-risk histological features were at pT1 stage, which shows that tumors with lower AJCC stage can also have risk of metastasis. This should alert pathologists to search for the presence of high-risk histological features in enucleated specimens of retinoblastoma even in cases with low AJCC stage. The treating clinician should also be vigilant regarding the risk of metastasis in these cases.

Conclusion

This study re-emphasizes the importance of the pathologist to carefully evaluate high-risk histological features in enucleated specimens for retinoblastoma in spite of lower AJCC stage and communicate the findings in time to the clinicians to determine the appropriate post-enucleation adjuvant therapy. Timely management could lead to higher chances of metastasis free survival and improve the quality of life in these young retinoblastoma patients.

Limitation of the study

The total number of retinoblastoma cases in this study is relatively small in number. We did not have access to the clinical details of the patients as all the cases were referred to our center for histopathological examination only, and the ophthalmological examination and enucleation was performed in a different center. Hence, the study did not include clinical details and correlation of the same. The status of metastasis was not evaluated as this is beyond the scope of the study.

References

Anon(2018). TNM8: The updated TNM classification for retinoblastoma. *Community Eye Health Journal*, 31(101), pp.34–34.TNM8: PMID: 29915471; PMCID: PMC5998398.

Biswas, J. et al., (2003). Histopathologic analysis of 232 eyes with retinoblastoma conducted in an Indian tertiary-care ophthalmic center. *Journal of Pediatric Ophthalmology and Strabismus*, 40(5), pp.265–267. PMID: 14560832.

Cozza, R. et al., (2009). Metastatic retinoblastoma: Single institution experience over two decades. *British Journal of Ophthalmology*, 93(9), pp.1163–1166. DOI:10.1136/bjo.2008.148932

Eagle, R.C.J., (2009.) High-Risk Features and Tumor Differentiation in Retinoblastoma. *Arch Pathol Lab Med*, 133(8), pp.1203–1209. doi: 10.1043/1543-2165-133.8.1203. PMID: 19653710.

Gupta R, Vemuganti GK, Reddy VA, Honavar SG. Histopathologic risk factors in retinoblastoma in India. Arch Pathol Lab Med.(2009) Aug;133(8):1210-4. doi: 10.1043/1543-2165-133.8.1210. PMID: 19653711.

Haik BG, Dunleavy SA, Cooke C, Ellsworth RM, Abramson DH, Smith ME, K.Z., (1987.) Retinoblastoma with anterior chamber extension. *Ophthalmology*, 94(4), pp.367–70.

Honavar SG, et al (2002). Post Enucleation adjuvant therapy in high-risk retinoblastoma. Arch Ophthalmol. 2002 Jul;120(7):923-31. doi: 10.1001/archopht.120.7.923. PMID: 12096963

Jubran, R.F. et al., (2004.) Approaches to Treatment for Extraocular Retinoblastoma: Children's Hospital Los Angeles Experience. *Journal of Pediatric Hematology/Oncology*,

26(1), pp.31–34. DOI: 10.1097/00043426-200401000-00011.

Kaliki S, Shields CL, Shah SU, Eagle RC Jr, Shields JA, L.A., (2011). Post Enucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. *Ophthalmology*, 129(11), pp.1422–7.doi: 10.1001/archophthalmol.2011.289. PMID: 22084213.

Karki, S. et al., (2015.) Retinoblastoma: An institutional experience. *Journal of Pathology of Nepal*, 5, p.723.https://doi.org/10.3126/jpn.v5i9.13780.

Kashyap, S. et al.,(2012). Clinical predictors of high risk histopathology in retinoblastoma. *Pediatric Blood and Cancer*, 58(3), pp.356–361. doi: 10.1002/pbc.23239. Epub 2011 Jun 30. PMID: 21721113.

Knudson, A.G. et al., (1976.) Chromosomal Deletion and Retinoblastoma. *New England Journal of Medicine*, 295(20), pp.1120–1123. doi: 10.1073/pnas.68.4.820. PMID: 5279523;

Pam, V., (2001) Ocular Tumours in Childhood. *Nigerian Journal of Surgical Research*, 3(1), pp.1–5.DOI: 10.4314/njsr.v3i1.12210

Parkin, D.M. et al., (1988). The international incidence of childhood cancer. *International Journal of Cancer*, 42(4), pp.511–520. doi: 10.1002/ijc.2910420408. PMID: 3170025.

Sastre, X. et al., (2009). Proceedings of the Consensus Meetings from the International Retinoblastoma Staging Working Group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. *Archives of Pathology and Laboratory Medicine*, 133(8), pp.1199–1202. doi: 10.1043/1543-2165-133.8.1199. PMID: 19653709.

Shields, C.L. et al., (1993.) Choroidal invasion of retinoblastoma: Metastatic potential and clinical risk factors. *British Journal of Ophthalmology*, 77(9), pp.544–548. doi:10.1136/bjo.77.9.544

Shields, C.L. et al., (1994). Optic nerve invasion of retinoblastoma. Metastatic potential and clinical risk factors. *Cancer*, 73(3), pp.692–698. PMID: 8299091.

Shields, C.L. et al., (2006.) The International Classification of Retinoblastoma Predicts Chemoreduction Success. *Ophthalmology*, 113(12), pp.2276–2280. PMID: 16996605.

Shields, C.L. & Shields, J.A., 2004. *Diagnosis and Management of Retinoblastoma*,

Uusitalo, M.S. et al., 2001. Evaluation of chemoprophylaxis in patients with unilateral retinoblastoma with high-risk features on histopathologic examination. *Archives of Ophthalmology*, 119(1), pp.41–48.PMID: 11146725.