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Clinical and histopathological correlation: A study on 334 eyes with retinoblastoma from Vietnam

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Abstract

Purpose: *To elucidate the clinical features which predict high-risk histopathological factors for subsequent metastatic disease as well as to report the incidence of these high-risk histopathological factors in a cohort of Asian patients with retinoblastoma.*

Design: *A retrospective and non-randomized sequential cases series.*

Methods: *A retrospective study was done on 334 eyes with retinoblastoma at Vietnam National Institute of Ophthalmology during a ten-year period (January 2004 – December 2013). All pathology specimens and medical records were reviewed and assessed for invasion and clinical signs.*

Results: *Among 334 eyes, 225 (67.4%) had high-risk retinoblastoma and 109 (22.6%) had non-high-risk features on histopathology. The high-risk histopathological features included anterior chamber seeding (48.2%), iris infiltration (14.7%), ciliary body involvement (14.1%), massive choroidal invasion (29.9 %), post-laminar optic nerve invasion (21.2%), optic nerve margin involvement (9.6 %), combined choroidal and optic nerve invasion (9.6%), scleral invasion (3.3%), and extra-scleral infiltration (11.4%). The significant clinical features in the high-risk group versus the non-high-risk group included hyphema (19.6% vs 3.7%, $p < 0.001$), pseudohypopyon (19.1% vs 6.4%, $p = 0.001$), iris neovascularization (25.3% vs 5.5%, $p < 0.001$), vitreous seeding (72.4% vs 37.6%, $p < 0.001$), staphyloma (24% vs 4.6%, $p < 0.001$) and scleritis (20% vs 3.7%, $p < 0.001$).*

Conclusions: *Clinical signs including hyphema, pseudohypopyon, iris neovascularization, vitreous seeding, staphyloma and scleritis were significantly associated with high-risk features on histopathology. Globe preserving methods should be used with caution in patients with these signs.*

Keywords*: Retinoblastoma, clinical, histopathological, high-risk*

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Introduction

Retinoblastoma (Rb) is the most common malignant intraocular tumor in children with an incidence of one in 15000-20000 live births.[1] In developed countries, the cure rate for children with Rb is greater than 95%.[2] However, the success rate is limited to less than 50% in developing countries because of late diagnosis and insufficient treatment.[3] The use of adjuvant chemotherapy after enucleation has been associated with increased survival of patients at risk of metastasis. Studies have shown that histopathologic factors may predict high-risk metastatic tumors. Those can be listed as anterior chamber seeding, iris invasion, ciliary body infiltration, massive choroidal infiltration, post-laminar optic nerve invasion, optic nerve margin involvement, combined choroidal and optic nerve infiltration, and sclera/extra-scleral invasion.[4-6]

In this study, we elucidate the clinical features which predict high-risk histopathological factors (HRFs) for subsequent metastatic disease as well as to report the incidence of these HRFs in a cohort of 334 eyes from Asian patients primarily enucleated for Rb. This research was performed at the Vietnam National Institute of Ophthalmology, which is a national treatment center for patients with Rb from the North of Vietnam, representing one third of the national population (93.39 million people).

Methods

This was a retrospective and non-randomized sequential case series. The study included all eyes with clinical retinoblastoma signs which have been primarily enucleated and pathologically confirmed during a ten-year period (January 2004 – December 2013).

Histopathological specimens were reviewed and grouped as high-risk and non-high-risk for subsequent metastatic disease.[4-6] High-risk features on histopathology were defined as the presence of anterior segment involvement (including anterior chamber seeding, infiltration of iris or ciliary body) (Fig. 1), massive (≥ 3 mm) choroidal invasion, post-laminar optic nerve invasion, optic nerve margin involvement, combined non-massive choroidal and prelaminar/laminar optic nerve invasion, or scleral/extra-scleral infiltration.[4-6] Choroid invasion was divided into three groups: no choroid invasion, focal choroid invasion (less than 3 mm in the maximum diameter (width or thickness) and without touching the sclera), and massive choroid invasion (3 mm or more in maximal diameter; width or thickness and/or touches the inner surface of the sclera) (Fig. 2). Extent of optic nerve invasion was divided into four groups: no optic nerve invasion, pre-laminar or laminar optic nerve invasion, post-laminar optic nerve invasion, and optic nerve transection (Fig. 3).

Tumor growth pattern was also recorded. Exophytic growth was defined as tumor growing outwards from the outer surface of the retina into the sub-retinal space toward the choroid. Endophytic growth was defined as tumor growing towards the vitreous cavity. Some lesions demonstrated a combined endophytic-exophytic

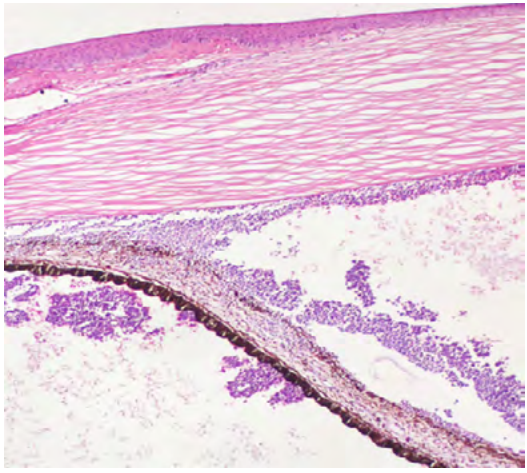

Fig. 1. Anterior segment invasion (HE stain, x40).

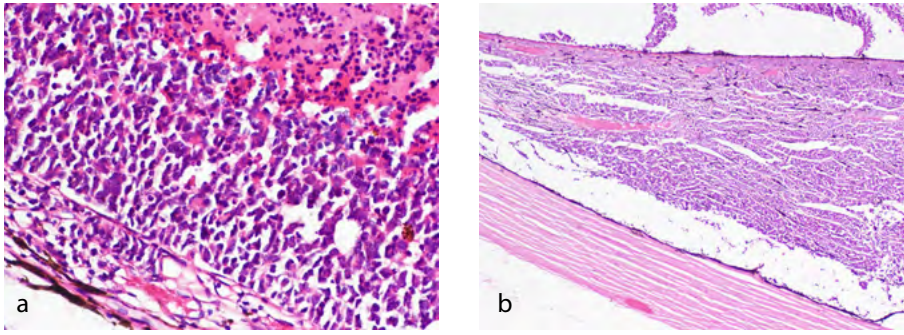

Fig. 2. Choroidal invasion. a (left). Focal choroidal invasion (HE stain, x100); b (right). Massive choroidal invasion (HE stain, x40).

pattern. Tumors were divided into two groups according to differentiation: differentiated and undifferentiated. Tumor differentiation was defined as appearance of with Flexner-Wintersteiner rosettes and/or fleurettes.[7] Tumor necrosis, calcification, and length of optic nerve were also noted.

Medical records were collected to review demographic and clinical signs. The demographic data included age at diagnosis (months), gender, hereditary pattern (sporadic or familial), and laterality. The clinical data included symptoms, intraocular pressure, hyphema, pseudohypopyon, dilated pupil, iris neovascularization, cataract, vitreous seeding, staphyloma, buphthalmos, phthisis bulbi, and scleritis at presentation.

Statistical analyses

Statistical analyses were performed using STATA 10.0 software. The Chi-square and Fisher's exact test were used to compare the qualitative data. A multivariate logistic-regression analysis was performed to identify the clinical predictors of HRFs on histopathology.

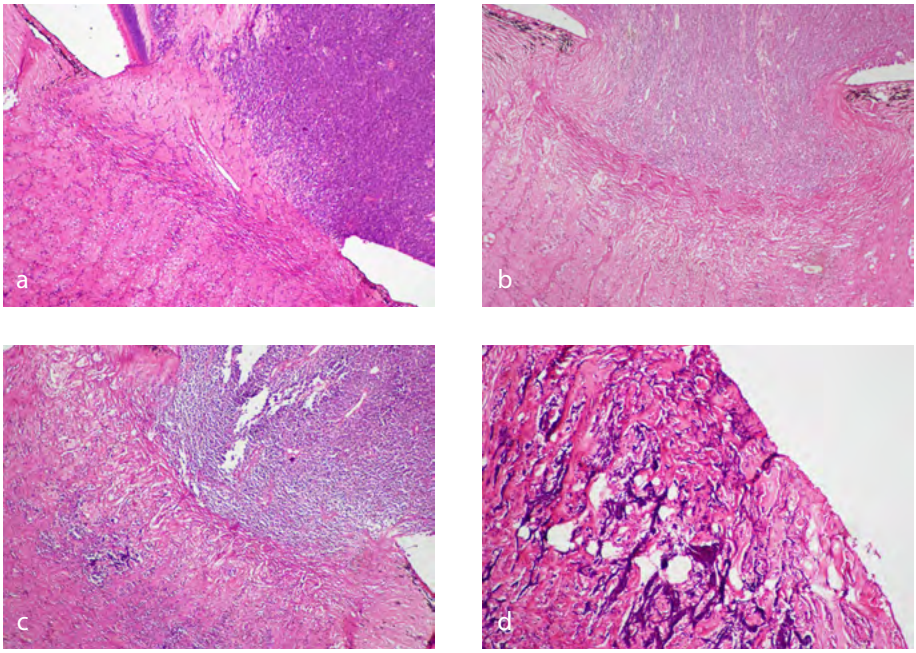

Fig.3. Optic nerve invasion (HE stain, x40). a. Prelaminar optic nerve invasion; b. Laminar optic nerve invasion; c. Poslaminar optic nerve invasion; d. Invasion of optic nerve transection.

Results

Basic demographics and clinical features

Overall, the median age of patients at diagnosis was 25.44 ± 15.84 months (25.56 ± 15.72 in high-risk versus 25.3 ± 15.36 months in non-high-risk group). Unilateral tumors were present in 80.5% of patients and bilateral disease was present in 19.5%. In the unilateral group, the right eye was involved in 54.7% cases and the left in 45.3% cases. Male/female ratio was 1.2 : 1. Ten patients (3.3%) had positive family history (eight in high-risk and two in non-high-risk group). All patients were of Asian racial background.

Leukocoria was the most common presenting symptoms in both groups, followed by decreased vision and strabismus. In medical consultation, vitreous seeding, staphyloma, and neovascularization iris are the most common clinical signs (Table 1).

Histopathological features

Histopathology details were available for all 334 primary enucleated globes. Of these 334 eyes, 225 (67.4%) had high-risk and 109 (32.6%) had non-high-risk features on histopathology.

Among high-risk cases, 101 (44.9%) eyes had one high-risk factor, 52 (23.1%) eyes

had two factors and 72 (32%) eyes had more than three factors. The histopathological high-risk features in these eyes included anterior chamber seeding (n = 161,48.2%), iris infiltration (n = 49, 14.7%), ciliary body involvement (n = 47, 14.1%), massive choroidal invasion (n = 100, 29.9%), post-laminar optic nerve invasion (n = 71, 21.2%), optic nerve margin involvement (n = 32, 9.6%), combined choroidal and optic nerve invasion (n = 32, 9.6%), scleral invasion (n = 11, 3.3%), and extra-scleral infiltration (n = 38, 11.4%). Among cases with microscopic residual disease, 32 (9.6%) involved optic nerve margin, 38 (11.4%) had extra-scleral soft tissue invasion. Length of optic nerve was about 5-8 cm in 71% cases. The data details are shown in Table 2.

There was no statistical difference between the proportion of tumor necrosis, tumor thickness, exophytic tumor and endophytic tumor in eyes with high risk compared to that in eyes without high risk features. The median tumor thickness was 15.89 mm in the high-risk group and 12.55 mm in the non-high-risk group. Overall, 155 of 334 cases (46.4%) had tumor calcification; corresponding figures in the high-risk and non-high-risk groups were 114 (50.7%) and 41 (37.6%), respectively. The rate of differentiated tumor was 48.9% (n = 110) for the high-risk group versus 41.3% (n = 45) for the non-high-risk group. Combined endophytic-exophytic growth presented in 78.7% (n = 177) versus 44.0% (n = 48) of eyes in high-risk and non high-risk cases, respectively (Table 1).

Correlation between histopathological and clinical features

The significant clinical features in the high-risk group versus non-high-risk group included hyphema (19.6% vs 3.7%, $p < 0.001$), pseudohypopyon (19.1% vs 6.4%, $p = 0.001$), neovascularization iris (25.3% vs 5.5%, $p < 0.001$), vitreous seeding (72.4% vs 37.6%, $p < 0.001$), staphyloma (24% vs 4.6%, $p < 0.001$), and scleritis (20% vs 3.7%, $p < 0.001$) (Table 1).

Clinical features that predict for individual histopathological high-risk factors are shown in Table 3. According to the collected data, clinical predictors for massive choroidal invasion included age more than two years at presentation, iris neovascularization, vitreous seeding, and buphthalmos. Age more than two years, staphyloma and cataract were significantly associated with post-laminar optic nerve invasion.

Discussion

There are three centers managing patients with Rb in Vietnam. This research was performed only at Vietnam National Institute of Ophthalmology, where children with Rb from the North of Vietnam were treated. Therefore, the study did not describe the whole picture of Rb in the country. Moreover, the absence of data on metastasis was also a limitation of this study. The study spanned ten years and 334 cases of Rb were reviewed. We included only primarily enucleated eyes and so the incidence of HRFs reported in our study represents the risk for metastasis in Rb at presentation unaffected by the effects of conservative interventions such as chemotherapy and laser. The mean age at diagnosis was 25.44 ± 15.84 months,

which was similar to other studies in developing countries,[8-10] but higher compared to those from the West.[11] Detecting high-risk features on histopathology is necessary because untreated high-risk retinoblastoma has a risk of systemic metastasis in 24% of patients and adjuvant chemotherapy reduces the risk to 0%-4%.[12,13]

In previous studies, the incidence of HRF cases varied from 18.5% to 41.4%.[4,5,14-17] In the current study, 67.4% cases had high-risk factors (Table 4). This has been attributed to a delay in presentation or diagnosis, which might be related to insufficient public awareness, lack of infant screening programs, and difficulties with access to medical services (*e.g*., long distances to travel to eye specialist services). Indeed we found it was not uncommon for some children with Rb to be taken to an herbalist instead of a hospital for primary treatment. Another issue we identified was artefact, which may have resulted in the significantly higher ratio of observed anterior segment invasion. Artefactual seeding is composed of small groups of tumor cells, usually with many necrotic cells present, inside natural spaces of the eye such as the anterior chamber.[6] Artefacts may occur at different stages in the routine collection of the tissues, fixation, processing, cutting, and staining of tissues. Therefore, great care is needed at every stage in the preparation of histology slides in order to get an accurate result.

In our series of 334 eyes, the clinical features predictive of high-risk retinoblastoma included hyphema, pseudohypopyon, neovascularization iris, vitreous seeding, staphyloma, and scleritis. In an analysis of 403 patients by Kaliki *et al.*, prolonged duration of symptoms of more than six months and secondary glaucoma might predict high-risk features on histopathology.[14] In another study of 326 enucleated eyes by Kashyap *et al.*, clinical predictors of high risk histopathology included age more than two years, lag period to diagnosis more than three months, hyphema, pseudohypopyon, staphyloma, and orbital cellulitis.[15]

In 2011, Kashyap *et al.* reported that predictors of post-laminar optic nerve invasion were older age at presentation, lag time to diagnosis greater than three months, and glaucoma. Meanwhile, predictors of massive choroidal invasion were cataract, staphyloma, and glaucoma.[15] Further, Shields *et al.* had found that raised intraocular pressure (IOP) and neovascularization iris (NVI) were predictors of choroidal invasion, while raised IOP, exophytic growth pattern, and tumor thickness more than 15 mm predicted optic nerve invasion.[18,19] In the current study, clinical predictors of massive choroidal invasion included age more than two years at presentation, neovascularization iris, vitreous seeding, and buphthalmos. Other signs such as age more than two years, staphyloma, and cataract were significantly associated with post-laminar optic nerve invasion.

In Northern Vietnam, the number of children presenting to our catchment center with Rb appears to be increasing (personal communication: hospital statistics since 2004). However, the management of these patients has been insufficient. Definitive diagnosis has relied upon detection of calcification using ultrasound and CT scan. MRI was rarely used to evaluate tumor invading eye structures. Lack of chemotherapy before and after enucleation may be associated with a poorer prognosis and increased mortality rate. Small tumors are particularly challenging,

as brachytherapy plaques and transpupillary thermo-therapy (TTT) are currently not available. Genetic analysis has not been a routine practice which made counselling and early diagnosis by screening nearly impossible. Additionally, there is currently no rehabilitation program available in this country, which is especially significant. Enucleations are generally performed without implant with resulting orbital deformity. Until recently, no telemedicine or tumor board meeting has been available to develop a multidisciplinary approach to retinoblastoma management in Vietnamese patients. With the advent of better information technologies at reduced cost, we are currently piloting a telemedicine approach through the Sydney Multidisciplinary Ocular Oncology MDT.

Conclusion

This is a retrospective clinico-pathological analysis of a large number of Asian eyes from Vietnam after primary enucleation for Rb. In our study, clinical signs including hyphema, pseudohypopyon, iris neovascularization, vitreous seeding, staphyloma and scleritis were predictors of high-risk features for metastasis on histopathology. Knowledge of these clinical correlates embedded within the framework of applying the formal international staging criteria[20] will assist ophthalmologists to better stratify patients to the most appropriate treatments, as these become available. These data suggest that within the context of current health care constraints within Vietnam; globe preserving methods should be used with caution in patients with these signs.

Conflict of interest

The authors declare that they have no conflict of interest.

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Tables

Table 1. Clinical features of 334 eyes with retinoblastoma.

Clinical and histopathological features		All eyes n = 334, n (%)	High-risk n = 225, n (%)	Non-high-risk n = 109, n (%)	P
Leukocoria		*280 (83.8)*	191 (84.9)	89(81.7)	0.16
Decreased vision		*48 (14.4)*	32 (14.2)	16 (14.7)	0.91
Strabismus		*42 (12.6)*	28 (12.4)	14 (12.8)	0.92
Eye pain		20 (6)	13 (5.8)	7 (6.4)	0.82
Red eye		15 (4.5)	12 (5.3)	3 (2.8)	0.2
Proptosis		13 (3.9)	10 (4.4)	3 (2.8)	0.4
Hyphema		48 (14.4)	44 (19.6)	4 (3.7)	*0.000*
Pseudohypopyon		50 (15)	43 (19.1)	7 (6.4)	*0.001*
Dilated pupil		19 (5.7)	14 (6.2)	5 (4.6)	0.4
Iris neovascularization		*63 (18.9)*	57 (25.3)	6 (5.5)	*0.000*
Cataract		23 (6.9)	16 (7.1)	7 (6.4)	0.55
Vitreous seeding		*204 (61.1)*	163 (72.4)	41 (37.6)	*0.000*
Elevated IOP		18 (5.4)	15 (6.7)	3 (2.8)	0.1
Buphthalmos		20 (6)	15 (6.7)	5 (4.6)	0.5
Staphyloma		*59 (17.7)*	54 (24)	5 (4.6)	*0.000*
Scleritis		49 (14.7)	45 (20)	4 (3.7)	*0.000*
Phthisis bulbi		2 (0.6)	2 (0.9)	0 (0)	-
Tumor calcification		155 (46.4)	114 (50.7)	41 (37.6)	*0.025*
Tumor necrosis		297 (88.9)	202 (89.8)	95 (87.2)	0.47
Differentiated tumor		155 (46.4)	110 (48.9)	45 (41.3)	0.19
Tumor growth pattern	Exophytic	67 (20.1)	44 (19.6)	23 (21.1)	0.74
	Endophytic	42 (12.6)	30 (13.3)	12 (11)	0.55
	Combined	225 (67.4)	177 (78.7)	48 (44.0)	*0.000*

Table 2. Histopathological high-risk factors of 334 eyes with retinoblastoma.

Histopathological features		Number (eyes)	Percentage (%)
Anterior chamber invasion	Anterior chamber tumor seeds	161	48.2
	Iris invasion	49	14.7
	Ciliary body invasion	47	14.1
Choroidal invasion	No	191	57.2
	Minor (< 3 mm)	43	12.9
	Massive(≥ 3 mm)	100	29.9
Scleral invasion	No	285	85.3
	Scleral infiltration	11	3.3
	Extrascleral involment	38	11.4
Optic nerve infiltration	No	48	14.4
	Prelaminar	118	35.3
	Laminar	65	19.5
	Postlaminar	71	21.2
	Optic nerve margin involvement	32	9.6

Table 3. Clinical predictors of Individual Histopathological High Risk Features (Multivariable Analysis, OR and 95% CI)

	AC	Iris Invasion	Ciliary body invasion	Massive choroidal invasion	Scleral invasion	Post-laminar ON invasion	ON cut end invasion	Extra scleral invasion
Age > 2years			0.3 (0.1-0.8)	0.3 (0.1-0.7)		0.7 (0.3-0.9)	1.6 (1.1-4.5)	
Hyphema	2.7 (1.2-48.7)	2.8 (1.1-89.8)	2.9 (1.1-130.7)					
Pseudohypopyon		1.5 (1.2-12.2)						
Dilated pupil			3.5 (1.1-11.2)					
Iris neovascularization		0.03 (0.00–0.3)		0.08 (0.003- 0.8)				
Cataract	3.6 (1.1-11.7)	3.7 (1.2-11.3)	4.3 (1.4-13.1)			4.7 (1.4-15.4)		
Vitreous seeding				1.3 (1.2-3.3)				
Elevated IOP	1,7 (1.5-5.8)							
Buphthalmos		5.4 (1.6-18.8)	6.0 (1.6-22.5)	17.3 (3.6-82.3)			11.1 (2.4-53.7)	12.7 (3.2-49.6)
Staphyloma					5.1 (1.7-37.9)	1.8 (1.3-12.4)		
Scleritis					17.1 (3.9-213.8)			
Phthisi bulbi								

Table 4. Comparison of incidence (%) of high risk factors in various reported series

Author	Country	Year	Number of eyes	Median age ate diagnosis (months)	AC invasion	Iris invasion	Ciliary invasion	Massive choroidal invasion	Scleral invasion	Post laminar ON invasion	ON cut end invasion	Extrascleral invasion
Kaliki *et al.*[14]	India	2015	403	27	6	3	4	17	5	18	1	2
Yousef Y *et al.*[16]	Jordan	2014	50	30	14	28	6	18	0	14	0	0
Kashyap *et al.*[5]	India	2012	609	30	10	10.7	6.7	24.6	13.7	16.1	7.4	4.1
Kashyap *et al.*[15]	India	2012	326	24	7	9	7	22	9	17	5.5	3.4
Eagle *et al.*[17]	US	2009	387		8			8.4		10.4	0.3	
Current study	Vietnam	2015	334	25.44	48.2	14.7	14.1	29.9	3.3	21.2	9.6	11.4