Histopathologic Risk Factors in Retinoblastoma in India

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• ***Context.*—The presence of histopathologic risk factors is associated with development of metastasis in a patient with retinoblastoma. Adjuvant chemotherapy administered to such patients decreases the risk of metastasis.**

***Objective.*—To analyze the incidence of histopathologic risk factors in our patient population and the clinical predictors of such risk factors.**

***Design.*—This is a retrospective case series, with review of clinical data and histopathologic slides, in 142 consecutive eyes enucleated for retinoblastoma between 1996 and 2002.**

***Results.*—Histopathologic risk factors were present in 54.2% of 142 eyes enucleated for retinoblastoma and included infiltration of iris (7%), ciliary body (9%), choroid (40%), optic nerve lamina cribrosa (11%), retrolaminar optic nerve (17%), optic nerve to the line of transection (8%), sclera (9%), and extrascleral structures (6%). On univariate analysis, histopathologic risk factors correlated with age greater than 24 months at presentation and with glaucoma and iris neovascularization at presentation. On multivariate logistic regression analysis, age greater than 24 months and iris neovascularization correlated with infiltration of the choroid, while iris neovascularization correlated with infiltration of the retrolaminar optic nerve.**

***Conclusion.*—Histopathologic risk factors are present in a significant proportion of patients enucleated for retinoblastoma and have identifiable clinical predictors.**

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Retinoblastoma is the most common primary ocular malignancy in children. While it is possible to salvage the eye and optimize the residual vision in less advanced tumors, enucleation is still the primary treatment of choice for advanced unilateral retinoblastoma. Enucleation is also the standard approach in patients with neovascularization of iris, secondary glaucoma and anterior chamber seeds, and in those who have failed conservative therapy.

Systemic metastasis occurs in less than 10% of patients with retinoblastoma in developed countries.[1] Postmortem studies have shown that central nervous system, orbital and cranial bones, long bones, and other viscera are the common sites for metastases.[1] Known risk factors for systemic metastasis include involvement of the choroid, orbital tissues, and retrolaminar optic nerve and involvement of the optic nerve to the line of transection.[2–8] The need to identify histopathologic risk factors (HRFs) for metastasis after enucleation and to provide appropriate adjuvant therapy has been emphasized.[9]

The incidences of HRFs and metastasis are likely to be higher in developing countries where patients generally present with advanced disease. However, there are no data available on the exact incidence of HRFs in eyes enucleated for retinoblastoma in developing countries. We reviewed our series of patients to determine the frequency of HRFs and analyzed the related clinical features that may predict HRFs.

METHODS

We retrospectively reviewed the records of all patients treated by us for retinoblastoma between January 1996 and February 2002. Those undergoing enucleation were included in the analysis after approval from the institutional review board. We excluded patients whose histopathologic slides were not available for review. These were patients seen by us before 1996 and those who had undergone enucleation elsewhere and were subsequently managed at our institute. As our patients include referred patients from all over the country, we believe the patients are likely to be representative of patients with retinoblastoma in India.

Patient data recorded were age and sex. The ocular features noted were laterality of the tumor, intraocular pressure, neovascularization of iris, presence of cataract, presence of massive tumor, or proptosis. We define massive tumor to be a tumor mass occupying more than 75% of the globe clinically or as observed by B-scan ultrasonography. The tumor characteristics noted included Reese-Ellsworth group, exophytic or endophytic tumor, presence of vitreous seeds, and presence of subretinal fluid. Details of chemotherapy regimen and dosage of radiation were noted if salvage of the globe was attempted before enucleation.

Routine histopathologic sections were processed and stained with hematoxylin-eosin. All histopathologic slides were reviewed for tumor differentiation and extent of ocular involvement. Tumors were classified as differentiated (presence of Flexner-Wintersteiner rosettes), undifferentiated, or necrotic. Involvement of the anterior segment structures were noted as invasion of anterior chamber angle, iris, or ciliary body (Figure 1). Choroidal invasion was categorized as massive if greater than 3 mm in diameter and/or with full thickness involvement of the choroid (Figure 2) or minimal if 1 to 3 mm in diameter and with partial thickness. The optic nerve invasion was judged as prelaminar, laminar, or retrolaminar (Figure 3) and to the line of transection of the nerve (Figure 4). Invasion of the sclera or any extraocular extension was noted (Figure 5). Cerebrospinal fluid (CSF) cytology and bone marrow biopsy were advised for all patients with infiltration of

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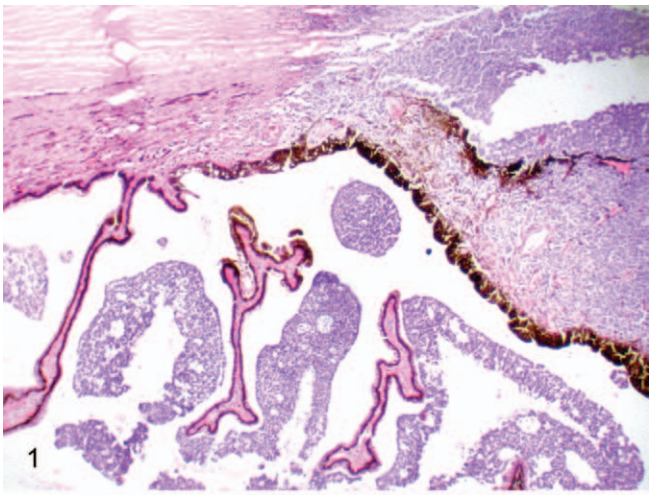
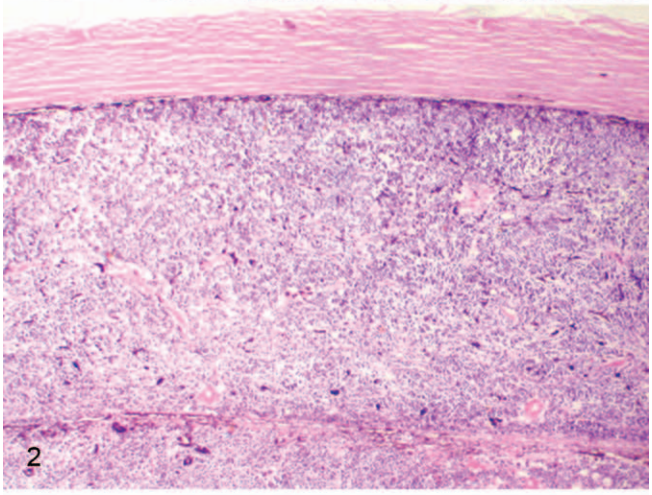




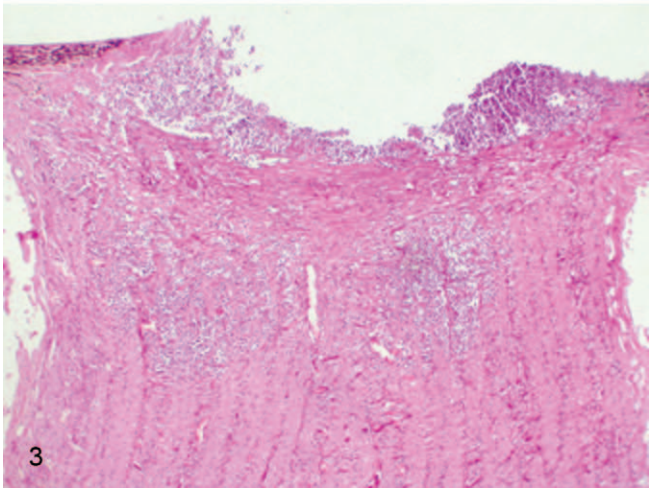
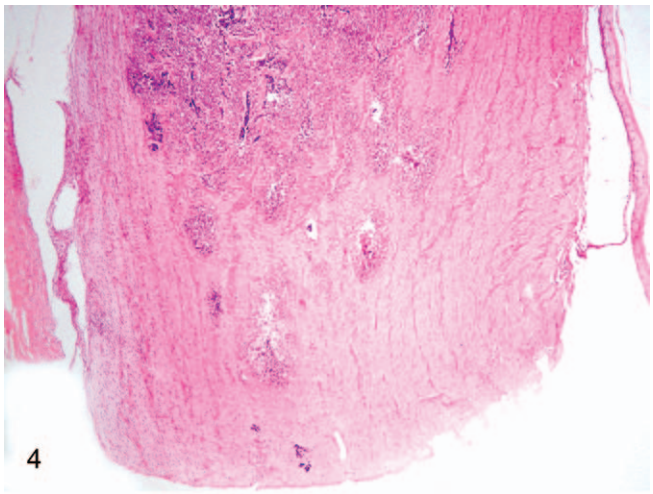


Figure 1. *Photomicrograph showing retinoblastoma tumor cells infiltrating iris, trabecular meshwork, and ciliary body (hematoxylin-eosin, original magnification ×40).*

Figure 2. *Photomicrograph showing involvement of choroid by tumor cells (hematoxylin-eosin, original magnification ×40).*

Figure 3. *Photomicrograph showing invasion of retrolaminar optic nerve by tumor cells (hematoxylin-eosin, original magnification ×40).*



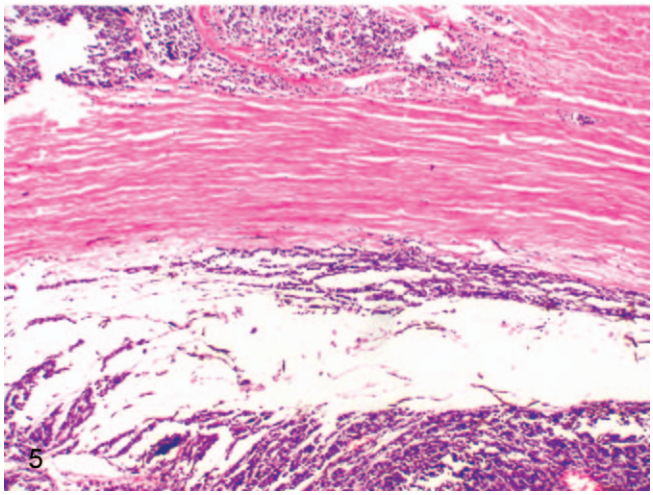


Figure 4. *Photomicrograph showing presence of retinoblastoma tumor cells at transection of optic nerve (hematoxylin-eosin, original magnification ×40).*

Figure 5. *Photomicrograph showing scleral and extrascleral extension of retinoblastoma (hematoxylin-eosin, original magnification ×40).*

iris, ciliary body, choroid, optic nerve lamina cribrosa, retrolaminar optic nerve, optic nerve to the line of transection, sclera, and extrascleral structures. These reports were reviewed for any such positive finding.

RESULTS

The study included 142 eyes of 140 patients, whose eyes were enucleated for retinoblastoma, out of a total of 265 patients with retinoblastoma seen during the study period. The median age of the patients at presentation was 24 months (mean, 29.74 months; SD, 17.97; range, 2–144 months). There were 83 (60%) male and 57 (40%) female patients. Bilateral retinoblastoma was present in 49 patients (35%). Median duration of symptoms was 1.5 months. Mean follow-up of our patients was 56.10 months with a median of 51.5 months (range, 0.25–156; SD, 40.21). Other modalities (chemoreduction and external beam radiotherapy) were attempted in 15 patients (10.71%) before enucleation. These patients most frequently had Reese-Ellsworth group Va and Vb tumors and an attempt was made to salvage the globe in the presence of bilateral disease.

Leukocoria was present at the initial examination in 119

eyes (83.8%). Other clinical features included glaucoma in 56 eyes (36.6%), neovascularization of iris in 39 eyes (26.6%), and cataract in 48 eyes (33.8%).

On review of histopathologic slides, 61 eyes (42.95%) had differentiated tumor, 71 (50%) had undifferentiated tumor, and 10 (7.04%) had necrotic tumor. Histopathologic risk factors were present in 77 (54.2%) of 142 eyes, and included infiltration of iris in 10 eyes (7%), ciliary body invasion in 13 (9%), anterior chamber angle seeds in 13 (9%), choroidal infiltration in 57 (40%), prelaminar optic nerve invasion in 24 (17%), optic nerve lamina cribrosa invasion in 15 (11%), retrolaminar optic nerve extension in 24 (17%), involvement of optic nerve to line of transection in 11 (8%), infiltration of sclera in 13 (9%), and extrascleral extension in 9 (6%) eyes. Of the eyes with choroidal infiltration, 18 (12.7%) showed minimal invasion and 39 (27.5%) showed massive invasion. Two of the 10 eyes with necrotic tumor (20%) showed HRF with choroidal invasion in one and retrolaminar optic nerve invasion in the other.

The total number of eyes showing HRFs, excluding those with only prelaminar tumor, was 77 (54.2%), with several eyes showing more than 1 feature. One HRF was present in 32 eyes, 2 HFRs were present in 27 eyes, 3 in 7 eyes, 4 in 5 eyes, 5 in 2 eyes, 6 in 2 eyes, and 7 in 2 eyes. All of the 9 eyes with extrascleral extension had scleral involvement. Twelve of 13 eyes with scleral involvement had massive choroidal invasion, while 1 had minimal choroidal invasion. Thirty-four eyes (23.9%) had choroid and optic nerve involvement and 16 eyes had laminar or retrolaminar invasion by tumor or invasion of the optic nerve to the line of transection, without choroidal involvement.

The extent of involvement of retrolaminar optic nerve was measured; the mean was 2.8 mm (SD, 2.4; range, 0.5–13) and the median, 2 mm.

Of the patients who were advised to undergo further diagnostic tests, 45 underwent lumbar puncture and bone marrow biopsy. Tumor cells were seen in the CSF in 1 patient at the time of enucleation.

In this group of patients, 5 (3.6%) had systemic metastases, and 1 had an orbital recurrence. One patient with involvement of choroid, sclera, and optic nerve to the line of transection and extrascleral extension on histopathologic analysis had CNS (central nervous system) metastasis at presentation, as seen on CSF cytology. Two more patients, 1 with involvement of the optic nerve to the transection line and 1 with extrascleral extension, had CNS metastasis during follow-up after an initial negative result with CSF cytology. One patient with massive choroidal and retrolaminar optic nerve involvement had bone marrow metastasis during follow-up, after initial negative results on bone marrow examination. One patient with massive choroidal involvement and retrolaminar optic nerve involvement had a metastasis to the opposite orbit. One patient with involvement of sclera had an orbital recurrence during follow-up.

STATISTICAL ANALYSIS

A univariate logistic regression analysis was done to measure whether any clinical characteristic was predictive of presence of histopathologic risk factors. Pearson χ^2 test and Fisher exact test were used. The clinical features analyzed included age, laterality, elevated intraocular pressure, presence of iris neovascularization, and presence of

Table 1. Clinicopathologic Correlation in Retinoblastoma

Clinical Feature and Histopathologic Factor	*P* Value
Univariate Analysis[a]	
Age >24 mo	
Undifferentiated tumor	<.001
Iris involvement	.20
Anterior chamber angle involvement	.74
Ciliary body involvement	.56
Choroidal invasion	.005
Prelaminar optic nerve invasion	.13
Laminar optic nerve invasion	.05
Retrolaminar optic nerve invasion	.43
Optic nerve to transection line involvement	.12
Scleral involvement	>.99
Extrascleral extension	.74
Elevated intraocular pressure	
Undifferentiated tumor	.04
Iris involvement	.17
Anterior chamber angle involvement	.29
Ciliary body involvement	.13
Choroidal invasion	.03
Prelaminar optic nerve invasion	.07
Laminar optic nerve invasion	.04
Retrolaminar optic nerve invasion	.23
Optic nerve to transaction line involvement	>.99
Scleral involvement	>.99
Extrascleral extension	.72
Iris neovascularization	
Undifferentiated tumor	.04
Iris involvement	.25
Anterior chamber angle involvement	.69
Ciliary body involvement	.31
Choroidal invasion	.002
Prelaminar optic nerve invasion	.004
Laminar optic nerve invasion	.005
Retrolaminar optic nerve invasion	.04
Optic nerve to transection line involvement	.71
Scleral involvement	>.99
Extrascleral extension	>.99
Unilateral disease	
Undifferentiated tumor	.03
Iris involvement	.74
Anterior chamber angle involvement	.28
Ciliary body involvement	.75
Choroidal invasion	.56
Prelaminar optic nerve invasion	.86
Laminar optic nerve invasion	>.99
Retrolaminar optic nerve invasion	>.99
Optic nerve to transaction line involvement	.75
Scleral involvement	>.99
Extrascleral extension	.16
Massive tumor	
Undifferentiated tumor	.60
Iris involvement	.04
Anterior chamber angle involvement	.02
Ciliary body involvement	.03
Choroidal invasion	.24
Prelaminar optic nerve invasion	.84
Laminar optic nerve invasion	.40
Retrolaminar optic nerve invasion	>.99
Optic nerve to transection line involvement	.70
Scleral involvement	.29
Extrascleral extension	.68
Multivariate Analysis	
Age >24 mo	
Choroidal invasion	.01
Iris neovascularization	
Choroidal invasion	.007
Prelaminar optic nerve invasion	.003
Laminar optic nerve invasion	.004
Retrolaminar optic nerve invasion	.03

[a] Pearson χ^2 test, Fisher exact test.

Table 2. Comparison of Histopathologic Risk Factors in Various Series

Source, y	Choroidal Invasion, No. (%)	Scleral Invasion, No. (%)	Extrascleral Extension, No. (%)	Retrolaminar Optic Nerve Involvement, No. (%)	Optic Nerve to Transection Line Involvement, No. (%)
Kopelman,[7] 1987 (n = 361)	361 (32.6)	42 (11.6)	70 (19.3)	67 (18.5)	137 (37.9)
Magramm,[6] 1989 (n = 841)	NA	NA	NA	66 (28)	51 (21)
Messmer,[2] 1991 (n = 499)	62 (12.4)	6 (1.2)	8 (1.6)	28 (5.6)	19 (3.8)
Shields,[5] 1993 (n = 289)	67 (23.1)	NA	NA	NA	NA
Shields,[4] 1994 (n = 289)	NA	NA	NA	17 (5.8)	2 (0.7)
Khelfaoui,[3] 1996 (n = 172)	73 (42)	14 (8)	8 (5)	33 (20)	17 (10)
Present series (n = 142)	57 (40.1)	13 (9.1)	9 (6.3)	24 (16.9)	11 (7.7)

Abbreviation: NA, not available; data not mentioned in published report.

massive tumor. These features were tested for their ability to predict tumor differentiation and HRF.

The clinical features at presentation that were statistically significant in predicting presence of HRFs were age greater than 24 months, elevated intraocular pressure, and iris neovascularization. Age at presentation greater than 24 months was associated with undifferentiated tumor ($P < .001$) and invasion of the choroid ($P = .005$). Raised intraocular pressure was associated with undifferentiated tumor ($P = .04$), invasion of choroid ($P = .03$), and laminar optic nerve involvement ($P = .04$). Iris neovascularization was associated with undifferentiated tumor ($P = .04$), choroidal invasion ($P = .002$), laminar optic nerve involvement ($P = .005$), and retrolaminar optic nerve involvement ($P = .04$). Massive tumor was more likely to involve the anterior segment structures: iris ($P = .04$), anterior chamber angle ($P = .02$), and ciliary body ($P = .03$). Unilateral presentation was associated with undifferentiated tumor ($P = .03$). A multivariate logistic regression analysis showed correlation of choroidal invasion with age greater than 24 months ($P = .01$) and iris neovascularization ($P = .007$). Iris neovascularization also showed correlation with laminar ($P = .004$) and retrolaminar optic nerve invasion ($P = .03$). The clinicopathologic correlation is presented in Table 1.

COMMENT

The histopathologic risk factors for metastatic spread of retinoblastoma include optic nerve invasion, particularly retrolaminar or to the transection line, and choroidal, scleral, and extrascleral (orbital) involvement.[2–8] The published rates of occurrence of these factors show a wide range: 7% to 56% for invasion of retrolaminar optic nerve and optic nerve to the transaction line[2–4,6,7]; 12% to 42% for choroidal involvement[2,3,5,7]; and 3% to 30% for scleral and extrascleral spread.[2,3,7] The incidence of HRFs has declined over time, possibly because of earlier detection of cases.[2] Our combined rate of histopathologic risk factors was 54.2% (confidence interval, 47%–63%), which is much higher than that found in the recent Western literature, with the exception of 1 series.[3] This difference may be due to comparatively late presentation of our patients, at a higher mean age, and with more advanced disease. Table 2 compares HRF occurrence for the current series with that of previously published series.

The degree of optic nerve invasion is associated with rate of survival; the incidence of metastasis rises sharply with retrolaminar involvement (14%–42%) and involvement to the transection line (41%–78%).[2–4,6] In our series, 52.1% of eyes had optic nerve involvement when all degrees of invasion of retinoblastoma, including prelaminar, were considered. The higher frequency of optic nerve involvement was probably due to the fact that, in our setup, patients at first presentation had a more advanced stage of ocular disease. The mean extent of retrolaminar optic nerve involvement on histopathologic examination was 2.8 mm; this measurement cannot be directly compared to the intraoperative optic nerve stump length, as formalin preservation would cause the tissue to shrink by about 30% to 40%. However, because 25% of eyes had retrolaminar invasion or invasion of the optic nerve to the transection line, it is advisable to obtain the longest possible optic nerve stump during enucleation.

Our case series had a 40% frequency of choroidal involvement, taking into account any degree of HRF involvement. In 18 eyes (12.7%), there was minimal involvement, with massive invasion of the choroid in 39 eyes (27.5%). We did not review serial sections of peripheral calottes for choroidal invasion, which may have yielded still higher rates of histopathologic risk factors. Our current practice is to study serial sections in eyes enucleated for retinoblastoma. Some authors[7] have suggested that isolated choroidal invasion is not a risk factor for metastasis. However, more recent studies[2–4] have found choroidal involvement to be a risk factor, especially if associated with optic nerve involvement.

On univariate analysis, we found that elevated intraocular pressure, neovascularization of the iris, and age greater than 24 months were predictors for histopathologic risk factors. The laterality of the tumor was not significantly associated with optic nerve or choroidal invasion. On multivariate analysis, only age greater than 24 months and iris neovascularization were predictive of choroidal invasion, and only iris neovascularization was associated with optic nerve invasion. Previously, Shields et al[4,5] have found that iris neovascularization and glaucoma are predictors of both optic nerve and choroidal involvement. Tumor thickness greater than 15 mm and exophytic tumors have also been associated with HRFs.[4,5] One would expect a larger tumor to involve more extensively the ocular structures; however, we did not find size to be significant in our study—except in anterior segment involvement—probably because most of our patients (77.5%) had massive tumors that almost filled the globe.

We also found that age at presentation greater than 24 months was a predictor for HRFs. Previously, age greater than 24 months has been associated with a greater mortality rate and more advanced Reese-Ellsworth staging but has not been correlated with HRFs.[10,11]

At initial examination of CSF and bone marrow, we had

only 1 positive result from 45 examinations of each of these specimens (2.2%). We request these investigations only for patients who show definite HRFs on enucleation such as choroidal, extrascleral, or retrolaminar optic nerve involvement. This low incidence is at variance with previous studies,[1] which reported positive yields of 4.3% with CSF cytology and 9.9% with bone marrow biopsy.

For patients with HRFs on enucleation, our current practice is to administer 6 cycles of adjuvant chemotherapy with vincristine, carboplatin, and etoposide, with additional external beam radiotherapy for patients with involvement of optic nerve to the transection line or scleral and extrascleral invasion. The protocol of chemotherapy is the same as that used for chemoreduction of intraocular retinoblastoma[12]; the protocol is changed to 12 cycles of high-dose chemotherapy for patients with involvement of the optic nerve to the transection line and scleral and extrascleral extension.[13]

This is the first large clinical series reporting the histopathologic risk factors associated with retinoblastoma for patients in a developing country. We found a higher rate of histopathologic risk factors and definite clinical predictors of such risk factors. Presence of any such clinical predictor in a patient warrants a high index of suspicion on the part of the pathologist as well as a meticulous evaluation of histopathology slides. The detection of the HRF and administration of appropriate adjuvant therapy may improve the chance of metastasis-free survival of the patients.

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