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Histopathological High-Risk Features and Clinical Outcomes in Retinoblastoma: A Prospective Observational Study from a Tertiary Eye Care Centre

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ABSTRACT: To evaluate the frequency of histopathological high-risk characters in enucleated eyes with retinoblastoma and find their correlation with clinical presentation, treatment characteristics and metastatic outcome. This was a prospective observational study of patients who were diagnosed to have intraocular retinoblastoma and underwent primary or secondary enucleation in a tertiary eye care center for a period of 18 months. Their clinical presentations, treatment and histopathological variables were recorded. Categorical variables were analysed using chi-square or Fisher exact tests; $P<0.05$ was considered statistically significant. Enucleated 50 eyes were included. Mean age at enucleation was 28.3±17.67 months (range 3 to 72); 25 patients (50%) were male. Leukocoria was the commonest presenting feature (47/50, 94%). Endophytic growth was present in 39 eyes (78%). Primary enucleation was performed in 19 eyes (38%) and secondary enucleation in 31 (62%). Histology showed differentiated tumour in 34 eyes (68%). Optic nerve invasion was identified in 36 eyes (72%): prelaminar 18 (36%), laminar 5 (10%) and retrolaminar 13 (26%). The optic nerve resection margin was involved in 4 eyes (8%). Choroidal invasion was massive in 29 eyes (58%), minimal in 15 (30%) and absent in 6 (12%). Orbital extension was found in3 patients during follow-up and 3 patients died from metastatic disease. Characteristic high-risk histopathological features were frequent in this cohort of advanced retinoblastoma. Systematic examination of enucleated specimens remains essential for risk stratification and postoperative management.

1. INTRODUCTION

Retinoblastoma is the most common primary intraocular malignancy of childhood, where 11 per million cases affected annually [1,2]; but in India approximately 2000 cases estimated every year [3]. Among pediatric cancers, retinoblastoma comprising 2.5 to 4% and significant variations observed from races and countries [4]. For avoiding the visual complications and preserving clear and unblurred vision, early diagnosis, newer advancement and multimodal treatment has markedly improved survival and globe salvage, in advanced stages enucleation remains important mode of management [5].

Histopathological evaluation of the enucleated globe is clinically important because selected pathological features shows increased frequency of extraocular dissemination and guide postoperative risk stratification [6]. Primary treatment is more

successful than chemosurgery who are non-necrotic and non-calcified tumour cells [7,8]. The enucleation comprised physiological and psychosocial squeal where the risk of extraocular tumor spread, psychological distress and body image concern are substantially impacting the quality of life [9,10].

International consensus recommendations emphasize histopathological examination of enucleated eyes, particularly evaluation of the optic nerve, choroid, sclera, anterior segment and extraocular extension [6]. Recent large cohorts continue to show strong prognostic value of pathological staging. In a global cohort of 1,411 primarily enucleated patients, tumor-related metastasis increased from 1% in pT1 disease to 43% in pT4 disease, while tumor-related mortality increased from 2 to 59% [11,12].

Indian studies have demonstrated substantial frequencies of high-risk pathology. In 403 Asian Indian patients undergoing primary enucleation, 36% were found to have at least one histopathological high-risk feature; of which postlaminar optic nerve invasion and massive choroidal invasion were prominent findings [13]. This study describes the clinicopathological profile of enucleated eyes, including both primary and secondary enucleations, and documents postoperative orbital and metastatic outcomes.

2. MATERIALS AND METHODS

This was a prospective observational study conducted at Aravind Eye Hospital, Madurai, India where eligible patients with clinically diagnosed intraocular retinoblastoma and underwent primary or secondary enucleation for the period of 18 months were included. Patients enucleated elsewhere and subsequently followed at the study centre were excluded.

Patients were diagnosed after detailed ophthalmic examinations including anterior-segment evaluation, dilated fundus examination under anaesthesia when required, B-scan ultrasonography and MRI of the brain/ orbits when needed. Eyes were staged using the International Classification of Intraocular Retinoblastoma used during the study period [14]. Written informed consent was obtained from a parent or guardian before any intervention.

Individual patients' treatment differed according to stage and included chemotherapy, focal therapy, radiotherapy and/ or enucleation. Nineteen eyes underwent primary enucleation and 31 secondary enucleation after chemotherapy. The institutional treatment protocol included vincristine, etoposide and carboplatin are followed for treating all cases [15].

Enucleated eyes were evaluated for tumour differentiation and high-risk features including anterior chamber/ iris/ ciliary-body involvement, choroidal invasion, optic nerve invasion, optic nerve resection-margin involvement, scleral invasion and extraocular extension. Optic nerve invasion was categorized as laminar, prelaminar or retrolaminar invasions [16].

Outcomes included postoperative orbital/ stump recurrence or extension and distant metastasis [17]. Continuous variables were summarized as mean±SD and categorical variables as frequency and percentage. Chi-square or Fisher exact tests were used for categorical associations. STATA version 14 was used; $P<0.05$ was considered statistically significant. Because only three metastatic deaths occurred, the dataset is not sufficiently adequate for reliable multivariable prediction of metastasis.

3. RESULTS AND DISCUSSION

Out of 50 patients included, 25 males and 25 females were recorded. Of which, right-eye involvement in 34 (68%) and left-eye involvement in 16 (32%) was occurred. The mean age at enucleation was 28.3±17.67 months (range 3 - 72; median 24 months). Leukocoria occurred in 47 patients (94%), strabismus in 8 (16%), orbital cellulitis in 5 (10%), buphthalmos in 3 (6%) and other complaints in 6. Some patients had more than one presenting complaint (Table 1).

Seven eyes had cataract, 5 had iris neovascularization and 3 had secondary glaucoma. Tumour focality was assessable in 13 eyes; out of which 12 (24%) were multifocal and 1 (2%) unifocal; focality was not assessable in 37 (74%). Growth was endophytic in 39 eyes (78%), mixed in 7 (14%), exophytic in 3 (6%) and diffuse infiltrative in 1 (2%).

Table 1: Demographic and clinical characteristics of the retinoblastoma patients (n=50)

Variables	N (%) / Mean±SD
Age group (in months)	**32.8±21.9**
3-25	19 (38)
26-50	20 (40)

51-75	11 (22)
Gender	
Male	25 (50)
Female	25 (50)
Laterality	
Right eye	34 (68)
Left eye	16 (32)
Age at enucleation (in months)	28.3±17.67
Leukocoria	47 (94)
Strabismus	8 (16)
Orbital cellulitis	5 (10)
Endophytic growth	39 (78)
Multifocal tumour	12 (24)

Primary enucleation was performed in 19 eyes (38%) and secondary enucleation in 31 (62%). All secondarily enucleated eyes had received chemotherapy. Mean chemotherapy duration before secondary enucleation was 8.16±11.93 months (range 1–48; median 3 months). Differentiated tumour was identified in 34 eyes (68%) and undifferentiated tumour in 16 (32%).

Optic nerve invasion was present in 36 eyes (72%): prelaminar in 18 (36%), laminar in 5 (10%) and retrolaminar in 13 (26%); it was not identified in 14 (28%). The optic nerve resection margin was free in 46 eyes (92%) and involved in 4 (8%). Choroidal invasion was massive in 29 eyes (58%), minimal in 15 (30%) and absent in 6 (12%). The study flow chart recorded anterior chamber invasion in 14 eyes (28%) and scleral invasion in 5 (10%) (Table 2).

Table 2: Enucleation and histopathological findings

Variables	N (%)
Primary enucleation	19 (38)
Secondary enucleation	31 (62)
Differential tumour	34 (68)
Undifferentiated tumour	16 (32)
Optic nerve invasion	36 (72)
Prelaminar invasion	16 (36)
Laminar invasion	5 (10)
Retrolaminar invasion	13 (26)
Optic nerve margin involved	4 (8)
Massive choroidal invasion	29 (58)
Minimal choroidal invasion	15 (30)
No choroidal invasion	6 (12)
Scleral invasion	5 (10)
Extraocular invasion	1 (2)

The original statistical tables did not demonstrate statistically significant associations between secondary glaucoma and optic nerve invasion (Fisher exact p=0.100), iris neovascularization and optic nerve invasion (p=0.205), secondary glaucoma and

optic nerve resection-margin involvement (p>0.99), iris neovascularization and resection-margin involvement (p>0.99), or secondary glaucoma and choroidal invasion (p=0.689). These findings should be reported as non-significant rather than interpreted as evidence of absence of association.

Table 3: Clinical-pathological association analyses

Comparison	Test	p value	Interpretation
Secondary glaucoma versus optic nerve invasion	Fisher exact	0.100	Not statistically significant
Iris neovascularization versus optic nerve invasion		0.205	
Secondary glaucoma versus resection margin		>0.99	
Iris neovascularization versus resection margin		>0.99	
Seconadry glaucoma versus choroidal invasion		0.689	

Orbital extension was documented in 3 patients (6%) during postoperative follow-up. Three patients died from metastatic retinoblastoma. Given the small number of metastatic events, individual high-risk features cannot be reliably assessed as independent predictors of mortality in this study.

This clinical investigation represented advanced retinoblastoma among pediatric group who are treated at a tertiary eye-care centre and includes both primary and secondary enucleations. The main finding was the high frequency of optic nerve and choroidal involvement in the enucleated eyes. The mean age at enucleation was 28.3 months, similar to several Indian and international series of enucleated retinoblastoma studies [18,19,20]. Leukocoria was the most common presentation (94%), consistent with previous studies in which leukocoria is the dominant presenting sign [21,22].

The high prevalence in an advanced-enucleation cohort also emphasizes that visible leukocoria may occur after substantial tumour growth. Optic nerve invasion occurred in 72% of eyes in this study. However, the distribution included a substantial proportion of prelaminar or laminar involvement; therefore, the 72% overall figure should not be equated with the frequency of the internationally recognized highest-risk postlaminar or transection-margin categories. In a large Indian primary-enucleation cohort, high-risk pathology was found in 36% and postlaminar optic nerve invasion in 49% of high-risk eyes [13].

International consensus recommendations support careful anatomical evaluation of optic nerve invasion and the cut end [23]. Massive choroidal invasion was present in 58% of eyes. This is higher than some large primary-enucleation series, likely reflecting the advanced nature of the present cohort and inclusion of secondary enucleations. The definition of massive choroidal invasion should be retained exactly as used by the original pathology protocol; if the original pathology records contain measured depth, this should be incorporated before submission. The primary-versus-secondary enucleation component is potentially useful for publication. Modern studies show that secondary enucleation cohorts can have different pathological profiles from primary enucleation cohorts.

A 2023 study of 121 eyes found similar overall high-risk pathology prevalence between primary and secondary enucleation, but primary enucleation was associated with more massive choroidal and postlaminar optic nerve invasion [24]. A much larger 2025 study of 295 secondarily enucleated eyes found high-risk histopathology in 17%, with older age, longer treatment duration and prolonged delay to enucleation associated with high-risk pathology [25].

The three metastatic deaths in the present series demonstrate the clinical importance of pathological risk assessment, but the number of events is too small to show independent prognostic associations. This is an important methodological limitation and should be stated explicitly. Large modern datasets demonstrate that pathological stage is strongly associated with recurrence, metastasis and death [12].

The study has a relatively small sample size and only three metastatic deaths, limiting power for outcome prediction. It is single-centre and includes only eyes selected for enucleation, so the findings cannot be generalized to all retinoblastoma. The study period predates several contemporary treatment approaches and modern pathological staging systems. Some variables were not assessable in all eyes, such as tumour focality.

4. CONCLUSION

Histopathological high-risk features were frequent among enucleated eyes with advanced retinoblastoma in this tertiary-care cohort. In this study optic nerve and choroidal invasions were frequent histopathological findings. Retrolaminar optic nerve invasion occurred in 26% and optic nerve resection margin involnement in 8%. This study supports systematic pathological examination after enucleation but does not provide sufficient statistical power to predict metastatic mortality, larger multicentre studies using standardized pathological definitions and contemporary AJCC staging are needed to determine which individual histopathological features independently predict metastatic outcome.

Conflict of interest: The authors declare that they have no conflict of interest in publishing this manuscript.

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Ethical Statement: Before the initiation of the study, the institutional ethical approval was obtained from the research and ethics committee. Written informed consent was obtained from the participants and confirmed that no risks or harm involved in participating in the study.

Author Contribution Statements

Mangaleshwari M: Conception, methods, and data collection, initial draft of the manuscript

Usha Kim: Supervision, reviewed and edited the manuscript

Sharavana Kumaran KB: Literature review, methods, data collection, Data curing, clinical data analysis and final draft

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