Histopathological Analysis of Retinoblastoma: Insights into Prognostic Factors and Chemotherapeutic Response

*Anjum R,[1] Roy SR,[2] Nasir TA[3]

Abstract

Background: Retinoblastoma is a highly aggressive ocular malignancy that mainly impacts young children. It is essential to comprehend the histopathological characteristics of this tumour to determine the most effective treatment approaches. Furthermore, recognizing high-risk histopathological factors can help predict the prognosis and guide treatment decisions for patients with retinoblastoma.

Objective: To analyse the histopathological features in eyes with retinoblastoma primarily treated by enucleation and those treated with chemoreduction. The goal is to identify high-risk factors and improve treatment modalities, prognosis, and reduce morbidity and mortality.

Methods: 40 enucleated eyes registered from October 2021 to October 2023 had been studied. Histopathological findings were evaluated according to age, sexlaterality, choroid, sclera, optic nerve, subarachnoid space involvement, necrosis, and calcification, degree of differentiation, chemotherapeutic effect and tumor regression after chemotherapy and histoprognostic factors. Grading and staging were done according to 8th AJCC classification of eye tumor.

Results: Rosettes, necrosis, calcification were common histological findings in this study. High risk factor assessment was very crucial in staging and prognosis. Chemotherapy treated eyes showed varying degrees of response.

Conclusion: Histopathological evaluation guide further management to prevent metastasis.

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1. **Dr. Rahat Anjum, Associate Consultant, Histopathology & Cytopathology, Apollo Imperial Hospitals, Zakir Hossain Road,Chattogram. anjum.r.dr@gmail.com*
2. *Dr. Soma Rani Roy, Consultant, Department of Oculoplasty and Ocular oncology, Chittagong Eye Infirmary.*
3. *Professor. Dr. Tareak Al Nasir, Senior Consultant and Laboratory Director, Islami Bank CentralLaboratory, Dhaka*

**For correspondence*

Introduction

Retinoblastoma is the most common intraocular malignancy in infancy and childhood arising from inner nuclear layer of retina accounting for 3% of all pediatric tumors.[1,2] The estimated incidence is about 01 in 15,000 to 20000 live births,[1] independent of race and sex.[3] Mutational inactivation of both alleles of RB 1 gene which maps to chromosome 13q14 and encodes retinoblastoma proteins are required to produce retinoblastoma.[2] The histologic findings in enucleated eyes detects few high risk features (HRF) that are associated with local recurrence and metastasis. Therefore standardized examination of enucleated eyes for utilization of chemotherapy and to evaluate prognostic factors are crucial.[4] The clinical assessment depend on the stage, size, and extent of tumor .[1,5] Tumor invasion of the anterior chamber, iris, ciliary body, massive choroidal invasion (>3 mm), optic nerve invasion, and extraocular tumor extensions are considered as high risk factor.[1,6] Assessment of high risk factors are requiredfor predicting metastasis.[1]

Methods

This study was prospective and descriptive, including all cases of retinoblastoma with or without chemotherapy. It includes enucleated eye specimens received in the Histopathology Department at Apollo Imperial Hospitals, Chittagong, between October 2021 and October 2023.

In our study total eye samples were 40 and among them 21 eyes were primarily enucleated that did not received any chemotherapy. The rest 19 eye were enucleated after receiving 3 to 5 cycles of neoadjuvant chemotherapy of vincristine, etoposide and carboplatin (VEC) schedule. The samples we received were grouped as E or D according to ICRB (International Classification of Retinoblastoma)

After receiving the tissue samples, we first conducted a thorough examination of all eye structures including the cornea, anterior chamber, iris, trabecular meshwork, choroid (above a certain thickness), sclera, extraocular structures, and the cut end of the retrolaminar optic nerve. We measured the length of the optic nerve after separating it to prevent contamination. We obtained slices containing the cornea, pupil, and optic disc by making two parallel slices from front to back, one on each side of the limbus. The orientation of the parallel slices was sagittal, oblique, or transverse depending on the tumor's location. The eyeball was then sectioned to describe the tumor's interaction with different structures, followed by hematoxylin and eosin staining. No immunohistochemistry was performed.

Patients' age, sex, laterality, high risk features classification, number of chemo reduction received, pathological staging was done according to AJCC pathological classification. Histologic type according to WHO classification, histoprognostic factors, evolutionwere noted.

The structures where tumor presence is regarded as an HRF are: anterior chamber, iris, trabecular meshwork , Schlemm's canal, ciliary body, choroid (above a certain threshold), sclera, extraocular structures, retrolaminar optic nerve (including the cut end). Retinoblastoma specimens with HHRFs indicate that the patient is at higher risk for metastasis (tumour spread) than a patient whose specimen does not have such features. Detection of HHRFs by the histopathologist influences management decisions. One or more solid nests of tumor cells fill or replace the choroid and had pushing infiltrating border was considered as choroidal invasion. A maximum of diameter of invasive foci 3 mm or more that may reach the scleral tissue were considered as massive choroidal invasion. Optic nerve invasion was

categorized intolaminar, post laminar or tumor at surgical margin. In post chemotherapeutic state, the retinoblastoma status was noted as tumor occupying greater than 50% of the eye, less than 50% of the eye, less than 5% or completely regressed. The histopathological parameters for regression status in retinoblastoma are classified as viable appearing retinoblastoma, regressed retinoblastoma, or regressed retinoblastoma with a well-differentiated component and retinocytoma-like features.

An infiltrate composed of poorly differentiated, mitotically active, and focally necrotic tumor cells with hyperchromatic nuclei and scant cytoplasm is considered a viable tumor component. Replacement of the tumor by a glial scar containing characteristic foci of calcified tumor cells is considered regressed retinoblastoma. Retinoblastoma with a well-differentiated component and retinocytoma-like features is composed of tumor cells with abundant cytoplasm, eosinophilic than retinoblastoma, and paucicellular. Other parameters of tumor regression include absence of mitotic figures, bland nuclei, foci of calcification within viable areas, and presence of photoreceptor differentiation.

Invasion of eye structure, necrosis, calcification were also noted. For analysis of necrosis and calcification it had been graded as, extensive (more than 50%), moderate (25-50%) and minimal (less than 25%).Tumors were divided into G1 to G4 on the basis of rosette formation. The degree of tumor differentiation, according to the American Joint Committee on Cancer (AJCC), is classified as follows: G1 for a tumor with areas of retinoma (flueurettes or neuronal differentiation); G2 for a tumor with many rosettes (Flexner-Wintersteiner or Homer-Wright); G3 for a tumor with occasional rosettes (Flexner-Wintersteiner or Homer-Wright); and G4 for a tumor with poorly differentiated cells without rosettes and/or extensive areas (more than half of the tumor) of anaplasia. Tumor anaplasia is a useful measurement that extends beyond standard histopathologic criteria for identifying retinoblastoma that does not display high-risk histologic features but still presents an increased risk of metastasis and may require adjuvant therapy.

Results

Most common symptom was leukocoria (62.5%) followed by strabismus. Most common clinical finding was creamy white tumor associated with vitreous seed and exudative retinal detachment. Complicated cases presented with iris neovascularization and secondary glaucoma.

Table I: Age and sex wise distribution

Age (Year)	Male	Female	Total (%)
0-1	01	01	02
1-2	06	04	10
3-4	08	05	13
4-5	06	03	09
>5	04	02	06
Total	25	15	40 (100)

Out of 40 cases 38 (95%) were unilateral and 02 (05%) were bilateral.

Table II: TNM staging of cases without chemoreduction (8th AJCC TNM classification)

Stage	No of cases	Percentage (%)
T1	07	33.33
T2	07	33.33
T3	05	23.80
T4	02	9.5
Total	21	100

In our series, 19 cases (47.5%) received chemoreduction and 21 cases (52.5%) were treated only with enucleation. Out of 40 cases 38 (95%) were unilateral and 02 (05%) were bilateral.

Out of 40 cases, 5 cases (12.5%) showed optic nerve involvement, 6 cases (17.5%) showed scleral invasion, 13 cases (32.5%) showed laminar involvement, and 11 cases (27.5%) showed post laminar involvement. Choroidal invasion was seen in 14 cases (35.0%). Two cases (14.29%) were determined to have massive choroidal invasion. Out of two cases, 01 was enucleated after chemotherapy and the other without any chemotherapy.

Out of 21 cases, 13(61.90%) cases were histologically graded as G3, 7 case s(33.33%) as G2 and single case(4.77%) graded as G4. Out of 13 G3 cases, 3 (23.07%) cases showed optic nerve cut end invasion. Choroid invasion was seen in 09 cases (42.85%), scleral invasion in 2 cases (9.5%), laminar involvement in 7 cases (33.33%), post laminar involvement in 6 (28.57%) cases.

In seven G2 cases, 2 cases (28.57%) showed choroidal invasion. 6 cases (85.71%) showed laminar involvement and post laminar involvement in 2 cases (28.57%).

According to the international retinoblastoma classification, ICRB group, out of 21 cases, 10 cases (47.61%) were classified into Group E. Group E cases showed choroidal invasion in 3 cases,2 cases with both laminar and post laminar involvement and single case with optic nerve cut end involvement. One case with only laminar involvement seen. Single case showed extra ocular fatty tissue infiltration.

Rosette formation was seen in 16 cases without chemotherapy and 13 cases after chemoreduction. Moderate rosette formations were mostly associated with G2 histological grade. Out of 40 retinoblastoma, 20 cases (50%) showed necrosis in eyes without chemotherapy. Extensive necrosis were seen in 6(30%) cases most were G3 tumor grade. 16 (40%) cases showed necrosis after chemoreduction. Extensive necrosis was seen in 07 (43.75%) cases after chemotherapy.

Five cases (12.5%) showed extensive calcification. Other findings include gliosis, hemosiderin laden histiocytes, fibrosis and hemorrhage.

Out of 19 (47.5%) eyes with chemoreduction, majority received 05 cycle chemotherapy.

Table III: Tumor regression after chemotherapy

Tumor regression status	Total eyes 19 (100)
>50 %	13 (68.42)
<50%	06 (31.58)
<05%	00(00)

Histopathological evidence of chemotherapeutic effects include varying degrees of necrosis and calcification .Out of 19 cases, 08 showed extensive necrosis, 7 showed moderate and 04 cases showed minimum necrosis. Rosettes were present in 13 (68.42%) cases. No histopathologic findings that could be considered consistent with chemotherapeutic toxicity were observed. Chemoreduction in cases with subretinal and vitreous seeds did not respond well. Biphasic component with tumor reduction and well differentiated component showed less shrinkage after chemotherapy.

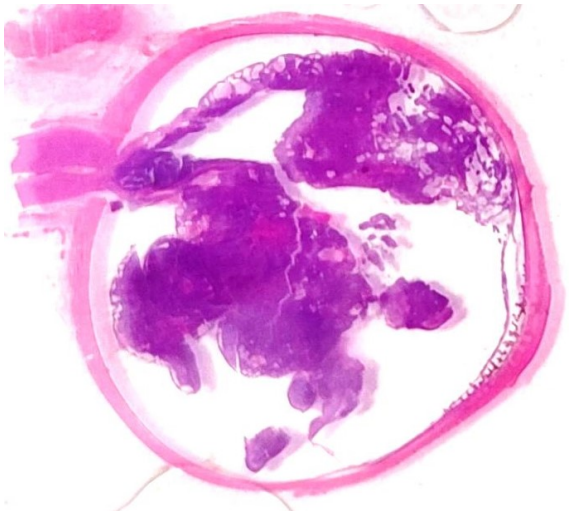

Figure 1. Showing cross section of eyeball with Retinoblastoma

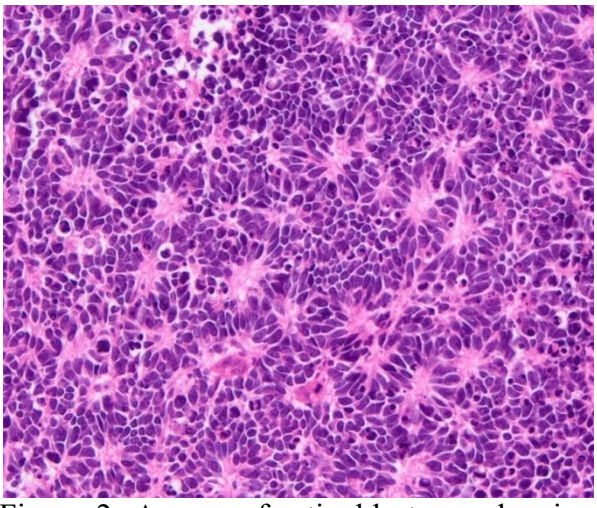

Figure 2. A case of retinoblastoma showing rossettes, formed by cell border and fine cytoplasmic extension without basement membrane (H&E) (20X)

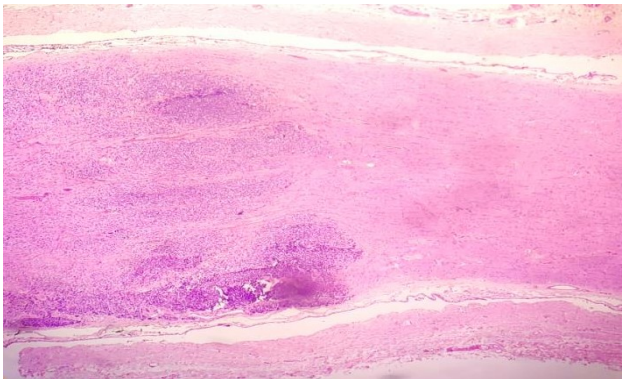

Figure 3. Infiltration of optic nerve by retinoblastoma cells (H&E stain) (20x)

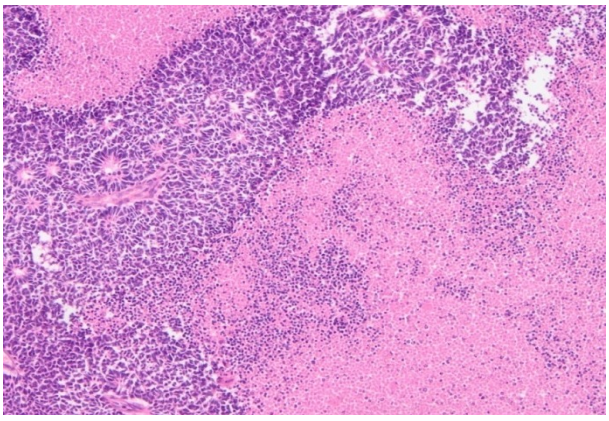

Figure 4. Eosinophilic areas of tumor necrosis (H&E) (20x)

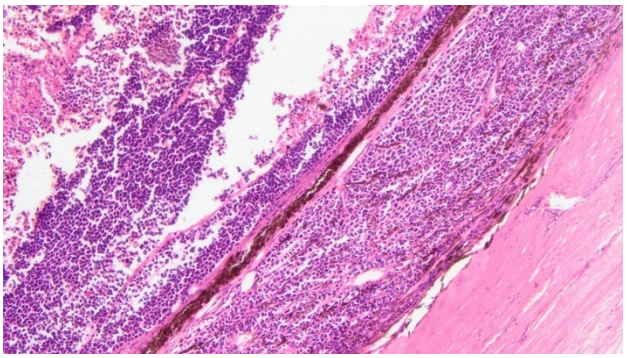

Figure 5. Showing choroidal invasion (H&E) (20x)

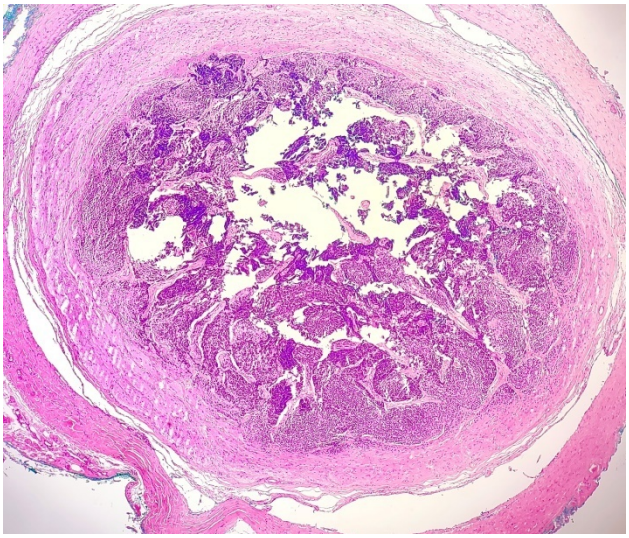

Figure 6. Showing involvement of cut end of optic nerve(H&E) (20x)

Discussion

Microscopically, Retinoblastoma showed small round hyperchromatic blue cells with scant cytoplasm in sheets, nests and trabeculaewith variable differentiation. Calcification and perivascular ischemic necrosis was common. Most commonly seen rosettes were Flexner-Wintersteiner rosettes with central lumen and basally located nuclei. Homer Wright rosettes were also seen that lack central lumen with nuclei displaced away from eosinophilic center.

In our series we have included eyes both enucleated primarily without receiving chemotherapy and eyes after chemoreduction. The tumor was unilateral in 95% cases and bilateral in 05% cases. Dasilva et al found 63.9% unilateral and 36.1% bilateral cases.[8] According to Shields more than 90% of RB cases were diagnosed before the age of five years.[9] 87.5% patient in our series were below five years. In our study we noted slight male predominance. Da silva et al and others noted female predominance in their series.[8,10] The literature does not find any gender predominance in retinoblastoma. The variation could be explained by the fact that our series was small.

The most common signs were leucocoria and strabismus.[7] In the series by Chebbi et al.,leukocoria was found in 58.5%.[10] Our study found similar findings.

In our study 47.5% received chemoreduction before enucleation. Da silva et al had 59.72% cases of chemoreduction before enucleation.[8] Neoadjuvant chemotherapy is used to reduce the tumors. In retinoblastoma, the indication of neoadjuvant chemotherapy is reducing the tumor size and also as a prophylaxis of metastasis.Itis given to the patents who presented bilaterally, unilateral group E or D tumor where there are possibility of eye salvage or parents are very much unwilling for enucleation, extraocular retinoblastoma, tumor extended to optic nerve (in MRI or CT scan), distant metastatic cases.

In the current study 33.33% were classified as pT1 and 33.33% as pT3. Karkis et al[4] found 58% pT1 followed by pT2 (22%). The staging of the disease was influenced by the onset of the disease, age at diagnosis, and time of enucleation.

In eyes without chemotherapy the mean tumor size was 1.5 cm which is similar to Da silvaet al.[8] 61.90% cases were histologically graded as G3, 33.33% as G2 and single

case(4.77%) graded as G4. Tumor's differentiation highly varies in different countries. The differentiation of tumors is related to whether the patient presented early or late. The significant difference in tumor differentiation between developed and developing countries has been associated with the availability of ophthalmologists and pediatricians in lower healthcare settings, better healthcare-seeking behavior, and reasonable socioeconomic status in developed countries.

Many showed higher incidence of poorly differentiated tumors. Most tumors showed well to moderately differentiated Retinoblastoma which is consistent with our studies.[1,10] Da Silva et al showed 48% moderately differentiated tumors.Differentiation of tumor doesnot appear to have a prognostic role in Retinoblastoma.[8]

The study by Ralph et al and others[11,12,13], report that retinoblastomas become less differentiated over time as additional mutations accumulate. It is poorly understood why rosettes progressively disappear and foci of photoreceptor differentiation persist in otherwise undifferentiated retinoblastomas. One explanation for the persistence of retinoblastomas after malignant transformation is the absence of significant degrees of apoptosis and cellular turnover in these highly differentiated retinal precursor neoplasms. There is no evidence that retinoblastomas can undergo redifferentiation.

Recurrence was observed in only 1 case Karkis et al[4] found 3 cases in their study series. Choroidal invasion was seen in 35.0%. Massive choroidal invasion was seen in 14.29%.Various studies have reported choroidal invasion in 12% to 62% of enucleated eyes with retinoblastoma.[5,14,15]

Table IV: Comparison of choroid and optic nerve invasion with other studies

Author	Country	Year	Choroid invasion	Massive choroid invasion	Optic nerve, post laminar or resection margin invasion
Wilson et al[15]	USA	2013	61.5%	25.7%	14.9%
Kashyap et al[16]	India	2011	47.5%	24.6%	23.5%
Orellena et al[17]	Venezuela	2009	NA	41%	40.4%
Mistry et al[5]	India	2020	87.80%	55.56%	39.02%. Cut margin 18.87%
Da silva[8]	Morocco	2022	52.7%	NA	27.9%
Karki et al[4]	Nepal	2015	38%	20%	08% post laminar Resected margin 06%
Present study	Bangladesh	2023	35%	14.29%	Optic nerve cut end-12.50% Laminar: 32.5% Post laminar 27.5%

Choroidal and optic nerve invasion reported incidences in the literature are variable in different geographic locations with their unique biological behavior. Enucleation time, reporting institute location, and standardized assessment techniques for enucleated specimens also contributed to variability.[18,19]

Patients with choroidal invasion were more likely to develop systemic metastasis compared to those with or without optic nerve invasion.Both optic nerve and choroidal invasion have shown strong prediction of metastasis in most studies.[8]

Extensive necrotic retinoblastoma is associated with high risk histopathological findings like choroidal and optic nerve invasion.[20,21]Yakoub et al [21] showed 44% choroidal invasion and 18% massive choroidal invasion and 14% cases with optic nerve involvement.

Rosette formation was seen in 40% cases without chemotherapy and 32.5% cases after chemoreduction. Stannard et al[22] showed rosettes were commonly seen in patients with early stage of disease and thus considered as good prognostic factor. Moderate rosette formation were mostly associated with G2 histological grade. Ralph et al [11] confirmed that degree of tumor differentiation is inversely proportional with the age in month of the patient.Numerous rosettes are found in eyes of younger infants.Poorly differentiated retinoblastomas are usually found in older children.

Out of 40 cases of Retinoblastoma, 50% showed necrosis in the eyes without chemotherapy. Extensive necrosis was seen in 30% of cases, most of which were G3 tumor grade.40% cases showed necrosis after chemoreduction. Extensive necrosis was seen in 43.75% cases after chemotherapy. Well differentiated component showed less shrinkage after chemotherapy which is consistent with Demirci et al who stated lack of chemoreduction to well differentiated tumor.[23] We could not exclude the possibility that chemoreduction might induce differentiation in retinoblastoma.

Conclusion

Histopathological analysis is highly valuable in staging, diagnosing, risk factor analysis and assessment of risk of metastasis. Retinoblastoma is a disabling condition with high mortality and morbidity. Attention should be given to evaluating retinoblastoma to detect high-risk features indicating the need for adjuvant therapy, which can lower the rate of metastasis and increase the survival rate A well-detailed macroscopic and microscopic report helps oncologists create an effective management plan for their patients. This plan determines whether the patient will require additional adjuvant chemotherapy following primary enucleation, or if they will need radiation therapy after secondary enucleation, having already received neoadjuvant chemotherapy. Making this decision is crucial for saving the patient's life, preventing recurrence, and reducing the burden on families affected by retinoblastoma.

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