Histopathological Analysis of Retinoblastoma at Tertiary Care Ophthalmic Centre

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Abstract

Introduction: Retinoblastoma is the most common intraocular tumour of childhood. The incidence has been estimated to be 1 in 15,000 to 1 in 34,000 births. All races and both sexes are affected equally Unilateral (70%) or bilateral (30%). Sporadic (60%) or familial (40%). The mean age at diagnosis is 18 months, and about 90% of cases are diagnosed before 3 years of age

Objectives: • Aim of this study is find out incidence rate of retinoblastoma from at M. J Institute tertiary care ophthalmic centre, civil hospital, Ahmedabad.

• Histopathological analysis for to establish relationship between age, degree of differentiation and high risk features.

• Histopathological analysis is essential for early diagnosis, treatment, improve prognosis, reduce morbidity and mortality.

Material & Method: 41 enucleated eyes received out of 41 prospectively registered case from june-2017 to june-2019 were included in study which was carried out at histopathology department, M.J western regional institute of ophthalmology, civil hospital Ahmedabad. Histopathological findings were evaluated according to age, sex, laterality, extent of invasion into vitreous, choroid, sclera, optic nerve, surrounding tissue, necrosis, calcification, degree of differentiation. Grading and staging were performed according to 8th AJCC classification of eye tumour.

Results: Out of 41 eyes, 11(26.83%) cases were under 2 year of age and 30(73.17%) cases were above 2 year of age. 23(56.1%) cases were female and 18(43.90%) cases were male, 19(46.34%) cases were found in right eye, 22(53.66%) cases were found in left eye , all cases were unilateral, vitreous seeding was observed in 33 (80.49%) cases, Choroid invasion was observed in 36(87.80%) cases, Optic nerve involvement was observed in 16(39.02%) cases, Necrosis and Calcification were observed in 31(75.60%) and 28 (68.90%) cases respectively. 20(48.78%) cases were found in pathological stage pT3 .

Conclusion: Rosettes, Necrosis and calcification were commonly observed findings in the study. Optic nerve and choroidal invasion were important prognostic factors for staging, prognosis and patient outcome. High incidence of pathological stage pT3 and poorly differentiated tumours were observed in this study. This could be due to late age of diagnosis.

Key Words: *Retinoblastoma, intraocular tumour, Choroid invasion; Optic nerve invasion, degree of differentiation, high risk factors.*

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Introduction

Retinoblastoma (RB), a rare tumour arising from the inner nuclear layer of the retina, is the most common primary intraocular malignancy in childhood and infancy [(1)]. It is generally believed to be congenital and derived

from primitive neuroectodermal cells exhibiting retinal differentiation [2-4]. Two mutations (hits), involving both alleles of *RB* at chromosome locus **13q14**, are required to produce retinoblastoma [5]. Knudson's 'two-hit hypothesis', has become a paradigm of tumorigenesis [6].

The incidence is estimated at about 1 in 15,000 to 20,000 live births [7-10]. This tumour accounts for 2.5 to 4% of all childhood tumours [11,14] , with no sexual predilection [12-14] .

The diagnosis is usually made before 3 years of age [11-14]. Bilateral cases are diagnosed in the first two years of life, occurring in 20 to 35% of cases [14]. The clinical picture depends on the stage of the disease, size and location of the tumour [11].

The form of growth of the neoplasia can be: exophytic, endophytic, mixed and diffuse [14]. Microscopically, retinoblastomas are composed of dense masses of small round cells with hyperchromatic nuclei and scanty cytoplasm [15]. Trabecular and nesting formations are common) [16]. The formation of rosettes described separately by Flexner and Wintersteiner, and "rapiers" can be considered forms of tumour cell differentiation into photoreceptors [11,13,14].

A characteristic feature of this tumour is its ability to grow faster than the available blood supply, and it is often found viable cell tufts with a central blood vessel ("pseudorosette") and necrosis and calcification from 90 to 110 micrometres [14].

Tumours showing an extreme degree of differentiation are designated as *retinocytomas* and regarded as benign [17]. These lesions carry the same genetic implications as conventional retinoblastomas; they present as small placoid, noninvasive lesions composed entirely of benign-appearing cells with numerous fleurettes, lacking necrosis or mitotic activity [17].

Invasion of the optic nerve and eye tunics are considered important histopathological findings related to prognosis [14,18].

Treatment depends on the staging and of the lesion, and enucleation remains the most common form of treatment [14,19].

Aim of this study was find out incidence rate of retinoblastoma, relationship between age, degree of differentiation and high risk factors at M. J Institute tertiary care ophthalmic centre.

Materials & Method

41 enucleated eyes received out of 41 prospectively registered case from june-2017 to june-2019 were included in study which was carried out at histopathology department, M.J western regional institute of ophthalmology, civil hospital Ahmedabad.

All reported cases were evaluated according to age in months at time of enucleation, sex, laterality and neoadjuvant chemotherapy.

The eyes were fixed in 10% neutral buffered formalin for at least 24 hours. Measurements of eyeball including optic nerve if present were taken first before section. Eyes were sectioned horizontally back to front begin from adjacent to optic nerve and end at cornea may passing through the centre of the tumour when possible. Macroscopic observation with stereoscopic magnifying glass (Leica MZ8) and photographic documentation of the specimen were performed.

The specimens (ring and distal optic nerve stump) were processed for histopathological study in tissue processor, embedded in paraffin blocks. Approximately 5 mm sections were made and hematoxylin-eosin (HE) stained slides were prepared for microscopic examination.

Microscopy was done and histopathological evaluation performed .Histological grades were given according to the degree of differentiation (percentage of Flexner-Wintersteiner rosettes present) and staging was done according to 8th AJCC classification of eye tumour. Sections were also evaluated according to degree of invasion in choroid, optic nerve, surrounding tissue, presence of necrosis and calcification.

Ethical Consideration: All procedures performed were in accordance with the ethical standards of the institution.

Results

Histopathological analysis of 41 enucleated eyes were received at histopathology department of M.J institute of ophthalmic care centre, Ahmedabad was done after all ethical approval by institute. Average incidence rate was found 6.65% per year.

Out of 41 cases, mean age at diagnosis 2-3 years(24

to 36 months) was found in 14(34.15%) of cases . 23(56.1%) cases were female and 18(43.90%) cases were male, 19(46.34%) cases were found in right eye, 22(53.66%) cases were found in left eye , all cases were unilateral, vitreous seeding was observed in 33 (80.49%) cases.

Table-1: Age and Sex Wise Distrebution

Sr. No	Age(Year)	Male	Female	Total	%
1	0-1	1	1	2	4.9
2	1-2	2	4	6	14.63
3	2-3	4	10	14	34.15
4	3-4	6	4	10	24.39
5	4-5	4	3	7	17.07
6	>5	1	1	2	4.88
	Total	18	23	41	100

Choroid invasion was observed in 36(87.80%) cases, when analysing degree of invasion was found in focal 10(27.78%), minimal 6(16.67%), massive 20(55.56%) cases.

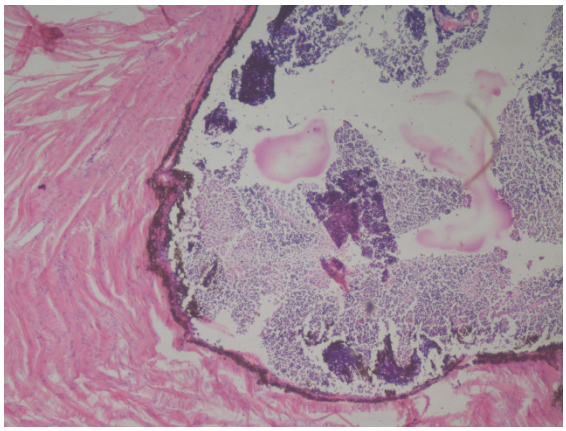
Table 2: Extent of Choroid Invasion

Sr. No	Choroid invasion	No. of cases	%
1	Focal	10	27.78
2	Minimal	6	16.67
3	Massive	20	55.56
	Total	36	100

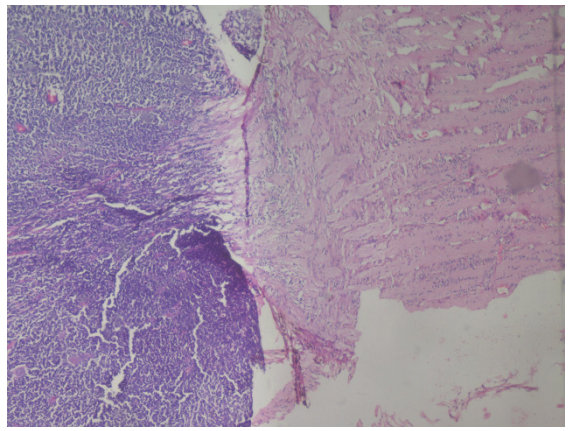
Optic nerve involvement was observed in 16(39.02%) cases. The degree of involvement was graded as table given below. 6 (37.5%) eyes were found with grade III –retrolaminar involvement without optic nerve margin invasion, 3(18.75%) eyes were found with grade Iv-optic nerve margin invasion.

Table 3: Optic Nerve Invasion Grade

Sr. No	Optic nerve invasion Grade	No. of cases	%
1	I	5	31.25
2	II	2	12.5
3	III	6	37.5
4	IV	3	18.75
	Total	16	100

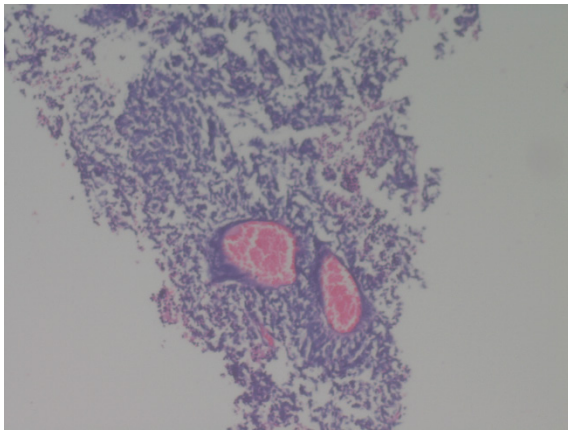


(A) Optic nerve invasion

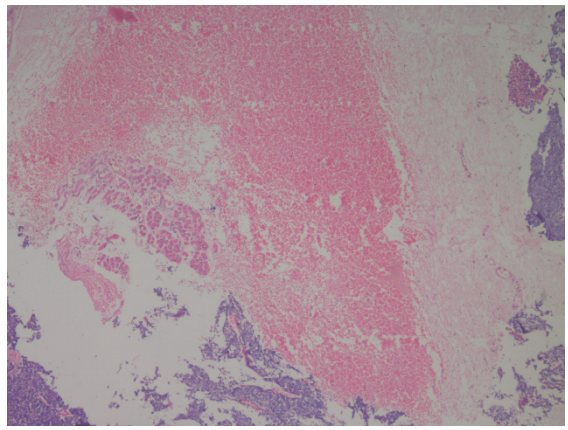


(B) Choroidal invasion

Necrosis and Calcification were observed in 31(75.60%) and 28 (68.90%) cases respectively. Basophilic deposits around vessels were found in 5(12.20%) cases.



(C) Necrosis



(D) Basophilic substance deposit around

Vessels

Anterior chamber involvement was observed in 1(2.44%) eye, scleral invasion was found with 1(2.44%) eye.

3(7.31%) enucleated eyes were received after post chemotherapy as primary treatment. When analysing chemotherapy taken eyes were moderately differentiated and better prognosis.

Table-4: Other Histopathological Features Wise Distribution

Sr. No	Parameters	No. of cases	%
1	Rosette	25	60.98
2	Pseudo rosette	33	80.49
3	Necrosis	31	75.60
4	Calcification	28	68.90
5	Basophilic substance around vessels	5	12.19
6	Anterior chamber invasion	1	2.44
7	Scleral invasion	1	2.44

Choroidal invasion and optic nerve invasion were staged according to 8th AJCC TNM classification, 20(48.78%) eyes were observed in pT3 stage.

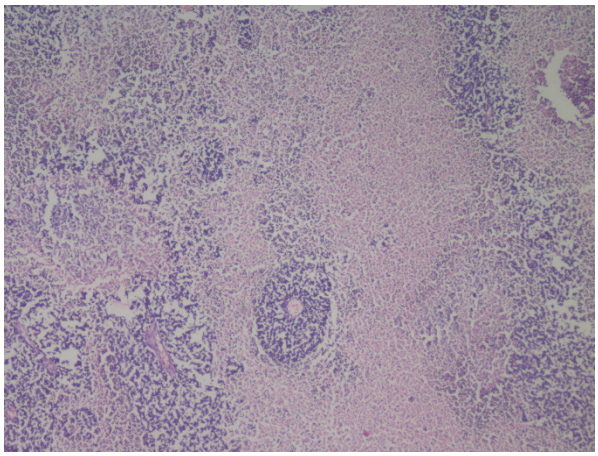
TABLE-5: TNM STAGE WISE DISTRIBUTION

Sr. No	Stage	No. of cases	%
1	pT1	5	12.20
2	pT2	14	34.15
3	pT3	20	48.78
4	pT4	2	4.89
	Total	41	100

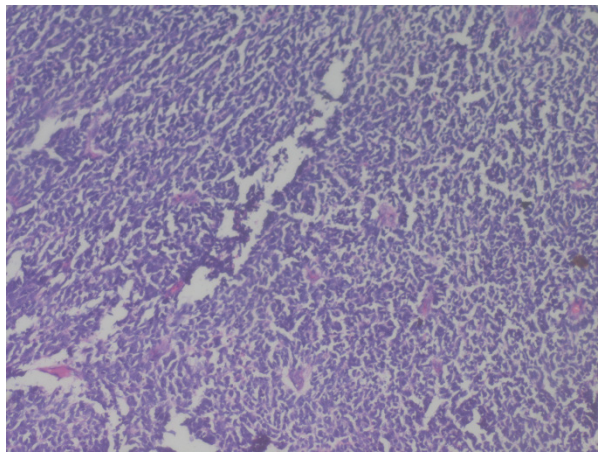
Degree of differentiation was defined as Grade. Out of 41 eyes, 25(61.0%) eyes were well differentiated and 5(12.20%) eyes were poorly differentiated.

TABLE-6: GRADE WISE DISTRIBUTION

Sr. No	Grade	No. of cases	%
1	Well	25	61.00
2	Moderately	11	26.82
3	Poorly	5	12.20
	Total	41	100



(E) Rosettes of tumour cells



(F) Nest and Cords of tumour cells

Discussion

In our present study mean age of enucleation was 2 to 3 years (24 to 36 months)

Compare with other study the average age of the patients at the time of enucleation was 22.7 months, ranging from 2 months to 96 months [14].

28 eyes studied from 27 patients, 13 (48.1%) were male and 14 (51.9%) female [14], present study Female were 23(56.1%) and male were 18(43.90%)

Bilateral and unilateral cases have the same fatality rate . Other study the tumour was bilateral in 13 (48.1%) cases and unilateral in 14 (51.9%) cases [14], in present study all cases were unilateral.

Grading of Optic nerve invasion (14):

When the optic nerve is not invaded, the mortality rate is approximately 8% .

Grade I: When it is vaded up to, but not involving, the lamina cribrosa (superficial involvement of the optic nerve head only), the mortality rate is approximately 10%

Grade II: When the invasion is up to and including the lamina cribrosa, the mortality rate is approximately 29%.

Grade III: When the invasion is beyond the lamina cribrosa, but not to the surgical margin, the mortality rate is approximately 42%. rade IV: When the invasion is to the line of transection or to the posterior point of exit of the central retinal vessels from the optic nerve, the mortality rate is approximately 67%

Post-laminar invasion of the optic nerve, even with free surgical margin (distal stump) (grade-III), is considered by most authors as a risk factor for the spread of the disease [14].

In the other study, 8 eyes (28.5%) with optic nerve invasion were found: 3 eyes with pre-laminar invasion, 4 eyes with laminar invasion and 1 eye with compromised surgical margin [14]. Khelfaoui et al found extraocular disease in 32% of cases with post-laminar invasion and free margin [14,20]. In Orellena et al, 4 2009 study 22/109 (20.2%) eye were found with retrolaminar invasion and 22/109 (20.2%) eyes were found with resected margin invasion[21].

In the present study, total 16(39.02%) eyes with optic nerve invasion were found: 6 (37.5%) eyes were found with grade III –retrolaminar involvement with optic nerve margin invasion, 3(18.75%) eyes were found with grade Iv-optic nerve margin invasion.

Choroid invasion as a risk factor for the spread of the disease is still questioned [14,22,23]. When choroidal invasion is slight, the mortality rate appears not to be affected; when the invasion is massive, the mortality rate is approximately 60%. Choroid invasion has been observed in different studies ranging from 12 to 62% of retinoblastoma-enucleated eyes [14,23].

In a study of 230 cases of retinoblastoma, choroid involvement was observed in 82% of the eyes, in another one study 64.2% of the eyes with choroid involvement were observed [14].

In Wilson et al, 28 2011a study 41/67 (61.5%) eyes were found with focal choroidal invasion and 17/67 (25.7%) eyes were found with massive choroidal invasion [21].

In the present study 36(87.80%) eyes with choroid involvement were observed. when analysing degree of invasion was found in focal 10(27.78%), minimal 6(16.67%), massive 20(55.56%) cases.

In other study, in 8 (28.5%) eyes there was simultaneous invasion of the eye tunics and optic nerve [14]. In present study, 16(39.02%) eyes were observed with simultaneous invasion of the eye tunics and optic nerve.

In other study 3(10.71%) cases were found in poorly differentiated stage [14], in present study, 5(12.20%) cases were found in poorly differentiated stage.

Conclusion

The prospective study held at M.J institute of ophthalmology during duration of June-2017 to June-2019.

The average incidence rate was found 6.65%. .

Mean age of enucleated eyes treated with primary treatment was 2- 3years (24 to 36 months).

There was no sex predilection for laterality.

Necrosis and calcification were two important prognostic factors found in 31(75.60%) and 28(68.90%) cases respectively.

Choroidal invasion and optic nerve invasion were found high risk factors in enucleated eyes treated with primary treatment 36(87.80%) cases and 16(39.02%) cases respectively.

Poor degree of differentiation in 5(12.20%) cases were found with advance age rather than early age of diagnosis. That would be due to late age of diagnosis.

Source of Funding: Self

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