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ARTICLE

High-risk retinoblastoma based on age at primary enucleation: a study of 616 eyes

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Abstract

Purpose To study the high-risk histopathology features of retinoblastoma based on age at primary enucleation.

Methods Retrospective study of 616 patients.

Results The mean age at presentation and primary enucleation for retinoblastoma was 34 months (median, 28 months; range, <1–455 months). Of these cases, 128 (21%) were aged ≤1 year, 149 (24%) were in the age group of 1–2 years, 117 (19%) in 2–3 years, 104 (17%) in 3–4 years, and 118 (19%) were >4 years of age at the time of enucleation. Bilateral retinoblastoma (34%; $p < 0.0001$) and buphthalmos (20%; $p < 0.0001$) were more common in children ≤1 year of age. Anterior chamber pseudohypopyon (15%; $p < 0.0001$) and vitreous seeds (53%; $p < 0.0001$) were more common in children aged >4 years. Based on 8th edition American Joint Committee on Cancer staging system, pT3 was less common in children ≤1 year of age (13%; $p < 0.001$). Based on histopathology, 38% patients had high-risk features including 24% children aged ≤1 year, 42% in the age group of 1–2 years, 34% in 2–3 years age group, 45% in 3–4 years age group, and 48% patients were >4 years of age. Post-laminar optic nerve infiltration (6%; $p = 0.02$) and massive choroidal infiltration (9%; $p = 0.04$) was least common in children ≤1 year of age. Over a mean follow-up period of 52 months (median, 36 months; range, <1–218 months), systemic metastasis and death occurred in 9% patients despite adjuvant systemic chemotherapy.

Conclusion The predominant high-risk histopathology feature of retinoblastoma varies with age at primary enucleation.

Introduction

Enucleation for retinoblastoma (RB) is a common modality of treatment in the developing countries owing to advanced intraocular disease at presentation. It forms the primary modality of treatment in nearly 35% patients with RB in India [1]. The definition of high-risk RB is not uniform across the oncology groups, though massive choroidal infiltration, post-laminar optic nerve infiltration, scleral and extrascleral tumour infiltration are considered as the high-risk histopathology features by most groups [2–6]. The occurrence of high-risk RB is twofold more common in Asian Indians with threefold increased risk of massive choroidal infiltration and fivefold increased risk of optic nerve infiltration compared with the West [7].

The age at detection of RB may vary in the developing and developed countries. However, early age at diagnosis of RB does not guarantee that the tumour is at an early stage and all eyes can be saved, though the proportion of patients needing enucleation may vary with age and stage of disease at presentation. Enucleation may be needed even in neonates with advanced intraocular RB [8, 9]. Based on age at presentation, the clinical signs and symptoms vary in cases with RB [8–11]. We hypothesize that the high-risk histopathological features of RB may also vary based on age at enucleation. In this study, we explore the differences in the high-risk histopathological features of RB based on age at primary enucleation.

Methods

This retrospective study was conducted at the Operation Eyesight Universal Institute for Eye Cancer, L V Prasad

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Eye Institute, Hyderabad, India. Institutional Review Board of L V Prasad Eye Institute, Hyderabad approved this study. All RB cases that underwent enucleation between the years January 2000 and June 2016 were reviewed. The patients who underwent primary enucleation for RB and had adequate clinical and histopathology data were included in this study. Those with inadequate data or had undergone secondary enucleation for RB were excluded.

Age at presentation, gender, hereditary pattern, tumour laterality, presenting complaints, and duration of symptoms were noted. The recorded clinical features included horizontal corneal diameter, intraocular pressure, anterior chamber findings (anterior chamber seeds/pseudohypopyon or hyphema), iris details (iris neovascularization, corectopia, posterior synechiae, ectropion uveae), lens status, evidence of orbital pseudocellulitis, and fundus details (tumour basal diameter, tumour thickness, subretinal fluid, subretinal or vitreous seeds, vitreous haemorrhage). Based on clinical features, the tumours were classified according to the Reese–Ellsworth Classification [12], International Classification of Retinoblastoma (ICRB) [13], and the 8th edition of American Joint Committee on Cancer (AJCC) staging system [14]. B-scan ultrasonography and orbital imaging (computed tomography or magnetic resonance imaging) findings were recorded. The details of treatment were recorded. The reason for enucleation was noted. In bilateral cases, the worse eye was enucleated as appropriate.

The following histopathology features were recorded: growth pattern (endophytic, exophytic, mixed endophytic-exophytic, diffuse infiltrating RB), tumour differentiation (well-differentiated, moderately differentiated, poorly differentiated, undifferentiated), anterior chamber seeding, iris infiltration, ciliary body infiltration, choroidal invasion [minor (<3 mm in greatest dimension) or major (>3 mm in greatest dimension of a single lesion or sum of all areas of choroidal invasion)], optic nerve infiltration (pre-laminar, laminar, post-laminar, or optic nerve transection), scleral infiltration, or microscopic extrascleral infiltration.

High-risk RB was defined as the presence of tumour seeds in the anterior chamber, iris/ciliary body infiltration, massive (≥3 mm) choroidal invasion, post-laminar optic nerve infiltration, optic nerve transection, combined pre-laminar/laminar optic nerve infiltration and minor choroidal invasion, or scleral/extrascleral tumour infiltration. All patients with high-risk RB were advised adjuvant systemic intravenous chemotherapy and those with involvement of optic nerve transection or scleral/extrascleral tumour infiltration were also advised additional external beam radiotherapy to the orbit. The details of adjuvant treatment, events of metastasis, and death were noted.

Statistical methods

The statistical analysis was performed using the software Origin v7.0 (OriginLab Corporation, Northampton, USA). The continuous data were summarized in mean, median and range, and categorical data in proportions. The continuous data were checked for normality by Shapiro–Wilk test and for equality of variance by Levene test. The continuous and categorical data were compared among various age groups by Kruskal–Wallis test and Chi-square test, respectively, and, for pair-wise comparisons, by Mann–Whitney test and Chi-square test respectively. A p value of <0.05 was considered statistically significant. For multiple pair-wise comparisons, Bonferroni correction was applied and p value of <0.0125 was considered statistically significant.

Results

A total of 916 patients underwent enucleation for RB during the study period. Of these, 288 patients underwent secondary enucleation and 12 patients had inadequate data and were excluded from the study. A total of 616 patients who underwent primary enucleation for RB were included in the study. Of these cases, 128 (21%) were aged ≤1 year, 149 (24%) were in the age group of 1–2 years, 117 (19%) in 2–3 years, 104 (17%) in 3–4 years, and 118 (19%) were >4 years of age at the time of enucleation.

The demographic and clinical features in Table 1. The mean age at presentation was 34 months (median, 28 months; range,<1–455 months). The mean duration of symptoms was 4 months (median, 1 month; range, <1–78 months). The time interval between the date of presentation and date of primary enucleation was less than 2 weeks in all cases. Of all cases, 57% patients were males and 43% were females. Bilateral RB (34%; $p < 0.0001$) and buphthalmos (20%; $p < 0.0001$) were more common in children ≤1 year of age. Anterior chamber pseudohypopyon (15%; $p < 0.0001$) and vitreous seeds (53%; $p < 0.0001$) were more common in children aged >4 years. Vitreous seeds (21%) were less common in children ≤1 year of age.

Based on AJCC (Table 2), pT3 was less common in children ≤1 year of age (13%; $p < 0.001$). Based on histopathology (Table 3), well-differentiated tumours were more common in children ≤1 year of age (56%; $p < 0.001$), and poorly differentiated tumours were commoner in children >3 years of age (65%; $p < 0.001$). Of all patients, 38% had high-risk features including 24% children aged ≤1 year, 42% in the age group of 1–2 years, 34% in 2–3 years age group, 45% in 3–4 years age group, and 48% patients were >4 years of age. The occurrence of high-risk histopathology features ($p = 0.06$) and the number of high-risk histopathology features ($p = 0.85$) were not statistically

Table 1 High-risk retinoblastoma based on age at presentation: demographics and clinical features

Feature	All ages ($n = 616$)	Age ≤1 year ($n = 128$)	Age 1–2 years ($n = 149$)	Age 2–3 years ($n = 117$)	Age 3–4 years ($n = 104$)	Age >4 years ($n = 118$)	p value
Age at presentation (months) mean (median, range)	34 (28, <1–455)	8 (9, <1–12)	20 (20, 13–24)	31 (32, 25–36)	43 (43, 37–48)	73 (62, 49–455)	N/A
Gender							
Male	352 (57)	79 (62)	80 (54)	67 (57)	56 (54)	70 (59)	0.95
Female	264 (43)	49 (38)	69 (46)	50 (43)	48 (46)	48 (41)	0.91
Hereditary pattern							
Sporadic	608 (99)	125 (98)	147 (99)	117 (100)	103 (99)	116 (98)	1.00
Familial	8 (1)	3 (2)	2 (1)	0 (0)	1 (1)	2 (2)	0.59
Laterality							
Unilateral	516 (84)	85 (66)	126 (85)	108 (92)	96 (92)	101 (86)	0.43
Bilateral	100 (16)	43 (34)	23 (5)	9 (8)	8 (8)	17 (14)	<0.0001[a]
Duration of symptoms (months) mean (median, range)	4 (1, <1–78)	3 (1, <1–44)	4 (2, <1–27)	4 (2, <1–36)	5 (1, <1–48)	4 (1, <1–78)	0.20
<6 months	539 (88)	118 (92)	129 (87)	99 (85)	87 (84)	106 (90)	0.99
>6 months	77 (12)	10 (8)	20 (13)	18 (15)	17 (16)	12 (10)	0.36
Buphthalmos	55 (9)	26 (20)	11 (7)	6 (5)	7 (7)	5 (4)	<0.0001[b]
Secondary glaucoma	205 (33)	35 (27)	45 (30)	35 (30)	44 (42)	46 (39)	0.37
Anterior chamber seeds/ pseudohypopyon	35 (6)	3 (2)	5 (3)	3 (3)	6 (6)	18 (15)	<0.0001[c]
Iris neovascularization	197 (32)	29 (23)	46 (31)	35 (30)	40 (38)	47 (40)	0.23
Hyphema	37 (6)	6 (5)	12 (8)	6 (5)	6 (6)	7 (6)	0.83
Ectropion uveae	133 (22)	23 (18)	30 (20)	23 (20)	26 (25)	31 (26)	0.67
Cataract	29 (5)	2 (2)	8 (5)	5 (4)	8 (8)	6 (5)	0.32
Largest tumour base (mm) mean (median, range)	19 (19, 5– 24)	19 (20, 10–24)	18 (19, 5–24)	19 (19, 10–24)	18 (18, 6–24)	18 (18, 8–24)	0.30
Tumour thickness (mm) mean (median, range)	15 (15, 3–24)	16 (16, 4–22)	16 (16, 3–23)	15 (16, 8–24)	15 (15, 3–22)	15 (15, 6–22)	0.38
Subretinal fluid	265 (43)	70 (55)	69 (46)	48 (41)	36 (35)	42 (36)	0.26
Subretinal seeds	130 (21)	28 (22)	30 (20)	27 (23)	24 (23)	21 (18)	0.92
Vitreous seeds	241 (39)	27 (21)	56 (38)	60 (51)	35 (34)	63 (53)	0.003[d]
Vitreous haemorrhage	67 (11)	8 (6)	17 (11)	8 (7)	16 (15)	18 (15)	0.11

N/A not applicable

[a]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from 1–2 years ($p = 0.006$), 2–3 years ($p < 0.001$), 3–4 years ($p < 0.001$) and >4 years ($p = 0.006$)

[b]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from 1–2 years ($p = 0.006$), 2–3 years ($p = 0.002$), 3–4 years ($p = 0.01$) and >4 years ($p = 0.001$)

[c]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only ages >4 years was significantly different from ≤1 year ($p = 0.001$), 1–2 years ($p = 0.002$) and 2–3 years ($p = 0.002$)

[d]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from 2–3 years ($p = 0.001$) and >4 years ($p < 0.0001$)

significant based on age at enucleation. Overall, post-laminar optic nerve infiltration was common in children at 1–2 years of age (23%), massive choroidal infiltration at 3–4 years (28%), and anterior chamber tumour seeding, iris infiltration, and ciliary body infiltration in children >4 years of age (14%, 9%, and 9%, respectively). Poorly differentiated tumours (15%; $p < 0.001$), massive choroidal invasion (9%; $p = 0.04$) and post-laminar optic nerve infiltration (6%; $p = 0.02$) were less common in children ≤1 year of age when compared with other age groups.

Of 237 patients with high-risk RB, 226 (95%) received adjuvant chemotherapy (Table 4). The remaining 11 patients did not receive adjuvant chemotherapy and were lost to follow-up. Overall, systemic metastasis and death

Table 2 High-risk retinoblastoma based on age at presentation: Tumour classification

Feature	All ages ($n = 616$)	Age <1 year ($n = 128$)	Age 1–2 years ($n = 149$)	Age 2–3 years ($n = 117$)	Age 3–4 years ($n = 104$)	Age >4 years ($n = 118$)	p value
ICRB classification							
Group A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
Group B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
Group C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
Group D	71 (12)	14 (11)	15 (10)	13 (11)	13 (13)	16 (14)	0.95
Group E	545 (88)	114 (89)	134 (90)	104 (89)	91 (88)	102 (86)	1.00
Reese–Ellsworth classification							
1A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
1B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
2A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
2B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
3A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
3B	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
4A	2 (<1)	1 (<1)	1 (<1)	0 (0)	0 (0)	0 (0)	0.65
4B	1 (<1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0.38
5A	272 (44)	82 (64)	66 (44)	42 (36)	49 (47)	33 (28)	0.007[a]
5B	341 (55)	45 (35)	82 (55)	75 (64)	55 (53)	84 (71)	0.03[b]
8th edition cT AJCC classification							
cT1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
cT2	30 (5)	5 (4)	7 (5)	5 (4)	7 (7)	6 (5)	0.91
cT3	586 (95)	123 (96)	142 (95)	112 (96)	97 (93)	112 (95)	1.00
cT4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	N/A
8th edition pT AJCC classification							
pT1	262 (43)	64 (50)	61 (41)	53 (45)	42 (40)	42 (36)	0.66
pT2	167 (27)	47 (37)	31 (21)	30 (26)	21 (20)	38 (32)	0.11
pT3	171 (28)	16 (13)	53 (36)	29 (25)	38 (37)	35 (30)	<0.001[c]
pT4	16 (3)	1 (<1)	4 (3)	5 (4)	3 (3)	3 (3)	0.58

ICRB international classification of retinoblastoma, *cT* clinical tumour, *pT* pathological tumour, *AJCC* American Joint Committee Classification, *N/A* not applicable

[a]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from ages 2–3 years ($p = 0.011$) and ages >4 years ($p = 0.001$)

[b]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from ages 2–3 years ($p = 0.008$) and ages >4 years ($p = 0.002$)

[c]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from 1–2 years ($p = 0.001$), 3–4 years ($p = 0.001$) and >4 years ($p = 0.007$)

occurred in 22 (4%) patients over a mean follow-up period of 52 months (median, 36 months; range, <1–218 months) and was comparable in all age groups. Of these 22 cases (Table 5), 21 (9%) cases had high-risk features on histopathology and all but one patient received adjuvant systemic chemotherapy. One patient had no high-risk features in the enucleated eye but died due to non-compliance to treatment for the contralateral eye, which progressed to develop extraocular tumour extension subsequently. Seven patients had received additional external beam radiotherapy to the orbit. The most common high-risk features in patients who died due to RB were post-laminar optic nerve infiltration with/without involvement of optic nerve transection ($n = 19$) and massive choroidal infiltration ($n = 17$). All patients ($n = 10$) with involvement of optic nerve transection died due to central nervous system involvement.

Discussion

The incidence of high-risk RB varies from 19 to 66%, with more cases in developing nations compared with the developed

Table 3 High-risk retinoblastoma based on age at presentation: histopathology features

Feature	All ages ($n = 616$)	Age <1 year ($n = 128$)	Age 1–2 years ($n = 149$)	Age 2–3 years ($n = 117$)	Age 3–4 years ($n = 104$)	Age >4 years ($n = 118$)	p value
Growth pattern ($n = 610$)							
Endophytic	287 (47)	57 (46)	64 (43)	59 (50)	54 (52)	53 (45)	0.90
Exophytic	241 (40)	59 (47)	68 (46)	42 (36)	33 (32)	39 (33)	0.36
Mixed	63 (10)	7 (6)	13 (9)	14 (12)	11 (11)	18 (15)	0.21
Diffuse infiltrating	18 (3)	2 (2)	3 (2)	2 (2)	6 (6)	5 (4)	0.27
Tumour differentiation ($n = 615$)							
Well-differentiated	134 (22)	71 (56)	31 (21)	11 (9)	8 (8)	13 (11)	<0.001[a]
Moderate differentiation	150 (24)	35 (28)	35 (24)	34 (29)	21 (20)	25 (21)	0.69
Poor differentiation	304 (49)	19 (15)	75 (51)	68 (58)	67 (65)	75 (64)	<0.001[b]
Undifferentiated	25 (4)	2 (2)	7 (5)	4 (3)	7 (7)	5 (4)	0.42
Anterior chamber tumour seeds	45 (7)	8 (6)	8 (5)	5 (4)	7 (7)	17 (14)	0.05
Iris infiltration	28 (5)	2 (2)	5 (3)	4 (3)	6 (6)	11 (9)	0.06
Ciliary body infiltration	29 (5)	4 (3)	5 (3)	2 (2)	7 (7)	11 (9)	0.06
Choroidal invasion	186 (30)	31 (24)	49 (33)	32 (27)	37 (36)	37 (31)	0.64
Minor (<3 mm)	66 (11)	19 (15)	14 (9)	10 (9)	8 (8)	15 (13)	0.45
Massive (≥3 mm)	120 (19)	12 (9)	35 (23)	22 (19)	29 (28)	22 (19)	0.04[c]
Optic nerve infiltration	279 (45)	51 (40)	74 (50)	55 (47)	47 (45)	52 (44)	0.89
Pre-laminar	60 (10)	16 (13)	15 (10)	9 (8)	9 (9)	11 (9)	0.83
Laminar	105 (17)	26 (20)	24 (16)	22 (19)	15 (14)	18 (15)	0.83
Post-laminar	103 (17)	8 (6)	34 (23)	20 (17)	21 (20)	20 (17)	0.02[d]
Optic nerve transection	11 (2)	1 (<1)	1 (<1)	4 (3)	2 (2)	3 (3)	0.43
Combination of pre-laminar/laminar optic nerve and non-massive choroidal involvement	27 (4)	9 (7)	3 (2)	4 (3)	3 (3)	8 (7)	0.20
Scleral infiltration	30 (5)	2 (2)	11 (7)	7 (6)	5 (5)	5 (4)	0.29
Extrascleral involvement	10 (2)	1 (<1)	4 (3)	4 (3)	1 (<1)	0 (0)	0.20
High-risk features of retinoblastoma	237 (38)	31 (24)	62 (42)	40 (34)	47 (45)	57 (48)	0.06
Number of high-risk features Mean (median, range)	2 (1, 1–7)	2 (1, 1–6)	2 (1, 1–5)	2 (1, 1–7)	2 (1, 1–7)	2 (1, 1–6)	0.85
1	150 (24)	23 (18)	40 (27)	24 (21)	29 (28)	34 (29)	0.42
2	43 (7)	4 (3)	9 (6)	8 (7)	11 (11)	11 (9)	0.25
3	24 (4)	2 (2)	6 (4)	3 (3)	3 (3)	10 (8)	0.08
4	11 (2)	1 (<1)	5 (3)	4 (3)	1 (<1)	0 (0)	0.14
5	5 (<1)	0 (0)	2 (1)	0 (0)	2 (2)	1 (1)	0.40
6	2 (<1)	1 (<1)	0 (0)	0 (0)	0 (0)	1 (1)	0.56
7	2 (<1)	0 (0)	0 (0)	1 (<1)	1 (<1)	0 (0)	0.47

[a]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from all the other groups (all $p < 0.001$)

[b]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from all the other groups (all $p < 0.001$)

[c]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from ages 1–2 years ($p = 0.008$) and ages 3–4 years ($p = 0.002$)

[d]Post-hoc analysis (Chi-square test with Bonferroni correction) showed that only age ≤1 year was significantly different from 1–2 years ($p = 0.001$) and 3–4 years ($p = 0.005$)

world [7]. In our study, the incidence of high-risk RB was 38%. High-risk RB was more common in children aged more than 4 years (48%) than children less than 1 year of age (24%), though the findings were not statistically significant.

In a study of 297 primarily enucleated eyes of RB, Eagle Jr. noted that there was a statistically significant inverse relationship between the age at enucleation and the degree of tumour differentiation [15]. The mean age at enucleation for

Table 4 High-risk retinoblastoma based on age at presentation: treatment and outcome

Feature	All ages ($n = 237$)	Age <1 year ($n = 31$)	Age 1–2 years ($n = 62$)	Age 2–3 years ($n = 40$)	Age 3–4 years ($n = 47$)	Age >4 years ($n = 57$)	p value
Adjuvant systemic chemotherapy ($n = 237$)	226 (95)	28 (90)	61 (98)	40 (100)	42 (89)	55 (96)	0.052
Systemic metastasis and death	22 (9)	3 (10)[a]	4 (6)	6 (15)	4 (9)	5 (9)	0.89

[a]One patient died due to non-compliance to treatment for the contralateral eye, which progressed to develop extraocular tumour extension. There were no high-risk features in the enucleated eye

Table 5 Details of patients who died due to retinoblastoma

Case	Age group	High-risk retinoblastoma	Details of high-risk features	Adjuvant chemotherapy	Adjuvant radiotherapy
1	<1 year	Yes	Massive choroidal invasion	Yes	No
2[a]	<1 year	No	N/A	N/A	N/A
3	<1 year	Yes	Anterior chamber seeding; ciliary body infiltration; massive choroidal invasion; involvement of optic nerve transection; scleral infiltration; extrascleral infiltration	Yes	No
4	1–2 years	Yes	Massive choroidal invasion; involvement of optic nerve transection; scleral infiltration; extrascleral infiltration	Yes	Yes
5	1–2 years	Yes	Massive choroidal invasion; post-laminar optic nerve infiltration; scleral infiltration;	Yes	No
6	1–2 years	Yes	Massive choroidal invasion	Yes	No
7	1–2 years	Yes	Massive choroidal invasion; post-laminar optic nerve infiltration	Yes	No
8	2–3 years	Yes	Massive choroidal invasion; post-laminar optic nerve infiltration; scleral infiltration; extrascleral infiltration	Yes	No
9	2–3 years	Yes	Massive choroidal invasion; post-laminar optic nerve infiltration; scleral infiltration; extrascleral infiltration	Yes	No
10	2–3 years	Yes	Massive choroidal invasion; involvement of optic nerve transection; scleral infiltration	Yes	No
11	2–3 years	Yes	Massive choroidal invasion; involvement of optic nerve transection; scleral infiltration; extrascleral infiltration	Yes	Yes
12	2–3 years	Yes	Anterior chamber seeding; iris infiltration; ciliary body infiltration; massive choroidal invasion; involvement of optic nerve transection; scleral infiltration; extrascleral infiltration	Yes	Yes
13	2–3 years	Yes	Massive choroidal invasion; involvement of optic nerve transection	Yes	Yes
14	3–4 years	Yes	Post-laminar optic nerve infiltration	Yes	No
15	3–4 years	Yes	Anterior chamber seeding; ciliary body infiltration; massive choroidal invasion; involvement of optic nerve transection; scleral infiltration	Yes	No
16	3–4 years	Yes	Anterior chamber seeding; iris infiltration; ciliary body infiltration; massive choroidal invasion; post-laminar optic nerve infiltration	Yes	No
17	3–4 years	Yes	Iris infiltration; ciliary body infiltration; massive choroidal invasion; post-laminar optic nerve infiltration	Yes	No
18	>4 years	Yes	Involvement of optic nerve transection	Yes	Yes
19	>4 years	Yes	Massive choroidal invasion; post-laminar optic nerve infiltration	Yes	No
20	>4 years	Yes	Involvement of optic nerve transection	Yes	Yes
21	>4 years	Yes	Post-laminar optic nerve infiltration	No	No
22	>4 years	Yes	Massive choroidal invasion; involvement of optic nerve transection	Yes	Yes

N/A not applicable

[a]One patient died due to non-compliance to treatment for the contralateral eye, which progressed to develop extraocular tumour extension. There were no high-risk features in the enucleated eye

tumours with abundant rosettes was 10 months, moderate rosettes was 18 months, fewer rosettes was 20 months, and for poorly differentiated tumours was 34 months [15]. Similar findings have also been reported by Madhavan et al. in their study of 170 eyes with RB [16]. Younger patients were commonly associated with well-differentiated tumours while older patients were commonly associated with poorly differentiated tumours. Similarly, in our study, well-differentiated tumours were more common in younger children (56% in children ≤1 year vs 11% in children older than 4 years; $p < 0.001$) and poorly differentiated tumours were more common in older children (15% in children ≤1 year vs 64% in children older than 4 years; $p < 0.001$). This suggests that well-differentiated tumours present earlier than poorly differentiated tumours. It is speculated that this could be related to either retinal cell affection at different levels of development or may represent tumour progression with time and subsequent dedifferentiation of well-differentiated tumours into poorly differentiated tumours [17].

In our study, there was difference in the most common high-risk histopathological feature based on age at enucleation. Post-laminar optic nerve infiltration was least common in children ≤1 year at 6% vs 17 to 23% in children older than 1 year, though the tumour basal diameter and thickness was comparable in all age groups. This finding could be related to the age-related changes in the biochemical composition of the extracellular matrix of lamina cribrosa, which is composed of collagen, elastin, and proteoglycans [17, 18]. With increasing age, there is age-related increase in total collagen and elastin, and decrease in type III to I collagen ratio and proteoglycan content [17, 18]. This results in age-related decrease in the resilience of lamina cribrosa [17, 18]. Better resilience to stress and pressure in very young children could be a protective factor against post-laminar optic nerve infiltration in children ≤1 year and decreasing resilience with age could result in relatively easy access of tumour cells through the weak lamina cribrosa to post-laminar region in eyes with stress secondary to posterior pole exophytic tumours. However, there was no consistent increase in post-laminar optic nerve infiltration with advancing age. In our study, the occurrence of optic nerve infiltration decreased after the age of 2 years. This could be related to direct correlation between the patient age and location of tumour [19]. It is known that the tumours follow a central-to-peripheral distribution with advancing age, and most posterior pole tumours are detected before the age of 2 years [19].

Similarly, massive choroidal infiltration was least common in children ≤1 year at 9% vs 19 to 28% in children older than 1 year in a setting of comparable tumour basal diameter and thickness in all age groups. Choroid is a vascular structure and is adherent to the sclera via numerous connective tissue strands, blood vessels, and nerves entering the choroid via the sclera. Thus scleral biochemical composition could influence the choroid behaviour. With increasing age, there is loss of collagen, elastin, and proteoglycans in the sclera resulting in a decrease in scleral elasticity [20]. High scleral elasticity and its influence on the overlying adherent choroid during very young age could be the cause of lesser chances of tumour infiltration to the underlying choroid.

Anterior chamber tumour seeding was more common in children older than 4 years at 14% compared with those ≤4 years at 4–7%. This finding could be related to increased chances of vitreous seeding in older children compared with younger children. In our study, vitreous seeding was more common in children >4 years (53%) compared with children ≤4 years (21–34%) ($p < 0.0001$). Free tumour cells in the vitreous or cell survival factors like VEGF and TGF-β expressed by the tumour cells can spread via the aqueous humour between the ciliary epithelium and vitreous base into the anterior chamber via the pupil and proliferate, presenting as anterior chamber pseudohypopyon [21].

Based on age at enucleation, post-laminar optic nerve infiltration was common in children at 1–2 years of age, massive choroidal infiltration at 3–4 years, and anterior chamber tumour seeding, iris infiltration, and ciliary body infiltration in children >4 years of age. This could be related to the central-to-peripheral distribution of RB lesions with advancing age [19].

Previous studies have shown that 24% of untreated children with high-risk RB develop systemic metastasis and adjuvant systemic chemotherapy minimizes the risk of systemic metastasis and death to 0–4% in patients with high-risk RB [3, 4, 7, 22]. In our study, 9% patients with high-risk RB died due to systemic or central nervous system metastasis, despite adjuvant systemic chemotherapy. This suggests that only 15% benefit from adjuvant systemic chemotherapy and the remaining 85% either receive unnecessary or ineffective treatment for high-risk RB. The most common high-risk feature associated with metastasis was post-laminar optic nerve infiltration, especially involvement of optic nerve transection, accounting for higher rate of systemic metastasis in this study. Despite aggressive treatment with chemotherapy and radiotherapy, involvement of optic nerve transection is associated with very high mortality of up to 80% [23–25]. Meticulous screening of optic nerve with pre-operative high-resolution magnetic resonance imaging of the orbit is mandatory prior to surgical planning.

In conclusion, the high-risk histopathology features vary based on age at enucleation. Earlier age at diagnosis and enucleation does not guarantee the absence of high-risk histopathology features, though the proportion of cases with high-risk features is less in younger children compared with older children. Optic nerve tumour infiltration and choroidal

involvement is less common in children ≤1 year of age compared with other age groups. This information is helpful in counselling the parents about the risk of high-risk features in eyes with advanced RB and need for further treatment based on age at diagnosis and/or enucleation.

Summary

What was known before

- High-risk features of retinoblastoma clinical features predicting high-risk retinoblastoma.

What this study adds

- The influence of age on the occurrence of high-risk retinoblastoma.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Kaliki S, Patel A, Iram S, Ramappa G, Mohamed A, Palkonda VAR. Retinoblastoma in India: clinical presentation and outcome in 1457 Patients (2074 Eyes). Retina. 2019;39:379–91.
2. Fabian ID, Stacey AW, Chowdhury T, Duncan C, Karaa EK, Scheimberg I, et al. High-risk histopathology features in primary and secondary enucleated international intraocular retinoblastoma classification group D eyes. Ophthalmology. 2017;124:851–8.
3. Kaliki S, Shields CL, Shah SU, Eagle RC Jr, Shields JA, Leahey A. Postenucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. Arch Ophthalmol. 2011;129:1422–7.
4. Kaliki S, Srinivasan V, Gupta A, Mishra DK, Naik MN. Clinical features predictive of high-risk retinoblastoma in 403 Asian Indian patients: a case-control study. Ophthalmology. 2015;122:1165–72.
5. Sreelakshmi KV, Chandra A, Krishnakumar S, Natarajan V, Khetan V. Anterior chamber invasion in retinoblastoma: not an indication for adjuvant chemotherapy. Invest Ophthalmol Vis Sci. 2017;58:4654–61.
6. Chantada GL, Dunkel IJ, de Dávila MT, Abramson DH. Retinoblastoma patients with high risk ocular pathological features: who needs adjuvant therapy? Br J Ophthalmol. 2004; 88:1069–73.
7. Kaliki S, Shields CL, Eagle RC Jr, Iram S, Shields JA. High-risk intraocular retinoblastoma: comparison between Asian Indians and Americans from two major referral centers. Retina. 2018;38:2023–9.
8. Kivelä TT, Hadjistilianou T. Neonatal retinoblastoma. Asia Pac J Oncol Nurs. 2017;4:197–204.
9. Abramson DH, Du TT, Beaverson KL. (Neonatal) retinoblastoma in the first month of life. Arch Ophthalmol. 2002;120:738–42.
10. Abramson DH, Notterman RB, Ellsworth RM, Kitchin FD. Retinoblastoma treated in infants in the first six months of life. Arch Ophthalmol. 1983;101:1362–6.
11. Shields CL, Shields JA, Shah P. Retinoblastoma in older children. Ophthalmology 1991;98:395–9.
12. Reese AB, Ellsworth RM. The evaluation and current concept of retinoblastoma therapy. Trans Am Acad Ophthalmol Otolaryngol. 1963;67:164–72.
13. Shields CL, Mashayekhi A, Au AK, Czyz C, Leahey A, Meadows AT, et al. The International Classification of Retinoblastoma predicts chemoreduction success. Ophthalmology. 2006; 113:2276–80.
14. Mallipatna AC, Gallie BL, Chévez-Barrios P, Lumbroso-Le Rouic L, Chantada G, et al. Retinoblastoma. In: Amin MB, Edge SB, Greene FL, Byrd DR, Brookland RK, et al., editors. AJCC Cancer Staging Manual. 8th ed. Switzerland: Springer; 2017. 819–31.
15. Eagle RC Jr. High-risk features and tumor differentiation in retinoblastoma: a retrospective histopathologic study. Arch Pathol Lab Med. 2009;133:1203–9.
16. Madhavan J, Ganesh A, Roy J, Biswas J, Kumaramanickavel G. The relationship between tumor cell differentiation and age at diagnosis in retinoblastoma. J Pediatr Ophthalmol Strabismus. 2008;45:22–5.
17. Albon J, Karwatowski WS, Avery N, Easty DL, Duance VC. Changes in the collagenous matrix of the aging human lamina cribrosa. Br J Ophthalmol. 1995;79:368–75.
18. Albon J, Purslow PP, Karwatowski WS, Easty DL. Age related compliance of the lamina cribrosa in human eyes. Br J Ophthalmol. 2000;84:318–23.
19. Abramson DH, Gombos DS. The topography of bilateral retinoblastoma lesions. Retina. 1996;16:232–9.
20. Rada JA, Achen VR, Penugonda S, Schmidt RW, Mount BA. Proteoglycan composition in the human sclera during growth and aging. Invest Ophthalmol Vis Sci. 2000;41:1639–48.
21. Crosby MB, Hubbard GB, Gallie BL, Grossniklaus HE. Anterior diffuse retinoblastoma: mutational analysis and immunofluorescence staining. Arch Pathol Lab Med. 2009; 133:1215–8.
22. Honavar SG, Singh AD, Shields CL, Meadows AT, Demirci H, Cater J, et al. Postenucleation adjuvant therapy in high-risk retinoblastoma. Arch Ophthalmol. 2002;120:923–31.
23. Magramm I, Abramson DH, Ellsworth RM. Optic nerve involvement in retinoblastoma. Ophthalmology. 1989;96:217–22.
24. Shields CL, Shields JA, Baez K, Cater JR, De Potter P. Optic nerve invasion of retinoblastoma. Metastatic potential and clinical risk factors. Cancer. 1994;73:692–8.
25. Kaliki S, Tahiliani P, Mishra DK, Srinivasan V, Ali MH, Reddy VA. Optic nerve infiltration by retinoblastoma: predictive clinical features and outcome. Retina. 2016;36:1177–83.