High-Risk Histopathology Features in Primary and Secondary Enucleated International Intraocular Retinoblastoma Classification Group D Eyes

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Short title: High-risk histopathology features in group D retinoblastoma.

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ABSTRACT

Purpose

To evaluate the rate and identify risk factors for high-risk histopathology (HRH) features in Group D retinoblastoma eyes enucleated as primary or secondary treatment.

Design

Retrospective analysis.

Participants

Sixty four enucleated Group D eyes (62 patients), of which 40 (40 patients) were primary and 24 (22 patients) secondary to other treatments.

Methods

Clinicopathologic correlation of consecutive group D eyes enucleated from 2002–2014. HRH features were defined as presence of anterior chamber seeds, iris infiltration, ciliary body/muscle infiltration, massive (≥3mm) choroidal invasion, retrolaminar optic nerve invasion, or combined non-massive choroidal and prelaminar/laminar optic nerve invasion.

Main Outcome Measures

HRH features, metastasis and death.

Results

Of the 64 Group D eyes, 37 (58%) were classified as cT2bN0M0H0, 24 (38%) as cT2bN0M0H1 and 3 (5%) as cT2aN0M0H1, according to the 8th edition cTNMH Retinoblastoma Staging. HRH features were detected in 10 (16%) eyes in the entire cohort; in 5 (13%) of the primary (pT3aNxM0 (n=2) and pT3bNxM0 (n=3); 8th edition pTNM) and 5 (21%) of the secondary (pT2bNxM0 (n=2), pT3aNxM0 (n=2) and pT3cNxM0 (n=1)) enucleated groups. Absence of vitreous seeds at presentation was the only predictive factor found for HRH features in the primary enucleation group (P=0.042), whereas none were found in the secondary group (P≥0.179). Anterior structures invasion (anterior chamber, iris, ciliary body/muscle) was detected significantly more after secondary enucleation (P=0.048). All patients with HRH features were treated with adjuvant chemotherapy and no metastases were recorded in a median follow-up time of 73.2 months (mean: 71.5, range: 13.7-153.0).

Conclusion

The choice of primary treatment for group D retinoblastoma should be carefully weighed, as, according to this study, 13% of eyes harbor HRH features at presentation, with absence of vitreous seeds being a potential risk factor. It is of special importance in Group D eyes being considered for non-systemic treatment, such as primary intra-ophthalmic artery chemotherapy. Secondary enucleated Group D eyes with HRH features more commonly involved anterior structures, warranting meticulous clinical and histological examinations for this subset of patients.

INTRODUCTION

The International Intraocular Retinoblastoma Classification (IIRC)[1] was introduced in 2005, in an era in which systemic chemotherapy has become the main treatment for intraocular retinoblastoma,[2–5] replacing the Reese-Ellsworth (R-E) classification.[6] The IIRC predicted chemotherapy success, with ≥90% salvage rate for groups A-C eyes and 47% salvage rate for D eyes.[7] Group E eyes are usually enucleated, due to irreversible ocular damage, but also due to the higher chance of adverse histology.[8–12] Such high risk histopathological (HRH) features, as tumour in the anterior chamber, uveal tract or optic nerve, are harbingers for metastatic spread, and therefore warrant adjuvant systemic chemotherapy to reduce the risk of death.[13]

The management of group D eyes then raises a dilemma: that some eyes undergoing an attempt at conservative therapy may actually have HRH, posing the risk of metastatic disease. A shift toward salvage therapy for group D eyes has occurred by use of systemic chemotherapy and lately by means of intra-ophthalmic artery chemotherapy (IAC).[14] The latter has been the subject of debate regarding systemic spread.[15] It has a high eye salvage rate,[16] but with selectivity to the eye there may be a potential risk of developing distant metastatic disease.[15] If such eyes are treated with systemic chemotherapy, then not only is intraocular disease successfully managed, but HRH features and distant disease treated at the same time. Many centers have favored systemic chemotherapy for Group D eyes for this reason, as first line globe retention therapy.

Most reports of HRH in the literature relate to group E eyes[13,17] or a heterogeneous mix of groups D and E. [8–12] In these studies, analysis of clinical features predicting HRH are not directly applicable to a physician trying to decide on systemic chemotherapy versus IAC or even enucleation for a group D eye. In the current study we aimed to understand better the clinicopathologic correlation between HRH and clinical factors in a large number of group D eyes. By analyzing eyes undergoing primary enucleation and secondary enucleation, where conservative therapy had been performed, we aimed to understand the clinical factors that might predict a low chance of HRH that might lead us to consider IAC as a first line conservative treatment.

METHODS

This was a retrospective chart review of all IIRC (Children's Hospital Los Angeles version)[1] group D cases from 10/04/2002 to 17/12/2014 that were managed at the London Retinoblastoma Service by means of primary enucleation, or initially with systemic chemotherapy, followed by adjuvant and salvage treatment, but eventually enucleated. During the study period, all children were examined and managed by 3 retinoblastoma specialists and histopathological evaluation of eyes that underwent primary or secondary enucleation was performed by 2 specialist pathologists. An ethical approval to carry out this study was granted by the Barts Health Trust institutional review board (number 6622).

Clinical data retrieved from medical charts included: age of presentation, disease laterality, family history of retinoblastoma, clinical features at presentation, genetic analysis results, type of primary, adjuvant and salvage treatments, detailed clinical data throughout follow-up, development of distant metastatic spread and death. In addition, all imaging scans performed at presentation and during follow-up were reviewed and analyzed.

Histopathology

For patients that underwent primary or secondary enucleation, after harvest of tumour tissue for genetic analysis, eyes were fixed in neutral buffered formalin and processed for light microscopy with sections stained with hematoxylin-eosin. Microscopic sections included the optic nerve and all ocular structures at multiple section levels of mid-globe and both calottes, and a transverse section from the surgical margin of optic nerve.

The histopathology reports of all patients that underwent enucleation (primary and secondary) were reviewed and analyzed. In cases in which HRH features were detected, the histopathology slides were pulled-out and re-analyzed. HRH features were defined as the presence of anterior chamber seeding, iris infiltration, ciliary muscle/body infiltration, massive (≥3 mm) choroidal invasion, retrolaminar optic nerve invasion, invasion of optic nerve surgical margin, combined non-massive choroidal and prelaminar/laminar optic nerve invasion, or scleral/extrascleral infiltration.[9]

TNM classification

After including all IIRC group D eyes and retrieval of data, eyes were classified according to the 8th edition AJCC/UICC clinical staging system (8th edition cTNMH).[18] In addition, enucleated high-risk retinoblastoma eyes were also classified according to the 8th edition AJCC/UICC pathological staging system (8th editions pTNM).[18]

Statistical Analysis

All calculations were performed using Microsoft Excel 2013 software (Microsoft Corporation, Redmond, WA) and SPSS software version 17.0 (SPSS, Inc., Chicago, IL). Risk factors to develop HRH features in primarily and secondarily enucleated eyes were calculated using Fisher's Exact Test and t-Test, for categorical and continuous variables, respectively. Comparison of HRH features between primarily and secondarily enucleated eyes was performed using Fisher's Exact Test. An alpha level of 0.05 and two-tailed p-values were used to determine statistical significance and an *a priori* decision was made that a multiple comparison correction would not be used.

RESULTS

During the study period, 104 group D eyes (92 patients) were managed at the London Retinoblastoma Service, 40 (38%, 40 patients (43%)) of which underwent primary enucleation and 64 (62%, 52 (57%) patients)) were treated initially with systemic chemotherapy. Of the latter group, 24 (38%, 22 patients (42%)) eyes underwent secondary enucleation, and these together with the 40 primary enucleated eyes comprise the study cohort. Hence there were 64 Group D eyes in this study.

Analyzing the whole study cohort, there were 36 (58%) males and 26 (42%) females, 19 (30%) presented with bilateral disease, 22 (35%) had positive *RB*1 mutation, 5 (22%) of which also had positive family history of retinoblastoma. The median age of presentation was 21.0 months (mean: 24.9, range: 0.6-144.0) and most common presenting sign was leucocoria (45 (72%) patients). Of the bilateral cases, the fellow eye was IIRC group A in 4 cases, B in 5, C in 2, D in 5 and E in 3. In terms of clinical examination at presentation, 6 (9%) eyes presented with multifocal disease, the retina was detached in 62 (97%) eyes, in 32 (50%) of which a total detachment was present, predominantly endophytic retinoblastoma growth pattern was present in 36 (56%) eyes and exophytic in 28 (44%), the optic disc was obscured in 58 (91%) eyes, a foveal tumour was present in 48 (75%) eyes, subretinal seeds in 22 (34%) eyes and vitreous seeds in 29 (45%) eyes. In terms of the 8th edition cTNMH, there were 37 (58%) eyes classified as cT2bN0M0H0, 24 (38%) as cT2bN0M0H1 and 3 (5%) as cT2aN0M0H1. The demographic, clinical variables, genetic analysis and 8th edition cTNMH classification subdivision of primary versus secondary enucleated D eyes is shown in **Supplemental Digital Table 1**.

HRH features were detected in 10 (16%) eyes in the entire cohort of 64 eyes. Of the 40 eyes that underwent primary enucleation, HRH features were detected in 5 (13%). **Table 1** summarizes the clinical features of these 5 eyes as compared to the 35 (87.5%) in which no HRH features were found. The only significant predictor for HRH features was the absence of vitreous seeds at presentation (P=0.042).

In the secondary enucleation group, the standard protocol of 6 cycles of intravenous vincristine, etoposide and carboplatin (VEC) was used in all patients treated with primary systemic chemotherapy. Additional adjuvant treatments in this cohort included diode laser and/or cryotherapy (11 (46%) eyes), plaque brachytherapy (5 (21%) eyes), EBRT (5 (21%) eyes), IAC (11 (46%) eyes), intra-vitreous chemotherapy (4 (17%) eyes) and second line systemic chemotherapy (2 (8%) eyes). In 5 (21%) of the secondary enucleated eyes HRH features were detected. Indications for secondary enucleation in these cases were progression of vitreous disease prior to the introduction of intra-vitreous chemotherapy in 2 cases (despite EBRT in both), widespread vitreous base relapse combined with diffuse subretinal hemorrhage in 1 case and tumour in the anterior chamber in 2 cases (**Figure 1**). **Table 2** summarizes the clinical features and management course of these 5 eyes as

compared to the 19 (79%) in which no HRH features were found. None of the variables were found to be significant predictors to develop HRH features (P≥0.179).

The detailed HRH features found in the 5 primarily versus 5 secondarily enucleated eyes, including the 8th pTNM classification, are shown in **Table 3**. Optic nerve and/or choroidal tumour invasion (**Figure 2**) was detected in 5 (100%) eyes that underwent primary enucleation as compared to 3 (60%) that underwent secondary enucleation (p=0.444). Superficial scleral invasion was detected in one secondary enucleated case. Regarding anterior structures tumour involvement (anterior chamber, iris, ciliary body and/or muscle), these were detected in none (0%) of the primarily enucleated eyes as compared to 4 (80%) eyes that underwent secondary enucleation (P=0.048).

The 5 patients diagnosed with HRH features following primary enucleation were further treated with 4 cycles of adjuvant VEC, and 5 patients diagnosed with HRH following secondary enucleation were treated with 4 cycles of ifosfamide, vincristine and doxorubicin (IVAd; n=3), topotecan, vincristine and doxorubicin (TVD; n=1) or thiotepa (n=1), depending on previous treatments and at the discretion of the managing medical oncologist. The whole cohort (N=64 eyes) was followed-up for a median time of 73.2 months (mean: 71.5, range: 13.7-153.0) and the 10 patients with HRH features for 78.6 months (mean: 79.7, range: 19.4-162.6). During this time, none of the patients has developed metastatic disease or died.

DISCUSSION

A choice of many treatments is available to the retinoblastoma specialist and several factors play a role in determining the opening strategy in treating this cancer. Much emphasis has been placed on conserving eyes that previously were enucleated as experience and technological knowledge has advanced, first with systemic chemotherapy, then IAC. The latter is an attractive option, with great success of globe retention, even in Group D eyes.[16] However, there is controversy regarding the possibility of metastasis and ultimately whether there is a risk of mortality.[15] The predictor of metastasis is tumour invasion to the choroid (> 3mm), optic nerve or anterior chamber, as these may act as portals outside the eye. While systemic chemotherapy will simultaneously treat these high-risk histopathology features and micrometastatic disease that has already left the eye, IAC will only treat the eye. The dilemma this poses is hardest in group D eyes, where the inclination is for conservative localized therapy with IAC, but the fear is risk of metastasis, as reported by Abramson et al. in 6%.[16] In this study we tried to better understand which clinical features would be able to predict HRH in group D eyes, and hence aid selection for IAC.

In the present study HRH features were detected in 10 (16%) eyes in the entire cohort. In the primary enucleation group 13% harbored high-risk histopathological features for systemic spread. All these patients were thereafter treated with 4 cycles of adjuvant intravenous chemotherapy and none has developed metastases at a median follow-up of 78.9 months. The rate of HRH features in primary enucleated D eyes found in the literature ranges from 13–33%, depending on the series and treating center (**Table 4**).[8–12] In 4 (80%) of these studies, rates ranged from 13-17%,[8–10,12] in keeping with our findings. All studies included both D and E eyes, in the latter of which HRH features were found to be more prevalent. In 3 (60%) of the studies in which clinical features to predict HRH features were investigated, IIRC group E, or typical features of this group (e.g. secondary glaucoma), were found to be significant. Hitherto HRH in group D eyes has not been assessed as a standalone group, so group E clinical features are not directly relevant when faced with deciding on treatment for a group D eye.

Our findings indicate that absence of vitreous seeds at presentation was the only significant predictive factor for HRH features in primary Group D enucleated eyes. No support for these findings was found in the literature. It could be hypothesized that the action of seeding into the vitreous may be associated with a decompressing effect on the main tumour on the choroid and/or optic disc. It could also be associated with growth pattern. However, our analysis indicated that endophytic versus exophytic pattern was a non-significant variable for HRH. Conversely, Palazzi et al.[19] found that primary enucleated eyes with exophytic retinoblastoma had significantly more choroidal invasion compared to endophytic retinoblastoma, though their study was conducted in the era of EBRT and enucleation, before the widespread introduction of chemotherapy. In our study, subretinal seeds were

present in the majority of both groups of primary enucleated eyes, with or without HRH features, and were not found to be a significant predictor for HRH.

In 21% of secondary enucleated eyes HRH features were detected. The rate of HRH features in this group are in keeping with those previously reported by Brennan et al. (HRH features found in 21% of groups B-D and 18% of group D secondarily enucleated eyes).[10] No clinical predictors to develop HRH features were found in the secondary enucleation group of eyes. Of note, all patients that underwent secondary enucleation had previously received systemic chemotherapy, potentially masking the extent of HRH features in this sub-population.[20] However, in contrast to being neoadjuvant therapy given immediately prior to enucleation, in most cases in the present study, enucleation was performed a long period after primary systemic chemotherapy was given, so presumably it had little or no masking effect.

The involvement of anterior structures by retinoblastoma, a HRH feature, occurred more commonly in the secondary enucleation group. Brennan et al. also found cases of anterior involvement after secondary enucleation, but in a cohort that included a range of IIRC groups, precluding a direct comparison to our results. In a previous report, we found anterior invasion on histopathology after failed IAC in 67% of IIRC groups B-D secondary enucleated eyes.[21] In the present study concentrating solely on group D eyes, previous IAC was not a significant factor for HRH features, nor for anterior structures involvement. Abramson and Gombos, in a study on the topography of retinoblastoma lesions in bilateral cases,[22] found that anterior tumours (i.e. anterior to equator) tended to develop at a later age or later in the course of disease, compared to posterior pole tumours. This idea supports our results, that more advanced or refractory retinoblastoma tumours propagate anteriorly. Anterior involvement following secondary enucleation was found in 4 eyes on histopathology, but detected clinically only in 2, in which tumour seeds were clearly seen in the anterior chamber. Retinoblastoma infiltrating into the ciliary body was more easily missed on clinical examination, further highlighting the need for a meticulous histopathology examination of all intraocular sites in enucleated retinoblastoma eyes.

Limitations of the study include its retrospective design. Nevertheless, we were able to retrieve detailed data from medical charts and imaging devices in a relatively large group D retinoblastoma cohort and to reassess histopathology slides of patients with HRH features. Some ambiguity exists in the clinical features in a group D eye such as tumour focality (unifocal versus multifocal), presence of retinal seeds, especially when a large retinal detachment is present, and tumour growth type (endophytic versus exophytic). Review of sequential imaging was found to be useful. Detection of vitreous seeds on clinical examination, in contrast, is an easier and presumably more accurate undertaking. Interestingly, this was found to be the only significant variable to predict HRH features. In this regard, with increased statistical testing, the family-wise error rate in general

increases.[23] However, given the small sample size, which works against the power of the statistical comparisons, we continue to believe that any statistically significant finding, even in the setting of an uncorrected p-value, could be clinically relevant and should be considered during future retinoblastoma research.

In summary, in this study, high-risk histopathology features were found in 13% and 21% of primary and secondary enucleated group D retinoblastoma eyes, respectively. All patients were treated with adjuvant systemic chemotherapy and no cases of distant metastases or deaths were recorded. Vitreous seeds at presentation were found significantly more in patients with no HRH features, potentially serving as a clinical sign to be taken into account when deciding on the mode of primary treatment. It is of additional importance in case eye-selective intra-ophthalmic artery chemotherapy is to be considered, as these patients may not be systemically protected. Anterior structures involvement was found significantly more after initial treatment with conservative chemotherapy compared to primary enucleation, in some cases only subclinically, proven on histopathology alone. Meticulous clinical examination is warranted as well as careful histopathology evaluation after a group D retinoblastoma eye is removed.

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REFERENCES

1. Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. *Ophthalmol Clin North Am*. 2005;18:41–53.

2. Gallie BL, Budning A, DeBoer G, et al. Chemotherapy with focal therapy can cure intraocular retinoblastoma without radiotherapy. *Arch Ophthalmol* (Chicago, Ill 1960). 1996;114:1321–8.

3. Kingston JE, Hungerford JL, Madreperla SA, Plowman PN. Results of combined chemotherapy and radiotherapy for advanced intraocular retinoblastoma. *Arch Ophthalmol* (Chicago, Ill 1960). 1996;114:1339–43.

4. Murphree AL, Villablanca JG, Deegan WF, et al. Chemotherapy plus local treatment in the management of intraocular retinoblastoma. *Arch Ophthalmol* (Chicago, Ill 1960). 1996;114:1348–56.

5. Shields CL, De Potter P, Himelstein BP, et al. Chemoreduction in the initial management of intraocular retinoblastoma. *Arch Ophthalmol* (Chicago, Ill 1960). 1996;114:1330–8.

6. REESE AB, ELLSWORTH RM. The evaluation and current concept of retinoblastoma therapy. *Trans Am Acad Ophthalmol Otolaryngol.* 1963;67:164–72.

7. Shields CL, Mashayekhi A, Au AK, et al. The International Classification of Retinoblastoma predicts chemoreduction success. *Ophthalmology*. 2006;113:2276–80.

8. Wilson MW, Qaddoumi I, Billups C, et al. A clinicopathological correlation of 67 eyes primarily enucleated for advanced intraocular retinoblastoma. *Br J Ophthalmol*. 2011;95:553–8.

9. Kaliki S, Srinivasan V, Gupta A, et al. Clinical Features Predictive of High-Risk Retinoblastoma in 403 Asian Indian Patients A Case-Control Study. *Ophthalmology*. 2015;122:1165–1172.

10. Brennan RC, Qaddoumi I, Billups CA, et al. Comparison of high-risk histopathological features in eyes with primary or secondary enucleation for retinoblastoma. *Br J Ophthalmol*. 2015;99:1366–71.

11. Yousef YA, Al-Hussaini M, Mehyar M, et al. PREDICTIVE VALUE OF TNM CLASSIFICATION, INTERNATIONAL CLASSIFICATION, AND REESE-ELLSWORTH STAGING OF RETINOBLASTOMA FOR THE LIKELIHOOD OF HIGH-RISK PATHOLOGIC FEATURES. *Retina*. 2015;35:1883–9.

12. Kaliki S, Shields CL, Rojanaporn D, et al. High-risk retinoblastoma based on international classification of retinoblastoma: analysis of 519 enucleated eyes. *Ophthalmology*. 2013;120:997–1003.

13. Kaliki S, Shields CL, Shah SU, et al. Postenucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. *Arch Ophthalmol (*Chicago, Ill 1960). 2011;129:1422–7.

14. Abramson DH, Shields CL, Munier FL, Chantada GL. Treatment of Retinoblastoma in 2015: Agreement and Disagreement. *JAMA Ophthalmol*. 2015:1–7.

15. Yousef YA, Soliman SE, Astudillo PPP, et al. Intra-arterial Chemotherapy for

Retinoblastoma: A Systematic Review. *JAMA Ophthalmol*. 2016 [Epub ahead of print].

16. Abramson DH, Daniels AB, Marr BP, et al. Intra-Arterial Chemotherapy (Ophthalmic Artery Chemosurgery) for Group D Retinoblastoma. *PLoS One*. 2016;11:e0146582.

17. Chawla B, Sharma S, Sen S, et al. Correlation between clinical features, magnetic resonance imaging, and histopathologic findings in retinoblastoma: A prospective study. *Ophthalmology*. 2012;119:850–856.

18. Mallipatna AC, Gallie BL, Chévez-Barrios P et al. Retinoblastoma. In: Amin MB, Edge SB, Greene FL, et al., eds. AJCC Cancer Staging Manual. 8th ed. New York: Springer; 2017.

19. Palazzi M, Abramson DH, Ellsworth RM. Endophytic vs exophytic unilateral retinoblastoma: is there any real difference? *J Pediatr Ophthalmol Strabismus*. 1990;27:255–8.

20. Zhao J, Dimaras H, Massey C, et al. Pre-enucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumor extension and increases death from metastasis. *J Clin Oncol*. 2011;29:845-51.

21. Pavlidou E, Burris C, Thaung C, et al. Anterior Segment Seeding in Eyes With Retinoblastoma Failing to Respond to Intraophthalmic Artery Chemotherapy. *JAMA Ophthalmol*. 2015;133:1455–8.

22. Abramson DH, Gombos DS. The topography of bilateral retinoblastoma lesions. *Retina*. 1996;16:232–9.

23. Stacey AW, Pouly S, Czyz CN. An analysis of the use of multiple comparison corrections in ophthalmology research. *Invest Ophthalmol Vis Sci*. 2012;53:1830-4.

FIGURE LEGEND

Figure 1 – (A) Color photograph (RetCam, City, USA) showing retinoblastoma tumour cells in the anterior chamber (arrow) and (B) histopathology of the same eye after secondary enucleation showing viable tumour cells attached to the iris and extending into the drainage angle and cornea.

Figure 2 – Histopathology after primary enucleation showing (A) choroidal and (B) retro-laminar invasion.

Figure 1A

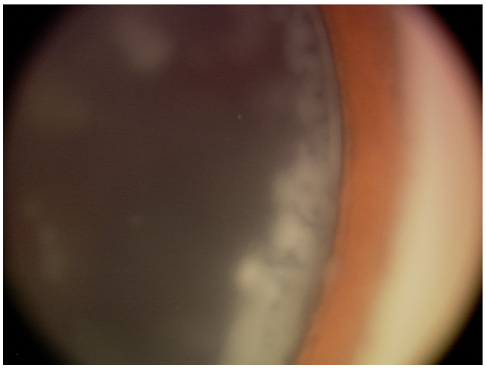

Figure 1B

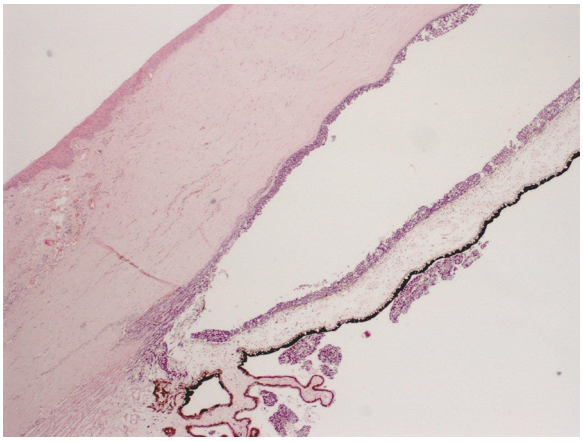

Figure 2A

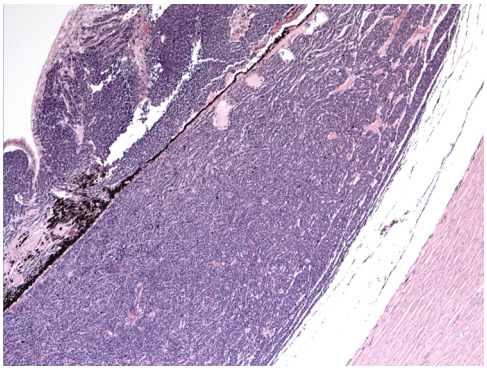

Figure 2B

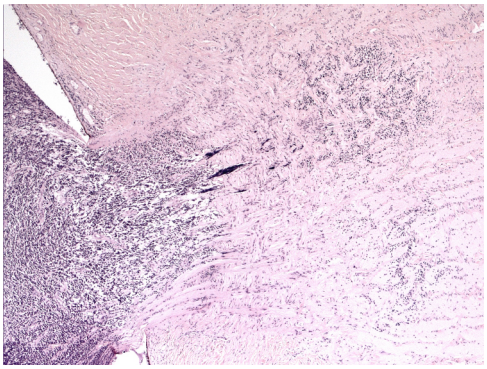

Table 1. Clinical features of 40 (40 patients) primary enucleated group D eyes: high-risk histopathology (HRH) features (n=5) versus no HRH features (n=35).			
Parameter	**HRH features (N=5)**	**No HRH features (N=35)**	**Significance**
Gender			P=0.642
Male	2 (40%)	20 (57%)	
Female	3 (60%)	15 (43%)	
Uni/bilateral retinoblastoma			P=0.338
Unilateral	4 (80%)	33 (94%)	
Bilateral	1 (20%)	2 (6%)	
Age of diagnosis (months)			P=0.683
Median (mean, range)	24.0 (24.0, 1.0-60.0)	22.0 (21.2, 4.0-36.0)	
Presenting signs			P=0.922
Leucocoria	4 (80%)	28 (80%)	
Strabismus	1 (20%)	6 (17%)	
Leucocoria and strabismus	0 (0%)	1 (3%)	
RB1 Blood mutation			P=1.000
Negative	4 (80%)	28 (80%)	
Positive	1 (20%)	7 (20%)	
Laterality			P=1.000
RE	3 (60%)	18 (51%)	
LE	2 (40%)	17 (49%)	
Tumour focality			P=1.000
Unifocal	5 (100%)	34 (97%)	
Multifocal	0 (0%)	1 (3%)	
Retinoblastoma growth pattern			P=0.155
Endophytic	1 (20%)	21 (60%)	
Exophytic	4 (80%)	14 (40%)	
Optic disc obscured			P=1.000
Not obscured	0 (0%)	2 (6%)	
Obscured	5 (100%)	33 (94%)	
Fovea involvement			P=0.490
Not involved	0 (0%)	2 (6%)	

Sub-foveal fluid	0 (0%)	6 (17%)	
Foveal tumour	5 (100%)	27 (77%)	
Retinoblastoma seeds			
No seeds	0 (0%)	0 (0%)	P=1.000
Sub-retinal	4 (80%)	21 (60%)	P=0.633
Vitreous	1 (20%)	25 (71%)	P=0.042

Table 2. Clinical features at presentation and initial management of 24 (22 patients) group D eyes that underwent secondary enucleation: high-risk histopathological (HRH) features (n=5) versus no HRH features (n=19).

Parameter	HRH Features (N=5)	No HRH Features (N=19)	Significance
Gender			P=0.608
Male	3 (60%)	14 (74%)	
Female	2 (40%)	5 (26%)	
Uni/bilateral retinoblastoma			P=0.568
Unilateral	2 (40%)	4 (21%)	
Bilateral	3 (60%)	15 (79%)	
Age of diagnosis (months)			P=0.303
Median (mean, range)	20.0 (42.6, 5.0-144.0)	10.0 (23.0, 0.6-108.0)	
RB1 Blood mutation			P=0.568
Negative	2 (40%)	4 (21%)	
Positive	3 (60%)	15 (79%)	
Laterality			P=1.000
RE	4 (80%)	14 (74%)	
LE	1 (20%)	5 (26%)	
Tumour focality			P=1.000
Unifocal	4 (80%)	15 (79%)	
Multifocal	1 (20%)	4 (21%)	
Retinoblastoma growth pattern			P=1.000
Endophytic	3 (60%)	11 (58%)	
Exophytic	2 (40%)	8 (42%)	
Optic disc obscured			P=0.179
Not obscured	2 (40%)	2 (11%)	
Obscured	3 (60%)	17 (89%)	
Fovea involvement			P=0.849
Not involved	1 (20%)	2 (11%)	
Sub-foveal fluid	2 (40%)	3 (16%)	
Foveal tumour	2 (40%)	14 (74%)	
Retinoblastoma seeds			
No seeds	0 (0%)	3 (16%)	P=1.000
Sub-retinal	4 (80%)	13 (68%)	P=1.000
Vitreous	3 (60%)	6 (32%)	P=0.326
Adjuvant/salvage treatments			
Diode laser/cryo	2 (40%)	9 (47%)	P=1.000
Plaque brachytherapy	2 (40%)	3 (16%)	P=0.271
IAC	3 (60%)	8 (42%)	P=0.630

Intra-vitreous chemotherapy	0 (0%)	1 (5%)	P=1.000
EBRT	2 (40%)	3 (16%)	P=0.271
Time to enucleation (months)			P=0.729
Median (mean, range)	27.1 (27.0, 17.0-39.9)	8.9 (22.9, 2.6-91.0)	
IAC – intra-ophthalmic artery chemotherapy; EBRT – external beam radiotherapy.			

Table 3. High-risk histopathological (HRH) features in primary (n=5) and secondary (n=5) enucleated group D eyes: type of HRH features and 8th AJCC pTNM Retinoblastoma staging.[18]

#	RE/LE	High-risk histopathological features							8th ed. pTNM
		CM/CB/iris/AC invasion	ON invasion			Choroidal invasion		Scleral/ extrascleral invasion	
			Prelaminar/ laminar	Retrolaminar	Transection	Non-massive	Massive		
Primary enucleation									
1	LE	-		+	-	-	-	-	pT3bNxM0
2	LE	-		+	-	+	-	-	pT3bNxM0
3	RE	-	+		-		+	-	pT3aNxM0
4	RE	-	-	-	-		+	-	pT3aNxM0
5	RE	-		+	-	+	-	-	pT3bNxM0
Secondary enucleation									
6	LE	CM, CB, iris & AC	-	-	-	-	-	-	pT2bNxM0
7	RE	CB & iris	-	-	-	-	-	-	pT2bNxM0
8	RE	CM, iris & AC	-	-	-		+	Superficial scleral invasion	pT3cNxM0
9	LE	Reaches but does not infiltrate CB	-	-	-		+	-	pT3aNxM0
10	RE	CB	+		-		+	-	pT3aNxM0

CM – ciliary muscle, CB – ciliary body, AC – anterior chamber, ON – optic nerve.

Table 4. High-risk histopathological (HRH) features in primarily enucleated retinoblastoma eyes: comparison to published reports in which group D eyes were included.

Authors	Year	Number of eyes (%)		Eyes with HRH features (% of IIRC group)		Clinical variables predictive of HRH features
		D	E	D	E	
Wilson et al.[8]	2011	47 (70)	20 (30)	6 (13)	10 (50)	IIRC group E
Kaliki et al.[9]	2015	50 (12)	353 (88)	8 (16)	137 (39)	prolonged duration of symptoms of >6 months and secondary glaucoma
Yousef et al.[11]*	2015	24 (57)	18 (43)	8 (33)	11 (61)	Higher 7th edition clinical TNM stage and more advanced ICRB group (i.e., group E eyes)
Brennan et al.[10] **	2015	54 (39)	86 (61)	8 (15)	37 (43)	NA
Kaliki et al.[12]	2013	87 (17)	432 (83)	15 (17)	102 (24)	NA
Fabian et al. (present study)	2016	40 (100)	-	5 (13)	-	Absence of vitreous seeds at presentation

IIRC – International Intraocular Retinoblastoma Classification.[1]

* Eight IIRC group C were also included in the study, in 1 of which HRH features were detected.

** One IIRC group B and 1 group C were also included in the study, in addition to 2 cases in which IIRC grouping was not available. In one of the latter cases, HRH features were detected.

Supplemental Digital Table 1. Primary versus secondary enucleation in 64 group D eyes: demographic, clinical variables, genetic analysis and classification according to the 8th edition cTNMH Retinoblastoma Staging.[18]

Parameter	Primary enucleation N=40 patients (%) N=40 eyes (%)	Secondary enucleation N=22 patients (%) N=24 eyes (%)
Gender		
Male	22 (55)	14 (64)
Female	18 (45)	8 (36)
Uni/bilateral retinoblastoma		
Unilateral at presentation	37 (93)	6 (28)
Bilateral at presentation	3 (7)	16 (72)
Age of diagnosis (months)		
Median (mean, range)	24.0 (23.7, 1.0-60.0)	12.5 (8.8, 0.6-144.0)
Family history of retinoblastoma		
Negative (sporadic)	40 (100)	17 (77)
Positive (familial)	0 (0)	5 (23)
RB1 Blood mutation		
Negative	32 (80)	6 (27)
Positive	8 (20)	16 (73)
Tumour focality		
Unifocal	39 (98)	19 (79)
Multifocal	1 (2)	5 (21)
Tumour dimensions (mm)		
Median (mean, range)		

Height	10.4 (10.4, 6.9-13.7)	11.5 (10.8, 5.1-13.9)
Base	14.2 (14.1, 9.2-17.5)	14.3 (13.6, 8.8-16.2)
Quadrants of retinal detachment		
No detachment	2 (5)	0 (0)
Local	6 (15)	4 (17)
1	3 (8)	1 (4)
2	3 (8)	3 (13)
3	8 (20)	2 (8)
4	18 (45)	14 (58)
Retinoblastoma growth pattern		
Endophytic	22 (55)	14 (58)
Exophytic	18 (45)	10 (42)
Optic disc obscured		
Not obscured	2 (5)	4 (17)
Obscured	38 (95)	20 (83)
Fovea involvement		
Not involved	2 (5)	3 (13)
Sub-foveal fluid	6 (15)	5 (21)
Foveal tumour	32 (80)	16 (67)
Retinoblastoma seeds		
No seeds	0 (0)	3 (13)
Sub-retinal	15 (38)	7 (29)
Vitreous	14 (35)	15 (63)
8th edition cTNMH		
cT2bN0M0H0	32 (50%)	5 (8%)
cT2bN0M0H1	8 (13%)	16 (25%)
cT2aN0M0H0	0 (0%)	0 (0%)
cT2aN0M0H1	0 (0%)	3 (5%)
Time to enucleation (months)		
Median (mean, range)	0.2 (0.3, 0.2-1.4)	16.2 (23.7, 2.6, 91.0)
Total follow-up time (months)		
Median (mean, range)	73.2 (68.8, 19.0-124.9)	73.2 (76.1, 13.7-153.0)