ORIGINAL ARTICLE

OCCURRENCE RATE OF HIGH-RISK HISTOLOGICAL FEATURES IN PRIMARY ENUCLEATION SPECIMENS OF PAEDIATRIC RETINOBLASTOMA PATIENTS: A LOCAL EXPERIENCE

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Background: Retinoblastoma, the most common primary intraocular malignancy in childhood, predominantly affects children under the age of five. With an incidence of approximately 1 in 15,000 to 20,000 live births globally, the condition sadly leads to over 3000 childhood deaths annually, with significantly higher mortality rates in Asia and Africa. This study, conducted in the Histopathology Department of Foundation University Medical College, Rawalpindi, over a one-year period (from 25th September 2019 to 24th September 2020), aimed to determine the frequency of high-risk histological features in primary enucleated eye specimens with retinoblastoma to guide therapeutic strategies. A cross-sectional study design was employed, utilizing non-probability consecutive sampling. **Methods:** After approval from ethical review board, all patients fulfilling the inclusion criteria were enrolled in the study. All the demographic details of the patients including age, gender, duration since the diagnosis and family history of retinoblastoma were obtained. The specimens of retinoblastoma received form eye department were marked. Freshly cut 3-5 microns thick sections stained with Haematoxylin and Eosin (H & E) were examined microscopically by a team of two histopathologists. The presence or absence of high-risk histological features was reported /recorded in the designed proforma. **Results:** A total of 150 patients were included in the study. The mean age of the patients was found to be 2.51±1.30 years. The gender distribution showed that most of the patients were males. The mean duration since diagnosis was 4.70±2.81 months. Positive choroid invasion was found in 25 patients (16.7%) and optic nerve invasion was found in 77 patients (51.3%). **Conclusion:** The study showed that optic nerve invasion was found in significant number of patients and was more prevalent than choroid invasion in patients with retinoblastoma. This will help our clinicians to better guide our patients regarding the prognosis of the disease and deciding the treatment options for the patients.

Keywords: Children; Eye; High risk Histology (HRH); Retinoblastoma

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INTRODUCTION

Retinoblastoma is the most common primary intraocular malignancy of childhood.[1,2] The majority of cases are seen in children aged <5 years. The incidence of retinoblastoma is approximately 1 in 15,000 to 20,000 live births worldwide. Unfortunately, more than 3000 children die of retinoblastoma every year, with mortality rates being significantly higher in Asia and Africa.[3–5]

Incidence of retinoblastoma showed variation in different regions of world. In Asia, highest number of cases were seen in Jordan followed by Saudi Arabia with lowest in Qatar. Similarly in Europe, highest incidence was seen in Belgium and lowest in Australia.[3] The treatment protocol is determined depending on tumour characteristics at the time of initial diagnosis. Conservative treatment modalities for retinoblastoma include systemic chemo-reduction, focal consolidation with laser photocoagulation, cryotherapy or thermotherapy, plaque brachytherapy, and local chemotherapy.[3] The surgical treatment options for retinoblastoma include neo-adjuvant chemotherapy in case of advanced disease followed by enucleation.(In case of advanced disease the treatment options incudes neoadjuvant chemotherapy followed by enucleation).[3,6] However, in most of the cases with relatively early presentation, enucleation is first line of management. Post-operative chemotherapy or

radiotherapy may also be given, but this decision is made after histological examination of enucleated specimen.[7–9] If the specimen has extra-ocular invasion and extension into choroid, iris or orbital nerve, it is called high-risk histological (HRH) features[8,10,11] and further chemotherapy or radiotherapy may be warranted. HRH are considered to be associated with more risk for metastasis and higher mortality and morbidity of the disease.[10]

This study was undertaken to identify HRH features of retinoblastoma in enucleated samples to help guide the clinicians in terms of prognosis and treatment options. There is a dearth of local studies on this topic hence arising the need for this study on local population.

MATERIAL AND METHODS

The study was designed as a cross-sectional analysis conducted over a one-year period, from September 25, 2019, to September 24, 2020, in the Histopathology Department of FUMC and the Ophthalmology Department of Fauji Foundation Hospital, Rawalpindi. A total of 150 patients were included in the study, with the sample size determined using a confidence level of 92%, a margin of error of 8%, and an expected frequency of choroid invasion of 44% among patients diagnosed with retinoblastoma. The sampling technique employed was non-probability, consecutive sampling. The inclusion criteria encompassed all patients who had undergone primary enucleation for retinoblastoma at Fauji Foundation Hospital during the study period. Exclusion criteria consisted of patients who had received pre-operative chemotherapy or radiotherapy, as such treatments could potentially alter the histologic features of the tumor, as well as patients who exhibited metastasis at the time of surgery, indicating an advanced stage of the disease, based on medical records.

After approval from ethical review board, all patients fulfilling the inclusion criteria were enrolled in the study. All the demographic details of the patients including age, gender, duration since the diagnosis and family history of retinoblastoma were obtained. Specimens in 10% formalin from eye department of FFH were received in pathology lab at FUMC. (Specimens were received from ophthalmology department of FFH in 10 percent formalin) Specimens were routinely processed followed by staining of 3–5 microns thick sections with Haematoxylin and Eosin (H & E) and were examined microscopically by a team of two histopathologists. The presence or absence of HRH features was reported by joint consensus and recorded in the designed *proforma*. All data was recorded on the *proforma* (attached).

The collected data was entered and analyzed accordingly using SPSS version 20. Mean and standard deviation was calculated for quantitative values like age and duration since diagnosis. Frequencies and percentages were calculated for qualitative variables like gender, family history of retinoblastoma and HRH features. HRH features were stratified for age, gender, family history of retinoblastoma and duration since diagnosis to deal with effect modifiers. Post-stratification chi-square test was applied taking $p<0.05$ as significant.

RESULTS

A total of 150 patients were included in the study. The mean age of the patients was found to be 2.51±1.30 years. The gender distribution showed that most of the patients in this study were male. The mean duration since diagnosis was found as 4.70±2.81 months. All of these variables are shown in table 1.

Regarding HRH features in these patients, choroid invasion was found in 25 patients (16.7%) and 125 patients (83.3%) did not show it. Also, optic nerve invasion was found in 77 patients (51.3%) and not seen in 73 patients (48.7%). Data was stratified for age, gender, duration since diagnosis and family history of retinoblastoma (tables 2 and table 3).

Table-1: Demographic details of patients (n=150)

Variable	No. & %
Age	
≤3 years	111 (74%)
>3 years	39 (26%)
Mean±SD (years)	2.51±1.30
Gender	
Male	88 (58.7%)
Female	62 (41.3%)
Family History	
Positive	13 (8.7%)
Negative	137 (91.3%)
Duration since diagnosis	
≤5 months	90 (60%)
>5 months	60 (40%)
Mean±SD (months)	4.70±2.81

Table-2: Stratification of optic nerve invasion with variables

	Optic nerve invasion		*p*-Value
	Yes	No	
Age groups			
≤3 years	48 (32%)	63 (42%)	0.001
>3 years	29 (19%)	10 (7%)	
Gender			
Male	49 (32.5%)	39 (26%)	0.204
Female	28 (18.5%)	34 (23%)	
Family History of Retinoblastoma			
Positive	5 (3%)	8 (5%)	0.331
Negative	72 (48%)	65 (44%)	
Duration since diagnosis			
≤5 months	36 (24%)	54 (36%)	0.001
>5 months	41 (27%)	19 (13%)	

Table-3: Stratification of choroid invasion with variables

	Choroid invasion		*p*-Value
	Yes	No	
Age groups			
≤3 years	18(12%)	93(62%)	0.803
>3 years	7(5%)	32(21%)	
Gender			
Male	13(9%)	75(50%)	0.458
Female	12(8%)	50(33%)	
Family History of Retinoblastoma			
Positive	0 (0%)	13 (9%)	0.092
Negative	25(17%)	112(74%)	
Duration since diagnosis			
≤5 months	6(4%)	84(56%)	0.000
>5 months	19(13%)	41(27%)	

DISCUSSION

Retinoblastoma, the most common primary intraocular tumour of childhood, arises in the developing retina. Most cells comprising the tumour histologically resemble the cells of an undifferentiated retina of the embryo called retinoblasts which tend to form Flexner-Wintersteiner rosettes.[11] This resemblance prompted Verhoeff to coin the term retinoblastoma,which later was adopted by the American Ophthalmological Society.

Tumour formation usually begins with mutation in both alleles of the retinoblastoma tumour suppressor gene RB1, followed by a series of other genetic alterations that correlate with the clinical stage and pathologic findings of the tumor.[11,12] Research on retinoblastoma has paved the way toward understanding many of the mechanisms in cancer genetics. Studies of preclinical models of retinoblastoma in the form of transgenic mice and xenograft animal models have significantly contributed to the development of effective therapies for this disease.

Retinoblastoma can occur as either bilateral or unilateral disease. Most bilateral tumours are caused by the presence of germline mutations of the RB1 gene.[12] Familial cases may exhibit second primaries like osteosarcoma and rhabdomyosarcoma close to irradiation fields. Unilateral retinoblastoma account for 75% of all cases, and the majority are thought to be the result of RB1 somatic mutations in the developing retina or new germline mutations .[11,12] Amplification of a region on chromosome 2p spanning the MYCN oncogene in ~10% of retinoblastoma. BCOR mutation is identified in ~13% cases. Retinoblastoma cases also exhibit somatic and germline mutations in p53and MDM2 pathways.

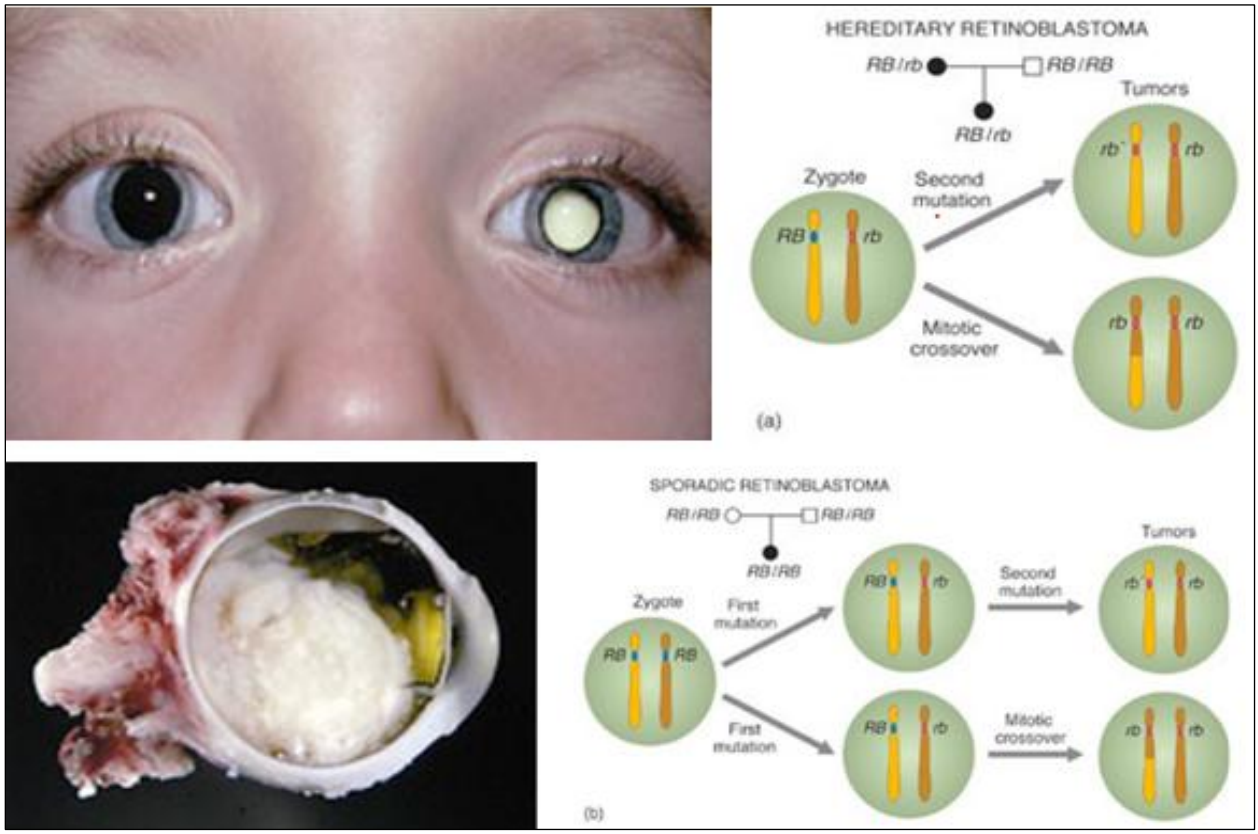

Figure 1: Genetics and types of Retinoblastoma

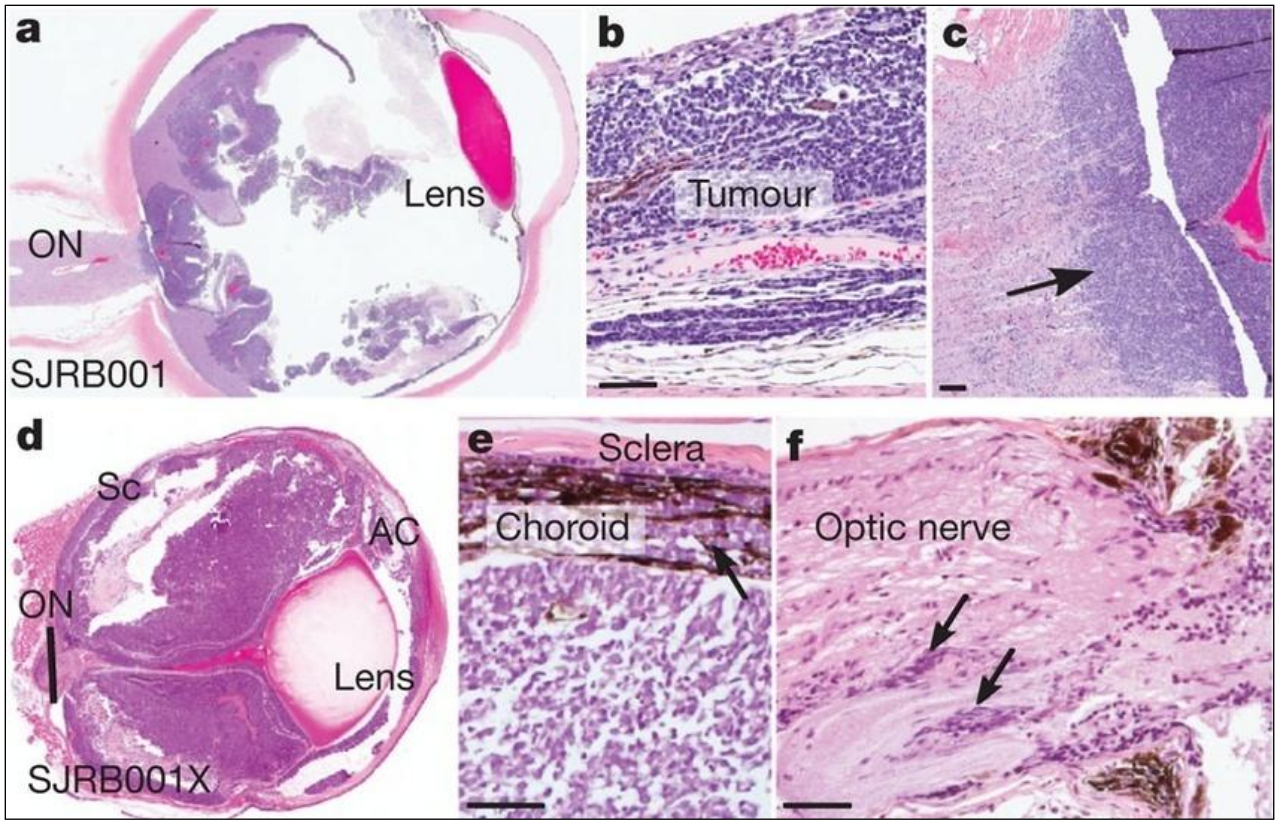

Figure-2: High risk histology in retinoblastoma

Retinoblastoma are classified in three types based on their growth pattern: Endophytic retinoblastoma (grows through the inner layer of the retina with vitreous seeding), Exophytic retinoblastoma (grows in between the layers of the retina causing retinal detachment) and Diffuse infiltrating retinoblastoma.[13] Most common clinical presentation of retinoblastoma is Leukocoria, followed by proptosis, corneal perforation, pain and ortbital cellulitis.[14–16]

A number of factors can affect the treatment options in retinoblastoma. Some of these include[15]:

- Whether tumours are in one or both eyes (Unilateral or bilateral tumours)
- The size and location of the tumor in the eye(s)
- The chance for saving vision in the eye(s)
- Whether the tumor is still confined within the eye(s) or (show extraocular spread)

Many children will get several types of treatment. The treatment options may include focal treatments (cryosurgery, thermography and brachytherapy), surgery, radiation, chemotherapy and stem cell transplantation.[17,18] Early diagnosis and treatment of retinoblastoma improve greatly the patient's survival rate and quality of vision. Intraocular retinoblastoma usually has a better prognosis than retinoblastoma with extra ocular spread.[19] Over the past years, many studies have attempted to identify prognostic factors, and most investigators have analyzed the effects of several risk factors on patient outcome. Choroidal invasion has been considered to be a classical risk factor, and it has even been stated that patients with choroidal invasion frequently develop systemic metastases; on the other hand, other authors have found that choroidal invasion is both more common and less dangerous than previously believed. In the same way, the historic data are consistent with an increased risk of metastases in the case of optic nerve involvement.[19,20]

The mean age of the patients was found to be 2.51±1.30 years in our study, 74% cases were <3 years and 26% >3 years. This is in concordance with the study by Nur Melani et al where mean diagnosis age was 2–3 years.[20] A study by Yaqoob N *et al* (also)showed median age presentation of 2 years.[21]

The gender distribution in our study showed male predominance with 58.7% compared to females (41.3%). This is in accordance with a study conducted by Yahaya JJ *et al* in Kampala Uganda whose male patients were 57.5% and females (42.5%)[21], contrary to (study) by Yaqoob N *et al*[22] which showed female predominance (54%) and a study by Alkatan HM *et al* showed almost equal distribution of cases in both genders[23].

The family history of retinoblastoma in our study was positive in 13 cases (8.7%) with rest 91.3% cases negative, comparable to study by Yahaya JJ *et al* who showed positive family history in 9.7% cases, all being females with bilateral tumor.[21] Contrary to our study, Yuliawati P and Ekawati NP study conducted at Sanglah general hospital showed no case out of 20 total patients to have positive family history.[24]

The reported incidence of the various histopathologic features in eyes enucleated for retinoblastoma is highly variable, and generally the incidence of choroid and optic nerve invasion in the developing world was higher than in the developed world. The overall reported incidence of optic nerve involvement beyond the lamina cribrosa and at the resection margin ranged from 6.5% to 40%, while the overall reported incidence of massive choroid invasion ranged from 12–41%. In our study optic nerve invasion was found in 77 patients (51.3%), with 32% cases in age group <3 years and 19% in > 3 years, predominantly males and overall 3% of those cases were also having positive family history as well, somewhat (comparable) to a study by Yuliawati P which showed optic nerve invasion in 60% cases.[24] A similar study conducted at a tertiary care hospital of Pakistan by Yaqoob N *et al* revealed optic nerve invasion in 75% cases which is quite high compared to our study.[22] Study by by Alkatan HM et al showed 68% positive cases of optic nerve invasion.[23]

Second major histological high risk feature in our study was Choroid invasion which was seen in 25 patients (16.7%) with 12% cases in <3 years age group and remaining in >3 years age, almost equally in both genders. None of these cases had positive family history of retinoblastoma. Alkatan HM *et al* found massive choroid invasion in 45% cases. Study by Yaqoob N et al showed 55.6% positive cases[22] followed by 60% cases in study by Yuliawati P *et al*.[24]

Nur Melani et al. in an analysis of 121 eyes found that almost 75% of enucleated eyes had HRF mainly massive choroid invasion and post laminar optic nerve invasion.[20] There is currently a consensus concerning the poor prognosis of microscopic extra scleral invasion and invasion of optic nerve resection line and/or subarachnoid space. However, the potential risks with other sites of extra-retinal involvement remain controversial. Multivariate statistical methodology that accurately analyzes the influence of multiple risk factors has recently been used and has shown that optic nerve and choroidal involvement are the two most useful prognostic factors. Adjuvant chemotherapy has been used to prevent metastases in patients with significant risk. Its value is clearly accepted in cases of microscopic extra scleral disease and/or involvement of the optic nerve resection line and/or subarachnoid space. However, uniform agreement on the need of chemotherapy with other forms of extraretinal involvement has not yet been achieved. Because chemotherapy may increase the risk of second malignancies, especially in patients with hereditary retinoblastoma, adjuvant therapy should be restricted to patients with a significant risk of orbital and/ or metastatic disease. A study by Nausheen Yaqoob *et al* assessed the histopathological changes in retinoblastoma cases treated with chemotherapy. Most common findings in these cases appeared to be necrosis, calcification and gliosis. Study also showed that patients with more than 2 high risk features have decreased survival rates in comparison to those with one or no HRF.[25]

There has been agreement that optic nerve invasion with resection line involvement and extra scleral involvement (i.e, microscopic orbital involvement) are highly predictive of death from retinoblastoma. However, there is considerable debate about the prognostic value of other cases of extraretinal involvement, such as choroidal invasion and retro- laminar optic nerve invasion. Adjuvant chemotherapy therefore remains controversial for these cases.

The definition of HRH has been debated, but most physicians agree that massive choroidal invasion with post -laminar optic nerve involvement (and) tumour present at the resection margin are associated with a high risk of metastatic disease and warrant further therapy.[25]

CONCLUSION

Optic nerve invasion was found in significant number of patients and was more prevalent than choroid invasion in patients with retinoblastoma. This will help our clinicians to better guide our patients regarding the prognosis of the disease and deciding the treatment options for the patients.

AUTHORS' CONTRIBUTION

NS: Literature search, conceptualization of study design, data collection, write-up. IKK: Literature search, data collection, data analysis, write-up. MN, HA: Data analysis, data interpretation. ZQ, MW: Data interpretation, proof reading.

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