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Histopathologic risk factors for metastasis in retinoblastoma seen in a tertiary eye center in South, South Nigeria

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ABSTRACT

Purpose: To analyze the frequency of histopathological high-risk factors (HRF) for systemic metastasis in retinoblastoma (RB) in our patient population.

Materials and Methods: This is a retrospective case series, with review of clinical data and histopathologic, immunocytochemistry slides, from a particular laboratory, of eyes enucleated for RB between 2006 and 2012.

Results: A total of 28 eyes with histopathologic reports confirming RB from a particular laboratory were seen between 2006 and 2012. The mean age at presentation was 33.68 ± 12.27 months (range: 6-55 months). 15 (53.60%) of the patients were males, and 13 (46.40%) were females. Approximately 12 (43%) of these patients presented with leucocoria, while the least frequent presentation was strabismus 2 (7%). The mean duration of symptoms at presentation was 7.07 ± 4.29 months. Grade E intraocular classification for RB was seen in 27 (96.40%) of cases. International staging classification of stages included Stage 1 (1 patient, 3.57%), Stage 2 (2, 7.14%), Stage 3A (4, 14.30%), Stage 3B: (6, 21.40%) Stage 4A (2, 7.14%) and Stage 4B (13, 46%). HRF that were predictive of metastasis were choroidal infiltration (20 patients, 71.40%), retrolaminar optic nerve (ON) invasion (17, 60.70%), invasion of the ON to transection 1 (6, 78.60%), scleral infiltration (23, 82.10%) and extra-scleral extension (13, 46.40%).

Conclusion: There is a high frequency of histopathological risk factors present in the patients with eyes enucleated for RB in this population. This finding is in agreement with suggestions of poor prognosis and high-mortality in this region, especially from the central nervous system metastasis.

Key words: *Cataract, high-risk factors, metastasis, optic nerve length, retinoblastoma, subarachnoid*

Introduction

The management of retinoblastoma (RB) is primarily focused on saving the life of the patient. Consequently, the ability to recover the organ (eye) or preserve vision (function) are the secondary and tertiary goals. The importance of identifying histopathological high-risk factors (HRFs) for metastasis after enucleation in RB and delivering appropriate adjuvant therapy has been emphasized [1].

Patients in developing countries are well noted for presenting late and with the most advanced form of the disease, resulting in a high incidence of HRF and metastasis. Data are lacking on the incidence of HRFs in eyes that have undergone the enucleation for RB in developing countries [2]. The diagnosis of HRF for systemic metastasis and the delivery of suitable and timely adjuvant therapy based on this knowledge, may increase the chance of metastasis-free survival of the children [3].

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Systemic metastases are seen to occur in <10% of patients with RB in developed countries [4]. The central nervous system (CNS), orbital and cranial bones, long bones, and other viscera are reported common sites for metastases [5]. The known ocular high-risk factors for systemic metastasis in RB include choroidal invasion, orbital tissues infiltration, and retrolaminar optic nerve (ON) tumor involvement and tumor infiltration of the ON to the line of transection [6].

This study aims to identify the frequency of clinical and histopathological risk factors for metastasis in children with RB seen in our environment.

Materials and Methods

The study was conducted as a cross-sectional review of clinical and histopathological records spanning from 2006 to 2001 for new patients who received enucleation treatment for RB, as performed by a single surgeon (RD), in the University of Calabar Teaching Hospital. Histopathological analysis of the enucleated specimen from these patients was conducted at the National Specialist Ophthalmic Pathology Service, Department of Histopathology, Royal Hallmshire Hospital in Sheffield, UK, under a collaboration agreement. We excluded patients whose histopathological slides were reported from any other hospital as many of these had incomplete data. Patient data recorded included age at presentation, gender and clinical features. All tumors were graded according to the international classification for RB, and staged appropriately. Details of the chemotherapeutic regimen and use of systemic steroids were noted.

Enucleation specimens were fixed in standard buffered formalin. The macroscopic description, block selection and the number of histological sections cut were in absolute accordance with the proceedings of the consensus meetings from the international RB staging working group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic [7].

Risk Factors in RB

All histopathological slides were assessed for the following:

- The presence or absence of viable RB.
- The rosette types present (e.g. Flexner-wintersteiner, homer-wright, fleurettes).
- The number of tumor foci.
- The extent of ON invasion (graded as pre-laminar, laminar and post-laminar).
- Tumor involvement of the ON surgical margin.
- Anterior chamber structures involvement (ciliary body, iris).
- Massive choroidal invasion.
- Focal choroidal invasion.
- Intra-sceral invasion.
- Extraocular spread.

The relevant and particular steps in the surgical technique for enucleation and excision of the ON for RB in children were performed using the technique described by Honavar, in which gentle traction can be applied to the recti muscles stumps prior to transecting the ON using a mosquito artery clamp. A 15° curved and blunt tip tenotomy scissors is introduced from the lateral aspect, and the ON is palpated with the closed tip of the scissors while maintaining gentle traction on the eyeball. The scissors is moved posteriorly to touch the orbital apex while strumming ON. The scissors is lifted by about 3 mm of the orbital apex. The blades of the scissors are opened to engage the ON, and the nerve is transected in most instances with one bold cut enucleation spoon and enucleation scissors are not used [8].

Data on the clinical features, surgical techniques, gross pathology and histopathology were entered into a spread-sheet for analysis. Analyses were performed using the software SPSS Statistics version 20 (IBM Corp. Armonk, NY, USA). Summary data were presented in tables and charts. Cross-tabulations were performed to ascertain possible associations between risk factors and occurrence of metastases.

Ethical clearance from the University of Calabar Ethical Review Committee was obtained, and the study adhered to the Helsinki declaration.

Results

A total of 28 eyes with histopathological reports confirming RB from a particular laboratory were seen between 2006 and 2012. The mean age at presentation was 33.68 ± 12.27 months (range: 6-55 months). 15 (53.60%) of the patients were males, and 13 (46.40%) were females. Approximately 12 (43%) of these patients presented with leucocoria while the

least frequent presentation was strabismus 2 (7%). Involvement of the right eye alone was more frequent than left eye or bilateral involvement (Table 1). The mean duration of symptoms at presentation was 7.07 ± 4.29 months. Table 1 shows the clinical features and bilaterality.

The grades and international staging for RB are shown in Table 2, which describes the collective information gathered by clinical information, imaging, systemic survey and histopathology from the study. Intra-ocular findings are detailed in Table 3. Microscopic features with evidence of metastasis to the subarachnoid space were seen in 15 patients (62.50%). High-risk factors for metastasis are reported in Table 4.

The mean length of the harvested ON fixed in formalin was 4.89 ± 4.15 mm (ranging from 0.5 mm to 15.0 mm). An ON of adequate length appeared to have been harvested in only two (7.10%) cases. The mean ON diameter was 7.0 ± 1.80 mm. Cataract was seen in 64.3% of children that presented, in 66% of patients with Grade E intra-ocular tumor and 22% in patients who had undergone chemoreduction.

Chemoreduction agents in a combination of carboplatin, etoposide and vincristine in six cycles were administered prior to surgery in four children. None of the children had undergone radiation therapy.

Discussion

The most common primary malignancy in children is RB [9]. In developed countries, children with RB rarely die from this intraocular cancer. The most probable cause of death is from related conditions such as trilateral RB and second cancers. This is contrary to

Table 1. Clinical case presentation of patients with RB (*n*=28).

Presentation	Description	Frequency (%)
Clinical presentation	Leucocoria	12 (42.9)
	Strabismus	2 (7.1)
	Painful red eye	4 (14.3)
	Orbital cellulitis	10 (35.7)
	Total	28 (100.0)
Bilaterality	Left eye	10 (35.7)
	Right eye	15 (53.6)
	Both eyes	3 (10.7)
	Total	28 (100.0)

RB: Retinoblastoma

Table 2. Grading and staging of the tumor (*n*=28).

Grade	Description	Frequency (%)
International classification of intra-ocular tumor	Grade A	1 (3.57)
	Grade E	27 (96.4)
	Total	28 (100.0)
International staging classification	Stage 1	1 (3.57)
	Stage 2	2 (7.14)
	Stage 3A	4 (14.3)
	Stage 3B	6 (21.4)
	Stage 4A	2 (7.14)
	Stage 4B	13 (46)
	Total	28 (100.0)

Table 3. Intraocular description (*n*=28).

Anatomic site	Description	Frequency (%)
Site of rupture	Cornea	2 (7.14)
	Posterior to the insertion of the EOM	2 (7.14)
	No evidence of rupture	24 (85.71)
	Total	28 (100)
Anterior chamber angle	Open	1 (3.57)
	Closed	25 (89.3)
	Narrow	2 (7.14)
	Total	28 (100)
Lens	Cataract	18 (64.3)
	Clear lens	1 (3.57)
	Displaced	7 (25.0)
	No documentation	2 (7.3)
	Total	28 (100)
Vitreous	Clear	1 (3.57)
	Cloudy	3 (10.7)
	Vitreous hemorrhage	1 (3.57)
	Tumor	23 (82.1)
	Total	28 (100)
Retina	Detachment	2 (7.14)
	Calcification	16 (57.1)
	Vascularization	7 (25.0)
	No further description	3 (10.7)
	Tumor	28 (100)
	Total	28 (100)
Orbital fat	Present	6 (21.4)
	Absent	22 (78.6)
	Total	28 (100)
Orbital soft tissue invasion	Present	22 (78.6)
	Absent	6 (21.4)
	Total	28 (100)

EOM: Extraocular muscle

Table 4. High-risk factors (*n*=28).

High-risk factor	Description	Frequency (%)
Anterior chamber seeding	Vascularization	6 (21.4)
	Invasion	21 (75.0)
	No evidence	1 (3.6)
	Total	28 (100)
Iris infiltration	Atrophic	2 (7.14)
	Not infiltrated	7 (25)
	Infiltrated	19 (67.8)
	Total	28 (100)
Ciliary body infiltration	Invasion	27 (96.4)
	No invasion	1 (3.57)
	Total	28 (100)
Choroidal invasion	Present	20 (71.4)
	Absent	8 (28.6)
	Total	28 (100)
ON invasion	Lamina cribrosa	3 (10.7)
	Retro lamina	17 (60.7)
	Orbital	2 (7.1)
	Disorganized	3 (10.7)
	No invasion	3 (10.7)
	Total	28 (100.0)
Scleral invasion	Present	23 (82.1)
	Absent	5 (17.9)
	Total	28 (100)
Extra-scleral spread	Present	13 (46.4)
	Total	28 (100)
Invasion of ON to transection	Present	16 (57.1)
	Absent	3 (10.7)
	No documentation	3 (10.7)
	Total	28 (100)

ON: Optic nerve

the situation in developing countries, where enucleation is still commonly required for management of this disease and 9-11% of patients present with metastasis [10-13]. Even though, there is a significant reduction in the incidence of enucleation in developed countries [14], this procedure is still often performed in developing countries and it is still the primary treatment of choice for advanced unilateral RB. Further, primary enucleation is the standard approach to be done in patients with neovascularization of the iris, secondary glaucoma and anterior chamber seeds, and in children for whom conservative therapy has failed [15]. Specific considerations have been advanced while enucleating an eye with RB, to include harvesting a long ON of about 15 mm, but never <10 mm [8]. Because up to 25% of eyes have retrolaminar invasion or invasion of the PN to the transection line, (it was up to 78.6% in our study) it is recommended that at surgery the longest possible ON stump should be obtained during enucleation. The importance of the harvested ON at enucleation for RB relates to identifying HRF such as invasion of the ON lamina cribrosa, retrolaminar ON invasion, and invasion of the ON transection [16]; these observations can predict the metastatic potential and the survival rate of the child with a tumor, and help in elucidating the response assessment criteria, which consequently determines the selection of patients for adjuvant therapy. Various surgical techniques have been described to enable to harvest this length of ON. However, it has been observed that varying lengths are obtained at surgery [8]. This variability was also observed in our study; furthermore, the frequency of a length below 15 mm was as high as 90%. Another study described the average extent of retrolaminar ON involvement seen on histopathological examination as 2.8 mm. It was reported that formalin preservation could cause the tissue to shrink by about 30-40%; therefore, this measurement may not be directly compared to the intra-operative ON stump length [17]. Most of the cases seen in our study were advanced cases with high-risk characteristics for metastasis; these HRF may also be responsible for the short length of the resected ON. Moreover, patients that undergo chemo reduction are also atrisk of having their tissues friable, further reducing the length of the ON during manipulations while trying to resect. Our study population is small; a larger sample size and further analysis of factors that may influence resection are required We need to know whether the factors responsible for systemic metastasis of RB are also responsible for the failed adequate excision length of the ON or whether there is a better surgical technique to produce the expected adequate length of ON required irrespective of the preservative. The incidence of ON involvement was higher in our study (89.20%) than in studies on patients from Ilorin, Nigeria, (65.20%) and India (29.50%) [9,18]. This high frequency of ON involvement is likely to result from the same or similar factors noticed in other studies in which a more advanced stage of ocular disease was seen

on the presentation.

As reported in several studies and similar to what was seen in our study, histopathologic HRF for systemic metastasis that we are reporting include: Involvement of the choroid, orbital tissues, and retrolaminar ON and involvement of the ON to the line of transection, scleral infiltration and extrascleral extension [Table 4]. The need to identify histopathologic risk factors for metastasis after enucleation and to provide appropriate adjuvant therapy has been highlighted by previous studies. Our study showed that histologic high-risk characteristics were associated with a 62.5% frequency of subarachnoid and 71.4% of choroidal invasion. Furthermore, combining the clinical, histopathological and systemic reviews of these patients showed that 46% of them were in the Stage 4B category, highlighting a CNS extension. Further classification of Stage 4 could not be documented due to the inability to perform computed tomography scans and magnetic resonance imaging in all patients. The mean age of presentation was >24 months in our study, which is a predictor for HRFs [17].

Clinical presentation of cataract was not noticed on clinical examination. However, on histopathological examination, features of cataract in these children were seen in 66% of these children in Group E intraocular classification and 22% had received at least six doses of standard chemo reduction drugs, while 25% had subluxation with clear lenses. These suggests an increased burden for cataract surgery in the population of children that survive as well as a modification of the surgical technique in children with subluxated lenses. This study is limited by its retrospective nature and the small number of patients.

Conclusion

Late presentation, a higher mean age, and more advanced disease are seen in this population. Multiple HRF for metastasis are present in a large proportion of these patients enucleated for RB. Determining the factors that influence the length of optic resection requires further investigation. While cataract, another sinister cause of leucocoria in children, appears to be an uncommon clinical feature of RB, it appears to be a common histopathological feature in Grade E RB seen in this population.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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