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ORIGINAL ARTICLE

The Role of Bone Marrow in Baseline Investigations for Advanced Intraocular Retinoblastoma

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ABSTRACT

Objective: To assess the rationale for bone marrow biopsy/aspiration as a part of a routine systemic workup in advanced intraocular retinoblastoma (RB) in relation to high-risk histopathological post-enucleation features.

Methods: A retrospective observational study was conducted at the Department of Pediatric Hematology and Oncology of the Children's Hospital, Lahore, Pakistan. Data regarding demographics, clinical presentations, staging workup, and treatment strategies of all the newly diagnosed retinoblastoma cases registered at our centre from January 2018 to December 2019 were collected. Statistical analysis was performed using IBM SPSS statistics version 22.

Results: Of the 61 patients, 41 (67%) showed unilateral disease, while bilateral and trilateral involvement was found in 17 (28%) and three (5%) cases, respectively. The mean age of presentation in our cohort was 25 months (median: 24 months; range: <1–72 months). Amongst 81 eyes (61 patients), tumours were limited to an intraocular location in 54 eyes (46 patients). In this group, 42 eyes (38 patients) underwent enucleation. Adverse histopathology was found only in nine (23.68%) patients. None of the patients with the intraocular disease, with or without adverse histopathological features in enucleated eyes, were found to have bone marrow (BM) involvement. For 15 patients with extraocular disease, five (33%) patients were found to have bone marrow involvement based on initial investigations.

Conclusion: Our study did not show any correlation between bone marrow positivity and advanced intraocular groups D/E RB. Therefore, BM evaluation can be avoided in this subset of patients with retinoblastoma at presentation, while stage III and IV patients (International Retinoblastoma Staging System IRSS) must be evaluated for metastasis. This will reduce the risks of biopsy to the patients and the burden on services.

Keywords: Retinoblastoma, Bone Marrow Involvement, Intraocular disease.

INTRODUCTION

Retinoblastoma (RB) is the most common childhood intraocular malignancy. The incidence has been reported to be one in 15,000–20,000 live births, resulting in almost 9,000 new cases recorded every year across the world.[1] Manifestations of the disease have been found to be bilateral in 40% of cases, which are either hereditary or in association with some genetic malignant predisposition syndrome. In 60% of the cases, the disease is unilateral.[2] There are wide presentation modes, ranging from leukocoria and strabismus to proptosis, buphthalmos, and red-eye.[3,4] In low-income countries, children mainly present with proptosis, buphthalmos, and red-eye, while leukocoria and strabismus are the main presenting features in high-income countries.[4-7] Early tumour recognition and advances in retinoblastoma management have played a pivotal role in a paradigm shift leading to improvements in survival and visual outcomes in high-income countries.[8-11] In contrast, increased lag time in low-income countries is not only a risk factor for extraocular disease spread but, in turn, reduces the survival rate to 50–70% from a 85–97% rate in high-income countries.[10,12-18]

Late presentation leads to clinically evident extraocular spread, thus compelling detailed systemic evaluation and extensive investigations to rule out distant metastases.[19] Not only do the staging investigations include invasive procedures, which cause trauma and emotional distress in the child, but also add to the financial costs.[20] Bone marrow is a common site of metastasis for several tumours. In RB, its involvement has been documented in 20% of cases.[21] Many centres in different countries, e.g. UK, Canada, and India, perform bone marrow aspiration/biopsies as a staging workup for varying levels of advanced intraocular disease.[22] If such investigations are considered the gold standard, this may delay treatment in low- and middle-income countries where they may not be performed in a timely manner.

We aimed to identify the rationale behind bone marrow biopsy/aspiration as part of a routine systemic workup in intraocular retinoblastoma (RB) by demonstrating the correlation between bone marrow (BM) involvement and high-risk histopathological features in a middle-income country that has a substantial number of patients with the intraocular and extraocular disease.

METHODOLOGY

A retrospective observational study was conducted in the Department of Pediatric Hematology/Oncology, the Children's Hospital and the Institute of Child Health, Lahore after obtaining permission from the Institutional Review Board. From January 2018 to December 2019, all newly diagnosed retinoblastoma cases during the study period were included, while all those who had incomplete staging workup, already received treatment, or developed recurrent disease were excluded. Informed consent was obtained from all parents. Initial evaluation included examination under anaesthesia, B scan ultrasound, MRI orbit and brain with contrast, cerebrospinal fluid examination, and bilateral bone marrow aspiration/biopsy from the posterior iliac crest after obtaining informed consent. Tumours were categorised into intraocular and extraocular groups based on clinical findings, radiological evaluation, CSF examination, and BM reports. All intraocular and extraocular retinoblastomas were further classified based on the International Intraocular Retinoblastoma Classification (IIRC) and International Retinoblastoma Staging System (IRSS), respectively.[23,24] In the Pediatric Oncology Unit at the Children's Hospital and Institute of Child Health (CH & ICH), we followed the UKCCLG protocol to treat RB.[22] According to this protocol, all patients diagnosed with group D or group E intraocular disease undergo bone marrow aspiration/biopsy as part of the staging workup, as bone marrow is a common metastasis site for

several tumours. Positivity of bone marrow involvement is confirmed on the basis of round blue cells being present in aspiration and/or biopsy. The treatment regimen was based on UKCCLG guidelines.[22] All patients with intraocular tumours were offered curative treatment, while those with extraocular extension were treated with palliative intent. For intraocular tumours, the treatment modalities varied based on the patients' condition and the course of the disease. Local therapy, systemic therapy, enucleation, and/or radiation therapy were used either alone or in combination. Neoadjuvant chemotherapy was given to all bilateral cases followed by enucleation for the worse eye with advanced group D/E disease. The better eye was enucleated if it did not respond to further conservative salvage treatment (laser photocoagulation, cryotherapy). The systemic chemotherapy given consisted of vincristine, etoposide, and carboplatin.[22] Enucleation was offered as a first-line treatment to all the patients with the advanced unilateral intraocular disease (group D/E). The histopathology of the enucleated eye was reviewed for adverse features, such as involvement of the anterior chamber, diffuse choroidal involvement, retrolaminar optic nerve involvement, disease at the cut end of the optic nerve, extra scleral extension, or a combination thereof for an ongoing management plan.

Data of 61 patients were collected regarding age; gender; family history; clinical presentation (leukocoria, red-eye, proptosis, squint, etc.); the lag time for treatment; tumour laterality (unilateral, bilateral, trilateral) and location; metastatic workup; treatment regimen and histopathological findings of enucleated eyes; and outcome. The data were analysed using the Statistical Package for Social Studies (SPSS; IBM, version 22 Corporate headquarters 1 New Orchard Road Armonk, New York 10504-1722 United States). The descriptive analysis included mean, standard deviation, median, and range and was computed for age. Frequency and percentage were computed for gender, clinical presentations, tumour laterality, and location. Results were presented in tabulated form.

RESULTS

During the study period, 72 patients were diagnosed with retinoblastoma. Eleven of them were excluded from the study owing to an incomplete baseline staging workup. Amongst the rest of the 61 patients (81 eyes), 41 (67%) showed unilateral disease, while bilateral and trilateral involvement was found in 17 (28%) and three (5%) cases, respectively (Table 1). In the total cohort, the mean age at presentation was 25 months (median: 24 months; range: <1–72 months), while in bilateral and unilateral cases, it was reported to be 24 months and 25 months, respectively (Table 2). The median lag time was eight months. The male to female ratio was 1.5:1, 1:1, and 2:1 in unilateral, bilateral, and trilateral cases, respectively. Out of 61 patients, seven had a positive family history. The most common presenting complaints were leukocoria (90%), loss of vision (79%), proptosis (25%), red-eye (28%), and strabismus (7%), as shown in Table 1.

Table 1: Demographic characteristics of retinoblastoma patients

Variable	n = 61	Percentage
Age at presentation (months)		
Mean	25 months	-
Median	24 months	-
Range	<1–72 months	-
Gender		
Male	36	59%
Female	25	41%
Presenting Complaints		
Leukocoria	55	90%
Loss of vision	48	79%
Proptosis	15	25%
Red eye	17	28%
Strabismus	4	7%
Tumour laterality		
Unilateral	41	67%
Bilateral	17	28%
Trilateral	3	5%

Table 2: Analysis of the presentation of retinoblastoma based on tumour location

Variable	Unilateral RB (n = 41)	Bilateral RB (n = 17)
Age at presentation (months)		
Mean	24 months	25 months
Family History n (%)		
Yes	2 (5%)	5 (29.4)
No	39 (95%)	12 (70.6%)
Gender n (%)		
Male	23 (56%)	11 (65%)
Female	18 (44%)	6 (35%)
Tumour location n (%)		
Intraocular	38 (92%)	8 (47%)
Extraocular	3 (8%)	9 (53%)
Orbital + CNS* involvement	3 (8%)	4 (23.5%)
Orbital + BM** involvement	0 (0%)	1 (11%)
Orbital + BM + CNS involvement	0 (0%)	4 (23.5%)

*CNS = Central nervous system, **BM = Bone marrow

Table 3: Studies investigating the role of bone marrow biopsy in baseline investigations for Retinoblastoma

References	Sample size (patients)	BM involvement in patients with intraocular disease	BM involvement in patients with extraocular disease	Enucleation is done in patients	Adverse histopathology in patients undergoing enucleation
Azar et al.[20]	123	0/121	0/2	106	7/106
Zacharoulis et al.[21]	54	0/54	-	36	11/36
Moscinski et al.[31]	60	1/58	0/2	--	--
Bakhshi et al.[35]	259	0/172	10/87	--	104/172
Pratt et al.[37]	126	0/109	3/17	--	--
Chantada et al.[38]	282	0/255	11/27	--	--
This study	61	0/46	5/15	38	9/38

Amongst 81 eyes of 61 patients, tumours were located intraocularly in 54 eyes of 46 patients. Out of these 46 patients, 38 had unilateral and eight had bilateral intraocular retinoblastoma. In the group with intraocular retinoblastoma, 51/54 (94%) eyes had advanced disease, group D or E. Thirty-eight patients (42 eyes) underwent enucleation (unilateral disease: 34 patients; bilateral disease: four patients). In the histopathology, adverse features were found in nine out of the 42 (22%) eyes. Bone marrow involvement was not found in any of the patients with intraocular disease, with or without adverse histopathology.

In the extraocular RB group, out of 15 patients, three (20%) had unilateral disease, nine (60%) bilateral, and three (20%) trilateral involvement. Amongst them, five patients had BM involvement in the baseline staging workup. Out of these five patients, four had combined involvement of orbit, CNS, and bone marrow, while one patient had only orbital disease along with bone marrow infiltration at the time of presentation.

DISCUSSION

Retinoblastoma is a progressive tumour that can involve extraocular sites; for example, orbit, central nervous system (CNS), bone, bone marrow and lymph nodes.[25] Patients with metastatic RB have a poor survival outcome. To identify the patients at high risk of extraocular disease, currently the International Retinoblastoma Staging System (IRSS) is widely

used.[23] The IRSS classifies RB into five stages, with stage IV being characterised by bone marrow involvement. The optic nerve is a major risk factor leading to CNS involvement, while scleral extension leads to distant metastases, including bone marrow and lymph nodes.[26,27] The unique structure of the vascular niche in bone marrow, composed of thin-walled and fenestrated sinusoidal vessels, makes it more vulnerable and a common site for metastases. This three-dimensional structure is not only favourable for the survival of cancer cells but also accelerates their proliferation.[25]

Our study was conducted to assess if bilateral bone marrow aspiration/biopsy at the time of presentation is essential for all RB patients. In his study, Bran reported haemorrhage, infections, persistent pain, and serious leak as adverse events in patients who underwent bone marrow aspiration and/or biopsy. He further concluded that though post-procedural complications are rare (0.08%), they may have a significant impact on patients.[28] Moreover, a series of studies ruled out bone marrow involvement in the absence of signs and symptoms of bleeding or infections, adverse histopathological features of enucleated eyes, extraocular involvement on radiologic studies, or recurrent disease.[20,29-31] Kashyap et al. assessed 326 enucleated eyes and reported that an age of more than 2 years and lag time of more than three months were the clinical predictors of high-risk histopathology retinoblastoma patients and suggested a poor outcome would result.[32] In our cohort, the mean age of presentation was 25 months, with a median lag time of eight months. We identified that 25% of eyes had extraocular involvement that may be due to delayed presentation and a long lag time. In 75% of eyes, tumours remained limited to the intraocular location, but 94% of the intraocular disease eyes had advanced disease, groups D or E. However, this is probably related to tumour biology rather than lag time, as even in countries with short median lag times (five weeks in the UK), 89% are in groups D and E.[33]

Khurshid et al. reviewed 30 patients to identify the pattern of complete blood count and bone marrow involvement.[34] Twenty patients presented with low haemoglobin levels at the time of presentation, but only three patients showed baseline BM involvement. Interestingly, all three of them had bilateral disease with metastasis.

Similarly, most of the patients in our study (78%) showed nutritional anaemia, but white blood cells and platelet counts were within normal limits. Five patients showed pancytopenia, out of which two patients presented with septicaemia and expired within 48 hours without receiving chemotherapy, and bone marrow aspiration/biopsy could not be done. The remaining three patients showed extraocular involvement.

In a study of 18 patients with metastatic RB by Gunduz et al., bone marrow involvement in five patients, either alone or involving CNS and/or bone concurrently were reported.[27] All of these patients showed adverse histopathology. A substantial statistical association between bone marrow involvement and choroidal invasion in enucleated eyes was also observed.[29]

In our study, 38 patients underwent enucleation and histopathology of enucleated eyes. Out of these, only nine patients showed adverse features. Out of these nine patients, five had focal choroidal invasion as well as retrolaminar involvement of the optic nerve; one patient had anterior chamber, diffuse choroidal, and retrolaminar involvement; and three patients had the disease at the cut end of the optic nerve along with diffuse choroidal invasion and anterior chamber involvement. All these patients showed normal bone marrow at the time of baseline staging workup. Table 3 details seven studies that have addressed the role of BM biopsy and metastases. We believe that the determination of whether the child has the intraocular or extraocular disease is crucial.

In a study conducted by Moscinki et al., bone marrow involvement was found in one patient in the intraocular group with extensive disease, i.e. Reese Ellsworth group IV eye (Pratt stage II). However, the authors had suspicions that this patient was under-staged pathologically, given the presence of massive orbital inflammation with the globe pressing up against the surrounding soft tissue.

In contrast to the reports of a substantial statistical association between bone marrow involvement and choroidal invasion,[29] no trend was established between positivity of bone marrow and adverse post-enucleation histopathological features, in particular between choroidal and retrolaminar or cut end optic nerve invasion, similarly to a study by Bakhshi et al,[35] who found metastases in CNS and/or BM in 7% of their cohort. However, none of the patients with stage 0 or I disease (complete resection histologically after enucleation) tested positive for metastases despite having high-risk histopathological features after enucleation. In other words, the bone marrow biopsy was not positive for the intraocular disease. It is possible that the choroidal invasion was associated with extraocular disease in the earlier studies and, in fact, that the extraocular disease was the more relevant factor.

Chantada et al. demonstrated that bone marrow involvement was an infrequent event at diagnosis without symptoms, signs, or histologic evidence of tumour dissemination in retinoblastoma patients. This reinforces a justification of not performing invasive workup routinely in stage I disease.[36] We echo these findings.

BM evaluation should be restricted to patients with extraocular disease,[37,38] as of 815 patients (including in this study; Table 3) with the intraocular disease, only one patient had a positive BM biopsy.[31] This single patient had a Reese Ellsworth group IV eye (Pratt stage II)[31] but presented with massive orbital inflammation with the globe pressing up against the surrounding soft tissue. Many clinicians would have described this patient as having extraocular disease and the patient may have been misclassified at the outset.

The Children's Cancer and Leukemia RB special interest group reviewed their guidelines for the management of children with advanced unilateral retinoblastoma after primary enucleation and proposed adjuvant chemotherapy in patients with an adverse histopathology.[39] According to these guidelines, an initial laboratory evaluation should include bone marrow aspiration and biopsy in disease groups D and E. The same recommendations were proposed for inclusion in the first draft of the National RB Guidelines of Pakistan. Our results suggest that this invasive procedure can be avoided in patients who have intraocular retinoblastoma. Our study's limitation is its small sample size, but it is comparable with several other studies in terms of positive biopsy results and a high proportion in the extraocular group (Table 3). Therefore, this study adds to the literature that found BM biopsies to most likely be unnecessary for intraocular disease.

CONCLUSION

Bone marrow studies are currently recommended in advanced intraocular retinoblastoma groups D/E in several countries. Our study did not find any correlation between bone marrow positivity and advanced disease with or without an adverse histopathology. A cautious approach would be to perform this invasive investigation when there is evidence of adverse histopathology and extraocular disease with full knowledge that a high percentage of children with adverse histopathology will have negative results. This work adds to the body of evidence that bone marrow biopsies can be safely avoided in many patients with retinoblastoma, saving significant time and expense before the initiation of treatment and reducing trauma to the child. The implications are important in all settings but particularly in low- and middle-income countries where the minimisation of unnecessary costs would allow more patients to be treated.

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Authors' Contributions:

HZ (Pediatric Hematologist Oncologist): Idea conception, design, data collection and analysis, write up, and final revision of the manuscript.

MF (HOD & Pediatric Haematology Oncology): Data analysis and final critical revision of the manuscript.

AM (Pediatric Ophthalmologist): Data collection and literature review.
SC (Pediatric Ophthalmologist): Literature review and write up of the manuscript.
JF (Pediatric Haematology and Blood Transfusions): Specimen collection, analysis, and write up of the manuscript.
AG (Pediatric Radiologist): MRI reporting and data collection.
SZ (HOD-Pathologist): Histopathology data verification.
MAR: Interpretation, literature review analysis, and writing the manuscript with final revisions.

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