Original Article

Correlation between Magnetic Resonance Imaging & Histopathological Findings In Retinoblastoma : A Prospective Study

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Background : Retinoblastoma is the most common primary intraocular malignancy of childhood has one of the best survival rate. Therefore, early and appropriate diagnosis is of the highest importance in survival of patients.The present study was undertaken to evaluate the incidence of high risk factors on histopathology in retinoblastoma and to determine the diagnostic accuracy of MRI in detecting tumor invasion and to correlate them.

Aims and Objectives : To determine sensitivity, specificity and accuracy of MRI in optic nerve invasion. To find if any correlation exists between MRI findings and high risk histopathological findings.

Materials and Methods : A non-randomized prospective study was conducted among 49 eyes of 49 patients clinically diagnosed as Retinoblastoma, who underwent MRI for staging of the disease. Treatment of all the patients were recorded and postoperative results were studied to determine the diagnostic accuracy of MRI in tumor invasion.

Results : Out of the 49 patients, their mean age of presentation was 30 months and Standard Deviation (SD) was 16.4 months. Among Histopathological findings, choroidal involvement was found in 77.5%,followed by optic nerve involvement in 42.9%, anterior chamber seeding in 12.2%, scleral involvement in 10.2%, and among MRI reports, optic nerve involvement was found in 32.6% cases, followed by choroidal involvement (12.2%), scleral Involvement (8.2%), anterior segment involvement (8.2%). Hence, there is a clear discrepancy seen in our study between MRI and HISTOPATHOLOGY findings in patients with Retinoblastoma.

Conclusion : It is concluded from our study that Though high-contrast MRI is considered to be an accurate tool for pre-treatment staging of Retinoblastoma, high risk features such as microscopic choroidal and optic nerve invasion are often missed on it. Since these are the important prognostic indicators of survival, we can't rely upon MRI only for further treatment in terms of Chemotherapy.

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Key words : Retinoblastoma, MRI, Histopathology in Retinoblastoma, Intraocular Malignancy.

Retinoblastoma is the most common primary intraocular malignancy of childhood[1]. It has two distinct clinical presentations: a bilateral or multifocal, heritable form (25% of all cases), characterized by the presence of germline mutation of Rb1 gene; and the other one being a unilateral or unifocal form (75% of all cases), 90% of which are non-hereditary[2]. The mean age at diagnosis is 18 months, which is 24 months for unilateral cases and 12 months for bilateral cases[3].

Retinoblastoma (RB) is potentially curable, but the prognosis for survival is heavily dependent on early stage diagnosis and appropriate therapy. Mortality is as high as >50% in rural areas that are lacking good medical care and in many cases, those who seek

> **Editor's Comment :**
> ■ Awareness, early diagnosis and accurate use of diagnostic tool can make huge difference in survival of this curable disease.
> ■ Our children are our future we should fighting harder to find cures for them.

treatment there are already at an advanced stage of the studies[4] from our country reveal a major population of affected children with delayed presentation having mortality as high as 24%[5]. The management of RB is highly individualized and complex and it needs a multidisciplinary team approach.

The modern methods of diagnosis now available are CT scan and Magnetic Resonance Imaging (MRI) for studying tumor characteristics and the extent of tumor invasion. The present study was undertaken to evaluate the incidence of High Risk Factors (HRF) on histopathology in retinoblastoma and to determine the diagnostic accuracy of MRI in detecting tumor invasion and to correlate them.

Aims and Objectives

Aim :

To correlate high risk histopathological findings with MRI findings and determine diagnostic accuracy of Magnetic Resonance Imaging (MRI) in detecting tumor invasion.

Objectives :

(1) To determine sensitivity, specificity and accuracy of MRI in optic nerve invasion.

(2) To find if any correlation exists between MRI findings and high risk histopathological findings.

Materials and Methods

Study Setting :

The study was conducted among 49 eyes of 49 patients clinically diagnosed as Retinoblastoma, who underwent MRI for pre-treatment staging of the disease. It was a non-randomized prospective study and the patients have been followed up periodically. Treatment of all the patients were recorded and postoperative results were studied to determine the diagnostic accuracy of MRI in tumor invasion.

Inclusion Criteria :

- All patients presenting at out patient department, clinically diagnosed with retinoblastoma IIRC Group D and Group E.
- Guardian giving informed written consent

Exclusion Criteria :

- Retinoblastoma patients IIRC Group A, B and C with salvageable vision or globe.
- Guardian unwilling for regular follow-up.
- Guardian not giving any informed consent.
- Dropped out cases and death of patient in between study.

Ethical measures were adhered to and after taking informed written consent from guardian, a careful ocular and systemic history was taken from each patient's guardian including name, age, sex, address, presenting complaints and their duration, birth history and family history of similar complaints.

Indirect ophthalmoscopy was performed under general anesthesia to examine the fundus with 360 degree scleral indentation.

An MRI of the orbit and brain was performed with a 1.5 Tesla system (MagnetomAvanto, Siemens, Erlangen, Germany), with the use of a standard head coil. Pre-contrast orbital images acquired were axial and sagittal T1- weighted images with repetition time (TR) msec/echo time (TE) msec of 350-380/8-10 and 3 acquisitions and axial T2-weighted images with TR/TE 3000- 3300/70- 100 and 1 acquisition. After intravenous injection of gadolinium based contrast material gadopentetatedimeglumine (Magnevist, Schering, Berlin, Germany), images acquired were TI-weighted axial, sagittal and coronal images (TR/TE 560-630/8-12, 3 acquisitions) with fat suppression. For fat suppression, frequency-selective technique was used. A slice thickness of 2 mm and interslice gap of 0.2 mm was used. Postcontrast MRI of the brain was performed and images acquired were axial TI-weighted images (TR/TE 380-420/10-12) and T2-weighted images (TR/TE 3200-3400/80-120), with a slice thickness of 5 mm. For imaging, spin echo sequences with a matrix of 256*256 and a resolution of 0.8*0.8 mm was used. For the patients who underwent chemoreduction, we have considered post chemotherapy MRI for the correlation study.

Primary enucleation was performed in patients having non salvageable eye or no potential of vision in unilateral cases of Retinoblastoma, while in phthisical globe with no residual lesion after chemoreduction underwent for secondary enucleation under general anesthesia. After the enucleation procedure, the eyeball was fixed in 4% formaldehyde for 24 hours, washed with alcohol for 24 hours and embedded in paraffin. After macroscopic examination, sections were prepared from the specimen and stained with hematoxylin and eosin for microscopic examination. Histopathological findings in terms of tumor differentiation, presence or absence of calcification and necrosis, vitreous seeding, anterior chamber seeding, Iris and ciliary body infiltration, extraocular muscles and scleral involvements, high risk characteristics like choroid and optic nerve invasion and extraocular involvement is noted and tumor graded according to AJCC[10]. Adjuvant chemotherapy was advised in a patient with High Risk Features (HRF) on histopathology. At our institute, consideration of high risk factors given to massive choroidal invasion (>3mm), optic nerve invasion, invasion of iris, anterior chamber and ciliary body.

Statistical Analysis :

To find out whether a correlation exists between two variables, to check if that correlation is actually valid and statistically significant and then to obtain the degree of the correlation, we need to use statistical correlation tests. In our dataset, we have several nominal (binary) variables and some continuous (scale) variables. To find out the correlation between 2 binary variables (eg, Involvement of Optic Nerve in Histopathological findings and Gender), we have used the Chi-square method.

Chi-square method assumes the null hypothesis

(h0) to be that no correlation exists between the two binary variables in question. It gives out the p-value of the test (also called Pearson's p), using which we can determine if there's a statistically significant correlation between our two binary test variables.

A p-value less than 0.05 usually indicates a statistically significant correlation. And then we find out the value of Phi Coefficient to know the degree of correlation. However, in certain cases of binary correlation tests where the number of data points available is not in large quantity and if the expected values of certain outputs are very low, the Pearson's p-value tends to lose its accuracy. In most such cases, it ends up suggesting a higher statistical significance than what it actually might be.

Such a scenario demands the use of Fisher's exact test, which works well even for a small dataset. Since our dataset consists of 49 patients, which is not very large, we have used Fisher's exact test in our binary correlation tests (wherever more than 20% of the expected values are low) to obtain highly accurate results. If the 2-sided significance value obtained through Fisher's exact test is less than 0.05 (5%), then we accept the correlation and proceed to find the degree of correlation using Phi Coefficient[7].

All the data was first entered in an Excel master-chart and subsequently analyzed using the statistical analysis software IBM SPSS.

OBSERVATION AND ANALYSIS

Out of the 49 patients who were part of this study, 27 (55%) were Males and 22 (45%) were Females. Their mean age of presentation was 30 months and Standard Deviation was 16.4 months.

A total of 41 (83.7%) cases were Unilateral and 8 (16.3%) were Bilateral. 3 patients (6%) had a positive family history of Retinoblastoma.

In our study, Leukocoria was the most common present symptom with 95.9%. Followed by Proptosis (26.5%), Squint (22.4%), Red Painful Eye (20.3%), Fungating Mass (10.2%) and Orbital Celluloitis (4.1%).

Among other clinical features, Secondary Glaucoma and Iris Neovascularization were present in 16.3% cases, followed by Ectropean Uveae (10.2%), Hyphema (8.16%), and Pseudohypopyon (2.1%).

Among Histopathological findings, choroidal involvement was found in 77.5%, followed by optic nerve involvement in 42.9%, anterior chamber seeding in 12.2%, scleral involvement in 10.2% and uveal involvement in 6.1%.

And among MRI reports, optic nerve involvement was found in 32.6% cases, followed by choroidal involvement (12.2%), scleral Involvement (8.2%), anterior segment involvement (8.2%) and ciliary body involvement (4.1%).

For the objective of identifying clinical predictors of high risk histopathology, we ran the correlation tests in IBM SPSS to figure out the authenticity of various MRI findings in indicating high risk Histopathological findings (Tables 1&2)(Figs 6a & 6b).

Following are some of the noteworthy results where appropriate correlation tests were performed to find out if a statistically significant correlation exists and if it does, what is the degree of the correlation.

DISCUSSION

In the present non randomized prospective study, we have managed to better define the relationship between MRI findings and high risk histopathological features at our centre. In our study, high risk factors included massive choroidal invasion (53%), iris invasion (2%), ciliary body invasion (6%), anterior chamber involvement (12%), scleral invasion (8%), pre laminar optic nerve invasion (42.9%), post laminar optic nerve invasion (36.7%), and invasion to the transection end of the optic nerve (18.4%). These figures are in partial agreement with Chawla, et al[8] for Indian patients wherein they obtained 25.3% massive choroidal invasion, 10% iris invasion, 9.3% ciliary body invasion, 4% anterior chamber involvement, 8% scleral invasion, 22.3% post laminar optic nerve invasion and 5.3% invasion to the transection end of the optic nerve.

Delayed presentation is responsible for higher incidence of high risk histopathological features in

Table 1 — The following table suggests optic nerve and choroidal involment seen in MRI findings and histopathological finding

	MRI	HISTOPATHOLOGY
Optic Nerve Involvement	16	21
Choroidal Involment	6	38

Table 2 — MRI Characteristics of 49 Eyes Compared with Histopathology Findings

Parameter	TP (n)	FP (n)	TN (n)	FN (n)	Accuracy (%)	Sensitivity (%)	Specificity (%)
ON INVASION :							
Prelaminar	8	4	24	13	65.3	38.1	85.7
Laminar	5	4	24	16	59.2	23.8	85.7
Postlaminar	5	5	25	14	61.2	26.3	83.3
Transection end	2	3	35	9	75.6	18.2	92.1
AC invasion	1	4	37	5	77.6	16.66	90.24
Choroid Invasion	2	1	9	36	22.4	5.4	90
Scleral/Extrascleral invasion	1	3	43	2	89.8	33.3	93.47

TP : True Positive, TN : True Negative, FP : False Positive, FN : False Negative
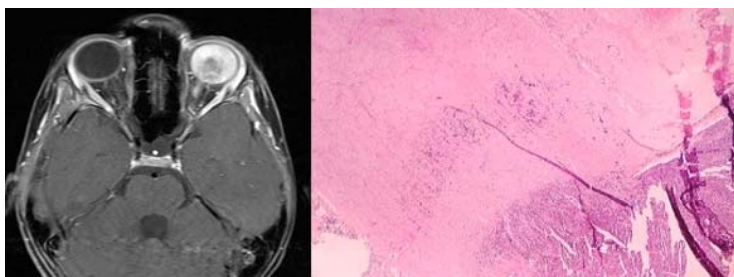ON : Optic Nerve, AC : Anterior Chamber

Fig 6a   **Fig 6b**
24-month-old female patient with Bilateral Retinoblastoma post Chemotherapy

Fig 6a — Axial post contrast T1- weighted magnetic resonance image showing a large tumour mass in the left globe with normal postlaminar optic nerve signal intensity

Fig 6b — This photograph shows pre-laminar, intralaminar and post laminar optic nerve invasion with transection end infiltration. (Stain : Hematoxylin and Eosin)

Asian patients compared to the west[9]. Similar observation was found in our study wherein we were able to find positive correlation between choroid involvement in histopathological study with age of presentation. We included the following high risk clinical features in our study: age at presentation, intraocular pressure, positive family history, iris neovascularization, proptosis and secondary glaucoma. It was observed that these parameters, individually, weren't able to correlate positively with a high risk histopathological finding; but when considered together, we managed to find their moderate positive correlation with choroidal involvement in histopathological findings.

MRI is useful in determining the important prognostic factors for survival, which makes its interpretation very critical. In our study, MRI's accuracy in detecting choroidal invasion was 22.4%, sensitivity was 5.4%, and specificity was 90%. While in a previous study[8], the accuracy was 57%-72%, sensitivity was 36%-100% and specificity was 40%-100%. These numbers indicate that MRI often fails to detect microscopic choroid invasion. This can be due to partial volume averaging effect that occurs because of 2 mm slice thickness in our study. The accuracy can be improved by reducing the slice thickness to 1mm. We obtained 89.8% accuracy in scleral or extra scleral invasion, while specificity in that case was 93.5%. These numbers are comparable to the study by Chawla, *et al*[6], wherein they obtained accuracy of 98.7% and specificity of 100%.

Suspected post laminar involvement in MRI can influence the results. In our study, the accuracy of MRI in predicting pre-laminar optic nerve invasion was 65.3% and specificity was 85.7%. In predicting postlaminar optic nerve invasion, accuracy was 61.2% and specificity was 83.3%. Only MRI findings cannot be relied upon to give chemotherapy to the patient, which is evident by the fact that we obtained no correlation in our Fisher's exact test between MRI findings and critical histopathological findings.

CONCLUSION

It is concluded from our study that delayed age at presentation is the clinical predictors of high risk histopathology findings in Retinoblastoma. Also, awareness about the disease among parents, and early diagnosis and treatment at primary and secondary health care services can make a huge difference in survival of this curable disease.

Though high-contrast MRI is considered to be an accurate tool for pre-treatment staging of Retinoblastoma, high risk features such as microscopic choroidal and optic nerve invasion are often missed on it. Since these are the important prognostic indicators of survival, we can't rely upon MRI only for further treatment in terms of Chemotherapy.

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