ORIGINAL ARTICLE

HISTOPATHOLOGICAL FEATURES OF RETINOBLASTOMA

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ABSTRACT

Objective: *To study the various histopathological features of retinoblastoma in patients operated in a local teaching hospital.*

Material and Methods: *This prospective, descriptive study was conducted at Eye "A" unit, Department of Ophthalmology, Khyber Teaching Hospital, Peshawar, Pakistan from 31st December, 2007 to 1st January 2009. Twenty Five children were registered in referral center for retinoblastoma. Based on the results of examination under anesthesia as well as other investigations (CT scan and ultrasonography), a management plan was drawn which also included enucleation in advanced cases. Thirteen patients were included for studies on histopathological features.*

Results: *Out of 13 patients, 8 (61%) were males, 5 (39%) were females ranging in age range from 1 year to 7 years. Mean age was 3.62±1.55 years. Nine (69.2%) cases had unilateral disease. Seven (53.85%) patients had differentiated tumor characterized by Flexner-wintersteiner rosettes, 4 (30.77%) patients had clear optic nerve invasion. Eight (61.54%) specimens revealed necrosis and 5 (38.46%) cases had pigmentation. Four patients with optic nerve invasion and 4 patients with no comments on optic nerve invasion in biopsy report, were offered chemotherapy and local radiotherapy. Rest of 5 patients having no optic nerve invasion, were not given any chemotherapy or radiotherapy after enucleation.*

Conclusion: *Majority of retinobalstoma had well differentiated tumours and about two third having suspician of optic nerve involvement required chemotherapy and radiotherapy. Histopathology report is crucial in devising further treatment plan after surgery.*

Key words: *Retinoblastoma, Histopathological Features, Optic nerve, Tumour.*

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INTRODUCTION

Retinoblastoma is a childhood ocular malignancy with an incidence between 1: 17,000 and 1: 20,000 live births. It presents below 05 years of age, and often in the first two years of life. Bilateral cases present at an earlier age, while sporadic unilateral disease presents around three years of age. The most common presentation is leukocoria comprising 60% of cases. It also presents as fungating mass, proptosis, glaucoma and hyphema. Plan of treatment depends on the stage of the disease and the visual status of the patient. Eyes with tumors, with no vision as well as advanced disease, need to be enucleated.

Histopathological parameters derived from microscopic examination of specimens, play key role in understanding the behavior of tumor, in each patient. Well differentiated tumors are characterized by flexner-wintersteiner rosettes. Necrosis in the tumor and optic nerve invasions are the crucial points in deciding future course of treatment.

Flexner in 1891 described one case of rosettes which was then designated as neuroepithelioma. Wintersteiner in 1897 studied rosettes in a larger series. Both authors advanced the opinion that rosettes represented an attempt to form rods and cones. Verhoeff adopted the term retinoblastoma, which was officially agreed by American Ophthalmological society in 1926.[1]

Shield JA et al studied metastatic risk of optic nerve invasion[2] and Khelfaoul F et al deliberated on histopathological risk factors in patients with retinoblastoma.[3]

While the management of retinoblastoma is a multidisciplinary approach, unfortunately histopathological report does not receive the required attention in this

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part of the world. Misplaced microscopic reports and use of histopathological reports only to designate the diagnosis, diminish the chances of survival in retinoblastoma patients, leading to the worst form of human misery.

Histopathological features – the accuracy of which originates at precise steps of surgical procedures during enucleation – carry a hidden message about prognostic parameters, and outcome of the disease process.

The differentiation of tumor, choroidal and optic nerve invasion as well as necrosis, guides the direction of correct treatment. Lack of awareness in this regard poses a direct threat to the lives of patients with retinoblastoma. Various studies have focused on the importance of histopathological features internationally.

Currently the importance of P-glycoprotein expression in relation to histopathological features and outcome of chemotherapy cannot be over emphasized.[4] The aim of this paper is to project important histopathological features in a series of retinoblastoma children, in order to highlight its importance in the follow up treatment.

MATERIAL AND METHODS

Thirteen children scheduled for enucleation were prepared for general anaesthesia and enucleation was done by surgeon with expertise in eye surgery for enucleation in retinoblastoma children. Eyeball was removed intact, with section of at least 10 mm optic nerve. Specimen was fixed in 10% formalin for 24 hours. The relevant proforma carrying, information about the laterality, identity of patient, clinical staging, and steps of surgical procedures was filled. Labeling of specimen container included the name and bed No. of patient. In the histopathology laboratory, specimen of the eyeball with tumour was grossly examined. Optic nerve margin was sampled first and then main tumor block was taken. Multiple longitudinal sections through the optic nerve head and optic nerve were taken with microtome in order to assess the degree of optic nerve invasion. The slides were prepared for examination after staining with hemotoxylin and eosin. A panel of consultant histopathologists made the observations under microscope.

Histopathologist reports included comments on Flexner-winter Steiner rosettes, pseudorosettes, fluorettes and differentiation. Optic nerve was examined and reported upon for any tumor cell invasion. Involvement of sclera and choroids were noted. Necrosis, calcification and pigmentation were recorded in the report. Documentation of microscopic pictures was done with the ancillary computerized camera attached to microscope. Consultant ophthalmologist critically analyzed the histopathological reports in the background of clinical parameters of the patients. Final report with regard to high risk cases was taken in to account to modify further mode

HISTOPATHOLOGICAL FEATURES AND DEMOGRAPHIC DATA IN 13 RETINOBLASTOMA PATIENTS

Case No:	1	2	3	4	5	6	7	8	9	10	11	12	13
Age in years	5	4	4	2	5	7	4	4	3	1	3	2	3
Gender	F	F	M	M	M	F	M	F	M	M	M	F	M
Laterality	U	B	B	B	U	U	U	U	U	B	U	U	U
Rosettes (F.W)	+	+	—	-	-	+	+	—	+	-	-	+	+
Fluorettes	+	+	+	—	-	+	-	+	-	-	-	+	+
Pseudorosettes	-	-	-	-	-	-	+	-	+	+	-	+	+
Scleral invasion	-	+	+	+	-	-	-	-	-	-	-	+	-
Choroidal invasion	-	-	-	-	-	-	+	—	+	+	-	+	-
Optic nerve invasion	-	+	-	-	-	-	Nc	+	Nc	+	Nc	Nc	+
Necrosis	+	+	+	-	+	+	+	-	-	-	+	+	-
Melanin Pigmentation	-	+	-	-	+	-	-	-	+	-	+	-	+

Legend:

M: Male F: Female +: Present

-: not present U: Unilateral F.W: Flexner wintersteiner

TABLE I

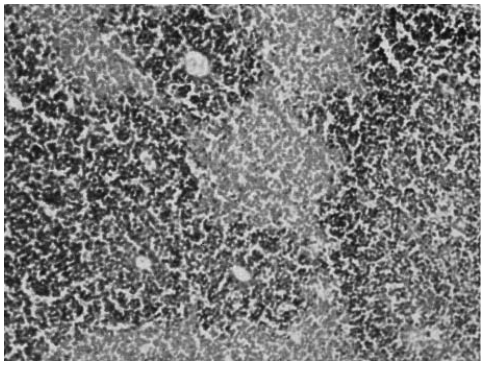
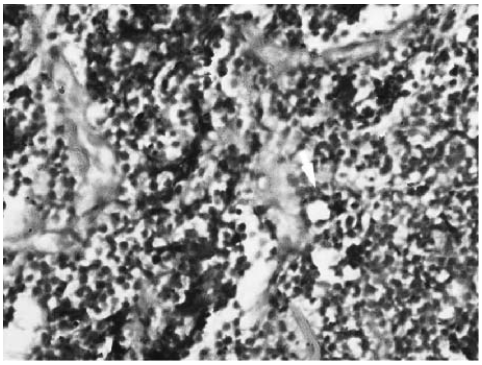
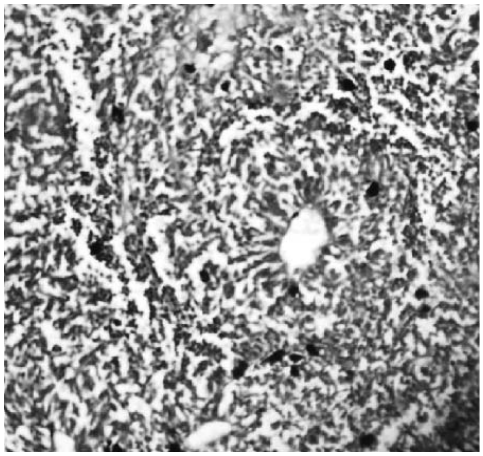
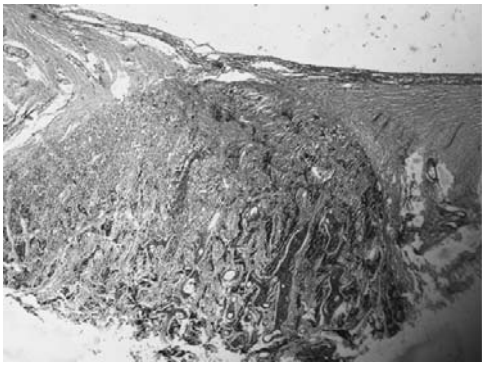

Fig. 1: Top Right: Showing Pseudo Rosettes And Area Of Necrosis In Retinoblastoma.

Fig. 2: Top Left: Showing Flexner Winterstenor Rosettes In Retinoblastoma.

Fig. 3: Bottom Right: Sun Flower Like Pseudo Rosettes With studeed Melanin Pigment In Retinoblastoma.

Fig. 4: Bottom Left: Optic Nerve Invasion By Tumor Cells In Retinoblastoma.

of treatment (chemothearpy and local radiotherapy) in perspective of prognostic implications. At the same time parents of the patients were kept informed about the further line of management, and a schedule of follow up was devised.

RESULTS

Clinical Data

Thirteen patients who underwent enucleation were included in the study. In this study 8 (61%) were males, 5 (39%) were females. Age range was 1 year to 7 years. Mean age was 3.62+1.55 years and Mode was 4 years. 9 (69%) were unilateral, 4 (21%) were bilateral. Seven (53%) presented with leukocoria, 3 (23%) with proptosis, 2 (15%) had endophthalmitis and 1 (7%) patient had glaucoma. 4 (31%) children had stage VI disease, 9 (69%) had stage V B according to Rees-Elsworth classification. Serum LDH was raised in 9 (69%) cases.

Microscopic Data

Examination of the histopathological reports of these 13 retinoblastoma patients revealed differentiated tumour in 7 (53%) cases and poorly differentiated tumour in 6 (47%) cases. There were significant number of Flexner- Wintersteiner rosettes in 7 (53%) cases.

Necrosis was noted in 8 (61%) cases. There was choroidal invasion in 4 (30%) cases. Optic nerve was not commented upon in 4 (30%) cases, which for the purpose of interpretation and treatment were considered as infiltration of optic nerve. 4 (30%) cases had optic nerve invasion. In 5 (40%) cases optic nerve was reported free of tumour. Pigment cells were noted in 5 (40%) specimens.

Four patients with clear optic nerve invasion and in another 4 (30%) specimens, optic nerve was reported to be missing or comments on the optic nerve were not recorded thus it was presumed as if optic nerve is involved. All these 8 patients were offered chemotherapy and orbital radiotherapy, while 5 patients with having no optic nerve invasion were not given any chemotherapy after enucleation. Chemotherapeutic drugs included etoposide, vincristine and carboplatin.

DISCUSSION

Retinoblastoma, if untreated is usually fatal, however in developed countries, five years survival rate of retinoblastoma is approaching almost 96.5%.[5] However delay in diagnosis and treatment and the additional misfortune of poverty in the developing world, is coupled with miss directed follow up in retinoblastoma patients. Lacunae in handling the valuable histopathological documents in these cases as well as lack of awareness further magnify the devastation.

Typical histological features in the form of Flexner Winterstenier rosettes as well Fleurettes are considered as evidence of higher differentiation of the tumor.[6] Microscopic retinoblastoma is usually creamy white with chalky area of calcification and yellow necrotic regions. The tumor grows intraocularly and may result in vitreous seeding. It may be multifocal, endophytic or exophytic growth. Tumor ischemia may lead to necrosis in retinoblastoma. Calcification is pronounced in these sites. All these features and also the extent of optic nerve invasion co-relates closely with outcome of the disease.

Owoeye Joshua FA et al, in their study on clinicopathological study on 20 retinoblastoma patients reported poorly differentiated tumor in 82.6% of specimen. Flexner Winterstener rosettes were noted in 17.4% of specimen. Optic nerve invasion was recorded in 69.6% of cases. Necrosis was noted in 87% of cases.[7]

In our present study of 13 enucleated eyes, the microscopic picture of 7 (53%) specimens demonstrated differentiated tumor and 6 (47%) as poorly differentiated. This finding is quite deviated from the above study pointing towards the differing behavior of tumor in terms of microscopic pictures in different groups of patients.

In a another series by Shield JA et al[6] in a retrospective study of 289 children with retinoblastoma who were treated initially with enucleation, showed a trend of correlation of optic nerve invasion with poorly differentiated retinoblastoma.

Nikolas E. Bechrakis et al[8] while studying histopathological features in relation to primary chemotherapy documented the existence of Flexner – wintersteiner rosettes with apparently viable tumor cells in 4 patients after four courses of chemotherapy.

In very early series by Algernon B. Reese, M.D,[9] between 1878 and 1929, out of 119 retinoblastoma patients who were enucleated, in 53% of the eyes the tumor had extended into the optic nerve beyond the lamina cribrosa. In 43% of the eyes the optic nerve was not severed at surgery beyond the tumor extension.

In our present series of 13 cases who were enucleated 4 (30%) cases had optic nerve invasion; in another 4 (30%) specimens, optic nerve was reported to be missing or comments on the optic nerve were not recorded; thus it was presumed as if optic nerve is involved; thus making it 60% of optic nerve invasion. Prophylactic chemotherapy and radiotherapy was given to all 8 patients. There was choroidal involvement in 4 (30%) cases and scleral infiltration in 4 (30%) cases. Magram I, et al[11] in their retrospective study of 814 patients reported 29.5% involvement of optic nerve. He also related this finding to the age of the patient at the time of diagnosis, thus co-relating it with survival.

A study of 298 retinoblastoma cases by Shield JA et al,[6] in 1994, reported involvement of 84 eyes (29%) with optic nerve invasion. There was infiltration of the tumor cells posterior to lamina cribrosa but not to the cut end of the optic nerve in 17 cases (6%). Only 2 cases (1%) had optic nerve invasion to the site of optic nerve transection. At the same time choridal invasion presented simultaneously, with optic nerve invasion and increased the risk of metastatsis.

The presence of necrosis in the microscopic picture of specimen is graded according to the percentage of the necrotic tumor area.

In another series by Chong, EM, et al,[12] who studied 43 patients with retinoblastoma between 1990 and 2001, authors reported 11 patients with extensively necrotic intraocular retinoblastoma while 2 patients died of metastasis (tumors is designated as exhibiting extensive necrosis if more than 95% of tumor cells and intraocular tissues were necrotic.). None of the 32 patients without extensive necrosis developed metastatic disease. The author concluded that extensive ocular tissue and tumor necrosis was associated with histologic high-risk prognostic factors for tumor metastasis and mortality.

In our current study, the histopathological specimen of 8 patients showed necrosis. However the degree of necrosis was not commented upon in report, on microscopy.

An interesting phenomenon in the form of significant number of pigment cells was noted in 5 specimens of retinoblastoma patients. There has been a mentioned of this phenomena in the case report by Smith PJ, Ablett GA, Sheridan JW, where histopathological examination of the enucleated eye of a 7 months old child revealed retinoblastoma with areas of rosette formation as well as focal areas of melanin pigmentation.[11] We have co-related this phenomena with advanced stage of the disease and late presenting age of the patient. The existence of pigment cells in histopathological picture of retinoblastoma needs to be further evaluated also in relation to progeny of this deadly childhood intraocular tumor.

CONCLUSION

In conclusion it is important that attention is to be given to the details of the histopathological report, par-

ticularly optic nerve invasion, differentiation, necrosis, calcification and choridal invasion. Lack of awareness and negligence in this regard is unacceptable and is a risk to the life of patients.

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CONFLICT OF INTEREST
Authors declare no conflict of interest