Histopathologic Risk Factors of Retinoblastoma: A Retrospective Study of 104 Enucleated Eyes

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Abstract

***Purpose*:** To introduce a novel classification system for the extent of choroid invasion and to analyze the incidence of histopathologic risk factors (HRFs) in the patients with retinoblastoma in China

***Methods*:** The clinical data of 104 enucleated eyes diagnosed with retinoblastoma were retrospectively reviewed, and the pathological re-examination of the enucleated eyes was conducted.

***Results*:** Overall, the HRFs were present in 53% of the 104 eyes. For choroid infiltration, type 1 was observed in 38 eyes as isolated, sporadic tumor cells, or suspected tumor cells with no obvious choroid thickening; type 2 in 26 eyes, as localized nest-like or nidulant tumor cells without obvious choroid thickening; and type 3 in 25 eyes, as lumpish, massive or dense invasion with or without obvious choroid thickening. The mean follow-up period was 27.6 months (median, 24.9; range, 8.3-65.7). During the course of the study, four patients died of recurrence or metastasis. Statistically significant differences between the proportional mortality ratios were observed in choroidal infiltration of Type 3 (P=0.003), invasion of postlamina (P=0.033), sclera (P=0.003), and optic nerve resection line (P=0.005) cases. Based on univariate and multivariate analysis, leukocoria was negatively correlated with HRFs (P=0.001, OR=0.21; P=0.010, OR=0.25).

***Conclusion*:** Clinically, the novel classification system for the extent of choroid invasion could function as a practical definition for choroid infiltration, and the HRFs are present in a significant proportion of patients enucleated for retinoblastoma in China.

***Keywords*:** Histopathology, Histopathologic Risk Factors, Retinoblastoma, China

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Introduction

Retinoblastoma is the most common intraocular malignancy in children.[1] With the application of chemoredution and focal adjuvant therapies, it is possible to halt the deterioration of the eyes and optimize the residual vision in eyes with less advanced tumors.[2] However, enucleation is still the primary treatment for the advanced retinoblastomas, specially for unilateral patients. Metastasis, following enucleation, is estimated to occur in less than 10% of the patients in developed countries.[3,4] Following the identification of histopathologic risk factors (HRFs) during postenucleation pathological examination, adjuvant chemotherapy and/or external beam radiation therapy (EBRT) ought to be prescribed to further reduce the risk of metastases. However, EBRT could produce the risk of generating second malignancies and facial bone hypoplasia,[5,6] hence chemotherapy is usually preferred.

It is well accepted that the HRFs include massive choroidal infiltration, invasion of the retrolaminar optic nerve, invasion of optic nerve resection line, scleral infiltration and anterior chamber invasion (iris infiltration, ciliary body infiltration) by tumor cells.[3,4,7-11] However, there are still controversies over the practical definitions of HRFs, specially choroidal infiltration.[12] Since the incidences rates of HRFs for retinoblastoma are likely to be higher in developing countries,[4] there are few reports in English on the definition and the frequency of HRFs among Chinese patients. Therefore, in this retrospective study, we conducted an investigation to set up a novel classification system for the extent of choroid invasion so that we could verify the frequency of HRFs by analyzing the histopathologic characteristics of retinoblastomas in Chinese patients.

Methods

We retrospectively reviewed the records of 104 patients treated for retinoblastoma between January 2005 and March 2009 at the Eye & ENT Hospital (EENT Hospital) of Fudan University, Shanghai, a major referral center for eye diseases. And the follow-up period began with the discharge from the first hospitalization and ended with the final appointment with each patient.

The records were all collected and evaluated carefully with consideration of onset age (month), sex, hereditary pattern, laterality (unilateral, bilateral), clinical features (leukocoria, red eyes, etc.) and the final outcome (survival, decease). The presurgical International Classification of Retinoblastoma (ICRB) system was used to evaluate each eye.[2] Generally, combined therapy (chemoreduction and/or focal therapies) were suggested to patients in stage A to C to preserve the eyeballs or to patients in stage D who insisted to preserve the eyeballs. Besides, adjuvant chemotherapy and EBRT were given to patients with choroidal invasion and/or optic nerve invasion. Hospital approval from the Institutional Review Board (IRB)/Ethics Committee was obtained.

All of the enucleated eyes were preserved in formaldehyde before they were fixed in paraffin blocks. Ten serial routine histopathologic sections taken from the largest cross section through the optic nerve of each eyeball were processed and stained with hematoxylin-eosin for histopathologic analysis. Two pathologists and one ophthalmologist separately examined samples and reached to a consensus by discussions if needed to identify tumor differentiation and ocular involvement. Choroidal invasion was categorized into 3 types: 1) isolated, sporadic tumor cells, or suspected tumor cells with no obvious choroid thickening; 2) localized nest-like or nidulant tumor cells without obvious choroid thickening; 3) lumpish, massive or dense invasion with or without obvious choroid thickening (Table 1). The extent of optic nerve invasion was defined as prelaminar, midlaminar, retrolaminar (postlaminar) and to the optic nerve resection line. Invasions of the sclera and anterior chamber including iris and ciliary body were also noted (Figure 1).

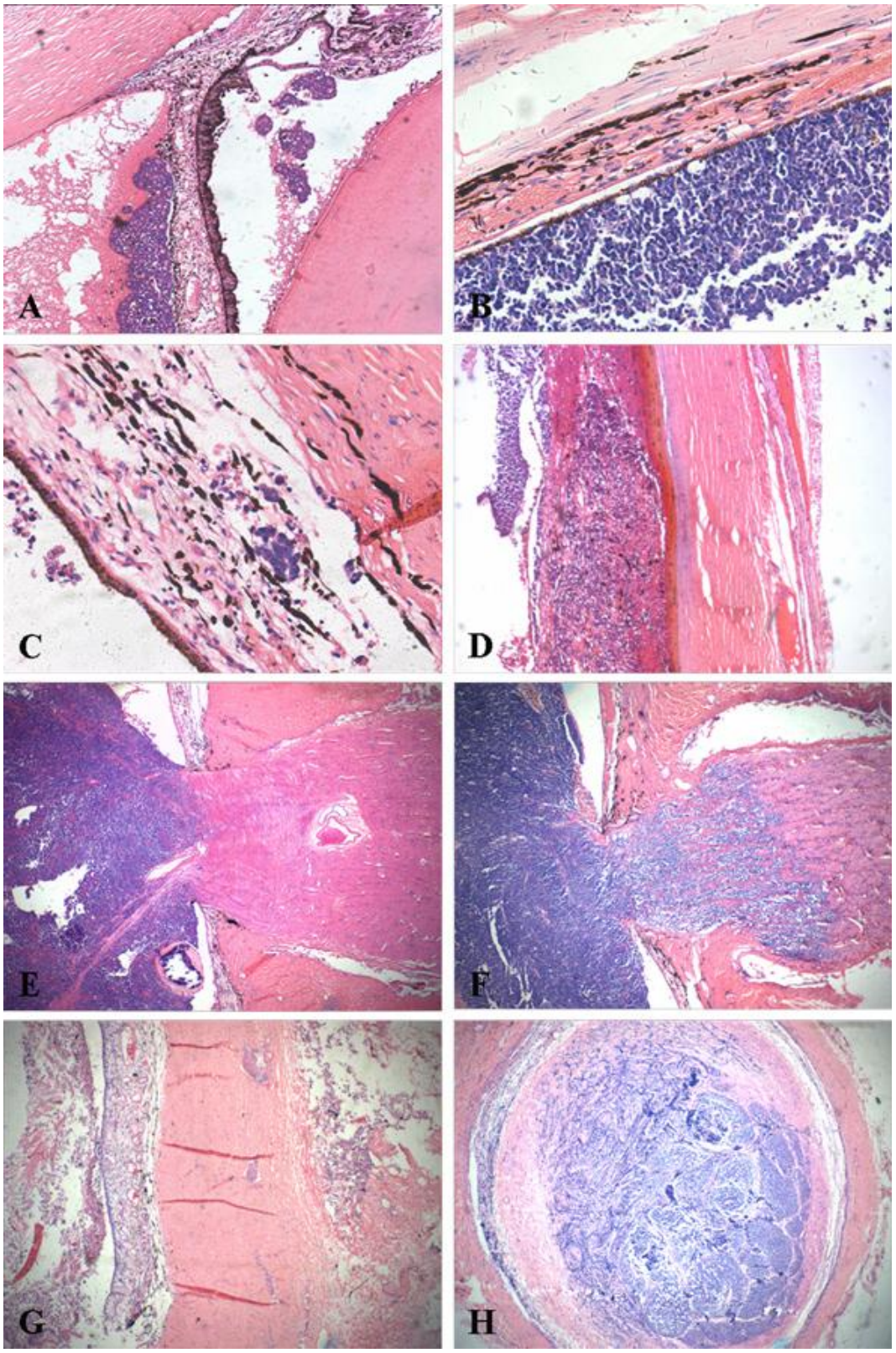

Figure 1. Photomicrograph showing the extent of ocular involvement in histopathologic sections of retinoblastoma, A: Anterior chamber infiltration; B, C, D: Type 1, type 2, and type 3 choroid infiltration, respectively; E: Infiltration of midlaminar optic nerve; F: Infiltration of retrolaminar optic nerve; G: Infiltration of sclera; H: Infiltration of optic nerve resection line. Stain, hematoxylin-eosin; original magnifications, ×50 (A, D), ×200 (B, C), ×25 (E, F, G, H)

Statistical analysis

Fisher's exact test was performed to analyze proportional mortality ratio in different pathological features to detect the HRFs in the 104 eyes, with the relative risk (RR) calculated as well. Univariate and multivariate logistic regressions analyses were applied to measure whether any clinical characteristic was predictive of the presence of HRFs; Pearson χ^2 test and Fisher exact test conducted in the univariate analysis. The analysis of the survival percentage was performed by Kaplan-Meier method, and also done between the groups with and without HRFs via Log-rank test. The level of significance was $P<0.05$.

Results

The sample of 104 patients was composed of 67 boys (64%) and 37 girls (36%), with the mean onset age 22.9 months (median, 19.5; range, 1.5-75). Unilateral retinoblastoma was found to have occurred in 82 (79%), and a positive family history was noted in 6 (6%). The mean lag time, between the first symptom and the first hospitalization, was 2.3 months (median, 1; range, 0-22).

It was found that leukocoria occurred in 73% of eyes; red eyes in 9% of eyes; impaired vision in 9% of eyes; strabismus in 6% of eyes; sore eyes in 2% of eyes; photophobia in 1% of eyes; and proptosis in 1% of eyes. Nine children had undergone chemoredution prior to enucleation, and 30 received chemotherapy or EBRT following enucleation. In the bilateral patients with one eye enucleated, attempts were made to salvage the fellow eye.

The review of the histopathologic slides showed 20% of eyes presented differentiated tumors and 80% were undifferentiated. Choroidal infiltration was observed in 89 eyes (86%), with 38, 26 and 25 of them falling under type 1, type 2 and type 3, respectively. The invasion ratio of prelamina, midlamina, postlamina and optic nerve resection line accounted for 41%, 24%, 22% and 12%, respectively. The infiltration of the sclera and anterior chamber (iris and ciliary body) were noted in 11 (11%) and 44 eyes (42%), respectively.

Between the surviving and deceased groups, there were statistical differences of proportional mortality ratio in Type 3 choroidal infiltration (P=0.003), postlaminar invasion (P=0.033), scleral invasion (P=0.003), and the invasion of optic nerve resection line (P=0.005) (Table 2). And the RR values over 3 were found in the groups with infiltrations of the postlaminar optic nerve, optic nerve resection line, sclera and anterior chamber, respectively. Thus, HRFs were designated as infiltrations of choroid (type 3), retrolaminar optic nerve, optic nerve resection line, sclera and anterior chamber.

The results indicate that the total number of the eyes presenting HRFs was 55 (53%), with 30 (29%) presenting more than one; 25 presenting one; 14 presenting two; 6 presenting three; 6 presenting four and 4 presenting five HRFs. In the last group, statistically significances between the outcomes were noticed (P=0.007). The logistical regression analysis failed to indicate clinicopathological correlations between the groups with and without HRFs in terms of gender, laterality, family history, onset age (greater than 12 months and less than 12 months), lag time (greater than 6 months and less than 6 months), as well as stage (group D and group E) (Table 3). Based on univariate and multivariate analysis, leukocoria was negatively correlated with HRFs (P=0.001, OR=0.21; P=0.010, OR=0.25).

In the study, the mean follow-up period was 27.6 months (median, 24.9; range, 8.3-65.7). And up until November 2009, 4 children died of metastatic disease (Table 4), the mean age at death of whom was 41.5 months (median, 40; range, 30-55.8), and the mean interval time, from enucleation to death, 12.7 months (median, 10.7; range, 4-25.5). The survival percentage was calculated by the Kaplan-Meier method, and no statistically significant difference was found between the two survival curves as indicated by the Log-rank test (P=0.069).

Table 1. Classification of choroidal invasion

Type	Features	Obvious choroid thickening
1	Isolated, sporadic tumor cells, or suspected tumor cells	No
2	Localized nest-like or nidulant tumor cells	No
3	Lumpish, massive or dense invasion	Yes/no

Table 2. Pathological characteristics and outcomes of 104 patients (104 eyes)

Anatomic site	Tumor invasion	Total 104 eyes No. (% in 104)	Survival 100 eyes No. (% in row)	Decease 4 eyes No. (% in row)	P*	RR†
Differentiation‡	Differentiated	21 (20.2%)	21 (100%)	0	0.580	
	Undifferentiated	83 (79.8%)	79 (95.2%)	4 (4.8%)		
Choroid	Yes	89 (85.6%)	85 (95.5%)	4 (4.5%)	>0.999	
	No	15 (14.4%)	15 (100%)	0		
Type 1		38 (36.5%)	38 (100%)	0	0.294	
Type 2		26 (25%)	26 (100%)	0	0.570	
Type 3		25 (24.0%)	21 (84%)	4 (16%)	0.003	
Lamina cribrosa	Yes	91 (87.5%)	88 (96.7%)	3 (3.3%)	0.409	0.43
	No	13 (12.5%)	12 (92.3%)	1 (7.7%)		
Prelamina		43 (41.4%)	43 (100%)	0	0.140	
Midlamina		25 (24.0%)	25 (100%)	0	0.570	
Postlamina		23 (22.1%)	20 (87.0%)	3 (13.0%)	0.033	10.04
Optic nerve resection line	Yes	12 (11.5%)	9 (75%)	3 (25%)	0.005	23.00
	No	92 (88.5%)	91 (98.9%)	1 (1.1%)		
Sclera	Yes	11 (10.6%)	8 (72.7%)	3 (27.3%)	0.003	25.36
	No	93 (89.4%)	92 (98.9%)	1 (1.0%)		
Anterior chamber	Yes	44 (42.3%)	41 (93.2%)	3 (6.8%)	0.308	4.09
	No	60 (57.7%)	59 (98.3%)	1 (1.7%)		

*: Fisher's exact test
†: RR: Relative risk
‡: Differentiation type included Homer-Wright rosettes, Flexner-Wintersteiner rosettes and photo receptor differentiation.

Table 3. Univariate and multivariate analysis of clinical factors predictive of histopathologic risk factors† in 104 eyes with retinoblastoma

Univariate Analysis		**HRFs (55 eyes)**	**HRFs-free (49 eyes)**	**P***	**OR**
Gender	Male	37	30	0.520	
	Female	18	19		
Laterality	Unilateral	42	40	0.511	
	Bilateral	13	9		
Family history	Sporadic	52	46	>0.999	
	Familial	3	3		
Onset age	<12M	14	16	0.516	
	>12M	41	33		
Lag time	<6M	46	45	0.246	
	>6M	9	4		
Stage	Group D	8	14	0.080	
	Group E	47	35		
Leukocoria	Yes	33	43	0.001	0.21
	No	22	6		
Multivariate Analysis				P**	OR
Leukocoria				0.010	0.25

†: The histopathologic risk factors: type 3 choroid infiltrations, invasion of the retrolamina, optic nerve resection line, sclera and anterior chamber
*: Pearson χ^2 test, Fisher exact test
**: Multivariate logistics analysis
OR: Odds ratio
HRFs: Histopathologic risk factors

Table 4. Information and pathological characteristics of four dead cases

Features		**Case 1**	**Case 2**	**Case 3**	**Case 4**
General information	Gender	Male	Male	Male	Male
	Age at diagnosis (M)	12	20	31	31
	Age at death (M)	37.3	30	42.7	55.8
	Interval time (M)†	25.5	10.3	11.1	4
	Laterality	Both	R	R	Both
	Family history	No	No	No	No
Stage	ICRB‡	L: D	R: E	R: E	L: C
		R: E	-	-	R: D
Treatments	Enucleation	R	R	R	R
	Chemotherapy	-	Refused	-	9 cycles
	EBRT	Refused	4500cGy	Refused	R: 5200cGy
Pathology	Differentiation	Undifferentiated	Undifferentiated	Undifferentiated	Undifferentiated
Tumor invasion	Type 3 choroidal invasion	Yes	Yes	Yes	Yes
	Retrolamina	Yes	Yes	No	Yes
	Sclera	Yes	No	Yes	Yes
	Optic nerve resection line	Yes	Yes	No	Yes
	Anterior chamber	Yes	Yes	No	Yes
Death detail		Metastasis, NQC	Cerebullar and buccal metastasis	Cerebullar metastasis	Metastasis, NQC

R: Right eye; L: Left eye; M: Month; NQC: Not quite clear
†: The interval time is the time between the enucleation and death.
‡: The International Classification of Retinoblastoma

Discussion

In previous ophthalmic literature reports, the HRFs for the metastasis of retinoblastoma were defined in a slightly different way. It is generally agreed that HRFs include massive choroidal infiltration, invasion of the retrolaminar optic nerve, invasion of the optic nerve up to the resection line, and scleral infiltration,[3,4,7-11] and in some reports the anterior chamber invasion (iris, ciliary body),[3,4,8,11] lamina cribrosa of the optic nerve.[3,4] All included reasons for this variation of diagnostic criteria was reported to be due to the small number of the patients within each subgroup of retinoblastoma patients. This pitfall existed even at the major ocular oncology centers, which made it difficult to define the HRFs of retinoblastoma.[8] The International Retinoblastoma Staging Working Group exposed major controversies concerning the definition of HRFs after enucleation, which resulted in a wide range of treatment proposals.[12] At this conference, however, there were only three Asian counties participating, India, Iran and Indonesia, out of the total of 28 countries from 4 continents included in the study.

In this present investigation, the HRFs were considered as type 3 choroidal infiltration (massive or dense invasion), invasion of the tumor to the postlaminar region to sclera, and the optic nerve resection line. Considering the limited sample size in the deceased group and short period of follow-up, the proportional mortality rate was chosen to analyze the HRFs. Since the incidence of RR of anterior chamber invasion was 4.09, this was considered a potential risk factor in our patients, hence a HRF in our study for retinoblastoma covered five risk factors, consistent with some other reports.[3,4,8,11]

Although choroidal infiltration is generally divided into minimal and massive invasion in the literature, the definition of the latter has not reached a consensus.[3,4,7,12] In general, its classification has been based on the thickness of infiltrations or percentage of the thickness of choroid,[12] which may underestimate the incidence of the HRFs, often due to the failure to make serial sections for a pathological diagnosis. In our study, we have chosen to analyze qualitatively the extent of invasion as a novel classification system, instead of employing the quantitative classification, to categorize choroidal infiltration into 3 types based on the morphology of the tumor cells. Morphological changes are likely to be generated before the invasion and thickening of choroid. However, this assumption needs to be supported by further evidence both pathophysiologically and clinically.

In our study, the rates of occurrence of massive or dense (type 3) choroidal infiltration, defined as many clusters of tumor cells linking up into a single stretch and obviously causing choroid thickening. The prognostic outcome was significantly different from type 1 and 2 of choroidal infiltration which is in agreement with some other reports.[7] In addition, although Type 1 and Type 2 of choroidal infiltrations showed no statistical correlation with the proportional mortality indicator, they may aid in the evaluation of the process of tumor growth and extension.

According to the univariate and multivariate analysis, the presence of leukocoria was negatively correlated with the presence of HRFs, suggesting that patients with leukocoria were not inclined to present HRFs. It has been reported that endophytic retinoblastomas were more likely to grow into the vitreous body or develop vitreous seeding rather than extend to the choroid or to the optic nerve.[13,14] Presumably, patients with endophytic tumors are more easily to be recognized for the leukocoria than those patients with exophytic ones. Leukocoria is a view of an outward extension of endophytic retinoblastoma. Therefore, the patients with leukocoria, unlikely present HRFs, and would have a better survival rate. However, this should be proven by further investigations.

As the first clinical investigation on a large scale reporting HRFs associated with retinoblastoma in China, we found that the incidence of HRFs was much higher in our country than that in the Western countries.[7,10] This fact has been also reported in other developing countries.[15,16] The combined rate of HRFs in this study was found to be 53%, similar to reported rate in India; 54.2%.[4] The difference between the rates of HRFs in developed and developing countries could be ascribed to a greater age at diagnosis and a longer lag time in the latter. It has been reported that the mean lag time in Brazil has

been more than 5 months,[16,17] which is more or less similar to the report from Beijing.[18] This is an essential factor related to the high incidence of HRFs and also high proportion of patients with advanced disease.

It is worth to emphasize that the mortality or recurrence rate seemed to be lower in our patients; 3.7% than in the developed countries, which is less than 10%.[3,4] The lower rate in our series could be explained as follows: the duration of follow-up was short as compared with that of other reports.[7]

Physicians typically administer salvage therapies such as adjuvant chemotherapy and/or EBRT to patients with HRFs to prevent metastasis. As reported by Shields, patients with histopathologic high-risk features including invasion beyond the lamina cribrosa, choroid, or sclera were at the greatest risk for metastasis.[19] In our study, the enucleated eyes had develop choroid invasion in 86% of cases, and the metastases risk could be significantly overestimated if they all were defined as patients with histopathologic high risk. EBRT could produce the risk of generating second malignancies and facial bone hypoplasia,[5,6] and chemotherapy could bring about potentials side effects such as marrow depression[20] and ototoxicity.[21] Therefore, the question arises that whether it is necessary to prescribe additional therapies to patients enucleated and present HRFs in anatomopathological reports . Major controversies still remain over the definition of HRFs, which, accordingly, results in a wide range of treatment regimen.[12]

In view of these circumstances, we suggest based on our statistical data that salvage therapies to be given to patients with the following HRFs: type 3 choroidal infiltration, invasion of the postlamina, sclera, and optic nerve resection line. In addition, it is suggested that patients with anterior chamber invasion (iris infiltration, ciliary body infiltration) exclusively are not recommended to receive salvage therapies.

Conclusion

In sum, this was the first large-scale clinical investigation reporting the HRFs of retinoblastoma in China, and we proposed a novel classification system for the extent of choroid invasion, statistically analyzed the HRFs, and found out that there was a higher rate of HRFs in developing countries. Some limitations of our investigation should be mentioned:

1) Some parents of the patients in advanced stages had given up the treatment, specially when enucleation was needed which can influence our end results and conclusions.
2) Almost two thirds of our patients were boys (64%). In the developing countries sick boys are more likely to be sent to a specialist center than sick girls which is unusual in the developed countries.
3) Short-term follow-ups, our conclusions need to be verified by further investigations at a larger scale and for longer clinical studies in the future.

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