Original Article

Characteristics of Germline and Non-germline Retinoblastomas

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Purpose: To discuss the clinical characteristics, treatment and outcomes of germline and non-germline retinoblastoma tumors.
Methods: A retrospective study was performed on retinoblastoma cases from 1979 to 2007. General characteristics of the patients, treatment modalities, histopathological findings and survival were compared in germline versus non-germline cases.
Results: We analyzed 557 cases of retinoblastoma with mean age of 32.2±22.0 months including 177 and 380 patients with germline and non-germline tumors, respectively. Germline cases were significantly different from non-germline counterparts in terms of mean age (24.7±17.7 vs 35.7±23.0 months), symptoms (leukocoria in 49.4% vs 62.9%), and outcomes (death in 40.1% vs 13.9%), respectively (P<0.001). In the germline group 66.5% and in non-germline group over 97% of patients had stage Va or higher (ICRB D-E disease). Disease-free survival was 48.6% for germlines cases versus 80.9% for non-germline patients (with mean follow up of 61.9 months, P<0.001). Histopathologically, more invasions to intraocular and extraocular tissues were seen with non-germline tumors of (66% vs 39.8%). Mortality rates in germline cases and non-germline were 40.1% and 13.9%, respectively (P<0.001).
Conclusion: Despite higher tumor staging in nongermline cases at the time of diagnosis and therefore more aggressive behavior of the tumor, germline cases had a higher rate of mortality during the follow up period.

Keywords: Childhood Tumors; Epidemiology; Retinoblastoma; Germline; Non-germline

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INTRODUCTION

Retinoblastoma is the most common tumor of the retina in children with an incidence of one case in 15,000 to 30,000 children in Western countries.[1] About 60% of all patients with retinoblastoma have the non-heritable form of the disease with normal life expectancy if the eye cancer is cured. In this type, the average age at diagnosis is about 24 months, the eye tumor is unilateral and the risk of other cancers is essentially equal to the normal population. In contrast, the other 40% of patients mostly have bilateral tumors, with the RB1 germline mutation and mean age of 12 month at the time of diagnosis; these patients have a heritable cancer predisposition syndrome.[2-5]

The term "germline mutation" in retinoblastoma implies that the genetic abnormality is present in all cells of the body whereas non-germline (somatic) mutations mean that only the tissue of concern harbors the mutation. Bilateral and familial retinoblastomas have germline mutations and are heritable; in contrast unilateral sporadic retinoblastoma is usually not heritable.[6-9]

Germline status, as compared to laterality, is of greater importance in terms of patient care.[6-9] Our English language literature review failed to reveal any study addressing the clinical characteristics of germline and non-germline retinoblastomas as separate groups. The aim of this report is to describe the clinical characteristics, treatments, histopathological findings and outcomes of retinoblastoma patients with germline versus non-germline tumors referred to our pediatric ocular oncology unit.

METHODS

This retrospective, comparative case series includes 557 consecutive retinoblastoma patients (734 involved eyes) diagnosed at Farabi Eye Hospital, a major referral eye care center in the country, from 1979 to 2007. Information was retrieved from the medical records which included age at first visit, duration of symptoms before presentation, symptoms, gender, tumor laterality, disease stage, family history, treatment, histopathologic findings and outcomes of therapy. Tumor stage was determined and recorded (Reese-Ellsworth classification system) by an experienced ophthalmologist (HC) at the initial examination under general anesthesia.[10,11] Because of the retrospective nature of this study and possible conflicts in the final results, we could not alter the Reese-Ellsworth classification system already present in the records to more up-to-date systems such as the International Classifications of Retinoblastoma Tumor (ICRB). However, small or medium-sized intraocular retinoblastomas (Reese-Ellsworth Groups I-III) may be considered as ICRB A-C and more advanced intraocular tumors (Reese-Ellsworth IV, V) as ICRB D and E.

Bone marrow aspiration and cerebrospinal fluid (CSF) evaluation were performed for 500 cases. Before the mid-1990's our main treatments for retinoblastoma were enucleation (for stages IV and V), photocoagulation (for localized small tumors), cryotherapy and external beam radiotherapy. Since the late 1990's, systemic chemotherapy and /or other adjunctive treatments (subtenon carboplatin, intravitreal injections and cryotherapy) were applied according to published protocols and stage of the disease at initial visit.[10-12] In general, exenteration was performed in patients with optic nerve or orbital involvement on CT scan imaging or histopathology reports. Chemotherapy included VC (Vincristine, Carboplatin), VEC (Vincristine, Etoposide, Carboplatin) or OPEC (Oncovorin, Cyclophosphamide, Etoposide, Carboplatin) protocols during the time.[13] All treated children were followed closely for five years and yearly thereafter.

In this study, we considered two groups of germline and non-germline patients. RB1 mutation testing was carried out only for 20 cases. Since in the literature more than 85% of bilateral cases were germline and more than 85% of unilateral cases are nongermline, all children with bilateral tumors and/or positive family history of retinoblastoma (either unilateral or bilateral) were considered as "germline" and all unilateral patients with no family history as "non-germline".[7]

In the analysis of staging, histopathologic findings and treatments, all affected eyes (including both eyes in bilateral cases) were included. Treatment was divided into two categories: surgical and non-surgical. SPSS software (version 16; SPSS, Chicago, IL, USA) was used for statistical analyses employing chi-square and t tests.

RESULTS

A total of 557 patients including 332 boys and 225 girls aged 0.5 to 168 (mean 32.2±22.0) months were included in this study. Unilateral tumors were present in 380 (68.2%) of cases and bilateral disease was present in 177 (31.8%) subjects. General characteristics, demographics, symptoms

Table 1. Demographic findings and outcomes in retinoblastoma patients

	Germline [177 (31.8%)]	Non-germline [380 (68.2%)]	P value*
Sex (%)			
Male	64.4	57.4	0.115
Female	35.6	42.6	
Mean age (month)	24.7±17.7	35.7±23.0	
<12	33.9	14.2	
12 - 24	31.1	27.9	
24 - 36	19.2	23.7	< 0.001
36 - 48	7.3	15.8	
48 - 60	2.8	7.4	
60 - 72	5.6	6.8	
> 72	0	4.2	
Duration of symptoms before specialist visit (month)	7.8±12.6	5.4±8.9	0.139
Signs & Symptoms (%)			
Leukocoria	49.4	62.9	0.002
Strabismus	13.6	9.2	0.193
Buphthalmous	7.9	9.7	0.485
Proptosis	14.7	8.7	0.066
Follow up (month)	51.7±61.3 (55)	64.5±65.0 (65)	0.148
Outcome (%)			
Dead	40.1	13.9	
Alive	26.6	58.9	
Unknown	33.3	27.2	< 0.001

*Independent sample t.test for continuous and chi square for categorical variables

and outcomes are compared between the two groups in Table 1. Mean follow-up duration was 61.9±65.0 (range 1-321, median 37.0) months.

Overall, 135 (24%) patients had family history of retinoblastoma at the time of presentation; 27 (4.8%) cases had family history of retinoblastoma in their parents or siblings; in 56 (10.1%) cases one or more cousins or aunts/uncles were involved and in 52 (9.3%) cases a remote family history of suspicious eye problems was present. Patients with family history of retinoblastoma in their parents, siblings, uncles, aunts and cousins were included in the germline group.

Germline cases (177 patients) were different from non-germline subjects (380 patients) in terms of mean age (24.7±17.7 vs 35.7±23.0 months), symptoms (leukocoria in 49.4% vs 62.9%), and outcomes (death in 40.1% vs 13.9%) (P<0.001 for all comparisons). Overall 332 of 557 cases (59.6%) were male; corresponding figures in the germline and non-germline groups were 114 (64.4%) and 218 (57.4%), respectively. Statistically nonsignificant male predominance (Table 1) was observed in our study population especially in the germline group (64.4% male subjects vs 35.6% female cases, P<0.05). The staging of the cases is presented in Table 2. Stage Va (ICRB D-E) or higher tumors were present in 66.6% of cases in the germline group versus 97.3% of the non-germline group; 75% of our patients had been diagnosed within 6 months from appearance of the first symptoms.

Table 2. Staging of the eyes with retinoblastoma tumors based on the first visit

Staging	Germline (n= 359) (%)	Non-germline (n= 375) (%)	P value
Ia	2.2	0	<0.001
Ib	3.1	0.3	<0.001
IIa	0.6	0.5	<0.001
IIb	5.8	0.5	<0.001
IIIa	0.6	0	<0.001
IIIb	12.3	1.1	<0.001
IVa	8.9	0	<0.001
IVb	0	0.3	<0.001
Va	34.5	62.9	<0.001
Vb	6.2	16.0	<0.001
Not evaluable	5.3	6.7	<0.001
Phthisis bulbi	6.7	0.8	<0.001
Orbital involvement	13.9	10.9	<0.001

Positive results for tumor cells in bone marrow aspirates were 11.6% overall, 8.5% in

germline cases (N=15) and 14.1% in non-germline subjects (N=31) (P<0.001). Cerebrospinal fluid (CSF) analysis revealed tumor cells in 3.2% overall, 2.3% (N=4) versus 3.7% (N=14) of cases in germline and nongermline cases, respectively (P<0.001).

In our study, 65.4% of eyes were enucleated and 7.6% of subjects underwent bilateral enucleation. As shown in Figure 1, in the non-germline group more patients required enucleation during the course of treatment.

Table 3 presents the histopathologic results. There was histological confirmation of retinoblastoma in 89% of our patients and a mild preponderance of undifferentiated forms in non-germline cases was present as compared to germlines tumors (P<0.001).

Of patients for whom survival data were available (N=402 with 61.9±65.0 months of mean follow-up), 68.8% (N=271) were alive at the end of the study. Disease-free survival was 48.6% for germlines cases versus 80.9% for non-germline patients (P<0.001). Histopathologically, more invasions to intraocular and extraocular tissues were seen with non-germline tumors of (66% vs 39.8%). Mortality ratio in germline cases

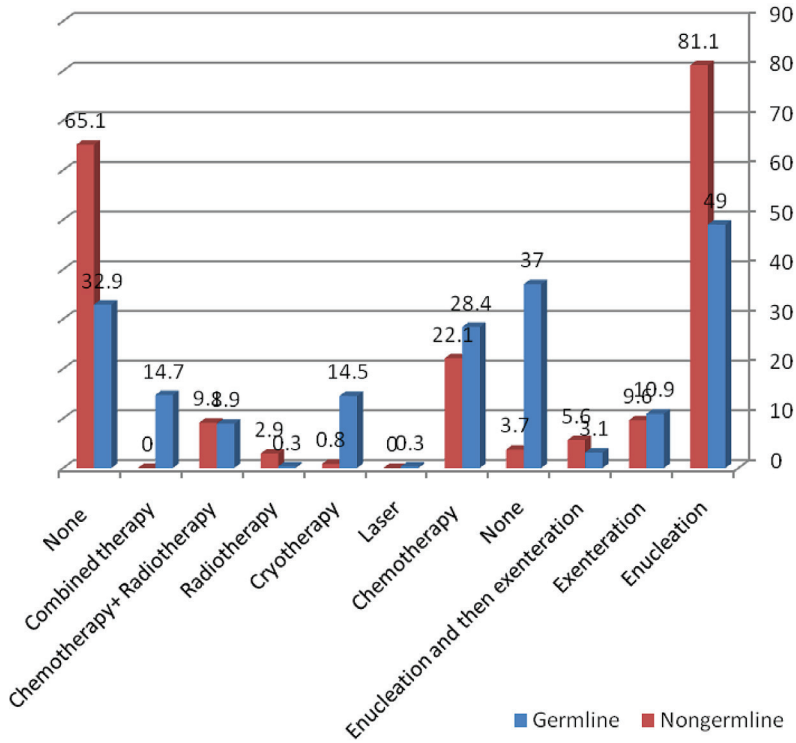

Figure 1. Surgical and non-surgical treatments for the eyes with retinoblastoma (Percent).

Table 3. Histopathology in the enucleated eyes with retinoblastoma

Histopathology (%)	Germline (n=359)	Non-germline (n=375)	P value
Undifferentiated	27.9	57.9	<0.001
Differentiated	19.2	25.1	<0.001
Unspecified retinoblastoma	52.9	17.0	<0.001
Choroid extension	15.0	18.9	0.030
Scleral involvement	5.0	8.5	0.023
Optic nerve head involvement	7.5	10.1	0.063
Surgical margin of optic nerve	7.5	18.9	<0.001
Iris involvement	4.5	8.0	<0.001
Ciliary body involvement	8.6	12.0	0.311
Orbital involvement	7.8	11.7	0.016

ON, optic nerve

was significantly higher than non-germline patients (40.1% vs 13.9%, P<0.001). We had no information about related and unrelated causes of death as well as second primary cancers in most of our cases. The rate of uveal invasion in our study was 18.9% and 15% in non-germline and germline tumors, respectively.

DISCUSSION

In the current series germline cases were different from non-germline subjects in terms of mean age, symptoms and outcomes. Stage Va or higher (ICRB D-E) was more common with non-germline tumors. Disease free survival less likely happened in germline group. Pathologically, more invasions to intraocular and extraocular tissue were seen in non-germline groups of patients and non-germline group had more undifferentiated form of the tumor. Although advanced cases were more in non-germline group, the death ratio was significantly higher in germline cases.

The mean age of the patients at diagnosis in the developing countries is higher than developed countries.[13-19] Like the previous studies, we observed that the mean age at diagnosis for germlines was lower than that observed in unilateral cases (24.7±17.7 vs. 35.7±23.0 months, P<0.001).[14,16,20-22] In our report, only 84.2% in germline groups and 65.7% in non-germline group of the patients were diagnosed before 3 years of age.

According to RE classification (ICRB D-E), patients in non-germline group were presented in more advanced stages comparing to the other group. In the United States, the percentage of patients with stage V (advanced intraocular disease) varies between 48% and 83% of the cases.[23]

We found that the mean time lag between first presentation of the disease and retinoblastoma diagnosis (delay in diagnosis) was comparably high in both groups, much higher in germline cases (Table 1). In few other studies, 50% of late diagnosis was because of specialists' wrong diagnosis.[14,24,25] 75% of our patients had been diagnosed within 6 months from the first symptoms appearance (Table1). This is shorter than the mean overall lag time of 8.3 months reported by Erwenne.[26] It is shown that advanced stage at the time of diagnosis and delayed diagnosis increases the risk of extraocular extension and diminishes the perspective for cure.[18,26]

Before 1980s, the enucleation ratio in patients with retinoblastoma was 96%, and between 1980 and 1990, this ratio decreased to 70–85%.[27] Recently, early diagnosis and eye-sparing treatment modalities have improved the patients' survival rate and have decreased the enucleation rate. In our study, enucleation rate is much higher in non-germline cases (81% vs 49%). This difference could be due to the diagnosis of non-germline cases in advanced stages. This retrospective study did not consider the chronologic changes in the type of treatments during three decades of study period.

Retinoblastoma is an eminently curable cancer and treatment is usually highly successful. The survival of patients with retinoblastoma has gradually improved over the years, in part because of the early diagnosis and multidisciplinary approach and management[2] varying in different regions of the world.[18,19,25,26,28] Of our followed cases, only 39.9% were alive with the mean follow-up time of 61.9±65.0 months (range 1-321 months) in germline group compared to 80.9% in non-germline group (P<0.001) with more mean follow-up time than other studies. We do not have any access to the cause of deaths and secondary tumor data in these patients but mortality in the bilateral cases was much more than unilateral cases (P<0.001). The reason of higher death rate in the germline mutation group (with a less advanced disease stage) could be due to the second primary cancers. To the best of our knowledge, there is no comparison between germline and non-germline cases regarding the mortality in English literature but the secondary and tertiary tumors are much more common in germline cases especially after third decade.[6-9] Majority of deaths from retinoblastoma occur within three years of diagnosis in developed countries[16]. We did not have any access to the time and causes of death in these patients in this study.

According to pathology results, the proportional percentage of undifferentiated

tumor was significantly higher in non-germline group (70%) comparing with germline group (59%). This could be due to the hypothesis that retinoblastomas become progressively less differentiated over a period of time.[29] Pathologic high risk features of retinoblastoma including massive posterior uveal invasion, retrolaminar optic nerve invasion, and any degree of concomitant optic nerve and posterior uveal invasion were more probable in non-germline group.[29] Uusitalo and coworkers reported an incidence of 9.3% for massive uveal invasion and 11.6% for retrolaminar optic nerve invasion in a combined series of patients from two centers.[30] Eagle reported that within the entire series of treated and untreated eyes, 20.4% eyes had high risk features. Of the 297 untreated eyes, 10.4% had retrolaminar optic nerve invasion and 8.1% had massive uveal invasion.[29] The uveal invasion in our study was 18.9% and 15% in non-germline and germlines, respectively, in series including both groups of treated and untreated eyes. Chantada et al reported that 48% of 224 Argentinean patients had at least 1 high-risk feature.[31] Biswas et al reported retrolaminar optic nerve invasion in 29% of 232 patients from India.[32] The reported incidence of choroidal invasion ranges from 15.2% to 62%.[32-38] In none of the studies the high risk features were evaluated in germline and non-germline cases. As Biswas, we believe that higher incidence of choroidal and optic nerve infiltration that we found in Iranian children in non-germline group could be due to delayed diagnosis or to a difference in the biologic behavior of this type of tumors.

The limitations of this study were retrospective nature of the study and lack of data of death cause. The genetic testing was not available for majority of the patients and some germline unilateral and non-germline bilateral patients have been included in our grouping.

In conclusion, non-germline tumors had higher tumor stages, more local invasion, more bone marrow involvement and CSF invasion, higher rates of enucleation and poorer histologic differentiation. However, germline tumors had a greater risk of mortality, pthisis bulbi, and orbital involvement despite earlier diagnosis and lower tumor stage at the time of diagnosis. Addressing all cases and their clinical and pathologic findings in this large series, mortality in the germline group was much higher than nongermline cases.

Conflicts of Interest

None.

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