Original article

Retinoblastoma: geographic distribution and presentation at a tertiary eye care centre in Kathmandu, Nepal

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Abstract

Introduction: Several aspects of retinoblastoma in Nepal remain enigmatic. **Objective:** To assess the demographic and geographic distribution, clinical presentation, and treatment methods of retinoblastoma at a tertiary level ophthalmic institution in Kathmandu, Nepal. **Materials and methods:** A retrospective analysis of all the patients diagnosed with retinoblastoma at Tilganga Institute of Ophthalmology from July 2004 to June 2008 was performed. The main outcome measures included region of residence, treatment options and histopathological findings. The histopathological analysis was performed on enucleated and exenterated specimens. **Statistics:** The statistical analysis was performed with SPSS Version 11.5. Descriptive statistics are represented as mean ± standard deviation. All tests were two-sided and the P-values of less than 0.05 were considered statistically significant. **Results:** Thirty patients presented with retinoblastoma during the study period. The mean age at presentation was 2.5 ± 1.6 years (range five months to seven years). Ten of the 12 patients who presented with bilateral retinoblastoma (83 %) were from the Terai region of Nepal. The ratio of unilateral to bilateral cases in the Terai region was 1:2. This differed significantly with the ratio in the hilly region (Fisher's Exact Test, p = 0.0012). The mean duration of symptoms before presentation was 2.5 3.2 months (range three days to 12 months). Twenty-four patients (80 %) presented with leukocoria. Eleven patients (36.6 %) presented with leukocoria as their only symptom. Ninety-seven percent of the patients underwent either enucleation (90 %) or exenteration (6.7 %) of at least one eye. **Conclusion:** Bilateral retinoblastoma is more prevalent in the Terai region of Nepal. The majority of the patients present with leucokoria and are treated with enucleation.

Keywords: Clinico-histopathology, geographic distribution of Nepal, retinoblastoma

Introduction

Although extremely rare in the general population, retinoblastoma (RB) remains the most common primary intraocular malignancy expressed in the pediatric patients amongst all populations (Shields, 1992). However, the incidence of retinoblastoma varies significantly according to socioeconomic status and between developed and developing nations (Stiller and Parkin, 1996). The rate of retinoblastoma in the United States is 10.9 per million in children under five years, and it is 9.1 per million in children under six years in New South Wales (Tamboli et al, 1990; Azar, et al, 2006). In the Netherlands, the rate of retinoblastoma has held steady at 1 per 17,000 live births since 1945 (Moll et al, 1997). In contrast, the rate of retinoblastoma in India is

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estimated to be as much as 4.4 times that of the United States (Schultz et al, 1993). A discrepancy between the presenting signs of retinoblastoma also exists between developing and developed nations. In Western countries, retinoblastoma is typically diagnosed early and leukocoria is the presenting sign in 40 - 60 % of patients (Pendergrass and Davis 1980; Suckling, Fitzgerald et al, 1982; Abramson et al, 1998). In Nepal, retinoblastoma is often not diagnosed until later stages, with proptosis and fungating mass comprising a higher proportion of the mode of presentation, and thus prognosis is often much worse due to increased likelihood that the tumor has spread to the optic nerve (Badhu et al, 2005; Saiju et al, 2006). Hence, the primary treatment in Nepal remains enucleation or exenteration of at least one eye, and, in cases of bilateral retinoblastoma, cryotherapy, photocoagulation, and chemotherapy in the eye with the less advanced stage of the disease (Saiju et al, 2006). Although studies have demonstrated a significant difference between the incidence and severity of RB in Nepal and the developed world, many aspects of RB in Nepal remain enigmatic.

We sought to elucidate the presentation and progression of retinoblastoma in Nepal by evaluating the demographic distribution, clinical presentation, and method of treatment in retinoblastoma patients. As Nepal is geographically divided into three distinct regions, according to elevation and land type, this study also classified patients according to their region of residence in order to identify any geographic trends. These regions are the Mountain Region (e.g. the Himalayas in the northernmost region of Nepal), the Hill Region (central region of Nepal), and the Terai region (low elevation marshland near the Indian border in the southern region of Nepal) (Shambu, 2007).

Materials and methods

A retrospective analysis was performed on all cases of retinoblastoma that presented at the Tilganga Institute of Ophthalmology, a Tertiary Eye Care Centre (TECC) in Kathmandu, Nepal, between July 2004 and June 2008. A detailed history of each patient that included presenting complaints, history and duration of present illness, family history, treatment history, age, sex, and place of residence was taken.

All patients were subjected to an external ocular examination. The state of the lids/adnexa, extra-ocular movement, and presence or absence of vitreous seeding were noted. Visual acuity was also recorded. Anterior segment evaluation was performed via Slit-Lamp Biomicroscopy (Haag-Streit Burn 900 or a Shin Nippon Hand-Held Slit Lamp). Fundus evaluation after full pupil dilation was performed with both direct and indirect ophthalmoscopy (Heine with Volk +20D). When tumors were present, their size, quadrant, number, and location were noted. IOP was measured using Perkin's tonometry.

Following diagnosis of retinoblastoma, the available treatments included enucleation, exenteration, cryotherapy, photocoagulation, radiotherapy and chemotherapy. Enucleation was indicated by tumor size greater than or equal to one half of the total retinal diameter. All patients with bilateral retinoblastoma received photocoagulation at the TECC. The patients were referred to an oncology unit of a nearby hospital for chemotherapy and radiotherapy. A complete histopathological analysis was performed on all enucleated/exenterated specimens. Specimens were classified according to the cell type (well versus poorly differentiated), optic nerve infiltration and orbital infiltration.

All data utilized in this study was collected via chart review and then entered onto a standardized form. Data was subsequently entered into Microsoft Excel 2007. All statistical analysis was performed with SPSS Version 11.5. Descriptive statistics were represented as mean ± standard deviation. All the tests were two-sided and the P-values of less than 0.05 were considered statistically significant.

Results

Thirty patients with retinoblastoma presented at the TECC during the study period. The mean age at

presentation was 2.5 ± 1.6 years (range five months to seven years). The mean duration of symptoms before presentation at the TECC for examination was 2.5 ± 3.2 months (range : three days to 12 months). Table 1 shows the mean age at presentation and the mean duration of symptoms for male and female and the laterality of involvement. The majority of patients (83 %) were three years old or younger. There were no significant differences between age at presentation or duration of symptoms prior to treatment between the male and female patients (p = 0.423 and p = 0.820, respectively).

Table 1: Mean age at presentation and duration of symptoms before presentation

	Male	Female	Uni-lateral	Bi-lateral	Total Average
Mean Age at Presentation (years)	2.3±1.3	2.8±2.0	2.4±1.6	2.8±1.8	2.5 ± 1.6
Mean Duration of Symptoms before Presentation (months)	2.3±3.2	2.7±3.2	3.0±3.9	1.7±1.3	2.5± 3.2

The only patient with bilateral retinoblastoma residing in the Hill region was from the far eastern part of the country near the Indian border. Bilateral RB was significantly more common in the Terai region, whereas unilateral RB was much more prevalent in the Hill region. Thirteen of the 18 patients (72.2 %) with unilateral retinoblastoma resided in the Hill region, and five (27.8 %) resided in the Terai region (Table 2). No patients with unilateral retinoblastoma resided in the Mountain region. When the Terai and Hill regions were compared, the association between the region of residence and bilateral involvement was highly significant (p = 0.0012, Fisher's Exact Test).

The patients were also classified according to the ethnic groups and religious affiliation. Eleven ethnic groups were represented amongst the 30 patients. In this study, only one of the 30 patients was Muslim, 23 were Hindus, and 6 were Buddhists.

Table 2: Regional distribution of laterality amongst the three regions of Nepal

Region	Unilateral	Bilateral	Combined Frequency (%)
Hill (Central)	13	1	46
Terai (Southern)	5	10	50
Mountain (Northern)	0	1	3.3
Combined Frequency (%)	60	40	

Out of 30 cases, 18 (60 %) had unilateral involvement and 12 (40 %) had bilateral involvement (Table 3). The male/female ratio was 1.3:1. All patients had a negative family history for retinoblastoma. There was no statistical difference between the proportions of males and females who developed unilateral versus bilateral retinoblastoma (p = 0.88).

Table 3: Distribution of gender and laterality

Gender	Laterality		
	Unilateral (n, %)	**Bilateral (n, %)**	**Total (n, %)**
Male	10 (33%)	7 (23%)	17 (57%)
Female	8 (27%)	5 (17%)	13 (43%)
Total	18 (60%)	12 (40%)	30 (100.0%)

Twenty-four patients (80 %) presented to the TECC with leukocoria. Of these patients, 11 (36.6 %) presented with leukocoria as their only symptom, and 13 had additional symptoms (Table 4). In all patients who presented with leukocoria, the mean duration of presenting symptoms was 2.6 ± 3.5 months, and in patients with leukocoria as their only presenting symptom, the mean duration of symptoms before presentation was 1.9 ± 1.6 months. Twelve patients (40 %) presented with a red eye, and this was associated with an average duration of symptoms of 3.2 months. With one exception, all patients presenting with red eye also presented with additional symptoms, including leukocoria and "other" symptoms. Twelve (40.0 %) patients presented with other symptoms (e.g. watery eye, swelling, tenderness, etc.) All patients who presented with "other" symptoms also presented with leukocoria or red eye.

Table 4: Frequency of presenting symptoms with duration of symptoms

Frequency (Percent)	**Presenting symptom**						
	Leukocoria only	Leukocoria total	Strabismus	Proptosis	Fungating mass	Red eye	Other
	11 (37%)	24 (80%)	1 (3.3%)	6 (20%)	1 (3.3%)	12 (40%)	12 (40%)
Mean duration of presentation (in months)	1.9±1.6	2.6±3.5	2.0 ± N/A	4.5 ± 4.5	1.0 ± N/A	3.1 ± 3.5	3.2 ±3.4

N/A: not applicable

Five of the cases in this study had their histopathology reports missed, and one patient diagnosed with unilateral retinoblastoma did not undergo surgery. Cases without the histopathology scores were otherwise reflective of the rest of the patient cohort in terms of age and time to treatment. Table 5 illustrates the histo-pathological findings in the remaining 24 cases. Poorly differentiated and well differentiated tumors were present in nearly equal proportions in this study. Ten of the 13 patients with poorly differentiated tumors had a unilateral retinoblastoma (Table 5). This distribution of poorly differentiated, unilateral tumors versus other tumors was not statistically significant (Fisher's Exact Test, p=0.12). Well differentiated tumors were present in near equal proportions in unilateral and bilateral cases. Patients with well differentiated tumors experienced symptoms for an average of 1.8 months before reporting to the TEC, whereas those with poorly differentiated tumors experienced symptoms for 3.4 months, but the difference was not statistically significant (p = 0.237, T-Test). Seven of the 9 patients with an affected optic nerve had unilateral retinoblastoma. Patients with an affected optic nerve experienced symptoms for 4.9 months on average before reporting to the TEC, while patients with an unaffected optic nerve only experienced symptoms for 1.3 months, and this difference was statistically significant (p = 0.009, T-Test). Both patients with orbital infiltration had unilateral retinoblastoma and had experienced symptoms for an average of seven months before reporting to the TEC. Well differentiated tumors presented at an earlier age than poorly differentiated tumors, and patients without optic nerve infiltration presented at an earlier age than those with optic nerve infiltration. The difference in age of presentation amongst patients with well differentiated tumors versus those with poorly differentiated tumors was not statistically significant (p = 0.0596, T-Test), but the difference in age between the patients with affected versus unaffected optic nerves was significant (p = 0.0037, T-Test).

Table 5: Histo-pathological findings with duration of symptoms and age at presentation

	Histopathology					
	Poorly differentiated	**Well differentiated**	**Optic nerve affected**	**Optic nerve unaffected**	**With orbital infiltration**	**Without orbital infiltration**
Frequency (percent)	13 (54%)	11 (46%)	9 (38%)	15 (63%)	2 (8.3 %)	22 (92%)
Unilateral	10 (42%)	5 (21%)	7 (29%)	8 (33%)	2 (8.3%)	14 (58%)
Bilateral	3 (12%)	6 (25%)	2 (8.3%)	7 (29%)	0 (0%)	8 (33%)
Mean duration of symptoms (months)	3.4±4.3	1.8±1.5	4.9±4.5	1.3±1.3	7.0±7.1	2.3±2.8
Mean age at presentation (years)	3.0±1.4	1.8±1.4	3.6±1.6	1.8±1.0	3.5±0.7	2.4±1.5

A total of 97 % of the patients underwent either enucleation (90 %) or exenteration (6.7 %) of at least one eye (Table 6). Even in cases when leukocoria was the only presenting symptom, all tumors occupied more than half of the retina. One patient did not undergo any treatment following diagnosis. All bilateral eyes received photocoagulation at the TEC in the less affected eye. Exenteration was performed in cases with extraocular involvement. In bilateral cases, the eye with the more advanced disease was treated surgically. Although all bilateral patients were referred for chemotherapy and radiotherapy, only 9 of the 12 bilateral patients (75.0 %) underwent chemotherapy, 6 (50 %) photocoagulation and 1 (8 %) radiotherapy. No patient in this study was elected to undergo cryotherapy. The patients with unilateral or bilateral retinoblastoma that had infiltrated optic nerve were referred for chemotherapy. Six of the seven of these patients underwent chemotherapy. Orbital extension was present in one case, and this patient underwent exenteration but did not undergo chemotherapy. One patient with unilateral retinoblastoma without optic nerve infiltration also underwent chemotherapy. This accounts for the seven unilateral patients who underwent chemotherapy.

Table 6: Frequency of treatments and treatment according to laterality

Laterality	Treatment				
	Enucleation (n, %)	Exenteration (n, %)	Photocoagulation (n, %)	Radiotherapy (n, %)	Chemotherapy (n, %)
Unilateral	15 (50%)	2 (6.7%)	1 (3.3%)	2 (6.6%)	7 (23%)
Bilateral	12 (40%)	0 (0%)	6 (20%)	1 (3.3%)	9 (30%)
Total	27 (90%)	2 (6.7%)	7 (23%)	3 (10%)	16 (53%)

Discussion

Whereas previous studies have found the distribution of bilateral to total cases of retinoblastoma to be around 20 - 30 % (Amozorrutia-Alegria et al, 2002; Kaimbo et al, 2002), this study documented a bilateral rate of 40 %. This is also higher than the 9 % - 24 % reported in previous studies from Nepal (Badhu et al, 2005; Saiju et al, 2006). Of particular interest is the unique geographic distribution of unilateral and bilateral tumors noted in this study. All cases of bilateral retinoblastoma are inherited, whereas about 90 % of unilateral cases arise sporadically (Vogel, 1979). The RB1 gene is located on chromosome 13q14 and retinoblastoma results when a deletion occurs at this locus. Hereditary (bilateral) retinoblastoma has an autosomal dominant pattern of inheritance. In Nepal, the discrepancy between occurrences of unilateral versus bilateral retinoblastoma in the Hill region and the Terai region was highly significant (Fisher's Exact Test, p=0.0012). Ten of the 12 patients who presented with bilateral retinoblastoma (83 %) were from the Terai region. Of all the patients who presented from the Terai region, 64 % had a bilateral retinoblastoma, which is nearly double the usual rate reported in developed countries (Vogel 1979; Stiller and Parkin, 1996; Moll et al, 1997) and more than triple the rate recorded in Nepal when considered as a whole (9 % - 24 %) (Badhu et al, 2005; Saiju et al, 2006). Of the 18 patients who presented with unilateral retinoblastoma, 13 (72 %) were from the hill region. Of these 13 patients, 8 were from Kathmandu or a district on its immediate border. Of all the patients who presented from the hill region, 86.7 % had a unilateral retinoblastoma. Although higher than the unilateral proportion of total cases reported outside of Nepal (60 - 70 %) (Vogel 1979; Stiller and Parkin 1996; Moll et a, 1997), this is within the range previously reported in Nepal (76 % - 91 %) (Badhu et al, 2005; Saiju et al, 2006).

In a study by Stiller et al, differences in the proportions of unilateral versus bilateral retinoblastoma were displayed according to

geography. However, these differences were classified according to the country, and for the United States, according to cultural background (e.g. white, black, Navajo Native American) (Stiller and Parkin, 1996). Our study is unique in that it demonstrates the significant difference in distribution of unilateral versus bilateral retinoblastoma between different geographic regions of the same country.

Recent studies by Orjuela et al (2000) and Palazzi et al (2003) have both shown the presence of HPV in retinoblastoma tumor tissue, thus suggesting a link between HPV infection and retinoblastoma. Although there are no studies of the prevalence of HPV in Nepal, several studies have demonstrated an increased incidence of HPV amongst infection in urban populations as opposed to rural populations (Stone et al, 2002; Kliucinskas et al, 2006). This could possibly account for the increased prevalence of unilateral retinoblastoma in Kathmandu and its surrounding areas. The prevalence of HPV in retinoblastoma in Nepal and the prevalence of HPV in Nepal as a whole are subject to further study.

Regarding the increased frequency of bilateral retinoblastoma in the Terai region, several factors may be involved. When compared with every district of the Hill region, excluding Kathmandu and Lalitpur, the districts of the Terai region have a substantially higher proportion of foreign-born inhabitants (Shambu RJ, 2007). As there are few terrain barriers separating the Terai region from northern India, a majority of these foreign inhabitants have probably migrated from India. Unlike from northern India, the Terai region is separated from the hilly region of Nepal, including the Kathmandu valley, by significant terrain barriers (e.g. high hills) and a lack of adequate transportation routes in the form of highways and railroads (Shambu, 2007). Thus, Nepal's natural landscape may have kept retinoblastoma carriers isolated in the Terai region.

According to the Integrated Regional Information Network (IRIN) and the CIWEC travel medicine center, with the exception of the Tharu ethnic group, who had natural resistance to malaria, the Terai region was largely uninhabited until the late 1950's when the USAID helped to establish a malaria eradication program in the region (CIWEC, 2006; IRIN, 2008). Following this, a mass migration occurred, and now the Terai region is the most populated region of the country. Unfortunately, information regarding the prevalence of retinoblastoma in the Terai region prior to 1960 does not exist. As considerable inbreeding amongst the Tharus probably occurred prior to the population influx of the late 1950's, measurement of the prevalence of bilateral retinoblastoma and its alleles amongst this ethnic group is a potential area for further study.

In this study, patients were also separated according to ethnic group. Amongst 30 patients, 11 ethnic groups were present. Hence, distribution according to ethnic group could not be measured with statistical validity.

Badhu et al (2005) suggested that the increased prevalence of bilateral retinoblastoma in the Terai region may be due to consanguineous marriages amongst the Muslim parents, who comprised a higher proportion of the patients with retinoblastoma in the Terai. In this study, one patient out of 30 was Muslim, 23 were the Hindus, and six were Buddhists. Hence, consanguineous marriage amongst the Muslim patients did not play a role in the increased rate of bilateral retinoblastoma reported in the Terai region in this study.

All of the aforementioned hypotheses for the geographical distribution of retinoblastoma in Nepal are subject to further study. The results of this study indicate that the alleles that cause bilateral retinoblastoma, or environmental factors that increase the penetrance of these alleles, may be present at a much higher rate in the Terai region of Nepal than in the Hill region.

Previous studies on retinoblastoma in Nepal reported that a significant proportion of patients experienced symptoms for an extended duration of time before they underwent examination. In one study, 42 % of patients had experienced symptoms for over 12 months before consulting

a physician, while in the other, 63 % of patients waited six months or longer. This study showed a significant decrease in the duration of symptoms before examination, with a mean lag time of 2.5 ± 3.2 months. Although not statistically significant, this study also found that female patients experienced symptoms longer before examination than male patients. As all patients considered in this study were minors, this could be due to treatment preference being given to males in the developing world, as suggested by Pearce et al (2001). The male preponderance present in the sex ratio (M: F = 1.3:1) was within the range previously presented in the studies from Nepal (Badhu et al, 2005; Saiju et al, 2006).

In this study, 11 patients (37 %) presented with leukocoria as their only symptom. These patients experienced symptoms for an average of 1.9 ± 1.6 months before reporting to the TEC. When considered with patients that reported multiple symptoms, 24 patients (80 %) reported with leukocoria. One patient (3.3 %) reported with strabismus and a fungating mass each, and six patients (20 %) reported with proptosis. These results contrast with previous studies of retinoblastoma in Nepal in which proptosis was the primary mode of presentation (40 %) and fungating mass was a more common mode of presentation (33 %) (Badhu et al, 2005; Saiju et al, 2006). Previous studies on retinoblastoma in Nepal also reported optic nerve infiltration rates of 48 % and 40 % (Badhu et al, 2005; Saiju et al, 2006). This study showed a slightly lower optic nerve infiltration rate of 38 %.

Early detection of retinoblastoma, when leukocoria is the only symptom present, is associated with a more favorable prognosis (Abramson et al, 2003). Hence, when considered with previous retinoblastoma studies conducted in Nepal, the decreased lag time between the onset of symptoms and examination that was recorded in this study, as well as the increased proportion of patients who reported to the TEC when leukocoria was their only symptom, may reflect improvements in public awareness of retinoblastoma and/or retinoblastoma screening provided by primary healthcare workers.

All of the patients in this study underwent enucleation or exenteration. This rate is consistent with previous studies from Nepal (Badhu et al, 2005; Saiju et al, 2006) but it is higher than the 75 % enulceation/ enxenteration rate in the United States (Shields et al, 1989). All eyes undergoing treatment in this study were either enucleated or exenterated because all tumors occupied greater than or equal to one-half of the retina.

Conclusion

This study found a significant association between region of residence and bilateral retinoblastoma in Nepal, but the cause behind this distribution could not be deduced. The decreased lag time between the onset of symptoms and examination that was recorded in this study, as well as the increased proportion of patients who reported to the TEC with leukocoria, may reflect improvement in public awareness of retinoblastoma and/or retinoblastoma screening provided by primary healthcare workers.

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