Retinoblastoma: A review

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Abstract

Retinoblastoma (RB) is a neoplasm of retinal origin and one of the life threatening paediatric ophthalmic conditions. Most common clinical presentations include leukocoria and strabismus. Diagnosis is made by indirect ophthalmoscopy aided by imaging techniques. The treatment of retinoblastoma is multidisciplinary and is aimed at saving lives, salvaging the globe and maintaining good vision. The use of neoadjuvant chemotherapy and focal treatments, such as laser photocoagulation, thermotherapy, cry therapy, and plaque radiotherapy are major globe preserving treatments in retinoblastoma. Mutation of the RB1 gene is one of the key factors in its initiation. There is considerable increase in knowledge regarding molecular pathology of this disease in recent years and the role of RB1 and other genes has also been hypothesized. However, a method of early detection still remains a challenge and is area of future research. Present review aims to summarize important aspects of retinoblastoma including its molecular genetics.

Keywords: Paediatric oncology, RB1, Retinoblastoma.

Introduction

Retinoblastoma (Rb) is a malignant tumour of the eye, typically presenting in the first 2–3 years of life, but found rarely in adult. It represents about 4% of all paediatric malignancies (Shields & Shields 2004). The incidence of Rb does not show geographic, ethnic or gender variations and about one case per 15,000–20,000 live births are detected worldwide, which corresponds to about 9000 new cases every year. However, its burden is found to be high in those regions which have high birth rates, such as in Asia and Africa (Ki vela 2009). Its incidence is quite high in India with an incidence of 28 cases per million populations in less than 5 years of age (National Cancer Registry Project, 1999-2000, Delhi). About 1200 new cases/year are diagnose in India, while in USA it is limited to 250-350 cases. The median age of disease presentation is under 12 months for heritable cases. However, for sporadic cases mean age is about 24 months (Abramson & Servodidio 1992). Disease presentation after the age of 6 years is extremely rare, but there are a few reports of cases in late ages also (Mietz et al. 1997, Parulekar 2010).

Pathology & Classification

Retinoblastoma cells are small and stain blue with haematoxylin and eosin. Rings of cells surrounding an empty lumen are known as Flexner-Winter Steiner rosettes. They are characteristic but not mandatory to make a diagnosis of retinoblastoma. Homer Wright pseudo rosettes, a ring of cells with an eosinophilic fibrillary centre, are also commonly found. Fleurettes are retinoblastoma cells that have undergone greater photoreceptor differentiation. Calcification and necrosis are common in these tumours. The initial classification system of retinoblastoma is Reese-Ellsworth classification system, proposed in 1963. This system classify disease in to 5 group based on chance of salvaging the affected eye (Resse & Ellsworth, 1963). Shields et al. (2004) introduced a new International Classification of Retinoblastoma based on basic clinical features allowing a more practical approach to judge results of chemo reduction. Later, the International Classification of Retinoblastoma was introduced in 2004 by retinoblastoma experts. Outline of this classification system is outlined in Table 1. In 2006, an International Retinoblastoma Staging System (IRSS) was developed which is summarised in Table 2 (Chantada et al. 2006). Futher, Chantada et al. (2013) compared this system with other classifications in a large cohort of 533 patients. They found IRSS to be considerable for prognostic factors at lower stages and being predictive of disease-free survival at higher stages of tumour.

Clinical Features

The most common and initial sign of retinoblastoma is leukocoria, an abnormal appearance of the retina and is first apparent when the tumour is still contained within the eye. After the first sign of leukocoria, Rb remains curable for 3–6 months (Dimaras et al. 2012). Other signs and symptoms may include proptosis (protrusion of the eyeball), strabismus, deterioration of vision, faltering growth or delayed development, poor visual tracking, glaucoma, and inflammation. In advanced stages, the tumour can spread to central nervous system, or may involve bones, lungs and abdominal solid organs (Parulekar 2010). In India, up to 35% Rb cases are presented with locally advanced or metastatic disease. Time from symptom onset to treatment initiation is about 8 months in our country, mainly due to lack of awareness, delay in timely referral, and lack of finances. This leads to high

mortality rate of up to 70% in most of the developing countries.

Table 1: **Table representing outlines of International Classification of Intraocular Retinoblastoma system**

Group	Subgroup	Specific features
A		Retinoblastoma ≤ 3mm in size and located more than 1.5mm from the disc and > 3mm from the foveola
B		Retinoblastoma > 3 mm in size Macular retinoblastoma location (≤3 mm to foveola) Juxtapapillary retinoblastoma location (1.5mm to disc) Clear sub retinal fluid ≤ 3mm from margin
C		Discreet retinoblastoma with/without sub retinal fluid ≤ 1 quadrant
	C_1	Sub retinal seeds ≤ 3mm from retinoblastoma
	C_2	Vitreous seeds ≤ 3mm from retinoblastoma
	C_3	Both sub retinal and vitreous seeds ≤ 3mm from retinoblastoma
D		Retinoblastoma with/without sub retinal fluid ≥ 1 quadrant and
	D_1	Sub retinal seeds > 3mm from retinoblastoma
	D_2	Vitrous seeds > 3mm from retinoblastoma
	D_3	Both sub retinal and vitreous seeds > 3mm from retinoblastoma
E		Extensive retinoblastoma occupying > 50% of globe or Tumour touching lens, Diffuse infiltrating tumour, tumour involving anterior segment, neovascular glaucoma, tumor necrosis with aseptic orbital cellulitis, phthisis bulbi or opaque media from haemorrhage.

Table 2: **Table representing classification system of International Retinoblastoma Staging System (IRSS)**

International Retinoblastoma Staging System	
Stage 0	Patients treated conservatively
Stage I	Eye enucleated, tumour resection complete on histopathology examination
Stage II	Eye enucleated, microscopic residual tumour present on histopathology examination 1. Invasion into extrascleral tissue 2. Invasion into cut end of optic nerve
Stage III	Regional tumor extension a. Orbital extension (orbital mass or thickening of optic nerve) b. Regional lymph node involvement (Preauricular and/ or cervical lymph node extension)
Stage IV	Metastatic a. Hematogenous metastasis (without CNS extension) 1. Single lesion 2. Multiple lesion b. Central nervous system extension 1. Prechiasmatic lesion 2. Central nervous system mass 3. Leptomeningeal and cerebrospinal fluid (CSF) disease

Diagnosis

Clinical examination remains most important factor for early diagnosis and successful treatment of the disease. Ophthalmoscopy evaluation under anaesthetic conditions, leads to visualization of the tumour, which is usually followed by imaging such as ultrasound and/or CT scan of the orbit (Ray et al. 2012). Ultrasonography with colour Doppler is helpful in cases where the ocular media is hazy making direct visualization of tumour difficult. MR imaging is usually done for evaluation of extra ocular/optic nerve invasion, subarachnoid seeding and intracranial involvement, and for diagnosis of rare cases of trilateral Rb (bilateral Rb

and pinealoblastoma). It is also used in distinguishing Rb from pseudo tumour conditions such as Coats' disease and other differentials (Mehta et al. 2012). Optic coherence tomography has been found useful in the diagnosis of cystic retinoblastoma (Shields et al. 2004). If metastasis is suspected, specific investigations including cerebrospinal fluid analysis, bone marrow biopsy and bone imaging should be conducted. The differential diagnosis includes other causes of leukokoria such as Coat's disease, retinal detachment, and retinopathy of prematurity, persistent hyperplastic primary vitreous, endopthalmitis, toxocarasis, astrocytic, hamartomas, medullo-epithelioma and congenital cataract.

Management

Management of Rb is quite complex and requires combined efforts of the ophthalmologist, paediatric oncologist, radiation oncologist and ocular pathologist. The therapeutic plan of Rb can be classified into conservative methods (aim to preserve the globe) and e-nucleation (globe is to be sacrificed). The driving force behind therapeutic strategies is to avoid e-nucleation and/or external beam radiation therapy and trend towards focal conservative treatment. Every effort is made to save the child's life with preservation of eye and sight, if possible. The first goal is survival, with maintenance of vision and salvage of the globe as important secondary goals. Conservative management includes chemo reduction, in which intravenous drugs are used for the reduction of tumour size. For early intraocular Rb, chemo reduction is the major globe preserving therapy (Friedman et al. 2000). The standard regimen consists of six cycles of standard doses of vincristine, etoposide and carboplatin, however agents, number and frequency of cycles, varies according to different protocols used (Mehta et al. 2012). A major drawback is its inefficiency in majority of cases with sub retinal/vitreous seeds due to recurrence, which require further management in the form of enucleation. Various focal treatments are available, which can be used either alone or in combination as per requirement. Focal therapies include laser photocoagulation, thermotherapy, cryotherapy, and plaque radiotherapy. Most of these therapies are employed for small tumours, especially those that have been reduced by chemo reduction. Cryotherapy is performed for small equatorial and peripheral Rb (3 mm in basal diameter and 2 mm in thickness) (Mehta et al. 2012). Along with cytotoxic effects, cryotherapy disrupts blood retinal barrier allowing influx of chemotherapeutic agents. Also, delivery of heat to the eye at 42°C to 60°C has also been used to complement chemotherapy (chemo-thermotherapy) or radiotherapy (thermo-radiotherapy) (Ray 2012). Laser photocoagulation is rarely used and is usually employed for small Rb posterior to the equator of the eye. This method is not employed in eyes receiving chemo reduction. It is performed using the indirect ophthalmoscopy argon or green diode laser. Commonly, it is repeated at 1-month intervals for three sessions (Shields 2004). Plaque radiotherapy is a method of brachytherapy in which a radioactive implant (Ruthenium-106 and Iodine-125) is placed on the sclera over the base of a retinoblastoma to irradiate the tumour transsclerally. Primary plaque brachytherapy is currently indicated in cases of chemo failure, tumour recurrence and where chemotherapy is contraindicated (Shields 2004).

E-nucleation (removal of the eye leaving the muscles intact) remains the treatment of choice in situations where the tumour has diffusely seeded or it is massive or if there is evidence of tumour invasion into the optic nerve, choroid, or anterior chamber with little hope of residual vision (Ray 2012). While enucleating an eye with Rb, minimal manipulation 'no-touch' surgical technique is practised, with special precautions to ensure that the eye is not accidentally perforated during surgery (Shields 2004). After surgical removal, enucleated eye is examined for macroscopic optic nerve and extra-ocular extension and should be sent for histopathological examination. Unfortunately, 75% of unilateral retinoblastomas in developing countries are enucleated since typically the disease is detected at an advanced stage (Ray 2012). EBRT (External Beam Radiation Therapy) is performed for those cases that are non-responsive to chemotherapy and in case of recurrence after completion of treatment. EBRT is also recommended in such cases where histopathological evidence suggests extra ocular spread, such as invasion of the resected margin of the optic nerve or sclera involvement (Mehta et al. 2012).

Molecular Genetics

Retinoblastoma has served as a model for understanding the heredity and genetics of paediatric cancer. Knudson proposed two hit theory regarding development of Rb (Knudson et al. 1971). Later researchers succeeded in identifying gene linked with this hypothesis (Benedict et al. 1983, Friend et al. 1986). RB1, the first tumour suppressor gene to be described, located on chromosome 13q14 encodes a 100-kDa nuclear phosphoprotein, RB. It effects cell cycle progression by inhibiting transcription factors of E2F family (Dyer et al. 2005). Inactivation of this gene by mutation has been reported in various human malignancies (Nevins et al. 2001, Cobrinik et al. 2005, Skapek et al. 2006). Also, loss-of function due to mutations in RB1 were shown to be a key initiating event prior to the development of retinoblastoma. Once cell completes mitosis, RB gets dephosphorylated by phosphatases (Knudsen et al. 2008).

Hypo phosphorylated RB reversibly represses the promoter regions of genes producing E2F transcription factors (Cobrinik et al. 2005, Sage 2012). Blockage of E2F inhibits progression of the cell cycle from G1 to S (Nevins 2001, Cobrinik et al. 2005, and Sage 2012). In

order for the cell to reach the key S phase, inactivation of RB1 is done through phosphorylation of RB via cyclin-dependent kinases (CDK). The highly phosphorylated RB is unable to inhibit the E2F promoter, and thus the cell is able to reach the S stage as it heads again toward mitosis (Knudsen et al. 2008). Loss of RB1 is compensated by increased expression of its related proteins and the activation of multiple pathways leading to apoptosis (Xu et al. 2014). Failure of compensatory mechanisms to operate on the RB/CDK pathway is has been observed in various cancers (Michael et al. 2015). Significant association of other genes have also been observed in development of retinoblastoma. Overexpression of MYCN oncogene has been found in about 3% of Rb (Felsher et al. 2013, Rush low et al. 2013, Theriault et al. 2014). Also, about 1% of retinoblastoma cases have overexpressed MYCN but no RB1 mutations. This suggests RB independent pathway of retinoblastoma development may also be present which needs to be explored (Rush low et al. 2013, Theriault et al. 2014).

Prognosis

Retinoblastoma patient's prognosis is directly related with size, presence of seeds and extension of the disease. Tumours confined to the eye can be cured; however metastatic disease or tumour involving extensive orbital or optic nerve is difficult to manage. The overall survival in patients with extra ocular disease is 50-70% (Jubran et al. 2004). Bakhshi et al. (2010) observed overall survival to be 50% at 18 month follow up in Indian setup. Prognosis of patients with Central Nervous System (CNS) metastasis is extremely poor even with aggressive therapy. However, patients with non CNS metastasis reported to have improved survival rates (50-75%) (Matsubara et al. 2005).

Follow-up

Child should be monitored for recurrence of primary retinoblastoma during follow up. During long periods, all patients with heritable RB1 mutations, or who have undergone chemotherapy, external-beam radiotherapy, or autologous peripheral haemopoietic stem cell transplant should be monitored for second primary tumours (Dimaras et al. 2012). Siblings and offspring of children with retinoblastoma should be examined every month until 3 months of age and then every 6 months until 3 years of age. Genetic counselling of patient's families with heritable Rb need to be done through the experts.

Conclusion and Future Prospective

Early diagnosis and therapeutics of retinoblastoma continues to be a challenge for clinicians. There has been considerable increase in our knowledge regarding management of retinoblastoma over the past few decades. Patients with intraocular retinoblastoma have better overall ocular survival in respect to those with extra ocular involvement. Treatment strategies have evolved a lot in recent years and new approaches are continuously been tested. One such model using recombinant adenovirus coding for the retinoblastoma gene was able to suppress proliferation in retinoblastoma cell lines (Demers et al. 1998). Use of chemotherapy models has permitted many children to maintain their eye(s) and avoid external beam radiotherapy while management of malignant tumour is still done by e-nucleation. Methods of early detection through biomarkers remains trust area for future research.

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