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Recent Developments in Retinoblastoma

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Abstract

Retinoblastoma represents 3% of all childhood cancers, and is the most common intraocular malignancy of childhood. It is fatal if untreated. The management of retinoblastoma has gradually evolved over the past few decades, with an aim to not only preserve life and eye, but also optimize residual vision. The treatment of retinoblastoma is multimodal, with chemotherapy, focal treatment including trans-pupillary thermotherapy (TTT), cryotherapy and laser photocoagulation, radiation therapy and surgery, all playing a vital role. Intravenous chemotherapy has been the mainstay of treatment for the past two decades, and still continues to be the most extensively used eye-saving modality of treatment. Periocular and intravitreal chemotherapy have specific indications in the management of retinoblastoma. Intra-arterial chemotherapy has emerged as a promising alternative for advanced and refractory retinoblastoma, both as a primary and secondary therapy. Recent advances in genetics of retinoblastoma have also helped in improving the overall clinical management of this malignancy.

Keywords: *retinoblastoma, Intra-arterial chemotherapy, Intravitreal chemotherapy, Periocular chemotherapy, genetics*

Introduction

Retinoblastoma was first described by Pawius in the 16th century.[1] But it was not until 1809 that retinoblastoma was discovered to originate from the retina, when Wardrop performed meticulous dissection on eyes with retinoblastoma, and called it fungus haematodes.[1] He suggested enucleation as a life-saving treatment for retinoblastoma, and it was the most acceptable therapy until the introduction of radiation therapy. X-rays to treat retinoblastoma was pioneered in the early twentieth century and was the sole eye-saving treatment until mid-90s when the use of chemotherapy in retinoblastoma was introduced. Since then, numerous advances in the eye-salvage treatment of retinoblastoma have evolved. However, enucleation is still indicated in advanced cases, and specific indications necessitate the use of external beam radiotherapy (EBRT). Herein we discuss some of the relevant aspects in the management of retinoblastoma that have had a paradigm change in the recent past.

Epidemiology of Retinoblastoma

The incidence of retinoblastoma is 1 in every 15000 to 18000 live births.[2] There is no variation in the number among different races, although there is a diversity among different countries. There are an estimated 5000 new cases worldwide annually, with India alone contributing to 1500-2000 cases. With increasing population in Asian and African countries, the number of retinoblastoma patients is also rising. Unfortunately, the mortality rate for retinoblastoma is also higher in these countries, owing to delay in diagnosis, advanced disease at presentation, lack of access to advanced medical facilities, and absence of standard management protocols.

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Genetics of Retinoblastoma

Retinoblastoma is a malignancy associated with somatic mutation or germline mutation.[2,3] Knudson proposed the two-hit hypothesis where he described the occurrence of two consecutive mutations for the conversion of a normal retinal cell into a malignant cell (Figure 1). In heritable retinoblastoma, the first mutation is in the germ cell, and this 'first hit' is carried in every cell in the body, making them prone not only for retinoblastoma, but also for other second cancers (most commonly pinealoblastoma, osteosarcoma and soft tissue sarcomas).[3] The 'second hit' occurring in the retinal cells during retinal development causes retinoblastoma. In non-heritable retinoblastoma, both hits occur in the retinal cell, and thus the mutation is confined to one single cell in the retina. Heritable retinoblastoma constitutes 30-40% of all retinoblastomas, while the rest 60-70% are non-heritable. One-fourths of the germline mutations are familial with autosomal dominant inheritance pattern, and the others are de-novo non-familial germline mutations.[3]

RB1 is a tumor suppressor gene that was identified in association with retinoblastoma and it validated the two hit hypothesis. RB1 gene is located in the long arm of chromosome 13 (13q), and most of the mutations are nonsense codons or frame shifts. Sometimes retinoblastomas are caused by genomic deletion of chromosome 13q, a syndrome known as RB1 gene deletion syndrome, where

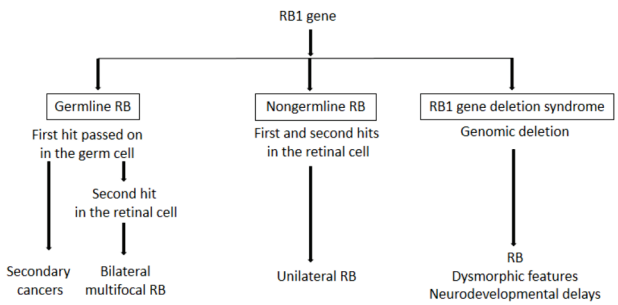

Figure 1: *Genetics of Retinoblastoma*

the affected individual has varying degrees of dysmorphic features and neurodevelopmental delays.[2]

Clinical Features

Retinoblastoma is usually diagnosed at an average age of 18 months, with 95% of children diagnosed by 5 years of age. Germline retinoblastomas can present as early as first month and sporadic retinoblastomas are detected at an average age of 24 months.[2] Retinoblastoma can be unilateral or bilateral. All bilateral cases are positive for germline mutation, whereas only 10-15% patients with unilateral retinoblastoma carry a germline mutation. The most common presenting symptom and sign is leukocoria. Strabismus is the second most common sign. The other common clinical features are as listed in (Table 1).

Table 1. Clinical Features of Retinoblastoma

Leukocoria
Strabismus
Poor vision
Red painful eye
Vitreous hemorrhage
Phthisis bulbi
Sterile orbital cellulitis
Proptosis

A child with a suspicious retinoblastoma is best examined under anesthesia for a detailed fundus evaluation. Retinoblastoma typically manifests as a unifocal or multifocal, well-circumscribed, dome-shaped retinal mass with dilated retinal vessels. Although initially transparent and difficult to visualize, it grows to become opaque and white. When small, the tumor is entirely intraretinal. As it enlarges, it grows in a three-dimensional plane, extending away from the vitreous cavity (exophytic) or towards it (endophytic).[2]

In the exophytic growth pattern, the tumor arises from the outer retinal layers and causes diffuse retinal detachment (Figure 2A). It is most often associated with numerous small subretinal seeds. In contrast, an endophytic retinoblastoma arises from the inner retinal layers, progressively fills the vitreous cavity, and causes vitreous seeding (Figure 2B). At times, the tumor maybe a combination of these two growth patterns. Diffuse infiltrating retinoblastoma is a rare pattern of presentation where there is no obvious mass, only a flat retinal infiltration, and is acalcific. It is generally seen in older children, and the incidence is less than 2%. Diffuse anterior retinoblastoma, a recent entity, is considered as an anterior variant of diffuse infiltrating retinoblastoma. It is thought to arise from the most peripheral parts of retina with anterior growth, and no retinal focus visible on examination.[4]

Patients with anterior extension of the tumor can present with white fluffy exudates in the anterior chamber resembling a hypopyon, called pseudohypopyon.[2] Neovascularization of iris and glaucoma are other clinical presentations seen in patients with advanced tumor (Figure 2C). Orbital cellulitis-like picture occurs when a large tumor undergoes necrosis and induces inflammation in and around the eye (Figure 2D). Retinoblastoma which has extended outside the confines of the eye is known as orbital retinoblastoma and this can occur when the tumor invades either the optic nerve, or full thickness of the sclera and beyond, and the patient generally presents with proptosis.

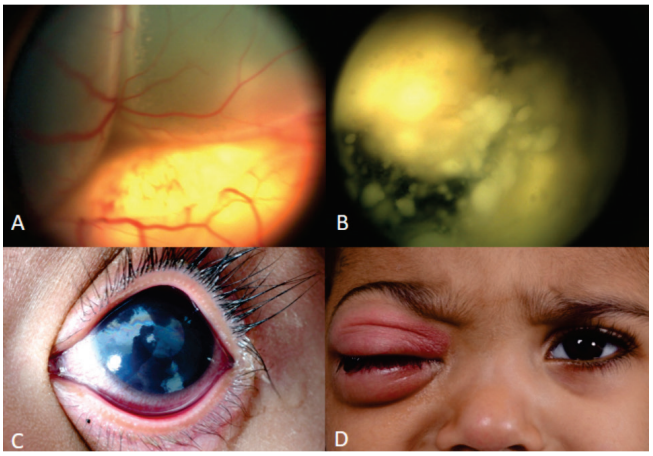

Figure 2: *Clinical presentation of retinoblastoma (A) Exophytic growth pattern with diffuse subretinal fluid (B) Endophytic growth pattern with diffuse vitreous seeds (C) Advanced retinoblastoma with neovascular glaucoma (D) Advanced retinoblastoma presenting as sterile orbital cellulitis*

Differential Diagnosis

The most important differential diagnosis is Coats' disease.[5] There are several other lesions that can simulate retinoblastoma and are known as pseudoretinoblastomas. The important differential diagnoses are listed in (Table 2).

Imaging

While the diagnosis of retinoblastoma is mostly clinical, ancillary tests like ultrasonography, fluorescein angiography

Table 2. Pseudoretinoblastoma

Coats' disease
Persistent fetal vasculature
Vitreous hemorrhage
Toxocariasis
Familial exudative vitreoretinopathy
Retinal detachment
Congenital cataract
Coloboma
Astrocytic hamartoma
Combined hamartoma
Endogenous endophthalmitis
Retinopathy of prematurity
Medulloepithelioma
X-linked retinoschisis
Incontinentia pigmenti
Juvenile xanthogranuloma
Norrie's disease

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(FA), optical coherence tomography (OCT), computed tomography (CT) and magnetic resonance imaging (MRI) aid in the documentation of the disease and differentiation of pseudoretinoblastomas from retinoblastoma (Table 3).[6,7] CT scan also helps diagnose extraocular extension, while

Table 3. Role of Multimodal Imaging in Retinoblastoma

Ret Cam	Wide angle fundus camera that is used in documenting fundus findings at each visit, and monitoring the treatment.
Ultrasonography	Detection of calcification, especially useful in establishing diagnosis in an opaque media. Also used in tumor thickness measurement and monitoring the effects of treatment.
Fluorescein Angiography (FA)	Performed on the RetCam using a filter. Helps in the visualization of capillary drop-outs, neovascularization, recurrences and occlusive vasculopathy following IAC.
Hand-held spectral domain OCT (SD-OCT)	Used in documenting early tumors, differentiation from pseudoretinoblastomas like astrocytic hamartomas, detection of small recurrences and assessment of fovea for visual potential
Computed Tomography (CT)	Detects calcification and aids in the identification of orbital and optic nerve extension, although less sensitive than MRI.
Magnetic Resonance Imaging (MRI)	Delineating the intraocular tumor extent and detection of optic nerve or scleral extension, and disease staging

MRI is most appropriate to detect optic nerve invasion and to screen for pinealoblastoma in heritable retinoblastoma.

Grouping and Staging

The grouping system is for retinoblastomas confined to the eye, where eye salvage is the end point, whereas the staging system is for predicting survival in patients with retinoblastoma. International Classification of Retinoblastoma (ICRB) was devised in 2003 and includes both grouping and staging.[8] The grouping is based on the tumor size, location, severity and presence of subretinal and vitreous seeds (Table 4).

Management

Management of a child with retinoblastoma is aimed at achieving the three sequential goals of life salvage, eye salvage and optimal vision. Management involves the identification of the tumor group and stage, decision-making regarding the appropriate therapeutic measure, and meticulous follow-up for monitoring the treatment progress and detection of any recurrence.

While intravenous chemotherapy remains the most extensively used modality of treatment, other tools available for the therapeutic intervention in retinoblastoma include chemotherapy using different delivery routes, focal treatment with cryotherapy, TTT, and laser photocoagulation, radiotherapy by teletherapy (external beam) or brachytherapy (plaque radiotherapy), and enucleation (Table 5).

Chemotherapy

In recent years, there has been a trend towards targeted

Table 4. International Classification of Retinoblastoma

Grouping	
Group A: Small tumor	Retinoblastoma ≤3 mm in size
Group B: Larger tumor	Rb >3 mm, Macular location (≤3 mm to foveola), Juxtapapillary location (≤1.5 mm to disc) Clear subretinal fluid ≤3 mm from margin
Group C: Focal seeds	Subretinal seeds ≤3 mm from retinal tumor Vitreous seeds ≤3 mm from retinal tumor Subretinal & Vitreous seeds ≤3 mm from retinal tumor
Group D: Diffuse seeds	Subretinal seeds >3 mm from retinal tumor Vitreous seeds > 3 mm from retinal tumor Subretinal & Vitreous seeds >3 mm from retinal tumor
Group E: Extensive retinoblastoma	Rb occupying 50% globe Neovascular glaucoma, Opaque media (from hemorrhage in anterior chamber, vitreous, or subretinal space) Invasion of postlaminar optic nerve, choroid (2 mm), sclera, orbit, anterior chamber
Staging	
Stage 0	Unilateral or bilateral retinoblastoma and no enucleation
Stage I	Enucleation with complete histological resection
Stage II	Enucleation with microscopic tumor residual (anterior chamber, choroid, optic nerve, sclera)
Stage III	Regional extension A. Overt orbital disease B. Preauricular or cervical lymph node extension
Stage IV	Metastatic disease A. Hematogenous metastasis 1. Single lesion 2. Multiple lesions B. CNS extension 1. Prechiasmatic lesiom 2. CNS mass 3. Leptomeningeal disease

Table 5. Decision-making in the Management of Retinoblastoma: Treatment Options

Primary tumor: Options for treatment	
Unilateral advanced (D, E)	IAC, IVC, Enucleation
Unilateral less advanced (A, B, C)	IAC, IVC, Focal therapy
Bilateral advanced (D, E)	IVC + POC
Bilateral less advanced (A, B, C)	IVC
Recurrent tumor: Options for treatment	
Solid tumor	IVC, IAC, Plaque radiation, Enucleation
Subretinal seeds	IVC, IAC
Vitreous seeds	IVitC

therapy to manage retinoblastoma with minimal adverse effects on surrounding normal retina and general systemic health. The use of intra-arterial chemotherapy (IAC), periocular chemotherapy (POC), and intravitreal chemotherapy (IVitC) has enabled to focus direct drug delivery to the tumor. Unlike IVC which can be used in the primary management of all retinoblastomas, IAC, POC and IVitC have specific indications.

Intravenous Chemotherapy

Currently, IVC is the most widely used treatment in India (Table 6). Used as a combination triple drug therapy of vincristine, etoposide and carboplatin, chemotherapy with focal consolidation achieves excellent success rates in the primary management of retinoblastoma. Chemotherapy alone can achieve an impressive tumor control in less advanced cases, with success rates of 100%, 93% and 90% in ICRB groups A, B and C, respectively (Figures 3A-D).[9,10,11] Rates of regression of retinoblastoma and eye salvage with standard triple-drug chemotherapy have been suboptimal for ICRB group D and E tumors. In group D eyes, approximately half of the eyes require either EBRT or enucleation for tumor control.[9] A combination of chemotherapy and radiation in eyes with vitreous seeds has yielded globe salvage rates varying from 22-70%.[12] Periocular carboplatin and topotecan injection also resulted in higher intravitreal drug level (Table 7). Transscleral penetration of posterior sub-Tenon carboplatin leads to augmented vitreous concentration. High-dose chemotherapy with concurrent periocular carboplatin has been tried as a primary management strategy, specifically in eyes with diffuse vitreous seeds.[13] This has led to better tumor control in advanced cases, with 95% eye salvage rate in eyes with focal vitreous seeds and a 70% eye salvage rate in those with diffuse vitreous seeds (Figure 4A-D).[13]

Table 6. Intravenous Chemotherapy

Procedure

IVC when given as a primary treatment for retinoblastoma causes reduction in tumor volume, and this is known as chemoreduction (CRD). Most commonly, a combination of three drugs of standard dose (SD) is used, although high dose (HD) may be necessary in advanced cases or tumors not responding to SD.

Drugs

Triple drug combination therapy of vincristine, etoposide and carboplatin (VEC) is employed, generally given 4 weekly for 6 cycles.

Day 1: Vincristine + Etoposide + Carboplatin

Day 2: Etoposide

Drug	SD-VEC (≥3 years of age)	SD-VEC (< 3 years of age)	HD-VEC
Vincristine*	1.5 mg/m2	0.05 mg/kg	0.025 mg/Kg
Etoposide	150 mg/m2	5 mg/kg	12 mg/Kg
Carboplatin	560 mg/m2	18.6 mg/kg	28 mg/Kg

*maximum dose < 2 mg

Indications:

(1) Primary tumor
(2) Recurrent tumor
(3) Recurrent subretinal seeds
(4) As adjuvant therapy in post-enucleation patients with high-risk features (discussed elsewhere)
(5) Orbital retinoblastoma
(6) As palliative therapy in metastatic retinoblastoma

Advantages:

(1) Long-term tumor control
(2) Reduces incidence of pinealoblastoma
(3) Reduces incidence of second cancers
(4) Reduces incidence of systemic metastasis

Disadvantages:

(1) Systemic side-effects including thrombocytopenia, leucopenia and anemia
(2) Allergic reactions to carboplatin and etoposide
(3) Long-term effects include hearing loss, renal toxicity and secondary leukemia

Table 7. Periocular Chemotherapy

Procedure:

POC is administered by posterior sub-Tenon injection of the chemotherapeutic drug in the quadrant closest to the location of the vitreous seeds. Innovative delivery systems for POC include the use of episcleral implants, fibrin sealants and nanoparticles of the drug

Drugs:

Carboplatin (1.5-2.0 mg)
Topotecan (1 mg)

Indication:

Advanced groups D or E with diffuse vitreous seeds in which a higher local dose of chemotherapy is desired

Advantages:

(1) Achieves rapid levels within the vitreous in 30 min and can last for hours
(2) Achieves doses that are six to ten times higher than that achieved by IVC

Disadvantages:

(1) Orbital and eyelid edema and ecchymosis
(2) Orbital fat atrophy
(3) Muscle fibrosis leading to strabismus.

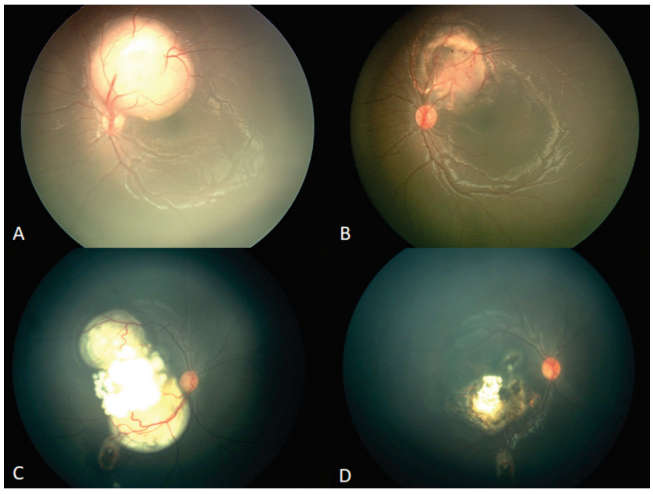

Figure 3: *Standard-dose chemotherapy in retinoblastoma (A) A group B eye (B) After 6 cycles of standard-dose chemotherapy (C) A group C eye with focal vitreous seeds (D) After 6 cycles of standard-dose chemotherapy*

Intra-arterial Chemotherapy

IAC for the treatment of intraocular retinoblastoma was first performed by Algernon Reese with direct internal carotid artery injection of the alkylating agent triethylene melamine in 1954. Suzuki & Kaneko described the technique of

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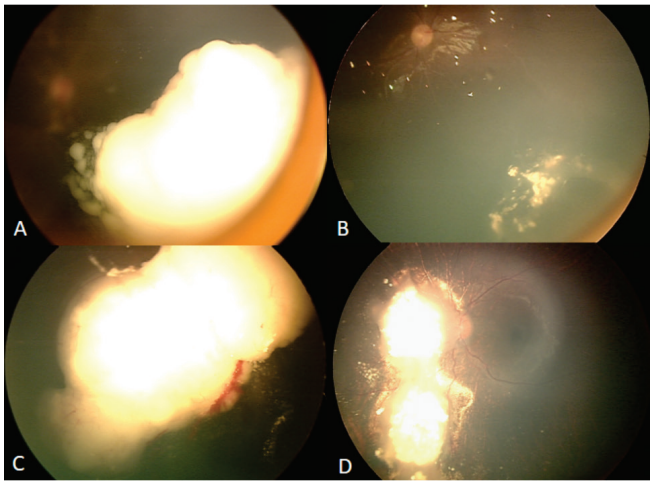

Figure 4: *High-dose chemotherapy in retinoblastoma with periocular chemotherapy (A) A group D eye with diffuse vitreous seeds (B) Clinical regression after 6 cycles of high-dose chemotherapy with 3 doses of concurrent periocular carboplatin (C) A group D eye with fine diffuse vitreous seeds (D) Complete regression after 6 cycles of high-dose chemotherapy with 2 doses of concurrent periocular carboplatin*

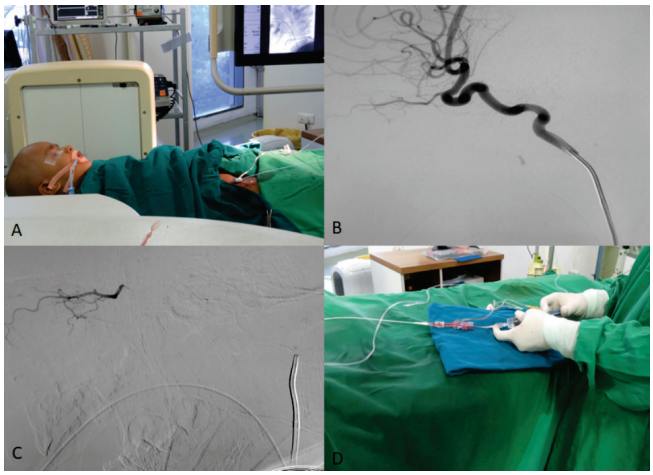

Figure 5: *Intra-arterial chemotherapy: Procedure in the cath lab (A) Patient under general anesthesia with a transfemoral cathether (B) An angiography performed at the beginning of the procedure, showing a patent internal carotid artery (C) An angiography performed with the microcatheter at the ostium of the ophthalmic artery, showing a patent ophthalmic artery (D) Infusion of the chemotherapeutic drug through the transfemoral cathether*

'selective ophthalmic artery infusion' (SOAI) in 2004 by the balloon technique, where a micro-balloon catheter is positioned by a transfemoral artery approach at the cervical segment of the internal carotid artery just distal to the orifice for the ophthalmic artery.[14] At this point, the balloon catheter is inflated, and chemotherapy is injected with flow thereby directed into the ophthalmic artery. The authors noted there are several small, but nevertheless important, branches proximal to the origin of the ophthalmic artery (i.e. cavernous branches of the ICA) into which infused chemotherapy could flow, and concluded that this infusion method is not truly selective. In 2006, Abramson and Gobin pioneered direct intra-arterial (ophthalmic artery) infusion or superselective intra-arterial chemotherapy or "chemosurgery".[15]

Patient is examined under anesthesia by the treating ocular oncologist. Documentation of each affected eye is performed by wide-angle fundus photography, FFA and B-scan ultrasonography. The decision to treat with IAC is undertaken in consultation with an ocular oncology team, an endovascular neurosurgeon and a paediatric oncologist.

The procedure is performed under general anesthesia using a sterile technique (Figures 5A-D). Nasal decongestion is achieved by topical decongestant drops or spray. Anticoagulation with intravenous infusion of heparin is delivered to a target activated clotting time of 2 to 3 times baseline. Through a transfemoral approach, the ipsilateral internal carotid artery is catheterized with a 4F pediatric guide catheter. The arterial anatomy is visualized with serial angiography runs, and the ostium of the ophthalmic artery is superselectively catheterized with a Prowler 10 microcatheter by the peep-in technique. A superselective injection through the microcatheter is performed to check adequate positioning and assessing the amount of reflux, if any, into the internal carotid artery before chemotherapy is injected. Each chemotherapy dose is diluted in 30 ml of saline and administered in a pulsatile fashion over 30 minutes to prevent lamination of medication and loss of dose to peripheral tributaries. Repeat angiography is performed immediately after the procedure to ensure patency of the vessels, and the catheter removed. At the end of the procedure, the heparin is reversed with intravenous protamine and hemostasis achieved with manual compression of the femoral artery upon removal of the catheter.[13] The child is monitored for 6 hours before discharge.

IAC has emerged as an effective treatment for advanced retinoblastoma (Figures 6A-D). It is increasingly being used in tumors as a primary treatment, especially in unilateral retinoblastoma. It can be used as a secondary therapy for those cases which have recurred or have not responded adequately to IVC (Table 8). Shields et al observed 94% globe salvage in group D eyes, and 91% vitreous seed regression, when IAC was used as a primary therapy.[16-18] In a study comparing 2-year ocular survival rate between naïve eyes with vitreous seeds (IAC as a primary therapy) and previously treated eyes with vitreous seeds (IAC as a secondary therapy), Abramson et al observed that IAC seemed to be more effective in eyes that have failed to respond to previous therapies. In an overall study on IAC in retinoblastoma, Abramson et al observed that eyes with vitreous seeds tend to require higher treatment sessions and doses, and multiple agents, as compared to eyes without vitreous seeds.[15]

Intravitreal Chemotherapy

Vitreous seeds are aggregates of tumor cells found in the avascular vitreous, which are relatively resistant to the effect of intravenous chemotherapy due to lack of blood supply (Table 9). These appear due to the disruption of the apical tumor either spontaneously (primary) or

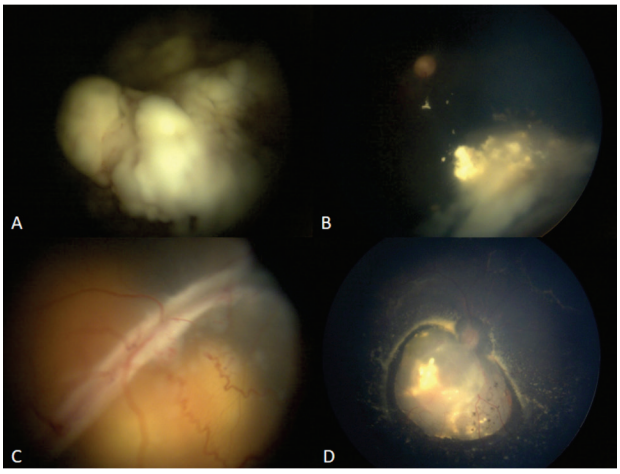

Figure 6: *Intra-arterial chemotherapy in advanced retinoblastoma (A) A group D eye with diffuse vitreous seeds (B) After 3 cycles of intra-arterial chemotherapy (C) A group E eye with a very large tumor and diffuse subretinal fluid (D) After 3 cycles of intra-arterial chemotherapy*

Table 8. Intra-arterial Chemotherapy

Procedure:
IAC involves the delivery of chemotherapeutic drugs directly in the eye through a fluoroscopy-guided microcatheter into the ostium of the ophthalmic artery, and is done in a cath lab by an interventional neuroradiologist. IAC can be a one-, two- or three-drug regimen, and each drug is delivered slowly over 30 min in a pulsatile fashion. It is repeated every 4 weeks, and most of the patients require 3 sessions to achieve complete tumor regression.

Drugs:
Melphalan is the most extensively used drug in IAC, and topotecan is added if there is extensive vitreous seeding. In advanced cases, three drugs including carboplatin are employed to ensure complete tumor control.
One-drug regimen: Melphalan (3-7.5 mg)
Two-drugs regimen: Melphalan (3-7.5 mg) + Topotecan (1-2 mg)
Three-drugs regimen: Melphalan (3-7.5 mg) + Topotecan (1-2 mg) + Carboplatin (15-50 mg)

Indication:
IAC can be used as a primary therapy, or secondary therapy in eyes which have not achieved tumor control after intravenous chemotherapy. In general, it is preferred in children older than 4 months of age without a germline mutation.
(1) Unilateral nongermline retinoblastoma
(2) Recurrent retinoblastoma following previous IVC or plaque radiotherapy
(3) Recurrent extensive subretinal seeds not controlled by IVC

Advantages:
(1) High intraocular concentration of the drug without associated systemic adverse effects of the drugs
(2) Shorter time for tumor control

Disadvantages:
(1) Expensive
(2) Difficulty with catheterizations
(3) Vitreous hemorrhage
(4) Branch retinal artery obstruction
(5) Ophthalmic artery spasm with reperfusion
(6) Ophthalmic artery obstruction
(7) Partial choroidal ischemia
(8) Optic neuropathy
(9) Complications associated with the technique including a risk for brain vascular events, hypoxia, hypotension and bradycardia

Table 9. Vitreous Seeds: Classification

Primary VS	Present at the initial diagnosis
Secondary VS	Those which appear during the course of treatment due to necrotic disruption of the tumor
Persistent VS	Primary VS which persist beyond chemoreduction
Recurrent VS	VS which appear after the completion of chemoreduction
Focal VS	Seeds located ≤ 3 mm from the main tumor
Diffuse VS	Seeds located >3 mm from the tumor
Free-floating VS	Seeds dispersed in the vitreous
Pre-hyaloid VS	Seeds present just anterior to the hyaloid membrane
Retro-hyaloid VS	Seeds present just anterior to the internal limiting membrane of the retina
Dust formation	Minute VS formed following the apical disruption of the tumor
Sphere formation	Balls of VS resulting from clonal expansion of dust
Cloud formation	Massive VS resulting from the disruption of the tumor

treatment-induced necrosis (secondary). Suboptimal concentration of chemotherapeutic agents in the vitreous results in persistence of vitreous seeds.[19] Refractory vitreous seeds are the persistent or recurrent vitreous seeds which do not respond to the standard treatment modalities. Persistent seeds are those which are present during chemoreduction, and continue to persist after the completion of chemoreduction.[20] Recurrent seeds are those which appear after the completion of chemoreduction. IVitC achieves higher drug concentration within the vitreous and effectively causes regression of vitreous seeds, without associated systemic side effects. IVitC in retinoblastoma was first introduced by Ericson and Rosengren using thiotepa in 1960. Methotrexate has also been tried as an intravitreal drug for retinoblastoma. In 1987, Inomata and Kaneko investigated the sensitivity of retinoblastoma to 12 anticancer drugs and found that the retinoblastoma cells were most sensitive to melphalan in-vitro. Melphalan is now the most extensively used drug to control the vitreous disease in retinoblastoma (Table 10). Munier et al discussed a potentially safe technique to perform intravitreal injections to prevent extraocular extension of the tumor.[21] They advocated the application of triple freeze-thaw cryotherapy at the injection site to prevent egress of the tumor cells in the needle track (Figure 7A-D).
With melphalan, vitreous seed regression ranging from 85-100% of eyes and globe salvage in 80-100% of eyes have been reported.[22-24] Intravitreal melphalan is given as a weekly injection until regression. The disadvantage of melphalan is that it is not stable in solutions, and has to be used within an hour of reconstitution of the drug. A combination of

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Table 10. Intravitreal Chemotherapy

Procedure:
Any intraocular procedure in retinoblastoma is generally avoided for fear of extraocular extension of the tumor. However, intravitreal injections by safety-enhanced technique has proven to prevent this risk. The injection site is carefully chosen after a thorough clinical examination to rule out the presence of tumor, vitreous seeds or subretinal fluid at the injection site. The injection is given using a 30 gauge needle by the transconjunctival pars plana route. After injecting the drug, the needle is withdrawn in the first ice ball formation of the chemotherapy followed by injection site triple freeze-thaw cryotherapy. This technique reduces the extraocular escape of any tumor cell through the needle track.

Drugs:
Melphalan is the most widely used drug in IVitC, and topotecan is generally added if there is extensive vitreous seeding. Topotecan may also be used as a single drug.
Melphalan (20-30 μg)
Topotecan (20-30 μg)
Combination: Melphalan (20-30 μg) + Topotecan (20-30 μg)

Indication:
(1) Recurrent diffuse or focal vitreous seeds
(2) Persistent diffuse or focal vitreous seeds

Advantages:
(1) High intraocular concentration of the drug without associated systemic adverse effects of the drugs
(2) Complications associated with POC avoided

Disadvantages:
(1) Extraocular extension of the tumor associated with an improper technique
(2) Risk of endophthalmitis

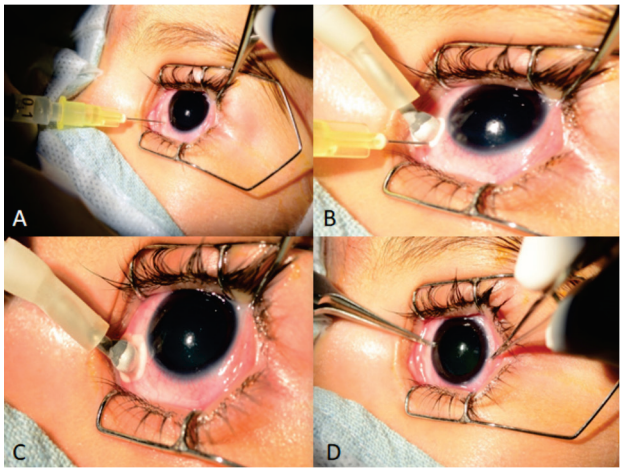

Figure 7: *Intravitreal chemotherapy: Safety-enhanced technique (A) Pars plana intravitreal injection of topotecan at a dose of 30 μg in 0.15 ml with a 30-gauge needle (B) Needle is withdrawn through the first ice ball of the cryotherapy (C) Triple freeze-thaw cryotherapy at the injection site (D) Forceps-assisted jiggling of the eyeball following the injection for an even dispersion of the chemotherapeutic drug*

intravitreal melphalan and topotecan has also been used to achieve excellent regression in refractory vitreous seeds. The authors have used topotecan as monotherapy in achieving vitreous seed regression in 36 eyes (Figure 8A-D). Topotecan is a very safe drug for intraocular use, is stable in solution and can be given as a 3-weekly injection.

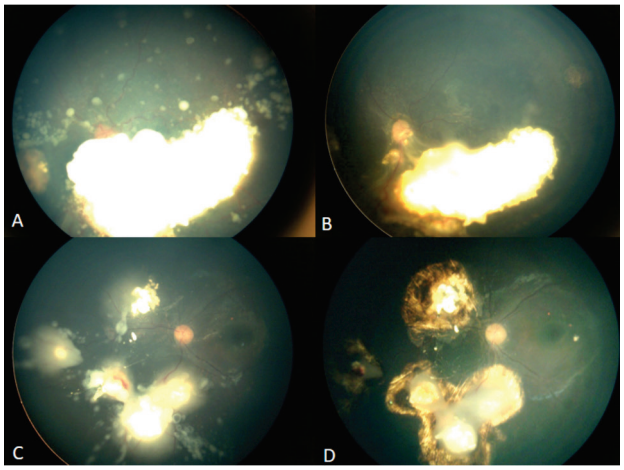

Figure 8: *Intravitreal chemotherapy with topotecan (A) Before and (B) after 2 doses of intravitreal topotecan injections in an eye with recurrent diffuse vitreous seeds after 9 cycles of chemotherapy (C) Before and (D) after 2 doses of intravitreal topotecan injections for a massive recurrence of diffuse vitreous seeds in a one-eyed patient*

Radiation Therapy

Retinoblastoma is a highly radiosensitive tumor, and radiation therapy can be curative. Radiation in the form of EBRT was the most popular globe-salvage therapy in retinoblastoma before the introduction of chemotherapy in 1990s. Although it is no longer the primary modality of treatment for retinoblastoma due to the associated complications, it has its own therapeutic indications (Table 11). Episcleral plaque radiotherapy is a form of brachytherapy wherein the source of radiation is placed on the episclera adjacent to the tumor, and the tumor absorbs radiation, sparing other healthy ocular tissues from the ill-effects of radiation (Table 12).[25]

Focal Therapy

Although episcleral plaque therapy may also be considered as a form of focal therapy, the term generally refers to the use of cryotherapy, TTT and laser therapy in the treatment of retinoblastoma. These are generally used for consolidation once the tumor has attained a considerably lower volume with chemoreduction, usually after 2 or 3 cycles, or for the treatment or small recurrent tumors of subretinal seeds.[26-28] However, they can also be used as the sole therapy for small retinoblastomas (Tables 13,14,15).

Enucleation

Enucleation is the oldest form of treatment for retinoblastoma, and is still indicated in advanced cases.[11] Unilateral disease with no salvageable vision is best treated by enucleation and the patient can be rid of the disease for life. Enucleation is a simple procedure, although special precautions need to be taken when handling an eye with retinoblastoma (Table 16). These are necessary to avoid accidental perforation that can potentially cause orbital seeding of the tumor. Use of a primary silicone or polymethylmethacrylate implant by the myoconjunctival technique provides adequate static and dynamic cosmesis. Porous polyethylene or hydroxyapatite

Table 11. External Beam Radiation Therapy

Procedure:
Numerous methods and protocols to treat retinoblastoma with EBRT have been described. Lens-sparing technique with electron beam and photon beam using a linear accelerator has been traditionally employed. Newer techniques using stereotactic radiation therapy (SRT), intensity modulated radiation therapy (IMRT) and proton therapy have been described. The standard dose is 40-45 Gy, generally given in fractionated doses over 3-4 weeks.

Indication:
(1) HRF on pathology after enucleation (described elsewhere)
(2) As a part of multimodal management in orbital retinoblastoma (described elsewhere)
(3) Tumor and/ or vitreous seeds refractory to other treatments

Advantages:
(1) Prevents orbital recurrence when given as an adjuvant therapy for indications 1 and 2
(2) Excellent long-term tumor control when used for refractory tumor/ vitreous seeds

Disadvantages:
(1) Orbital hypoplasia
(2) Secondary cancers in the field of radiation
(3) Cataract
(4) Dry eye syndrome

Table 12. Episcleral Plaque Brachytherapy

Procedure:
Radioisotopes like Iodine-125 and Ruthenium-106 that emit radiation are used for the treatment of various ocular tumors including retinoblastoma. I-125 emits gamma radiation, and Ru-106 beta radiation, which can penetrate the tumor. For this, the radioisotopes are loaded on an applicator (gold or silver), and the plaque sutured onto the episclera. The duration of the treatment is calculated by a radiation physicist, and the plaque is removed at the end of the treatment duration.

Indication:
(1) Recurrent tumor that is > 3 mm in thickness which is not suitable for treatment by other forms of focal therapy (TTT, cryotherapy or laser photocoagulation)

Advantages:
(1) Direct treatment of the tumor with minimal scarring
(2) Deeper penetration as compared with other forms of focal treatment
(3) Single treatment session
(4) Surrounding healthy tissue is not effected
(5) Overlying focal vitreous seeds are also treated simultaneously

Disadvantages:
(1) Not effective in large recurrent tumors
(2) Not ideal in multifocal recurrences
(3) Delayed radiation-related complications like radiation retinopathy

implants don't offer additional advantage unless pegged, and these are best avoided if a child is likely to need adjuvant chemotherapy or EBRT following enucleation since fibrovascular integration of these implants would be impeded. An enucleated eyeball is always submitted for pathology to assess for high risk factors (HRF). In a landmark paper by Honavar et al, the need for adjuvant chemotherapy has been emphasized to reduce the risk of secondary orbital recurrence and systemic metastasis.[29] The incidence of

Table 13. Cryotherapy

Procedure:
Transscleral cryotherapy involves freezing the tumor under visualization using indirect ophthalmoscopy. The cryoprobe tip is centered directly under the tumor and the ice ball formed on freezing should adequately cover the tumor and any focal vitreous seeds. Triple freeze-thaw cycles of cryotherapy are generally applied. Cryotherapy destroys the tumor cells mechanically by disruption of the cell membranes during thawing of the intracellular ice crystals. Typically the treatment is repeated every 3-4 weeks.

Indication:
(1) Peripheral tumors <4 mm in diameter and <3 mm in thickness
(2) Subretinal seeds

Advantages:
(1) Treatment of focal vitreous seeds overlying the tumor

Disadvantages:
(1) Large area of retinal scarring
(2) Retinal breaks

Table 14. Transpupillary Thermotherapy

Procedure:
In thermotherapy, hyperthermia generated by infrared radiation at subphotocoagulation levels destroys the tumor. A slow and sustained temperature range of 40 to 60 degree C within the tumor is generated using a semiconductor diode laser (810 nm) delivered as a 1300-micron large spot and long burn duration (1 minute) with indirect ophthalmoscope delivery system. The tumor is heated until it turns a subtle gray. Complete tumor regression can be achieved in over 85% of tumors using 3-4 sessions of thermotherapy. Using indocyanine green dye to sensitize the tumor for TTT (ICG-enhanced TTT) is an effective alternative for tumor control, particularly for small tumors that show suboptimal response to standard TTT.

Indication:
(1) Small tumors which are 4 mm in diameter and 2 mm in thickness
(2) Subretinal seeds

Advantages:
(1) Synergistic combination of thermotherapy with chemoreduction protocol (chemothermotherapy), with heat application amplifying the cytotoxic effect of platinum analogues

Disadvantages:
(1) Focal iris atrophy
(2) Focal paraxial lens opacity
(3) Large area of retinal scarring
(4) Retinal traction and serous retinal detachment

metastasis was 4% in those who received adjuvant therapy, compared with 24% in those who did not. Hence when HRF is positive, adjuvant treatment with chemotherapy and/ or EBRT is indicated (Table 17). Adjuvant chemotherapy consists of a combination of vincristine, etoposide and carboplatin given 4-weekly for 6 cycles.[29]

Orbital Retinoblastoma

Orbital retinoblastoma is an advanced form of retinoblastoma seen mostly in developing countries of Asia and Africa. The incidence varies among different countries, and is in the range of 18-40%.[30] Orbital disease can be classified as listed in (Table 18). Primary orbital

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Table 15. Laser Photocoagulation

Procedure:
Photocoagulation using argon green laser (532 nm) delivered with an indirect laser delivery system causes tumor apoptosis. Overlapping spots on the tumor edge are placed at a power setting of 250-350 mw for 0.3-0.5 seconds. The treatment destroys the tumor by restricting the blood supply to the tumor and also by hyperthermia. Typically the treatment is repeated every 3-4 weeks

Indication:
(1) Small posterior tumors which are 4 mm in diameter and 2 mm in thickness

Advantages:
(1) Can be used when TTT is not available

Disadvantages:
(1) Retinal traction and serous retinal detachment
(2) Retinal vascular occlusion
(3) Retinal hole
(4) Large area of retinal scarring
(5) Not ideal while the patient is on active chemoreduction as it restricts the blood supply to the tumor thus reducing the intra-tumor concentration of the chemotherapeutic agent

Table 17. High-Risk Features in Retinoblastoma

High Risk Features on pathology where adjuvant chemotherapy is indicated

- Anterior segment invasion
- Ciliary body infiltration
- Massive choroidal invasion (invasion ≥ 3 mm in basal diameter or thickness)
- Full thickness scleral extension
- Extrascleral extension
- Retrolaminar optic nerve invasion
- Optic nerve invasion at line of transection
- Combination of optic nerve infiltration till any level (pre-laminar/ laminar/ retrolaminar) and choroidal infiltration (any thickness)

High Risk Features on pathology where adjuvant radiotherapy is indicated (in addition to chemotherapy)

- Full thickness scleral extension
- Extrascleral extension
- Optic nerve invasion at line of transection

retinoblastoma is the orbital extension of the disease which is evident at presentation either clinically or radiologically. Most of the patients present with proptosis, or a large fungating mass which bleeds on touch. Tumor necrosis causes inflammation of the surrounding tissues and the patient may present with sterile orbital cellulitis. Secondary orbital retinoblastoma occurs in an enucleated socket after an uncomplicated surgery. It may present as an orbital mass with an unexplained displacement of the implant, or a palpable orbital mass. Accidental retinoblastoma occurs in the event of an inadvertent perforation of the eye harboring retinoblastoma. This can occur due to improper enucleation technique, or various intraocular surgeries in an eye with unsuspected intraocular retinoblastoma. Overt orbital retinoblastoma refers to previously unrecognized extrascleral or optic nerve extension discovered during enucleation as an episcleral nodule, or an enlarged and inelastic optic nerve with or without nodular optic nerve

Table 16. Enucleation

Enucleation is the removal of the eyeball, and is usually followed by replacement of the orbital volume using one of the several types of implants available including acrylic, silicone and hydroxyapatite implants, each with their own advantages and limitations.

Enucleation by the myoconjunctival technique with a silicone orbital implant is a safe and cost-effective procedure with prosthesis motility comparable to biointegrateble implants while minimizing the complications. This technique may also be used in those requiring periorbital radiotherapy following surgery.

- The surgery is usually performed under general anesthesia.
- A lateral canthotomy is performed.
- A 360 degree peritomy is done using a blunt tipped Westcott scissors, cutting as close to the limbus as possible.
- The underlying posterior Tenon's layer is undermined in all four quadrants in a spreading action using a blunt tipped tenotomy scissors.
- Each of the recti muscles is identified, hooked and double-tagged, first with 6-0 silk suture and then with 6-0 Vicryl suture. 6-0 silk sutures serve as traction sutures while 6-0 Vicryl sutures would later be used to suture the muscles through the conjunctiva.
- Each of the recti muscles is then transected at a point between the two sutures using a radiofrequency probe.
- Superior oblique and inferior oblique muscles are transected and allowed to retract posteriorly.
- A conjunctival relaxing incision is made for easy manipulation.
- The eyeball is then prolapsed between the blades of the speculum.
- With a forward traction on the eyeball using the 4 silk sutures, a gently curved blunt tipped tenotomy scissors is passed along the lateral wall and the optic nerve is strummed along its length.
- With one bold cut, the optic nerve is transected just a little anterior to the superior orbital fissure, to gain a good optic nerve length and at the same time to avoid injuring the superior orbital fissure contents.
- After achieving adequate hemostasis, an appropriate sized silicone orbital implant is placed posterior to posterior Tenon's.
- Posterior Tenon's is closed with interrupted 6-0 vicryl sutures.
- Each of the Recti muscles is sutured through the conjunctiva in its respective fornix, and these sutures are called the myoconjunctival sutures.
- Anterior Tenon's is closed with interrupted 6-0 Vicryl sutures.
- Conjunctival closure is done in a continuous key-suturing pattern with 6-0 Vicryl suture.
- An appropriate sized conformer is placed and a median tarsorrhaphy done with 6-0 Vicryl suture.
- The suture tarsorrhaphy is removed after 1 week and a prosthesis can then be placed in the socket after 6 weeks.

sheath. Microscopic orbital retinoblastoma is identified on histopathological examination of the enucleated eyeball as full thickness scleral infiltration, extrascleral extension or invasion of the optic nerve.

The presence of orbital disease is generally known to carry a poor prognosis. Orbital disease increases the risk of systemic metastasis by 10-27 times and the mortality rates range from 25 to 100%.[30] However, with an intensive multimodal management and careful monitoring, patients with orbital

Table 18. Orbital Retinoblastoma: Classification

1. Primary Orbital Retinoblastoma
Clinical or radiologically detected orbital extension of an intraocular retinoblastoma at the initial clinical presentation, with either optic nerve involvement or scleral extension of the tumor.

2. Secondary Orbital Retinoblastoma
Orbital recurrence following uncomplicated enucleation for intraocular retinoblastoma, presenting as unexplained displacement, bulge or extrusion of a previously well-fitting conformer or a prosthesis.

3. Accidental Orbital Retinoblastoma
Inadvertent perforation, fine-needle aspiration biopsy or intraocular surgery in an eye with unsuspected intraocular retinoblastoma are considered as accidental orbital retinoblastoma.

4. Overt Orbital Retinoblastoma
Previously unrecognized extrascleral or optic nerve extension discovered during enucleation as an episcleral nodule, or an enlarged and inelastic optic nerve with or without nodular optic nerve sheath.

5. Microscopic Orbital Retinoblastoma
Full thickness scleral infiltration, extrascleral extension or invasion of the optic nerve on histopathologic evaluation of an eye enucleated for intraocular retinoblastoma.

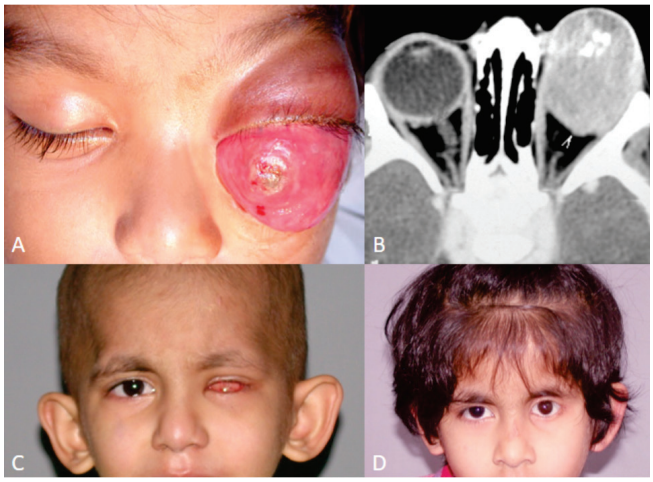

Figure 9: *Multimodal management in orbital retinoblastoma (A) External photograph of primary orbital retinoblastoma taken during examination under anesthesia (B) Axial computed tomography image displaying extraocular extension of the intraocular tumor (C) After 12 cycles of doses of high-dose chemotherapy, external beam radiotherapy and enucleation (D) Healthy child cured of orbital retinoblastoma, with a well-fitting prosthesis*

disease are known to do well (Table 19) (Figures 9A-D).

Metastatic Retinoblastoma

With an incidence of less than 5% of all retinoblastoma cases, metastatic retinoblastoma is most often seen in developing countries. It usually occurs as a relapse following enucleation for intraocular retinoblastoma, especially in those who had high risk pathologic features.[31] Most commonly, metastasis occurs to the central nervous system (CNS), bone and bone marrow. The metastasis occurs in one of the three ways-

Table 19. Orbital Retinoblastoma: Treatment

Baseline investigations:
- CT or MRI to assess the tumor extent
- Bone marrow biopsy
- Cerebrospinal fluid cytology

Treatment:
Multimodal management involving chemotherapy, surgery and radiation therapy is employed. Chemotherapy is essential for chemoreduction and to prevent systemic metastasis, surgery to reduce the tumor load and clear the orbit of most of the tumor, and radiation to take care of the residual disease and prevent orbital recurrence.

Primary Orbital Retinoblastoma
- Neoadjuvant high dose chemotherapy is given for 3 cycles
- Residual disease is assessed by CT or MRI
- If orbital retinoblastoma has resolved, enucleation is performed. If orbital retinoblastoma has not resolved, no surgery is done at this stage and 3 more cycles of high dose chemotherapy are given
- Residual disease is again assessed by CT or MRI
- If orbital retinoblastoma has resolved, enucleation is performed at this stage. In case there is residual orbital disease even after 6 cycles, exenteration is performed
- EBRT given to the orbit (45-50 Gy)
- Adjuvant high dose chemotherapy are given for 6 or 9 cycles, to complete a total of 12 cycles

Secondary Orbital Retinoblastoma
- Neoadjuvant high dose chemotherapy is given for 3 cycles
- Residual disease is assessed by CT or MRI
- If orbital retinoblastoma has regressed significantly, excision of the residual mass is performed. If orbital retinoblastoma has not resolved, no surgery is done at this stage and 3 more cycles of high dose chemotherapy are given
- Residual disease is again assessed by CT or MRI
- If orbital retinoblastoma has resolved, excision of the orbital mass is performed at this stage. In case there is significant orbital disease even after 6 cycles, exenteration is performed
- EBRT given to the orbit (45-50 Gy)
- Adjuvant high dose chemotherapy are given for 6 or 9 cycles, to complete a total of 12 cycles

Accidental Orbital Retinoblastoma
- If the intervention is limited such as a needle biopsy and the tumor is not advanced, high dose chemotherapy is given for 6 cycles and the patient carefully monitored at frequent intervals
- If the intervention is limited such as a needle biopsy and the tumor is advanced, enucleation with an en bloc excision of the conjunctiva at the needle site is performed and adjuvant high dose chemotherapy is given for 6 cycles and the patient carefully monitored at frequent intervals
- If the intervention is extensive such as pars plana vitrectomy, enucleation with an en bloc excision of the conjunctiva overlying the ports is performed and adjuvant high dose chemotherapy is given for 6 cycles and the patient carefully monitored at frequent intervals
- EBRT may be given in each case depending on the nature of the disease, type and extent of the intervention and the findings at subsequent follow-ups, a decision best left on the treating doctor's expertise

Overt Orbital Retinoblastoma
- If an extrascleral extension is macroscopically visualized during enucleation, special precaution is taken to excise the nodule completely along with the eyeball, also with the overlying Tenon's capsule in the involved area

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- If optic nerve extension is suspected during enucleation and the nerve stump obtained is short, extra effort to excise an additional length is made
- In both cases, EBRT is given followed by 12 cycles of adjuvant high dose chemotherapy

Microscopic Orbital Retinoblastoma

- If microscopic full thickness scleral involvement and/ or extrascleral extension and/ or optic nerve involvement up to the level of transection is detected, EBRT is given followed by 12 cycles of adjuvant high dose chemotherapy

Follow-up

- CT or MRI 6-monthly to look for tumor recurrence
- Bone marrow biopsy 6-monthly
- Cerebrospinal fluid cytology 6-monthly

by direct dissemination into the CNS via the optic nerve, choroidal invasion and hematogenous spread, or orbital extension with lymph node involvement and hematogenous spread. Bony metastasis, usually involving the long bones or the craniofacial bones, causes non-tender palpable mass. Cerebrospinal fluid cytology, bone marrow evaluation and whole body imaging are done in all cases of metastatic retinoblastoma for staging the disease. Use of high-dose chemotherapy with autologous stem cell rescue (ASCR) has offered some encouraging results. However, most of the experience is in stage 4a disease that does not involve the CNS. The use of radiotherapy and intrathecal chemotherapy for CNS lesions have been recommended, although the prognosis for such advanced metastatic retinoblastoma continues to remain grim.[31]

Prenatal Genetics

To prevent transmission of the disease from parents to offspring, genetic testing for germline mutations can be done at specialized laboratories. RB1 is the only gene that is implicated in retinoblastoma. However, there are different types of mutations affecting this gene. Direct DNA sequencing detects 75% of the mutations, and PCR amplification detects yet another 20% of the mutations. Peripheral blood lymphocytes or tumor tissue, when available, are sampled for the detection of the mutation.

In heritable retinoblastoma, once the mutation is identified in the lymphocytes, the presence of the same mutation is tested in the fetus (sibling or offspring) by chorionic villus biopsy or amniocentesis. If the mutation is found, a decision to terminate the pregnancy can be made.

In non-heritable retinoblastoma, if the tumor tissue is available from an affected individual, it can be sampled to detect the type of mutation. If the same mutation is also found in the blood of the patient, the individual is positive for germline mutation and an offspring can be tested for the same mutation. However, if no mutation is found in the blood, the tumor is nongermline (sporadic), without any risk of transmission of the disease to the offspring. In case no tumor tissue is available, lymphocytes are sampled for the type of RB1 mutation, but the interpretation of a negative result in these cases is difficult. Either the patient has a sporadic retinoblastoma, or a germline mutation that escaped detection by the currently available techniques.

Preimplantation genetic testing for carriers of mutation involves the identification of RB1 mutation in a blastomere (8-cell embryo) which is obtained by in vitro fertilization (IVF) technique. The small material is amplified by polymerase chain reaction (PCR) and the blastomere without the RB1 mutation maybe implanted for a successful pregnancy.[3]

Conclusion

The management of retinoblastoma revolves around having a sound knowledge of the disease, choosing the best treatment for the patient among the various available options and careful monitoring for recurrences. Enucleation should be performed when deemed necessary in advanced retinoblastoma with no visual prognosis, without needless overenthusiasm for globe salvage in advanced tumors. Specific precautions during the surgery, use of a primary implant for cosmesis, and post-enucleation evaluation of histopathologic HRFs and adjuvant therapy, as appropriate, achieve optimal life salvage. Primary focal therapy with laser, TTT or cryotherapy for peripheral tumors can be used for ICRB group A tumors in visually noncritical locations. IVC continues to be the standard treatment for ICRB groups B to D, and for bilateral retinoblastoma. Appropriate use of high-dose protocol and concurrent POC can help salvage group D and E eyes with diffuse vitreous seeds. IAC is a very promising treatment with high success for advanced retinoblastoma, but the cost factor must also be taken into consideration. IVitC should be performed with safety-enhanced technique. Radiation therapy should be employed only when indicated. Retinoblastoma has a very high cure rate, and is best managed in an integrated retinoblastoma clinic under the watchful monitoring of an expert ocular oncologist. The recent advances in management of retinoblastoma and a holistic approach have rendered it eminently curable - prognosis for life salvage is now around 98%, with 90% eye salvage and 80% vision salvage.

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