

Review Article

Received on: 14-03-2015
Accepted on: 26-03-2015
Published on: 15-04-2015

Corresponding Author:

* **Pramod Singh Khatri**,
Head of Department and Programme
Coordinator, Dept. of Clinical
Research, Amity Medical School,
Amity University, Haryana, India.



*Email Id-
pramodsinghkhatri@gmail.com

Retinoblastoma –A Molecular Dream

Pramod Singh Khatri*

ABSTRACT

Retinoblastoma is an uncommon eye tumor of adolescence that emerges in the retina. It is the most well-known intraocular distortion of childhood; with an occurrence of 1/20,000–25,000 live births. The two most recurrent manifestations uncovering retinoblastoma are leukocoria and strabismus. Iris rubeosis, hypopyon, hyphema, buphthalmia, orbital cellulites and exophthalmia might likewise be detected. Sixty five percent of retinoblastomas are unilateral and mostly are non-genetic (identified within two year). Retinoblastoma is bilateral in 35% of cases (identified within one year). All bilateral and multifocal unilateral structures are innate. Innate retinoblastoma constitutes a cancer predisposition disorder: a subject unavoidably conveying a RB1 gene mutation has 90% risk of developing retinoblastoma but on the other hand are at higher risk of developing various cancers. Identification is made by funduscopy, Ultrasound, MRI and CT scan. Managing patients with retinoblastoma must consider the different aspects of the malady: the visual impairment, the conceivably hereditary nature of the illness and the life-time risk.

Key-words: childhood cancer, genetics, retinal blastoma, tumor suppressor gene, 13q deletion.

Cite this article as:

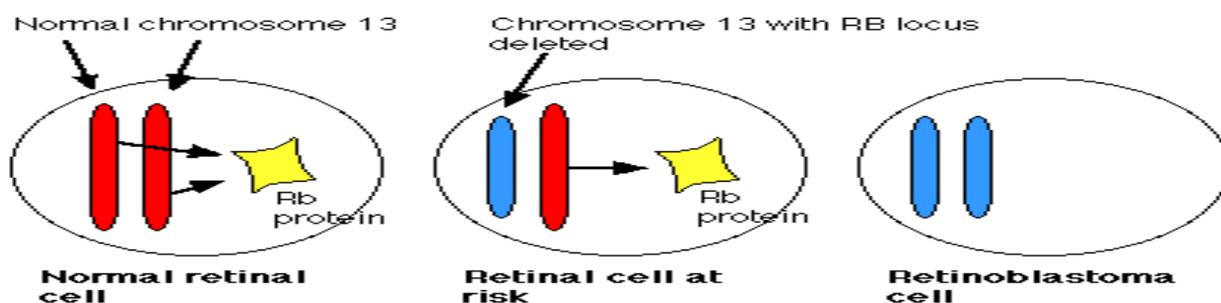
Pramod Singh Khatri*, Retinoblastoma – A Molecular Dream, Asian Journal of Pharmaceutical Technology & Innovation, 03 (11); 2015. www.asianpharmtech.com

Introduction

Retinoblastoma has assumed an important role in our comprehension of inherited predisposition to cancer. Retinoblastoma happens as a consequence of germline, inherited transformations in 40% to 45% of subjects¹, while alternate cases are brought about by somatic, nonhereditary transformations. Clinical perceptions of age at finding, tumor type, tumor laterality, and family history drove Knudsen in year 1971 to hypothesize the 2-hit theory, a scientific model that anticipated the presence of recessive cancer genes or tumor suppressor genes (TSG)². Integral to this theory was the idea that both cell alleles of the alleged retinoblastoma (RB) gene must be deactivated in an emerging retinoblast for harmful mutations. Ensuing work affirmed the Knudsen speculation by recognizing the RB gene on chromosome 13q14 and demonstrating that this gene is transformed in retinoblastomas, and also in different sorts of cancer. The RB gene was subsequently settled as the prototypical tumor suppressor gene (TSG). Most retinoblastoma families exhibit autosomal predominant inheritance with complete penetrance & high expressivity. Latest developments in our comprehension of the structure and function of the retinoblastoma protein (pRB) now give new experiences into the molecular premise of this low-penetrance type of retinoblastoma. These bits of knowledge have enhanced the precision of demonstrative testing and family genetic counseling for retinoblastoma and may in the end support in securing new treatment procedures³.

In many families with retinoblastoma, 90% of gene bearers develop eye tumors (high penetrance), and most influenced subject develop different two-sided tumors (high expressivity). Nonetheless, in a few families a huge extent of transporters stay unaffected (reduced penetrance), and numerous influenced people have just unilateral retinoblastoma or benign retinocytomas (decreased expressivity). Different mechanism for low-penetrance retinoblastoma have been suggested, including immunologic variables, DNA methylation, epigenetic mechanism, host resistance factor, a second retinoblastoma locus, and modulator genes. However, recent indication supports the thought that most low-penetrance retinoblastoma results from transformations at the RB gene locus. The way of these variation changes has been the subject of extreme attention, and latest intuitions into the molecular function of pRB now permit more prominent comprehension of the molecular premise of low-penetrance retinoblastoma⁴.

Figure 1:- formation of Retinoblastoma cell



Retinoblastoma is the most well-known intraocular disease in children. Associated invasion of retinoblastoma incorporate metastatic illness, choroidal attack, and neovascularization. Notwithstanding, present medicines (e.g. chemotherapy) bring about entanglements including, yet not constrained to, neutropenia, sickness, thrombocytopenia and risk for second malignancies (e.g., acute myeloid leukemia(AML)).Enucleation is by and large performed in around 25% of the instances of intraocular retinoblastoma because of advanced illness.

All bilateral forms, and also 15% of unilateral form are identified as inherited or de novo transformation of the RB-1 gene, limited on chromosome 13q14 (*figure 1*). Normally the patients present with leukocoria (white pupil reflection) or a squint.

Retinoblastoma is treatable. On the off chance if identified while still restricted to the globe and if there are no metastatic risk variables, the youngster will almost dependably survive after proper treatment .The safeguarding of visual capacity relies upon visual protection, beginning tumor volume, the anatomical connections of the tumors to the macula and optic plate and the antagonistic impacts of the medicines (vitreous hemorrhage, cataracts). In the vicinity of metastatic risk factor, adjuvant therapy regimens are generally connected to avoid life threatening reversion⁵⁻⁶.

Determination of retinoblastoma is normally made by fundoscopy and Ultra sound. The ophthalmologist ordinarily performs both examinations. In all cases intratumoral calcifications can be recognized by Ultrasound giving high certainty rate in regards to diagnosis. Different tumor parameters (laterality; number, area and size of tumors; tumor seeding to vitreous, sub retinal space) can be assessed with these methods. These are essential for gathering the retinoblastoma and to guide therapeutic conclusion. Further analytic imaging assumes a significant part in deciding the nearby extent and for recognizing related brain variations, i.e. intracranial tumor augmentation, possible midline intracranial primitive neuro ectodermal tumor (PNET) and brain distortions in patients with 13q deletion disorder.

Progressive treatment techniques (evasion of enucleation and outside beam radiation treatment) can be effective in the early phases of retinoblastoma and in a few patients with advanced intraocular ailment. The choices for eye-safeguarding treatment have fundamentally enhanced amid these years and are principally in light of tumor lessening with chemotherapy, and are typically consolidated with laser coagulation, cryotherapy or radioactive plaque. Lately, specific ophthalmic artery infusion of a chemotherapeutic agent got to be accessible as an extra treatment alternative for locally advanced illness. As a result, more kids are dealt without histopathological affirmation and without evaluation of risk components for infection spread and prognosis. Along these lines, imaging is vital in local staging⁷.

Cytology of Retinoblastoma

The RB1 gene, made out of 27 exons, encodes for a 110 kDa phosphoprotein (pRB). pRB, a tumor silencer gene, is a controller at the cell cycle check point in the middle of G1 and entrance into S stage. In the knock out mouse model of retinoblastoma, scientists have shown that pRB is needed for suitable exit from the cell cycle of retinal progenitor cells. Various studies have shown that other molecular procedures, notwithstanding the loss of pRB, are vital for tumorigenesis (chromosomal addition +1q, +6p; chromosomal lose -16, -16q, -17, -17p).

A novel hypothesis about the potential mechanism of retinoblastoma tumor development was anticipated in light of the perception that spontaneous unilateral retinoblastoma may be more regular in non- industrial nations. Scientists in a study assessing the vicinity of oncogenic human papilloma virus (HPV), inferred that pRB inactivation could be brought on to some degree by the oncoprotein HPV E7. Environment (low folate intake amid pregnancy) was additionally proposed to assume a part in the risk of retinoblastoma event due to the expanded occurrence of unilateral retinoblastoma in developing nations⁸⁻⁹.

Hereditary counselling

Hereditary counseling ought to be proposed to each guardian having a kid with retinoblastoma and to patients having a familial history of retinoblastoma. Amid this discussion, the progress of management of retinoblastoma and foreseen examination plan for testing ought to be introduced to the family (figure 2). An investigation of the kid's parent and familial history, and direct and indirect molecular studies ought to be performed by the geneticist.

Multifocal nature of the tumor, vicinity of psychomotor hindrance and a malformative disorder, and vicinity of a familial history of retinoblastoma and retinoma recommend a hereditary predisposition for the illness. The danger of transmission is a capacity of the familial history and the sort of retinoblastoma. If there should be an occurrence of inherited retinoblastoma, the danger of transmission is half. In the event of unilateral, unifocal, non-familial retinoblastoma, the danger of transmission is 5%¹⁰.

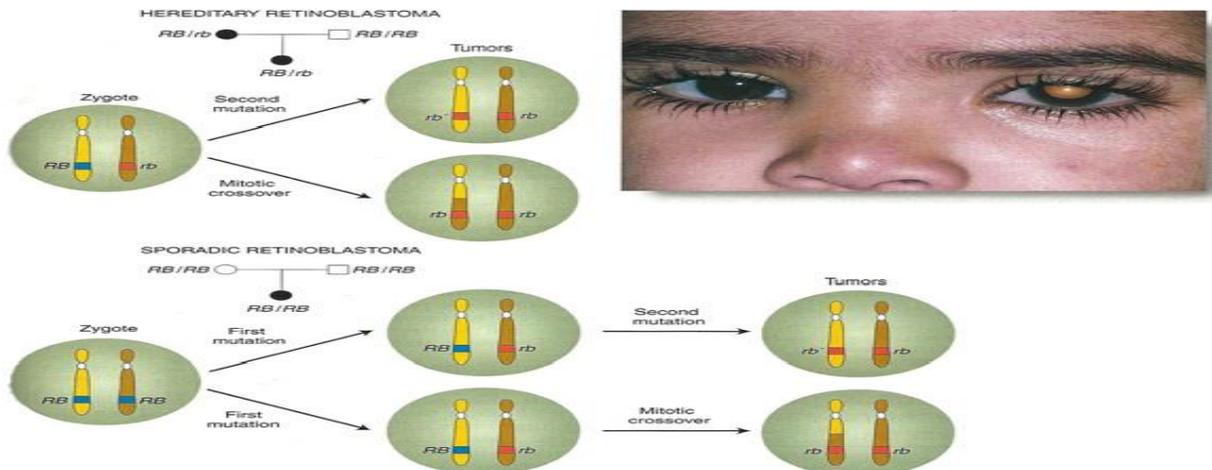
Hereditary investigation in influenced kids may incorporate the accompanying molecular tests:

- Direct scan for an established transformation of the RB1 gene performed on the DNA - The transformation detection rate is high in innate forms. No special transformation or "hot spot" has been distinguished in the RB1 gene.
- Indirect exhibit of the allele conveying the transformation in instances of familial history - This test comprises of distinguishing intragenic or RB1 flanking markers normal to all influenced individuals from the family.

- Tumor loss of heterozygosity assessment - This strategy obliges tumor material and permits determination of which allele is remaining and conveying the transformation.

Amid this consultation, patients ought to be educated of the dangers of transmission and of second primary tumor development. At present, there is no proof that a specific anomaly of the RB1 gene is connected with a higher danger of second cancer¹¹⁻¹².

Figure 2:-Familial versus sporadic retinoblastoma



Single Nucleotide Polymorphisms related with development of Retinoblastomas

Since TP53 is seldom transformed in retinoblastoma, different mechanism of p53 inactivation in these tumors have been found, including the genomic addition and overexpression of key inhibitors of p53 action, MDM2 and MDM4 (figure 3). MDM2 was the first modifier gene recognized in retinoblastoma, when scientists distinguished a T>G transversion SNP at nucleotide 309 in the MDM2 promoter (rs2279744) to be profoundly connected with the frequency of bilateral and unilateral retinoblastoma in RB1 transformation carrier families. This allele presents improved translation of mRNA prompting overexpression and aggregation of the MDM2 protein, adequately revoking the capacity of the p53 protein.

The p.Arg72Pro substitution in p53 protein (c.215G>C,) diminishes the capacity of p53 to prompt apoptosis, basically bringing functional inactivation. In development of retinoblastoma, a huge relationship of the Pro/Pro variation of p.Arg72Pro has been reported, while just a frail negative affiliation was seen with MDM2-309¹³.

MDM4, an alternate key controller of p53 movement discovered to be gained and overexpressed in retinoblastomas is likewise a hereditary modifier in retinoblastoma. Genotype investigations of 104 retinoblastoma patients found that both the MDM2 rs2279744G (versus T) and MDM4rs4252668C (versus T) SNPs were available at a higher recurrence in control patients, while MDM2 rs2279744TG and GG genotypes, and the MDM4 rs116197192G allele were available at high recurrence in retinoblastoma patients and connected with poor survival¹⁴.

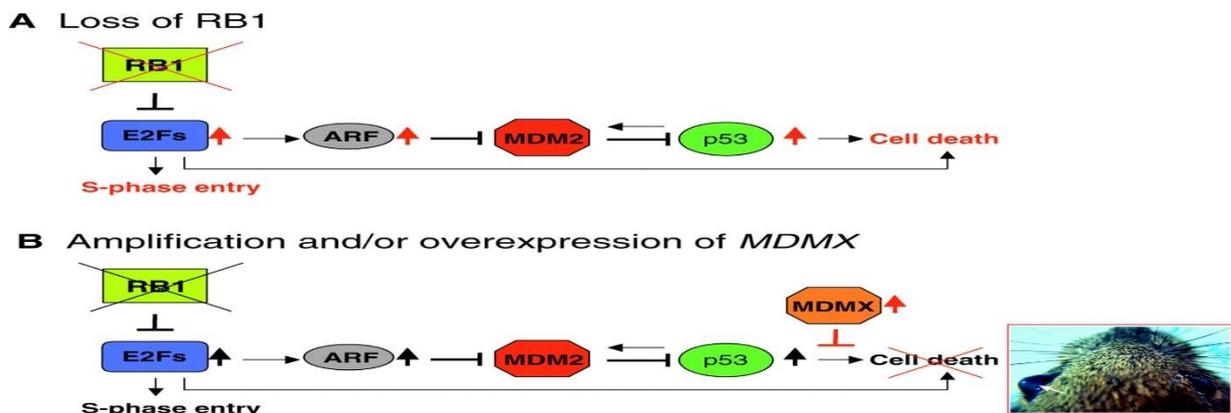
Methylation investigation of retinoblastomas

Methylation of the RB1 promoter was initially shown in 1989 by Greger et al., who recognized CpG 106, an island covering the promoter and exon 1, to be methylated in some retinoblastomas, accordingly silencing gene expression. From that point forward, different CpG islands inside the RB1 promoter and gene have been recognized and described in retinoblastomas, exhibiting an epigenetic part to RB1 inactivation and resulting development of retinoblastoma. Methylation of the RB1 promoter is the causative M1 in 8% of unilateral non-germline tumors.

Atypical methylation of extra genes has likewise been demonstrated in retinoblastomas. RASSF1A, a tumor silencer included in microtubule stability, is inactivated by promoter hyper methylation in anywhere in the range of 60 to

80% of retinoblastomas in examination to typical retinal tissues. It is inactivated by methylation in numerous cancers. MGMT, encoding an O⁶-alkylguanine-DNA alkyl transferase, was likewise discovered hyper methylated, yet in a littler extent of retinoblastomas (58% and 35% in two studies)¹⁵

Figure 3:- Interactions between Rb, MDMX and p53 during retinoblastoma formation



p16^{INK4A} (CDKN2) has long been embroiled as a tumor silencer in retinoblastoma development. Recently, scientists has examined p16^{INK4A} expression and promoter methylation in an accomplice of retinoblastomas alongside fringe blood from both subjects and their parents. 55% of retinoblastoma patients demonstrated a down regulation of p16^{INK4A} interpretation in blood. In over a large portion of these, one of the parents had the same down regulation of p16^{INK4A} in their platelets. Interestingly, methylation investigation of the CDKN2 promoter in this cohort study uncovered that subjects and parents concealing the same transformation demonstrated promoter hyper methylation, proposing that this modification could be heritable, and consequently could turn into a novel susceptibility marker for these subjects¹⁶.

Candidate genes as reconnaissance markers and remedial targets

Gene particular duplicate number losses or gains and changes in gene articulation, for example, KIF14, MDM4, MYCN, DEK, E2F3, CDH11, miR-17~92, and SYK have shown significance in retinoblastomas by means of numerous lines of confirmation . These genes have shown utilitarian significance in cell lines, creature models and patient tumors, and may be connected with development or poor results. These genes could be created into markers that would encourage observation of tumor repeat or metastasis (as indicated for RB1¹⁰⁹, KIF14 and E2F3¹⁵), and additionally being the focus of new treatments to treat repeating retinoblastomas¹⁷⁻¹⁹.

Management including treatment

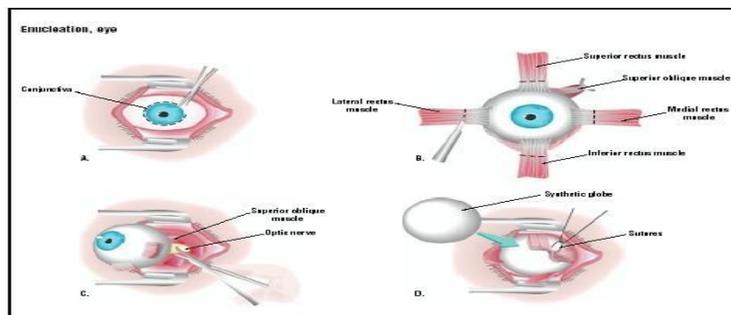
Enucleation

Enucleation remains the essential treatment for everything except a couple of unilateral cases that have a tendency to present with advanced infection in light of visual conservation in the ordinary contralateral eye. Advances in method and permeable implant materials have brought about great motility, incredible cosmesis, and a low expulsion rate. Subjects who get chemotherapy shortly after enucleation may be at higher risk for disease and expulsion (figure 4).

Enucleated globes are analyzed histopathologically for extra ocular tumor augmentation and expansion into the optic nerve. It is basic that the enucleating specialist take a long (10-cm) bit of optic nerve as tumor at the cut margin of the nerve gives a poor anticipation. Discovering tumor cells past the lamina cribrosa of the nerve (the way out of the nerve from the globe) is an evidence for prophylactic chemotherapy²⁰.

In eyes treated at first with other eye-saving modalities, there is dependably the danger of resulting enucleation because of repeat of tumor; visually impaired, agonizing eyes creating secondary difficulties of radiation, e.g., neovascular glaucoma; and deformed globes because of the late impacts of radiation or incessant retinal separation.

Figure 4:-Process of Enucleation



Exenteration

It is an amazingly distorting surgery. Forthright exenteration is currently outdated. It is right now performed just in those instances of primary/repetitive orbital infection that neglect to react to NACT.

External beam radiotherapy

EBR still assumes a part in the administration of retinoblastoma. Radiation is demonstrated even with diffuse vitreous seeding (Reese-Ellsworth stage 5B), failure of chemotherapy or different modalities or bigotry of chemotherapy. The guardian of kids treated with radiation ought to comprehend that there is a noteworthy danger (more or less 30% over 40 years) of the development of SMNs (sarcoma) inside and outside the turf of radiation.

Nearby difficulties of EBR incorporate facial disfigurements brought on by the discontinuance of orbital bone development, keratopathy and dry eye, cataract, and radiation injury to the retina and optic nerve bringing about visual misfortune. The last can bring about proliferative retinopathy, neovascular glaucoma, and extreme vision misfortune²¹⁻²³.

In any case, radiation harm to the visual structures can be challenging to oversee. Additionally, it may actuate secondary tumor in the field of radiation, with a reported 30-year combined rate for second tumors in bilateral retinoblastoma treated with light at 35% contrasted with 6% in the individuals who did not get radiation.

Plaque Brachytherapy

Plaque brachytherapy is a safe distinct option for enucleation in eyes with intraocular melanoma. Nonetheless, restricted radiation treatment as isotope-impregnated episcleral plaques is utilized sparingly as a part of eyes with RB. Large tumors in extra macular areas are suitable for this treatment²⁴.

The plaques, comprising radioactive iodine-125, are stitched to the eye for 2-4 days, taking into consideration radiation to the basic choroid, retina, and tumor. Neighborhood intricacies incorporate diplopia, cataract, and radiation retinopathy. In eyes already treated or simultaneously with chemotherapy, there is a high danger of radiation retinopathy, constraining the value of this modality.

Photocoagulation/Cryotherapy

Cryotherapy is appropriate for small anterior RB tumors. A triple freeze thaw technique, in which the tumor is enclosed in a restricted ice ball three times in quick succession, is powerful in creating prompt cell lysis and putrefaction of tumor tissue. Cryotherapy can result in shrinkage of the sclera, and extensive treatment inclines the developing eye to nearsightedness and builds the risk of retinal separation²⁵⁻²⁶.

Direct photocoagulation of little tumors is regularly utilized amid chemotherapy or afterward for little repeats or new tumors. The broad availability of aberrant ophthalmoscope laser delivery systems makes the utilization of laser energy to small posterior and foremost tumors simple, safe, and powerful. The precise nature of the laser permits the treating doctor to confine harm to the tumor and none of the encompassing (typical) retina. Limited smolders of the iris, especially the papillary edge, are uncommon.

EBRT in extra ocular retinoblastoma

Local EBRT is a vital part of the treatment protocol for annihilation of lingering ailment in the orbit. The common dose of radiotherapy given to the included orbit is 40-45 Gray for 4-5 weeks. There is no role for prophylactic cranial illumination. The incorporation of optic chiasma in the radiation field is unclear and is practiced by few eye hospitals²⁷.

The molecular flagging pathways in retinoblastoma and possible clinical implications

The typical retinoblastoma protein ties to and represses the translation element E2F, consequently ending transcription of E2F target genes, which are in charge of cell cycle movement. It additionally ties to histone deacetylase (HDAC), which brings about silencing of transcription. Mutated retinoblastoma protein brings about uncontrolled cell multiplication and, eventually cancer. Little advance has been made toward the development of focused therapies to profit retinoblastoma. The potential focuses for molecular treatment include:

Nutlin 3A

Nutlin 3A is a little molecule inhibitor of the MDM2/MDMX and p53 interaction, which was discovered to have the capacity to affect cell death in human retinoblastoma cell lines. Further, when utilized as a part of blend with topotecan, it executed human retinoblastoma cell lines. There was an 82-fold diminishment in tumor load in mice when Nutlin 3A was infused subconjunctivally with topotecan²⁸.

Inhibitors of HDAC

HDAC inhibitors may be especially helpful on the grounds that they instigate cytotoxicity to tumors specifically. Recently, it was discovered that certain HDAC inhibitors diminish cell survival in human retinoblastoma cell lines and altogether lessened tumor burden in both rodent and mouse models of retinoblastoma²⁹⁻³⁰.

Molecular inhibitors of the oncogene N-myc

N-myc is increased in 10% of human retinoblastoma cases and, consequently, such inhibitors may demonstrate helpful in the treatment of a subset of retinoblastoma patients.

Chemotherapy

The blend of etoposide phosphate, vincristine sulfate, and carboplatin is imbued like clockwork, normally for six months. Inconveniences of the medications incorporate neutropenia, weakness, listening misfortune and later leukemia³¹.

Chemo reduction

The point of chemo reduction is to diminish the tumor volume in huge intraocular tumors (ICRB Group B to D), along these lines permitting utilization of less-harming and more targeted therapeutic measures keeping in mind the end goal to protect vision and evade enucleation. The most well-known chemotherapy protocol utilizes vincristine, etoposide and carboplatin. Satisfactory tumor decrease obliges two to six cycles of chemotherapy. Taking after two cycles of chemotherapy, scientist have reported a 35% reduction in the mean base breadth, half lessening in the tumor thickness and complete determination of sub-retinal liquid in 76% cases. They have likewise reported a 25% repeat of sub retinal seeds when under six cycles were administered contrasted with 0% repeat in cases who got six cycles of chemotherapy. Therefore, it is accepted that under six cycles of chemo reduction may not be sufficient to totally wreck sub-retinal seeds. Likewise, central treatment is crucial to counteract tumor recurrence³²⁻³⁴.

Periocular chemotherapy

The significant issue with systemic chemotherapy is the recurrence of vitreous and sub retinal seeds. It is accepted that this may happen in view of constrained penetration of systemically directed chemotherapeutic agents into the sub retinal and vitreous spaces, which are avascular. Utilization of local delivery procedures (Periocular) permits higher compelling doses at these locales, securing powerful treatment while constraining the systemic reactions of

chemotherapy. Most centers use carboplatin in a fluid vehicle (same as utilized for systemic treatment). Despite the fact that Periocular carboplatin has been accounted for have guaranteeing remedial benefit in the treatment of group C and D retinoblastoma, it is connected with serious adverse events, including visual motility changes, orbital fat rot, extreme pseudo preseptal cellulitis and ischemic putrefaction with decay of the optic nerve bringing about blindness³⁵.

Intra-arterial chemotherapy

Lately, intra-arterial infusion of the chemotherapy drug melphalan in retinoblastoma patients has been attempted. Scientist have been examining intracarotid administration of chemotherapy. However, in their studies, numerous eyes additionally got external beam radiotherapy (EBRT) and intravitreal melphalan, making it hard to decide how compelling the intra-arterial infusions would be if utilized alone³⁶. Clinical Information from a stage I/II clinical trial has shown great results after direct intra ophthalmic artery infusions of melphalan. They utilized particular catheterization of the ophthalmic vein, subsequently hypothetically evading lethality to the brain. Vision balanced out or enhanced in all except one of the nine subjects enrolled in the clinical study³⁷.

Numerous patients, then again, likewise got supplementary thermotherapy, laser, brachytherapy or EBRT, making it hard to figure out if this system is successful in expanding the globe rescue rates all alone. Notwithstanding its striking control for retinoblastoma, especially repetitive tumor seeds taking after different treatments, a few scientist feel that intra-arterial chemotherapy ought to be utilized with alert. Recently, scientist have assessed the poisonous impacts of irradiation from fluoroscopy amid intra-arterial chemotherapy for retinoblastoma, and have advised that amassed irradiation toxic impacts taking after various sessions of intra-arterial chemotherapy could be cataractogenic and potentially cancer-causing.

Chemoprophylaxis

A few studies have demonstrated that vicinity of certain histopathological highlights in the enucleated eyes is connected with a higher risk of systemic metastasis or nearby recurrence. Adjuvant treatment in these cases lessens the probability of neighborhood recurrence and far off metastasis. Histopathological highlights, which are utilized as a sign for six cycles of adjuvant chemotherapy at most eye centers, incorporate attack of front chamber, iris, ciliary body, choroid (huge), sclera and optic nerve past lamina cribrosa. Furthermore, extra scleral and optic nerve cut-end association is named microscopic residual ailment and is treated with 12 cycles of adjuvant chemotherapy and EBRT³⁸⁻⁴⁰.

Chemotherapy for locally propelled disease

NACT is utilized to diminish the tumor size, took after by enucleation, neighborhood radiotherapy and adjuvant chemotherapy, to finish an aggregate of 12 cycles. NACT prompts quick lessening in tumor mass furthermore controls micro metastatic malady. The decrease in tumor mass empowers most patients to experience enucleation rather than cosmetically distorting and more perplexing exenteration.

Most eye center at present utilization standard dosages of vincristine, carboplatin and etoposide (VEC) for neo adjuvant and adjuvant chemotherapy. Then again, information relating to utilization of high-dosage versus standard dose of carboplatin is inadequate without any randomized control trials looking at the same. The general survival reported in patients with extra ocular infection is 50-70% in patients with extra ocular retinoblastoma. Data from India demonstrates a general survival of half a year and a half of post liminary. The role of intracranial prophylaxis with intrathecal chemotherapy or cranial illumination in patients with generally propelled retinoblastoma has not been tentatively assessed. Results in review studies with or without intrathecal chemotherapy have been comparative. Due to absence of confirmation, the utilization of intrathecal chemotherapy as prophylaxis to avoid central nervous system metastases in locally propelled retinoblastoma stays exploratory⁴¹⁻⁴³.

High dosage chemotherapy and autologous stem cell transplant

Autologous stem cell transplant with high-dose chemotherapy remains the main corrective choice for metastatic sickness. There have been just few case reports in the literature with respect to the utilization of this modality for

the treatment of metastatic malady. Children with non CNS illness (bone or bone marrow) have better outcomes when contrasted with patients of CNS metastasis. Long term survival of 50-75% has been accounted for in patients with non CNS disease in a couple of arrangement. The medications generally utilized as a feature of high-dosage chemotherapy incorporate mixes of carboplatin, etoposide, cyclophosphamide, melphalan and thiotepa. The part of allogenic transplant stays investigational⁴⁴.

Closing Remarks:-

Notwithstanding the Identification of the RB1 gene and the current understanding into the function of pRB, the comprehension of the sequences that prompts human retinoblastoma is still deficient. Latest advancement of new animal models of retinoblastoma will build the learning on tumorigenesis and give a chance to create treatment strategies. Despite the fact that retinoblastoma has a decent prognosis in industrialized nations, mortality because of development of a second tumor stays high. Development of a non-mutagenic treatment, (for example, photodynamic treatment) could be fascinating, especially in the event of hereditary retinoblastoma. Gene therapy for treatment of retinoblastoma is still at under evaluation.

In developing nations, retinoblastoma is sadly accompanied by a high death rate because of an altogether postponed diagnosis made at advance stages of the illness. Next Generation Sequencing (NGS) specifically holds the promise of read profundity that could potentially distinguish mechanism of merged evolution of gene transformations and gene duplicate number changes. This kind of innovation can possibly uncover the identity of starting versus movement changes, encouraging diagnosis and remedial management. While NGS is being utilized as a device for transformation revelation, its current sensitivity and precision for distinguishing RB1 transformations is still not published, not to mention clinically accepted. Despite the fact that NGS technologies promise to be more reasonable for clinical mutation testing for retinoblastoma, recognition of a wide range of transformation has yet to be illustrated.

Conflicts of Interest Statement:

The Authors declare no conflicts of interest.

References:-

- (1) Orjuela M, Castaneda VP, Ridaura C, Lecona E, Leal C, Abramson DH, Orlow I, Gerald W, Cordon-Cardo C. Presence of human papilloma virus in tumor tissue from children with retinoblastoma: an alternative mechanism for tumor development. *Clin Cancer Res*. 2000;6:4010-4016.
- (2) Orjuela MA, Titievsky L, Liu X, Ramirez-Ortiz M, Ponce-Castaneda V, Lecona E, Molina E, Beaverson K, Abramson DH, Mueller NE. Fruit and vegetable intake during pregnancy and risk for development of sporadic retinoblastoma. *Cancer Epidemiol Biomarkers Prev*. 2005;14:1433-1440. doi: 10.1158/1055-9965.EPI-04-0427.
- (3) Brisse HJ, Lumbroso L, Freneaux PC, Validire P, Doz FP, Quintana EJ, Berges O, Desjardins LC, Neuenschwander SG. Sonographic, CT, and MR imaging findings in diffuse infiltrative retinoblastoma: report of two cases with histologic comparison. *AJNR Am J Neuroradiol*. 2001;22:499-504.
- (4) Kaufman LM, Mafee MF, Song CD. Retinoblastoma and simulating lesions. Role of CT, MR imaging and use of Gd-DTPA contrast enhancement. *Radiol Clin North Am*. 1998;36:1101-1117. doi: 10.1016/S0033-8389(05)70234-7.
- (5) Houdayer C, Gauthier-Villars M, Lauge A, Pages-Berhouet S, Dehainault C, Caux-Moncoutier V, Karczynski P, Tosi M, Doz F, Desjardins L, Couturier J, Stoppa-Lyonnet D. Comprehensive screening for constitutional RB1 mutations by DHPLC and QMPSF. *Hum Mutat*. 2004;23:193-202. doi: 10.1002/humu.10303.
- (6) Harbour JW. Overview of RB gene mutations in patients with retinoblastoma. Implications for clinical genetic screening. *Ophthalmology*. 1998;105:1442-1447. doi: 10.1016/S0161-6420(98)98025-3.
- (7) Lohmann DR, Brandt B, Hopping W, Passarge E, Horsthemke B. The spectrum of RB1 germ-line mutations in hereditary retinoblastoma. *Am J Hum Genet*. 1996;58:940-949.
- (8) Lohmann DR. RB1 gene mutations in retinoblastoma. *Hum Mutat*. 1999;14:283-288. doi: 10.1002/(SICI)1098-1004(199910)14:4<283::AID-HUMU2>3.0.CO;2-J.
- (9) Blanquet V, Turleau C, Gross-Morand MS, Senamaud-Beaufort C, Doz F, Besmond C. Spectrum of germline mutations in the RB1 gene: a study of 232 patients with hereditary and non hereditary retinoblastoma. *Hum Mol Genet*. 1995;4:383-388.

- (10) Aerts I, Pacquement H, Doz F, Mosseri V, Desjardins L, Sastre X, Michon J, Rodriguez J, Schlienger P, Zucker JM, Quintana E. Outcome of second malignancies after retinoblastoma: a retrospective analysis of 25 patients treated at the Institut Curie. *Eur J Cancer*. 2004;40:1522-1529. doi: 10.1016/j.ejca.2004.03.023.
- (11) Kleinerman RA, Tucker MA, Tarone RE, Abramson DH, Seddon JM, Stovall M, Li FP, Fraumeni JF., Jr Risk of new cancers after radiotherapy in long-term survivors of retinoblastoma: an extended follow-up. *J Clin Oncol*. 2005;23:2272-2279. doi: 10.1200/JCO.2005.05.054.
- (12) Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veeck L, Abramson DH. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. *Am J Ophthalmol*. 2004;137:18-23. doi: 10.1016/S0002-9394(03)00872-9.
- (13) Murphree AL. Intraocular Retinoblastoma: the Case for a New Group Classification. *Ophthalmol Clin North Am*. 2005;18:41-53. doi: 10.1016/j.ohc.2004.11.003.
- (14) Moscinski LC, Pendergrass TW, Weiss A, Hvizdala E, Buckley KS, Kalina RE. Recommendations for the use of routine bone marrow aspiration and lumbar punctures in the follow-up of patients with retinoblastoma. *J Pediatr Hematol Oncol*. 1996;18:130-134. doi: 10.1097/00043426-199605000-00006.
- (15) Corson TW, Gallie BL. One hit, two hits, three hits, more? Genomic changes in the development of retinoblastoma. *Genes Chromosomes Cancer*. 2007; 46:617-634.
- (16) Bowles E, Corson TW, Bayani J, Squire JA, Wong N, Lai PB, Gallie BL. Profiling genomic copy number changes in retinoblastoma beyond loss of *RB1*. *Genes Chromosomes Cancer*. 2007;46:118-129.
- (17) Zielinski B, Gratiyas S, Toedt G, Mendrzyk F, Stange DE, Radlwimmer B, Lohmann DR, Lichter P. Detection of chromosomal imbalances in retinoblastoma by matrix-based comparative genomic hybridization. *Genes Chromosomes Cancer*. 2005; 43:294-301.
- (18) Corson TW, Gallie BL. *KIF14* mRNA expression is a predictor of grade and outcome in breast cancer. *Int J Cancer*. 2006; 119:1088-1094.
- (19) Corson TW, Huang A, Tsao MS, Gallie BL. *KIF14* is a candidate oncogene in the 1q minimal region of genomic gain in multiple cancers. *Oncogene*. 2005; 24:4741-4753.
- (20) Corson TW, Zhu CQ, Lau SK, Shepherd FA, Tsao MS, Gallie BL. *KIF14* messenger RNA expression is independently prognostic for outcome in lung cancer. *Clin Cancer Res*. 2007;13:3229-3234.
- (21) Theriault BL, Pajovic S, Bernardini MQ, Shaw PA, Gallie BL. Kinesin family member 14: an independent prognostic marker and potential therapeutic target for ovarian cancer. *Int J Cancer*. 2012; 130:1844-1854.
- (22) Laurie NA, Donovan SL, Shih CS, Zhang J, Mills N, Fuller C, Teunisse A, Lam S, Ramos Y, Mohan A, Johnson D, Wilson M, Rodriguez-Galindo C, Quarto M, Francoz S, Mendrysa SM, Guy RK, Marine JC, Jochemsen AG, Dyer MA. Inactivation of the p53 pathway in retinoblastoma. *Nature*. 2006; 444:61-66.
- (23) Orlic M, Spencer CE, Wang L, Gallie BL. Expression analysis of 6p22 genomic gain in retinoblastoma. *Genes Chromosomes Cancer*. 2006; 45:72-82. [PubMed: 16180235]
- (24) Marchong MN, Chen D, Corson TW, Lee C, Harmandayan M, Bowles E, Chen N, Gallie BL. Minimal 16q genomic loss implicates cadherin-11 in retinoblastoma. *Mol Cancer Res*. 2004;2:495-503.
- (25) Marine JC, Jochemsen AG. Mdmx and Mdm2: brothers in arms? *Cell Cycle*. 2004; 3:900-904.
- (26) Wade M, Li YC, Wahl GM. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat Rev Cancer*. 2012; 13:83-96.
- (27) Laurie NA, Shih CS, Dyer MA. Targeting MDM2 and MDMX in retinoblastoma. *Curr Cancer Drug Targets*. 2007; 7:689-695.
- (28) Guo Y, Pajovic S, Gallie BL. Expression of p14ARF, MDM2, and MDM4 in human retinoblastoma. *Biochem Biophys Res Commun*. 2008; 375:1-5.
- (29) To KH, Pajovic S, Gallie BL, Theriault BL. Regulation of p14ARF expression by miR-24: a potential mechanism compromising the p53 response during retinoblastoma development. *BMC Cancer*. 2012; 12:69.
- (30) Carleton M, Mao M, Biery M, Warrenner P, Kim S, Buser C, Marshall CG, Fernandes C, Annis J, Linsley PS. RNA interference-mediated silencing of mitotic kinesin KIF14 disrupts cell cycle progression and induces cytokinesis failure. *Mol Cell Biol*. 2006; 26:3853-3863.
- (31) Gruneberg U, Neef R, Li X, Chan EH, Chalamalasetty RB, Nigg EA, Barr FA. KIF14 and citron kinase act together to promote efficient cytokinesis. *J Cell Biol*. 2006; 172:363-372.
- (32) Madhavan J, Mitra M, Mallikarjuna K, Pranav O, Srinivasan R, Nagpal A, Venkatesan P, Kumaramanickavel G. KIF14 and E2F3 mRNA expression in human retinoblastoma and its phenotype association. *Mol Vis*. 2009; 15:235-240.
- (33) Markowski J, Oczko-Wojciechowska M, Gierek T, Jarzab M, Paluch J, Kowalska M, Wygoda Z, Pfeifer A, Tyszkiewicz T, Jarzab B, Niedzielska I, Borgiel-Marek H. Gene expression profile analysis in laryngeal cancer by high-density oligonucleotide microarrays. *J Physiol Pharmacol*. 2009; 60(Suppl 1):57-63.

- (34) Markowski J, Tyszkiewicz T, Jarzab M, Oczko-Wojciechowska M, Gierek T, Witkowska M, Paluch J, Kowalska M, Wygoda Z, Lange D, Jarzab B. Metal-proteinase ADAM12, kinesin 14 and checkpoint suppressor 1 as new molecular markers of laryngeal carcinoma. *Eur Arch Otorhinolaryngol.* 2009; 266:1501–1507.
- (35) Ahmed SM, Theriault BL, Uppalapati M, Chiu CW, Gallie BL, Sidhu SS, Angers S. KIF14 negatively regulates Rap1a-Radil signaling during breast cancer progression. *J Cell Biol.* 2012;199:951–967.
- (36) Basavarajappa HD, Corson TW. KIF14 as an oncogene in retinoblastoma: a target for novel therapeutics? *Future Med Chem.* 2012; 4:2149–2152.
- (37) Ganguly A, Nichols KE, Grant G, Rappaport E, Shields C. Molecular karyotype of sporadic unilateral retinoblastoma tumors. *Retina.* 2009; 29:1002–1012.
- (38) Mairal A, Pinglier E, Gilbert E, Peter M, Validire P, Desjardins L, Doz F, Aurias A, Couturier J. Detection of chromosome imbalances in retinoblastoma by parallel karyotype and CGH analyses. *Genes Chromosomes Cancer.* 2000; 28:370–379.
- (39) Gratias S, Schuler A, Hitpass LK, Stephan H, Rieder H, Schneider S, Horsthemke B, Lohmann DR. Genomic gains on chromosome 1q in retinoblastoma: consequences on gene expression and association with clinical manifestation. *Int J Cancer.* 2005; 116:555–563.
- (40) Sampieri K, Amenduni M, Papa FT, Katzaki E, Mencarelli MA, Marozza A, Epistolato MC, Toti P, Lazzi S, Bruttini M, De Filippis R, De Francesco S, Longo I, Meloni I, Mari F, Acquaviva A, Hadjistilianou T, Renieri A, Ariani F. Array comparative genomic hybridization in retinoma and retinoblastoma tissues. *Cancer Sci.* 2009; 100:465–471.
- (41) Woo CW, Tan F, Cassano H, Lee J, Lee KC, Thiele CJ. Use of RNA interference to elucidate the effect of MYCN on cell cycle in neuroblastoma. *Ped Blood Cancer.* 2008; 50:208–212.
- (42) Barr FG, Duan F, Smith LM, Gustafson D, Pitts M, Hammond S, Gastier-Foster JM. Genomic and clinical analyses of 2p24 and 12q13-q14 amplification in alveolar rhabdomyosarcoma: a report from the Children's Oncology Group. *Genes Chromosomes Cancer.* 2009; 48:661–672.
- (43) Bayani J, Zielenska M, Marrano P, Kwan Ng Y, Taylor MD, Jay V, Rutka JT, Squire JA. Molecular cytogenetic analysis of medulloblastomas and supratentorial primitive neuroectodermal tumors by using conventional banding, comparative genomic hybridization, and spectral karyotyping. *J Neurosurg.* 2000; 93:437–448.
- (44) Hui AB, Lo KW, Yin XL, Poon WS, Ng HK. Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization. *Lab Invest.* 2001;81:717–723.