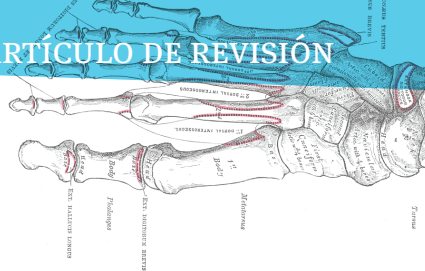


An update on retinoblastoma

by Azahara Herrera Espinosa

Estudiante de 4º de Medicina. Universidad de Valencia.

RETINOBLASTOMA IS A RARE CONDITION AFFECTING MAINLY INFANTS AROUND THE WORLD. BY AN EXHAUSTIVE SEARCH, THE AUTHOR AIMS TO PROVIDE HERE AN UPDATE IN THIS DISEASE, REVIEWING THE MOST IMPORTANT ASPECTS IN CLINICAL DIAGNOSIS, ASSESSMENT AND PATHOLOGICAL FEATURES. ALSO, POINTING OUT THE NEWEST THERAPIES AVAILABLE, FROM THE VERY BEGINNINGS OF ITS TREATMENT TO THE CURRENT OPTIONS THAT WE NOWADAYS HAVE AT OUR DISPOSAL.

Introduction

Retinoblastoma is a rare neuronal tumour in the general population, although the commonest one amongst children[1]. It has long been known that a mutation of the RB1 gene is needed to develop this cancer, although this may be developed by inheritance or by acquisition later in life. Note that the inherited ones are bilateral, and sometimes appear associated with pinealoblastoma, being called "trilateral retinoblastoma" which has a poorer prognosis. Incidence may be affected by factors like age: Little, Kleinerman *et al*[2] analysed the differential age incidence by using a fully stochastic model which shown that "knockout of the two alleles of the RB1 gene is necessary and may be largely sufficient for the development of retinoblastoma", a conclusion which is clearly supporting Knudson's two-hit hypothesis. As Kroll, Carpenter *et al*[3] show, its incidence increases as the diagnosis procedures become more advanced: that may be due to an under-recording of retinoblastoma and other childhood tumours and cancer cases in the past. Despite of being a malignant tumour, its survival rate in the developed countries is currently very high, as it happens with other paediatric tumours, reaching a 93% rate of remission. Cheung[4] identified several socioeconomic barriers affecting this prognosis: residence –urban or rural areas– together with African American descent or race increase the outcome differences between patients.

The goal of this review is to make an approach to what we know up-to-now about retinoblastoma and to elucidate the feasible paths investigation may take from this point of departure.

Methods

In order to develop this document several selection criteria were applied: the selected search engine was PubMed, thus having access to MEDLINE and other databases content. Results were filtered so only papers from 2008 to today have been accepted, most of them being published between 2010 and 2014. By this method, older papers were rejected. Free, open-source online/PDF articles and those which access is possible due to the Universitat de València institutional subscription fees have been selected, thus discarding any paid, non-affordable resources. No language selection criteria have been applied, although most of the information here used has been written in English.

Mendeley Desktop –software tool– was employed for bibliographic references organisation according to Vancouver style guide of referencing.

Results

Clinical manifestations

For diagnosing retinoblastoma it is very important to assess all possible familial history around this tumour and related ones –retinoma and other RB mutation-related conditions–, apart from other risk factors, medications, surgeries, type of delivery of the newborn and others that may influence. The physician will observe mainly leukocoria in the patient (see *Figure 1*)[5], being unilateral if acquired or bilateral in inherited cases. Also strabismus due to loss of fixation and proptosis can be found and, in advanced cases, even an extra ocular extension of the tumour mass. It is convenient to take a photo of the current external appearance of the affected eye to further analyse the evolution of the patient once treated and in successive meetings with the doctor. From this point, a slit lamp exploration is necessary, even recurring to anaesthesia in poorly cooperative patients. This exploration will allow the ophthalmologist/paediatrician to appreciate whether or not there's any anterior chamber affection –from neovascularisation on the iris to tumoral invasion. Intraocular pressure should be assessed–.

Apart from all these tests, the physician should most importantly perform an indirect ophthalmoscopy in order to document the location, the size and other features of interest for starting the most appropriate treatment. By far, this is the most important tool for diagnosing retinoblastoma, as it allows its stage determination. Early tumours appear as grey, round or ovoid thickenings of the retina, with translucent properties, which leads to a loss of contrast between the lesion and the normal fundus, making the diagnosis more difficult. As the tumour grows in stage, it does in size, changing its colour to a yellowish tone with evident vessels irrigating the mass. On occasion, calcifications may be visible by this method. In the most advanced cases, a retinal detachment can be detected, and the retinoblastoma transforms from a regular to an irregular border mass with a notable consistency[5].

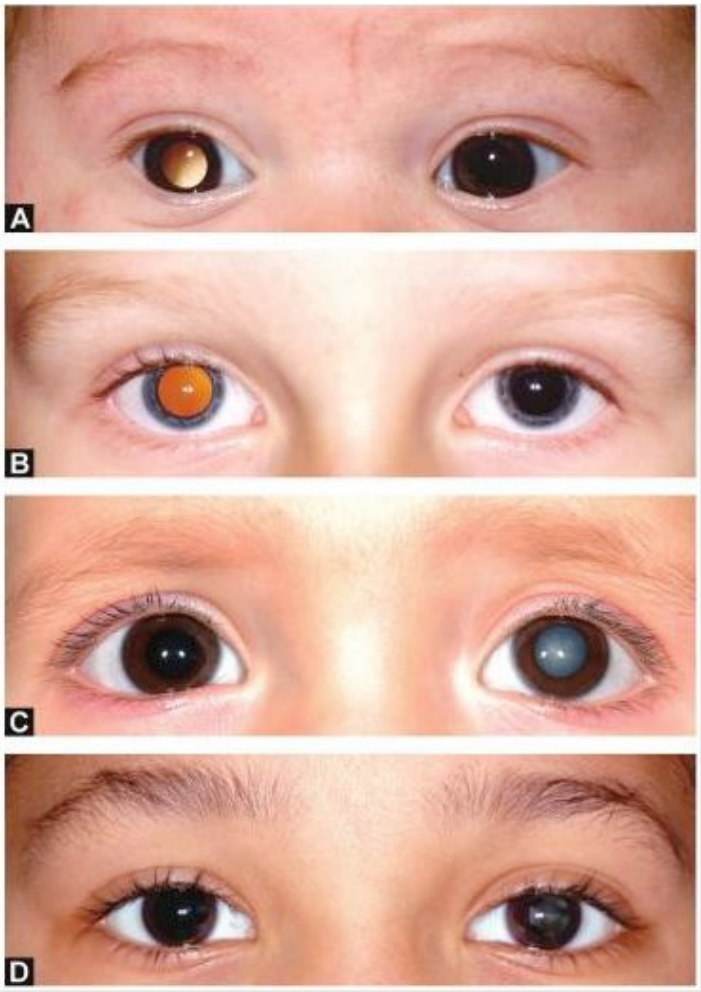
Imaging techniques

Among imaging techniques, ultrasonography gains a relevant role as it allows the confirmation of the presence of any intraocular mass, demonstrating the possible calcifications that can appear in a retinoblastoma. Brain and eye MRI are also performed in this case and in any orbit pathologies and pinealoblastoma suspicion until age 5 years. The value of this MRI technique is demonstrated in many studies such as the one performed by Rodjan *et al*[6], where the pinealoblastoma imaging presentation possibilities are recorded: in this study, the majority of the pinealoblastomas were related to RB inherited mutations, a huge proportion were actual trilateral retinoblastoma cases, and in most of them the pinealoblastoma showed a cystic appearance, although there were also solid ones.

Some retinoblastoma cases may associate orbital cellulitis, as stated in Chawla, Duraipandi and Sharma study[7]. In these cases an MRI is also very useful as it allows a proper differentiation of this inflammation phenomenon from a true tumoral invasion, determining a different patient management, treatment and prognosis.

Screenings

As this malignancy has a very powerful genetic basis, regular fundus screening for children at risk has been suggested as a good tool for improving the outcome in this kind of tumours, according to Rothschild *et al*[8].



▲ ***Figure 1***. *Images A to D show different leukocoria types. A corresponds to retinoblastoma, while B is the characteristic xantocoria from Coats disease, C is a caesiocoria due to a congenital cataract and D is caused by a medulloepithelioma of the cilliary body. Extracted from Kaliki S, Shields CL. Differential diagnosis of Retinoblastoma. In: Ramasubramanian A, L Shields, C. Retinoblastoma. 1st ed. New Delhi: Jaypee Brothers Medical Publishers; 2012. 47-61.*

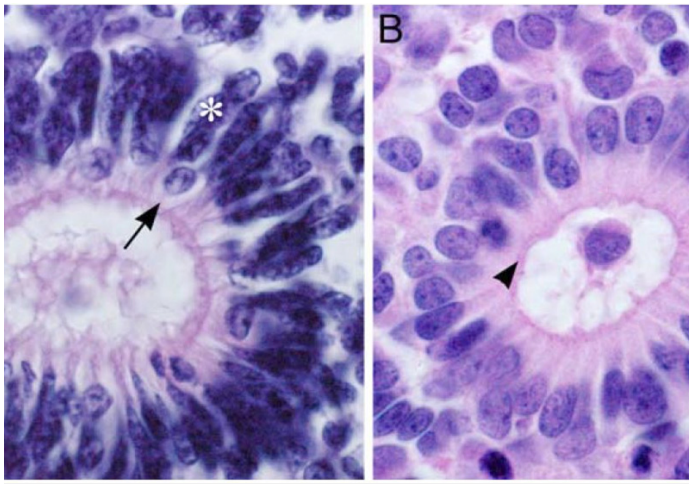
Histological features

Morphologically both inherited and acquired retinoblastomas are equal. By the observation of the sample, the pathologist will detect both non-differentiated and well-differentiated elements. On one hand, the tumours which have a good and early retinal differentiation are constituted by small round cells with hyperchromatic nucleus, which form Flexner-Wintersteiner rosettes (see *Figure 2*)[9]; on the other hand, fleurettes –formed by cells which origin lies on photoreceptors– can be seen as a sign of advanced photoreceptor differentiation. These Flexner-Wintersteiner rosettes are formed by cuboidal cells surrounding a central light containing glycosaminoglycans, with its nucleus facing the basal pole of the cell. All the structure is externally limited by an eosinophilic external limiting membrane as the one that is found in a histologically normal retina sample. In

other patients it is possible to find Homer-Wright rosettes, which are a primitive neuroblastic differentiation structures composed of a ring of nuclei around a central filamentous axis. Note that Homer-Wright rosettes are not only present in retinoblastoma, but they also appear in neuroblastoma and others. Apart from these rosettes and fleurettes, there will be tumoral cells surrounding the vessels plus necrosis in avascular regions of the mass, which is a sign of malignancy, and dystrophic eosinophilic calcification areas. There is normally no evidence of any infection, but Human Papilloma Virus (HPV) presence has been documented in Korean patients (Ryoo *et al*[10]).

Pathology studies as the one elaborated by Eagle[11] have stated that the differentiation degree seems not to correlate with retinoblastoma prognosis but to differ amongst patients with varying ages. By this way, typical Flexner-Wintersteiner rosettes are more common in the youngest patients whilst Homer-Wright ones appear in older people. The true prognostic factors, it is, the ones that indicate an adjuvant chemotherapy for a specific patient will then be the massive uveal and the retrolaminar optic nerve invasion. In the light of these facts, histology appears to play an important role in treatment decision.

It is histologically relevant to know that in the last years retinoblastoma seems to have a clear precursor: retinocytoma, also called retinoma, which behaves like a premalignant lesion. Its development is common in acquired retinoblastoma patients, where an eye is affected with a true retinoblastoma while the other one has a retinoma. Several studies show evidence supporting this connection. Barbosa *et al*[12] found shared RB1 mutations and both phenotypic and genotypic associations between them in their cohort, although these last ones –intronic deletions mostly seemed– to appear only in Latin American people, so it remains unclear whether or not these have an important pathogenic role. Older studies, such as the one from Dimaras *et al*[13] in 2008 already stated that the loss of RB1 was able to also induce non-proliferative retinoma development in affected patients and, furthermore, if it was then completed with increasing genomic instability –external aggressions to the person's genome– this non-proliferative form could evolve to retinoblastoma. Since then, other genetic studies have been performed in order to find further relationships between retinocytoma and retinoblastoma.



◄ ***Figure 2***. *Comparison between a non-retinoblastoma (A) and a genuine retinoblastoma rosette. Note that in the second case it is not possible to differentiate cones and rods, while in the first a cone (long arrow) and a rod (white asterisk) can be detected. Extracted from Chan A, Lakshminrusimha S, Heffner R, González-Fernández F. Histogenesis of retinal dysplasia in trisomy 13. Diagnostic Pathology 2007; 2: 48.*

As more studies around this tumour are done and more knowledge is gained, it is important to establish a differential diagnosis with potentially similar lesions in the eye which could lead to misdiagnosis and even an unnecessary enucleation. This is the case of retinal dysplasia, which can be provoked by vitamin deficiencies, viral infections or genetic defects. As it also forms rosettes, it is important to note the different features of these histological lesion and retinoblastoma itself. In a dysplastic rosette it is possible to discern the cell line from which each cell may arise, as there are cone-like and rod-like cells composing the rosette, with specific nuclei shapes remembering the original cell from which they come. In the case of a true retinoblastoma rosette, this is not observable. All the diseases gathered as "pseudoretinoblastomas" have to be considered for the differential diagnosis: from hereditary conditions –X-linked retinoschisis– to developmental –congenital corneal opacity– or inflammatory disorders due to infections, and other tumours; acquired diseases and other pathologies affecting the eye, such as Coats disease.

Treatment

Treatment of this condition includes several possibilities according to the characteristics of each specific case, from age to stage or other elements.

Enucleation was the first historic therapeutic approach for retinoblastoma patients, granting a radical cure in most cases but also a very important sequel as losing the eye is for sight function.

Radiation was then applied in order to avoid unnecessary enucleations: first external radiotherapy was applied, but as techniques advanced this one was substituted and/or combined with systemic chemotherapy.

As systemic treatments involve serious systemic adverse effects, local therapy earned relevance in order to avoid them: diathermy, which is currently known as a treatment for specific joint conditions, was applied in retinoblastoma treatment, as laser photocoagulation and cryotherapy were. This last one preferred for anterior tumours and those in the eye globe equator.

From all these experience, the current treatment has arisen, consisting on stereotactic radiotherapy and IMRT to keep the eye and avoid systemic complications. Several radioactive seeds have been employed for brachytherapic procedures, being Ruthenium-106 and Iodine-125 preferred over Cobalt-60. Ultimately chemoreduction –it is, applying chemo then brachytherapy to avoid external RT– has been developed, with good results, although alarming side effects such as myelogenous leukaemia and other severe ones appeared with etoposide usage. This chemotherapic reduction, despite the adverse effects, has been proven as a good way to avoid enucleation, as Künkele *et al*[14] study states.

Testing of intraarterial application of melphalan –better known for amyloidosis treatment, but also useful in this condition– has been successfully applied. Venturi *et al*[15] have performed 140 super-selective ophthalmic infusion treatments with this technique, earning great results, especially when this was applied after another therapy, it is, in a combination regimen. Other studies –Bracco *et al*[16] also show similar results–.

After melphalan another alternatives appeared, as intracarotid triethylene melamine. This last one provided great outcomes, and has been then stated as a good primary and salvage treatment avoiding systemic toxicity.

In the last two years, new therapies are starting to be used: the tandem therapy, as presented by Liu *et al*[17] consisting on the combination of immunotherapy and chemotherapy, has reached higher levels of cytotoxicity in retinoblastoma increasing its apoptotic rate, thus protecting the healthy tissue around. Zhang *et al*[18] have successfully used KZ-41 –a novel quinic acid derivative– in order to prevent retinal endothelial cell apoptosis without inhibiting the desired retinoblastoma cell death which is triggered by actions on the p38 signalling pathway with melphalan treatment. This KZ-41 protected REC against ICAM-1 up-regulation due to melphalan, then avoiding p38 MAPK/ICAM-1 signalling-dependent healthy cells apoptosis. By these new advances, the goal is to be able to specifically target and attack tumour cells avoiding all possible collateral damage to healthy retina, which will improve the visual function outcome of the affected eye.

In extraorbital invasion, exenteration was the primitive treatment, but nowadays the tendency is to try keeping the orbit intact by applying chemotherapy to reduce the tumour size until it allows a safe enucleation.

Discussion and conclusions

Retinoblastoma, as it is a common tumour in the infancy, has been investigated deeply till today. Despite that, the scientific community still keeps discovering new facts about it, such as those exposed in this work. This leads to the need of clearly understanding its characteristics in order to perform a proper diagnosis, avoiding the mistake of confusing it with other similar nosological entities as those described. In order to avoid this problem, imaging techniques have grown important, so has histology done –helping us discover retinoblastoma precursor–.

The treatment of this condition is currently the most changeable part of information about retinoblastoma that we dispose of. Immunology seems to have a future important role in the treatment of retinoblastoma, as it already has in other medical specialties: advances in this field will contribute helping the treatment to be more cell-specific by antibody usage, thus avoiding damage to healthy tissues and other adverse effects.

One of the main important goals of the treatment is trying to avoid enucleation and, in advanced cases, exenteration –although this last scenario is less common today–. Because of this, further studies on screening techniques for early retinoblastoma detection should be performed.

Aknowledgements

The author wants to thank Mº Dolores Pinazo-Durán (President of SIRCOVA Spain, ophthalmologist, researcher and associate professor at the University of Valencia) for her counselling during the development of this paper.

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