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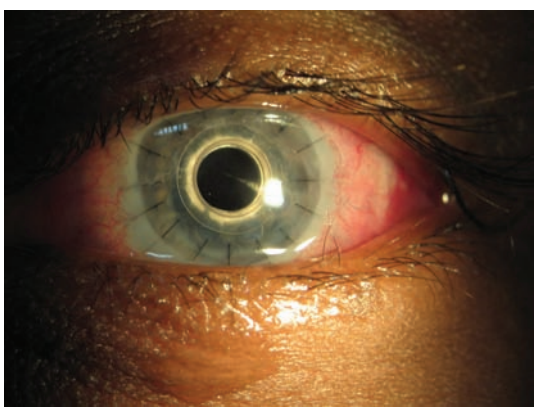

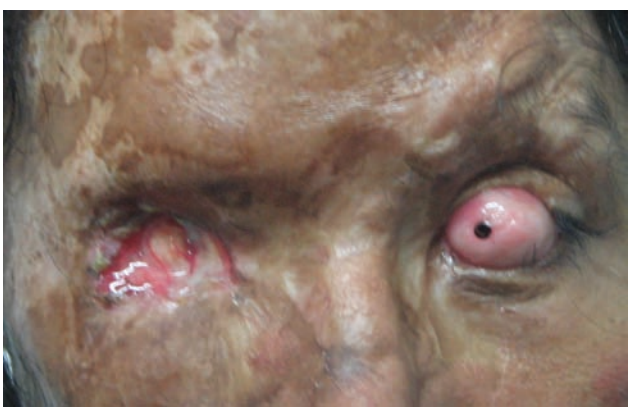

SANKARA NETHRALAYA

Editorial

Dear readers and contributors

This issue carries a perspective article on the presence of Mycobactrium tuberculosis DNA in the donor eyeball of a case of acute posterior multifocal placoid pigment epitheliopathy and practical pearls on the diagnosis and managing the orbital lymphomas. An intriguing puzzle follows. The continuing series on biostatistics presents yet another chapter. An interesting insight to the wide use of kerato prosthesis in corneal disorder concludes this issue.

Happy reading
Dr Shubhra Goel
Editor
February 2012

Mycobacterium tuberculosis DNA in the donor eyeball of a case of acute posterior multifocal placoid pigment epitheliopathy

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ABSTRACT

To report the histopathology and nested polymerase chain reaction of the detection of *Mycobacterium tuberculosis* genome from paraffin sections of the donor eye of a case of acute posterior multifocal placoid pigment epitheliopathy (APMPPE) diagnosed 35 years ago.

Method: Case report.

Design: The clinical findings, histopathology and polymerase chain reaction (PCR) of paraffin section of donor eyeball. The patient died of renal failure and her eyes were donated.

Results: Nested PCR of paraffin sections of the donor eye in this patient was positive for *M. tuberculosis*. Microscopy revealed the absence of photoreceptor segments with focal RPE atrophy and proliferation.

Conclusion: APMPPE in endemic countries could be due to *M. tuberculosis*.

Keywords: Acute posterior multifocal placoid pigment epitheliopathy, Polymerase chain reaction, *Mycobacterium tuberculosis*, *renal vasculitis*

INTRODUCTION

Acute posterior multifocal placoid pigment epitheliopathy (APMPPE) is an inflammatory chorioretinopathy and is a subset of white dot syndrome. It occurs in young women and is associated with systemic disease.[1]

CASE PRESENTATION

A 78-year-old woman had presented with blurring of vision and floaters in the right eye and headache 35 years ago. She had been diagnosed to have APMPPE about 10 years prior to her visit to our outpatient department. Examination revealed a visual acuity of 6/9 in the right eye and 6/6 in the left eye. Color vision using Ishihara's pseudoisochromatic plates was normal. Slit lamp examination was normal, and no relative afferent papillary defect was noted. Fundus examination was done by one of the authors (S.S.B.) who is a trained vitreoretinal surgeon, and healed lesions of APMPPE were identified in the right eye. Left eye was found to be normal. Automated plotting using size 3 white object was normal and did not suggest any field defects. She was a known hypertensive with blood pressure of 180/150 mmHg and was on treatment with antihypertensives. She developed renal disease subsequently and died due to renal failure. Her eyes were donated, and examination of the eyeball was done. The slides were submitted for histopathological study, immunohistochemical and immunoflourescence staining.

Fifty consecutive sections were taken, and microscopic examination revealed subretinal changes characterized by ribbon-like material, which represents artifact changes in autopsied eyes. The RPE apical processes were artifactiously detached at a few sites. Pigment granules were seen to be adherent to the detached photoreceptor outer segments in the space between retina and RPE. In one of the sections, a chorioretinal adhesion was seen, and a histopathological diagnosis of chorioretinal scar was made (Figure 1). In a few areas, outer and inner segments of photoreceptors were absent with focal areas of atrophy and focal proliferation of RPE. Sclerotic retinal vessels were observed and some of which were occluded, consistent with renal disorder changes in vasculitis. Ziel Neelsen staining for Acid fast bacilli was negative.

Molecular biological study for the detection of *Mycobacterium tuberculosis* genome was carried out on the eyeball tissue sections using two sets of primers targeting MPB64[2] gene and IS6110[3] region. DNA was extracted from six sections of the tissue which were of 10-μm thickness. DNA extraction was done using Qiagen Kit method (Germany). The nested PCRs were carried out according to the methods described by Therese et al.[2] and Wang et al.[3] for MPB64 gene and IS6110 region, respectively. Nested PCR amplification was carried out in the presence of two negative controls: one for sample extraction and another as reagent control, and DNA extracted from *M. tuberculosis* H37RV was used as a positive control for each run. The 200 bp specific PCR product was separated by electrophoresis and visualized on ultraviolet transillumination and photographed. The tissue sections were

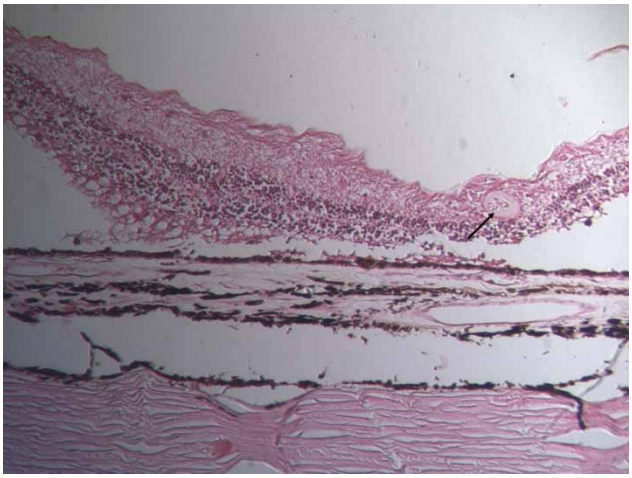

Figure 1. Photomicrograph showing focal breakdown of RPE and chorioretinal adhesion (hematoxylin and eosin × 200). Arrow: thickened retinal arteriole. Inset showing retinochoroidal adhesion (hematoxylin and eosin × 40).

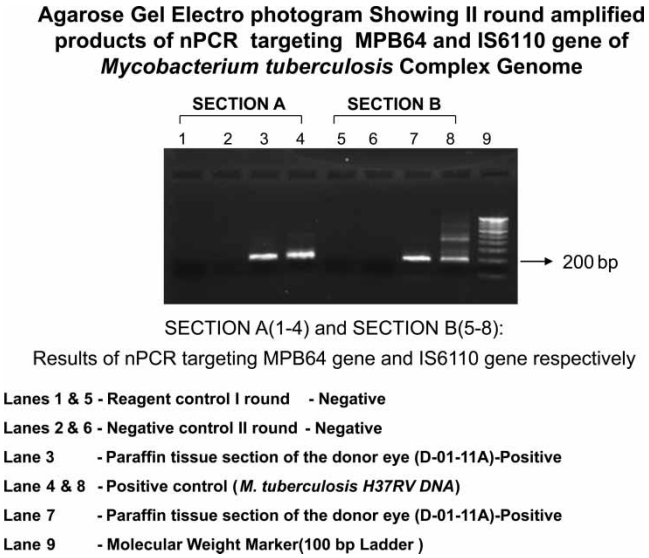

Figure 2. Agarose gel electrophotogram showing the amplified products of nPCR targeting MPB64 and IS6110 region of *M. tuberculosis* complex genome.

positive for the detection of *M. tuberculosis* DNA targeting both MPB64 gene and IS6110 region. Both nPCR targeting MPB64 gene and IS6110 region were positive with 200 bp specific amplified product, indicating the presence of *M. tuberculosis* DNA in the tissue sections (Figure 2, sections A and B).

Immunoflourescence study revealed the presence of photoreceptor cells in a ribbon-like pattern and internal photoreceptor retinal binding protein was present in these structures.

DISCUSSION

Although largely believed to be autoimmune in origin, speculations exist to the possible etiology of APMPPE.[4] The presence of *M. tuberculosis* authenticates the fact that infections such as tuberculosis certainly need to be considered as a causative agent or a trigger. Serpiginous choroidopathy has been found to have an association with *M. tuberculosis* and localization of this microbe in the retinal pigment epithelium (RPE) in eyes with retinal and uveal involvement has been reported by Rao et al.[6] Gass suggested an acute cellular response of the RPE and choroid to an unknown, possibly viral agent in triggering the inflammation in APMPPE. The nephropathy that eventually developed in this patient proves or purports the pathogenesis based on delayed hypersensitivity reaction reflected as microscopic renal vasculitis culminating in nephropathy and renal failure. Dhurni et al. have suggested that identifiable systemic disorders such as nephritis, cerebral angitis and erythema nodosum[6] due to vasculitis are the underlying common etiology of such inflammations. The coexistence of retinal vasculitis[7] causing APMPPE and renal disease suggestive of renal vasculitis in this patient could be due to vascular disturbance, but further complicated by the detection of *M. tuberculosis* genome which could itself be the cause of vasculitis. Histopathological evidence of APMPPE in a donor eyeball has not been reported in the past but in this case histopathological, microbiological and molecular biological methods have been adopted to confirm the same in this patient. These tissue sections thus suggest that the evidence of *M. tuberculosis* in association with APMPPE as evidenced by nested PCR[8] could be an etiology for this disease which is considered autoimmune. In conclusion, in a country which abounds with tuberculosis, a likely etiology of tuberculosis in APMPPE needs to be considered.

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Intraocular lymphoma: a few pearls

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DEFINITION

The term 'intraocular lymphoma' (IOL) describes an extranodal lymphoid malignancy arising from different parts within the eye and comprises of the following types:

1. Primary vitreo-retinal: the most common type, high-grade B-cell with worst prognosis.
2. Primary choroidal: low-grade B-cell similar to extranodal marginal zone lymphomas.
3. Primary iridal and ciliary body: considered very rare, either from T- or B-cell and have poor prognosis.
4. Secondary choroidal: metastasis from systemic lymphomas.

EPIDEMIOLOGY

PIOL was initially described as a reticulum cell sarcoma in 1951. Vitreo-retinal lymphoma is now considered a subset of primary central nervous system (CNS) lymphoma as it is concurrently found to be present in 15–20% cases and nearly 60–80% of PIOL ultimately develop CNS involvement. Also the behaviour of the tumour and survival of these patients have been found similar to PCNSL. CNS involvement has been theorized to be due to passage of malignant cells through the optic nerve or seeding through veins or as a result of common integrin expression as a target in either tissue. It forms a small percentage of the intracranial tumours with an incidence of 1 in 100,000 which has slightly increased after its association with AIDS.

CLINICAL PICTURE

It usually affects either gender in their fifth decade and is bilateral in around 80%. It is a frequently misdiagnosed entity and is thus considered a masquerader. The patient presents with floaters and blurring of vision which can rarely be associated with pain and redness. It thus initially gets treated as uveitis with minimal response to steroids. A high index of suspicion is required especially in such age group in cases with persistent vitritis. Anterior chamber might show minimal reaction with keratic precipitates. Vitreous picture is a hallmark though not very specific with large cellular clumps, strands or sheets with a hazy view of the fundus. They can develop subretinal infiltrates causing retinal pigment epithelial detachments which are more pathognomonic. Sometimes they can develop large creamy infiltrates with vasculitis and haemorrhages mimicking retinitis. Rarely iris infiltrates or isolated subretinal mass may be noted.

Cognitive or behavioural changes such as aphasia, paresis and seizures are early symptoms indicating CNS lesions.

DIFFERENTIALS

1. Posterior uveitis
2. Endophthalmitis
3. Retinitis
4. Subretinal abscess
5. Intermediate uveitis.

INVESTIGATIONS

1. FFA: Lesions appear as punctate hypofluorescent lesions in early phase which fluoresce in late phases. Absence of perivascular staining and cystoid macular oedema helps us to differentiate it from uveitic entities.
2. OCT: It can pick up smallest of lesions and confirm the level of lesions as sub-RPE, thus helping us to rule out retinal causes.
3. Ultrasound: It can be helpful in the cases of dense vitreous haze to pick up low-to-moderate reflective membrane echoes within the vitreous. Sometimes, subretinal mass lesion can be associated and give a clue to an eye harbouring a tumour.
4. MRI: It confirms the diagnosis and helps staging of disease. It shows a characteristic pattern of multifocal periventricular lesions which homogenously enhance with contrast. AIDS-associated cases, on the other hand, show ring enhancement similar to toxoplasma lesions.
5. Lumbar puncture: Lymphoma cells disseminate through the CSF and can be picked up on cytology which is diagnostic of CNS lymphoma.
6. Diagnostic vitrectomy: It is essential to get a pathological diagnosis prior to starting the laborious treatment protocol of lymphoma especially when MRI is not conclusive or CSF is negative. The undiluted vitreous sample needs to be immediately transported and examined by an experienced pathologist as it usually has many necrotic cells, T-cells or fibrin which can confuse the picture. If the vitreous sample is negative, a biopsy from the chorioretinal lesion can be taken for the specimen.

Cytology on an electron microscope shows these cells to be large lymphoid cells with scanty basophilic cytoplasm,

hypersegmented nuclei and prominent nucleoli. They lack cellular cohesion and cytoplasmic processes unlike metastatic lesions of the choroid.

Immunohistochemistry or flow cytometry helps detect the phenotype of the cell through B-cell markers like CD-19 or -20.

Molecular analysis like demonstration of IgH gene rearrangement is thought to be a strong evidence of this tumour and is considered responsible for its unique monoclonality.

Finally, these tumour cells secrete interleukins such as IL-10 which can be measured in the vitreous or CSF and a ratio of IL-10 to IL-6 of more than 1 has been found significant for its diagnosis.

TREATMENT

1. *Systemic chemotherapy*: high-dose methotrexate (8 g/m^2) intravenously is the mainstay of treatment for both CNS or ocular lymphoma. Cytosine arabinoside such as methotrexate has better intraocular penetration and can be used in cases with extensive involvement or those refractory to single drug. In spite of this, the tumour shows only partial response as the intraocular levels are 100-fold lower than the serum with high rate of recurrence.
2. *Intrathecal MTX*: it can be given as an adjuvant with intravitreal injections to have specific target action and has shown similar efficacy for either tumour–brain or eye. Dose is around 6–12 mg.
3. *Radiotherapy*: lymphoma cells are sensitive to radiation and thus it was the first-line treatment modality until the efficacy of methotrexate was known. It can either be isolated to the eye or along with whole-brain radiation for concurrent CNS lymphoma. It can also be used as an adjunct with chemotherapy.
4. Intravitreal injections
   (a) *Methotrexate*: local adjunctive therapy with intravitreal methotrexate has been well experimented and is more efficacious than systemic, especially in cases with only ocular involvement. It is given as 400 μg in 0.1 ml with an half-life of 48–72 h. The protocol followed is of two injections per week for a month as induction phase, weekly consolidation injections for a month or two, and subsequently a maintenance phase of monthly injections to complete 1 year. Clinical remission is usually reached after an average of 6.4 injections, with 95% of the eyes needing 13 injections or less to be cleared of malignant cells. The only complications noted have been corneal epitheliopathy which could disappear after stopping the drug and progression of cataract. The emerging problem is of drug resistance which will be seen as a non-responsive eye and needs a change of the drug.
   (b) *Rituximab*: It is a humanized monoclonal antibody inhibiting CD-20, a selective marker of B-cells, and has been approved by FDA in 1997 for treatment of NHL's of B-cell origin. CD-20 is a surface antigen which is neither shed nor internalized and is not found unbound in circulation and thus forms an ideal target for selective action. The vast majority of vitreo-retinal lymphoma and PCNSL are B-cells in origin and express CD-20 antigen, thus making rituximab an ideal drug for intraocular as well as intrathecal adjuvant therapy. Its penetration through the blood–brain barrier is poor and hence intravitreal injection is the preferred route of administration. The molecular weight of rituximab is similar to bevacizumab (145 kDa) which has been safely used within the eye and also it has been experimentally found to penetrate all layers of the retina thus expected to act equally well on the deeper infiltrates. Doses of intravitreal injections of 1 mg in 0.1 ml have been found efficacious and well tolerated. The half-life has been found to be 4.7 days and hence biweekly injections are recommended. There is good evidence of the efficacy of this drug in fresh and recurrent cases as well as good tolerance in the eye but due to lack of long-term studies, the protocol for dosage, duration and number of injections is yet inconclusive. The exact mechanism of how it destroys the B-cells is not yet understood. Thus, its use is still reserved for cases with methotrexate resistance or as an adjuvant therapy.
5. *Enucleation*: it maybe reserved for those with a painful blind eye secondary to the malignancy although they can still develop CNS lymphoma during the course of disease and need to be followed up for the same.
6. *Experimental*: membrane Fas-ligands activate innate immunity to eliminate the immune privilege of the eye. Immunotoxin HA22 is a recombinant protein with good target specificity. Other monoclonal antibodies such as daclizumab, alemtazumab or efalizumab also seem to have potential therapeutic value.

CONCLUSION

IOL is a masquerader and needs a high degree of suspicion in elderly uveitic cases. Diagnosis and staging of the disease can help in individualizing the management protocol. Since IOL was first recognized, its treatment have gradually evolved from enucleation through radiotherapy to chemotherapy. The survival is usually 2–5 years and death is due to CNS involvement. The results of biological therapy are encouraging, with no significant adverse effects reported. It seems that in the future, treatment of ocular lymphoma will rely more and more on local biological methods of treatment such as rituximab and other drugs that are currently at the basic research level.

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Puzzle

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INTRODUCTION TO PUZZLE

A 10-year-old boy presented with painless, progressive cystic swelling in supero-medial bulbar conjunctiva with positive transillumination of the left eye for 1 month (Figure 1). There was no history of ocular trauma, pain, decrease in vision, squint, diplopia or previous surgery. The rest of the examination of both the eyes was within normal limits.

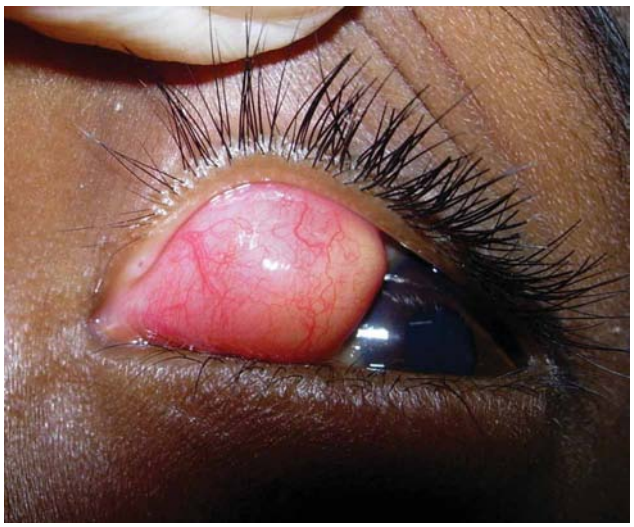

Figure 1

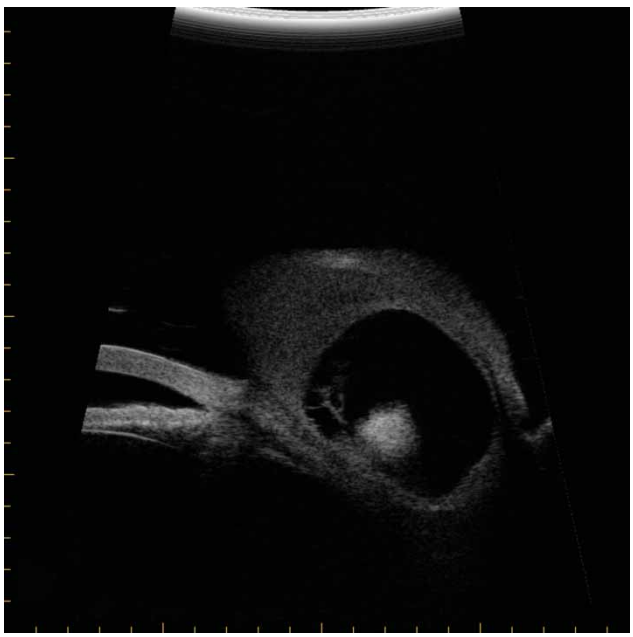

Figure 2

What is the Clinical Condition? What investigation has been performed?

WHAT IS YOUR DIAGNOSIS?

Answer available at page 9.

Introduction to Biostatistics-10

Part II. Sample Size

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INTRODUCTION

In the last issue, we dealt with various parameters that influence the sample size calculation required for a study. Now, in this issue, we will review the methods for its calculation in some simple, yet common cases. The number of individuals to include in a research study, the sample size of the study, is an important consideration in the design of many clinical studies. Sample size is closely tied to statistical power, which is the ability of a study to enable detection of a statistically significant difference when there truly is one.

A number of formulae have been devised for determining the sample size depending upon the availability of information. A few methods are given below.

DIFFERENT METHODS

1. Suppose if the study involves collection of data which is qualitative in nature, then the following formula can be adopted:

$$n = \frac{4pq}{L^2},$$

where p is the proportion of the sample, L the allowable error which should not exceed 10 or 20%.

Example: Death rate of measles in children is found to be 10%. What should be the size of the sample needed to find the death rate of measles?

Solution:

Step 1: Given $p = 10$, $q = 90$ and $L = 1$.

Step 2: Formula

$$n = \frac{4pq}{L^2},$$

where p is the proportion of the sample, L the allowable error which should not exceed 10 or 20% of the positive charter.

Step 3: Calculation

$L = 10\%$ of $p = 10 * 10/100 = 1$.

Therefore, $n = \dfrac{4 * 10 * 90}{1 * 1} = 3600.$

2. If the study involves two variables which are qualitative in nature, then one should use the below formula:

$$N = \frac{\left(Z_{\alpha/2}\sqrt{2\pi(1-\pi)} + Z_{\beta}\sqrt{\pi_1(1-\pi_1)+\pi_2(1-\pi_2)}\right)^2}{(\pi_1-\pi_2)^2},$$

where π_1 and π_2 are the anticipated proportions in the two populations, $\pi = (\pi_1 + \pi_2)/2$.

Example: A prospective study to observe mortality and morbidity pattern related to hyaline membrane disease in preterm babies with and without dexamethasone treatment is proposed. What is the required sample size if the anticipated survival is 80% in the treated group and 50% in the untreated group? Let us assume that the investigator wants a 5% level of significance and statistical power of 90%.

Step 1: Given $\pi_1 = 0.80$ and $\pi_2 = 0.50$, that give $\pi = 0.65$.

For 95% confidence and 90% power, the values of $Z_{\alpha/2}$ and Z_{β} are 1.96 and 1.28, respectively.

Step 2: Formula

$$N = \frac{\left(Z_{\alpha/2}\sqrt{2\pi(1-\pi)} + Z_{\beta}\sqrt{\pi_1(1-\pi_1)+\pi_2(1-\pi_2)}\right)^2}{(\pi_1-\pi_2)^2},$$

where π_1 and π_2 are the anticipated proportions in the two populations, $\pi = (\pi_1 + \pi_2)/2$.

Step 3: Calculation

Here $\pi_1 = 0.80$ and $\pi_2 = 0.50$, $\pi = 0.65$, $Z_{\alpha/2} = 1.96$, $Z_{\beta} = 1.28$.

Therefore,

$$n = \frac{\left(1.96\sqrt{2*0.65(1-0.65)} + 1.28\sqrt{0.8(1-0.8)+0.5(1-0.5)}\right)^2}{(0.8-0.5)^2}$$

Here, $n = 50.88$.

3. For a study with quantitative data collection, the following formula can be used:

$$n = \frac{Z^2_{\alpha/2}}{L^2}\sigma^2,$$

where $Z^2_{\alpha/2}$ is the table value and L the specified precision of the estimate on either side of the mean.

Example: Earlier experiment by Cobalt therapy for a tumor showed reduction. The SD of weights of earlier experiment is 6.1 g. We wish to be 95% confident that we will not deviate by more than 0.5 g in estimating the true mean weight of tumor organ. What is the sample size?

Solution:

Step 1: Given standard deviation = 6.1, $L = 0.5$, confidence level = 95%.

Step 2: Formulae

$$n = \frac{Z^2_{\alpha/2}}{L^2}\sigma^2,$$

where $Z^2_{\alpha/2}$ is the table value and L the specified precision of the estimate on either side of the mean. Here σ is the population SD (can be estimated from a pilot study).

Step 3: Calculation

Here $\sigma = 6.1$, $L = 0.5$ and $Z_{\alpha/2} = 1.96$.

Therefore,

$$n = \frac{1.96^2}{0.5^2}(6.1)^2 = 571.78 = 572.$$

4. Similarly, if the study has two variables and data are quantitative in nature, then one should use the below formula:

$$n = \frac{Z^2_{\alpha/2}}{L^2}(\sigma_1^2 + \sigma_2^2),$$

where $Z^2_{\alpha/2}$ is the table value and L the specified precision of the estimated difference on either side of the mean difference. Here σ_1 and σ_2 are the population SD of the two populations (can be estimated from a pilot study).

Example: The mean (SD) cholesterol level in Group A and Group B are 4.5 (0.90) mmol/L and 4.2 (0.62) mmol/L, respectively. The mean difference in cholesterol between the groups is 0.22 mmol/L. Determine the sample size at 95% confidence interval.

Solution:

Step 1: Given

Groups	Mean	SD
A	4.5	0.90
B	4.2	0.62

Step 2: Formula

$$n = \frac{Z^2_{\alpha/2}}{L^2}(\sigma_1^2 + \sigma_2^2),$$

where $Z^2_{\alpha/2}$ is the table value and L the specified precision of the estimated difference on either side of the mean difference. Here σ_1 and σ_2 are the population SD of the two populations (can be estimated from a pilot study).

Stpe 3: Calculation

$L = 0.22$ and $Z_{\alpha/2} = 1.96$.

Therefore, the sample size is

$$n = \frac{1.96^2}{0.22^2}(0.90^2 + 0.62^2) = 94.8\text{or}95.$$

SUMMARY

A study that is insufficiently precise or lacks the power to reject a false null hypothesis is a waste of time and money. A study that collects too much data is also wasteful. Therefore, studies must be adequately powered to achieve their aims, and appropriate sample size calculations should be carried out at the design stage of any study. Attention to sample size will hopefully result in a more meaningful study whose results will eventually receive a high priority for publication.

The next issue will deal with the confidence interval and its importance in a study.

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ANSWER FOR PUZZLE

A 10-year-old boy presented with painless, progressive cystic swelling in supero-medial bulbar conjunctiva with positive transillumination of the left eye for 1 month (Figure 1). There was no history of ocular trauma, pain, decrease in vision, squint, diplopia or previous surgery. The rest of the examination of both the eyes was within normal limits.

DISCUSSION

Cysticercosis is infestation with the larval form (cysticercus cellulosae) of the pork tapeworm *Taenia solium*. The condition is endemic in Africa, southeast Asia, Central and South America and India.[1] Diagnosis of cysticercosis is usually made on history, clinical examination, radiological (USG, CT, MRI scan) investigations and by histopathology, when it can be excised. CT scans not only confirm the diagnosis, but also helps to rule out associated neurocysticercosis. The heterogeneity of clinical presentations of cysticercosis can present a diagnostic dilemma to the clinician. While the most common site of localization reported in Western studies is in the posterior segment,[2] in the Indian literature the ocular adnexa are the most common site,[3] constituting 63% of all cases.[4] Extra-ocular cysticercosis can present as cystic lesions in the subconjunctival area, with or without involvement of extra-ocular muscles. UBM is a useful adjunctive technique to diagnose subconjunctival cysticercosis as it was seen that it could be picked up excellently on imaging.

UBM is an established and accessible imaging modality of lesions of anterior segment. High-frequency UBM provides high-resolution *in vivo* imaging of the anterior segment in a noninvasive fashion enabling visualization of structures previously hidden from clinical observation. The technology for UBM, originally developed by Pavlin, Sherar and Foster, is based on 50- to 100-MHz transducers incorporated into a B-mode clinical scanner.[5]

Higher-frequency transducers provide finer resolution of more superficial structures, whereas lower-frequency transducers provide greater depth of penetration with less resolution. The commercially available units operate at 50 MHz and provide lateral and axial physical resolutions of approximately 50 and 25 μm, respectively. Tissue penetration is approximately 4–5 mm.

Treatment of cysticercosis is initiated by oral steroids along with cysticidal drugs to control the inflammation elicited by the dying cyst. The therapeutic efficacy of oral albendazole for extra-ocular cysticercosis has been reported to be good.[6]

For non-resolving or residual subconjunctival cysts, surgical excision remains the mode of treatment. To conclude, UBM is a useful addition in non-invasive armamentarium of diagnostic modalities in suspected cases of subconjunctival cysticercosis in establishing accurate diagnosis and enabling us to initiate early treatment.

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The authors gratefully acknowledged Dr. Aditya Neog for his help.

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Keratoprosthesis for corneal disorders

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INTRODUCTION

Bilateral end-stage ocular surface disorders continue to remain a challenge to ophthalmologists. Apart from end-stage surface disorders, patients with multiple failed grafts, eyes with retained silicone oil following vitreoretinal procedures, high-risk pediatric grafts form a subset wherein conventional penetrating keratoplasty is likely to fail. With very limited options for management, keratoprosthesis forms the last resort for visual rehabilitation in these eyes.

The earlier models of keratoprosthesis were associated with a high risk of prosthesis extrusion and severe intraocular inflammation. With major advances in the field of material designs, surgical techniques and modifications in postoperative regimen, new models of keratoprosthesis (Kpros) have changed the overall outcomes, with expanding indications and better acceptance of these devices.

CLASSIFICATION

Keratoprostheses can be broadly classified into:

1. biocompatible – Dohlman–Duane (Boston Kpro types 1 and 2);
2. biointegrated – Pintucci keratoprosthesis;
3. biological – MOOKP (modified osteo-odonto keratoprosthesis).

The use of a particular type of keratoprosthesis depends on the underlying etiology and associated patient factors. Among the various types of prostheses available today, these can be termed as, at present, the most commonly used/reported devices.

IDEAL KERATOPROSTHESIS

It should have properties that surpass the quality of natural cornea. These include:

– good optical quality with reduced aberrations and improved field,
– excellent biointegration,
– resistance to infections,
– good drug penetration,
– reliable measurement of intraocular pressure and
– long lasting.

INDICATIONS

Keratoprosthesis is usually performed in one eye, preferably the eye with poor vision, in patients with bilateral visual handicap not correctable by any other measures. The other eye termed as the "spare eye" is reserved for future surgery, in the case of any deterioration in the operated eye.

It is the general recommendation that the visual acuity in the better eye is less than 1/20 for the procedure to be performed or up to the range of 2/10, provided the patient understands the potential risks and benefits of the surgery.

These conditions include:

1. multiple graft failures,
2. Stevens Johnson syndrome,
3. ocular cicatricial pemphigoid (advanced),
4. chemical or physical injuries and
5. total limbal stem-cell deficiency with surface keratinization.

The Boston keratoprosthesis type 1 is chosen for moist eyes with a good blink mechanism and absence of underlying immunological condition for better results. The MOOKP, Boston type 2 and the Pintucci keratoprosthesis are the prosthesis of choice for severe surface disorders with keratinization with an underlying immune etiology.

BOSTON KERATOPROSTHESIS

Design

The Boston Kpro is available in type I and type II formats. The type I design is used much more frequently than the type II which is reserved for severe end-stage dry eye conditions and is similar to the type I except it has a 2 mm anterior nub designed to penetrate through a tarsorrhaphy. The type I format will be discussed here as it is more commonly used.

The Boston type I KPro is designed like a collar button with a front plate, a stem and a back plate. It is manufactured from medical-grade polymethylmethacrylate (PMMA). During surgery, it is usually incorporated into a corneal transplant, which is then sutured into the patient's cornea in a standard manner.

The device comes in three parts: the front plate with the stem, the back plate and a locking ring. Since 2003, a locking ring that can be snapped into a groove on the stem behind the back plate has been added to prevent possible unscrewing of the plate *in vivo*. In 2007, a threadless design was introduced which simplified the assembly and produced less damage to the donor graft when the device was assembled during the surgical procedure.

– The front part consists of a front plate with a diameter of 5 or 6 mm.
– The back plate is substantially larger than the front plate and has a diameter of 8.5 mm. There are 16 holes with a diameter of 1.17 mm. The holes are necessary to allow aqueous access to the overlying cornea for nutrition (3). The combined surface area of the holes is about 18 mm^2. Another smaller back plate is also available and is useful in small children. It has a diameter of 7.0 mm, with eight holes each with a diameter of 1.3 mm.
– Just behind the back plate area is a groove for the locking ring to snap into. The locking ring is made up of medical grade titanium and it has a radial cut to facilitate snapping it into place.

The total antero-posterior height of the assembled device is about 3 mm, depending on the steepness of the curvature of the anterior surface.

Technique

1. A corneal graft (usually 8.5 mm in diameter) is prepared, and a central 3.0 mm hole is trephined with a dermatological punch.
2. For stability, the front part of the KPro is placed upside down on a patch of adhesive, which accompanies the device.
3. The graft with the central hole is slid over the KPro stem. An accompanying small pin, hollowed in one end, can be used to gently push the graft down to the front plate.
4. Viscoelastics are applied to the posterior surface of the graft.

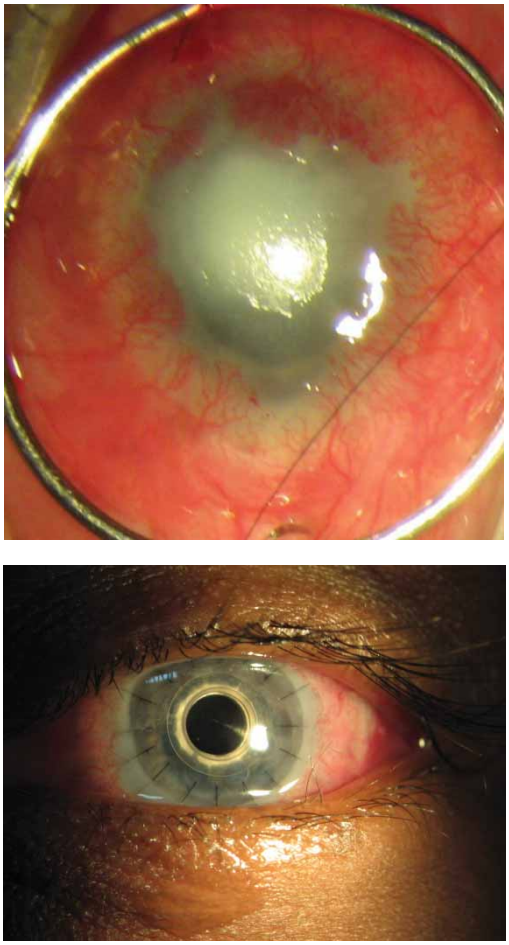

Figure 1. (a) Bilateral chemical injury following tenonplasty and large-diameter lamellar keratoplasty in acute phase. (b) 3 years after Boston type 1 Kpro with BCVA of 6/6.

5. The back plate is placed on the stem without any rotating movement.
6. The locking ring is pressed onto the lead stem with a finger. The hollowed pin is used to press the ring firmly down into the groove, usually with an audible snap. The assembly is inspected for correct position of the ring.
7. The graft–prosthesis combination is removed from the adhesive and stored in the eye bank solution while the patient's eye is prepared.
8. A trephine with 0.5 mm smaller diameter than the prepared graft (usually 8.0 mm) is used for the patient's cornea. Any required cataract extraction, vitrectomy, iris manipulation, etc., are performed before the graft–prosthesis combination is transferred to the corneal opening and sutured in place. Present preference is to use 12 or 16 10-0 or 9-0 nylon sutures, knots buried.

Finally, a soft contact lens, usually a Kontur lens (accompanies the device), 16.0 mm in diameter and 9.8 mm in curve, plano, is placed on the eye as the last step of the operation (Figure 1a and b).

Postoperative regimen constitutes long-term combination of fluoroquinolone and reconstituted vancomycin eye drops along with topical lubricants and tapering doses of steroid drops.

MODIFIED OSTEO-ODONTO KERATOPROSTHESIS (MOOKP)

Design/rationale

The MOOKP principle hinges on the use of a single rooted tooth as a biological carrier for a PMMA cylinder. This complex, the osteo-odonto acrylic lamina, constitutes the dentine of the tooth along with the surrounding alveolar bone (maxillary or mandibular) with the dento-alveolar ligament in between. The periosteum covering the bone is secured to the cornea and sclera by means of a fibrovascular covering, which is developed in the period when it is placed in a subcutaneous pouch in the cheek. The mucosa, harvested from the cheek, lines the surface over the OOAL. The PMMA cylinder is glued through a central opening within the confines of the avascular dentine in an effort not to violate the continuity of the dento-alveolar ligament which forms an integral and crucial part of the lamina. The ligament, in fact, not only maintains the vitality of the dentine by transmitting nutrients to it and by alveolar bone exchange, but also attracts the epithelium of the buccal mucosa towards itself, as happens with the teeth, attaching itself to it as if it were a real epithelial tissue, thus preventing bacteria from infecting the lamina and subsequently propagating to the eye. This epithelial seal also decreases the formation of a retroprosthetic membrane and subsequent extrusion of the cylinder or the OOAL.

The canine forms the most ideal single rooted tooth of choice by virtue of its broad width and girth. The incisor and premolars can also be used in cases of unsuitable canine.

The width of the dentine determines the diameter of the cylinder that can be implanted, which in turn determines the field of vision.

Technique

The procedure is performed in the following stages.

Stage 1. This is further divided into three stages:

– *Stage 1A*: Preparation of the globe for MOOKP procedure
  This involves total iris removal, and intracapsular cataract extraction with anterior vitrectomy. A tectonic penetrating keratoplasty is performed in cases with corneal thinning or perforation. Intraoperative fundus evaluation allows for the assessment of possible visual potential based on posterior segment findings and aids deciding about proceeding with the further stages of the procedure.
– *Stage 1B*: Mucus membrane grafting
  In this stage, the cheek mucosa measuring 3 cm is harvested. A 360° peritomy is done and rectii are tagged. This is followed by a Bowmanectomy and draping of the oral mucosa on to the ocular surface, securing it to the rectii.
– *Stage 1C*: Preparation of OOAL
  The maxillary canine is chosen if appropriate. The tooth is removed into with the surrounding bone and fashioned into a lamina measuring approximately 15 mm × 10 mm × 3.5 mm. A central hole in the dentine is drilled, measured according to the diameter of the cylinder, which is secured using acrylic resin. This lamina is then placed in a subcutaneous pouch prepared in the contralateral cheek for it to develop its fibrovascular covering.

Stage 2. Implantation of OOAL into the eye after 2–3 months.

Stage 2 involves removal of the lamina from the pouch followed by reflecting the mucosa from the ocular surface. The centre of the cornea is marked and trephined to allow the cylinder to be placed inside. The mucosa is reflected back and a central opening made for the cylinder to project out.

Cylinder details

The PMMA cylinder is usually 8.5-mm long with the anterior length being 6 mm and the posterior length being 2.5 mm. The anterior and posterior segments are differentiated by a thickness difference of 0.4 mm, creating a ledge abutting against the dentine surface of the lamina. The biometry of the cylinder is determined by the variable posterior radius of curvature which in turn is determined by the axial length of the eye. The anterior radius of curvature remains fixed at 16 or 20 mm. The diameter of the cylinder usually ranges from 3.5 to 4.5 mm (Fig 2a, final appearance of the eye and 2b).

Figure 2. (a) 10 days after chemical injury. OD, phthisical OS-tenonplasty with skin graft with tarsorrhaphy followed by annular perilimbal scleral patch graft. (b) 4 years after MOOKP with BCVA of 6/24.

PINTUCCI KERATOPROSTHESIS

Design

Technique

The technique is very similar to the MOOKP except that the tooth is not required to be harvested and the Dacron mesh surrounding the PMMA cylinder gets covered with fibrovascular tissue in the subcutaneous pouch.

PREOPERATIVE EVALUATION

Preoperative assessment is aimed at determining the potential visual acuity as well as the eligibility of the patient for undergoing the procedure.

Ophthalmic evaluation

History taking

– *Age at onset of visual loss*. Loss of vision prior to the age of 4 or 5 years may indicate poor prognosis due to dense amblyopia.
– Previous intraocular surgeries.

Visual acuity

Presence of light perception is mandatory to undergo the procedure. Inaccurate projection of rays can be indicative of nerve damage, especially secondary to glaucoma and it is necessary to carry out an electro-physiological examination (VEP). The eye with poor visual acuity is chosen for the procedure, unless contraindicated, particularly if the better eye has partial self-ambulatory vision.

Slit lamp evaluation

It helps assess the anterior segment details in terms of corneal thickness, anterior chamber depth, presence of peripheral anterior synechiae (PAS) and lens status.

Digital IOP evaluation

Finger tension is probably the most reliable method of evaluation of intraocular pressure in these eyes. Glaucoma is more common preoperatively in chemical injuries and needs to be controlled and it must be decided as to what is to be done prior to the keratoprosthesis procedure.

Ultrasound examination

A detail of posterior segment integrity is obtained from the ultrasound examination. The optic nerve head status is also determined. The presence of retinal detachment warrants management of the same with assessment of potential visual acuity, based on optic nerve status, and the retinal health to decide on proceeding with the Kpro surgery. A combined A scan determines the axial length of the eye which helps in calculating the diopotric power of the prosthesis to be implanted.

Silicone oil-filled eyes

Presence of silicone oil in the eye does not deter the procedure and 5000 centistokes silicone oil is preferred if oil removal is not contemplated. In oil-filled eyes, the axial length of the fellow eye is considered; if not, a standard axial length of 23 or 24 mm is taken for calculating the prosthesis power.

Ultrasound biomicroscopy

This test is indicated only in cases of inability to study the anterior segment details on slit lamp due to corneal opacity in order to image angle structures and the presence of PAS.

Electrodiagnostic tests

Only in cases of doubtful light perception, electroretinogram and visual evoked responses can aid in the decision regarding the patient being a candidate for the procedure or not.

PREOPERATIVE EVALUATION SPECIFIC FOR BOSTON KPRO

It is important to ensure a good blink mechanism, adequate lid closure and eye wetting. It is equally important to rule out the presence of any underlying immune etiology in these patients. Preoperative counseling should emphasize on the need for strict compliance with medications, maintenance of hygienic conditions and the need for regular follow-up. This is due to the risk of infection, especially in our country with a tropical climate.

PREOPERATIVE EVALUATION SPECIFIC FOR MOOKP PINTUCCI KPRO

Oral assessment

Mucosa

Tobacco chewing and sometimes smoking can cause significant mucosal damage leading to mucosa-related complications. Hence, it is imperative to stop these well in advance (minimum 3 months) before harvesting the mucosa. The primary mucosal insult in cases of Stevens Johnson syndrome can alter the normal pathophysiology, thus predisposing to mucosal ulcerations and necrosis in the postoperative period.

Periodontitis

This factor has to be identified, graded and treated accordingly in order to avoid persistent inflammation in the form of osteitis after placing the lamina in the eye, which, in turn, could lead to recurrent vitreous inflammation.

Tooth

The canines are assessed for the presence or absence of any fillings, caries and the amount of exposed crown. The teeth,

adjacent to the chosen canine, have to be free of any focus of infection.

Mouth opening

It is extremely important to assess the degree of mouth opening, especially in cases of chemical injuries involving contractures of the cheek skin, not only for assessing the ease of oral endotracheal intubation, but also for the adequacy of exposure of the cheek mucosa for its harvesting. In cases of severe cheek contracture with restricted mouth opening, releasing the restriction will need to be done primarily with nasal endotracheal intubation.

Orthopantomography

Orthopantomography further helps in the assessment and selection of a suitable tooth.

Spiral computed tomography (CT) scan

This investigation gives detailed information regarding the size of the canine (length, width and thickness), the proximity of the apex to the maxillary sinus/mental foramen, the density of bone surrounding the dentine and the interdental space.

GENERAL HEALTH ASSESSMENT

The overall systemic health of the patient should permit for multiple general anesthesia procedures.

Blood grouping and cross-matching with blood being reserved during harvesting the tooth and mucosa for emergency blood transfusion in the case of blood loss is mandatory.

PSYCHOLOGICAL COUNSELING

Offering psychological counseling to patients and their family is important in the patients' acceptance of the procedure in terms of its outcomes, both visual and cosmetic. To this effect, discussions are held with family members along with photographs of operated patients. If possible, patients and family members are allowed to interact with operated patients to better understand the process from the patient's point of view.

INFORMED CONSENT

An informed consent is obtained from the patient and the next of kin following a complete assessment of the patients' fitness and willingness to accept all the pros and cons of the procedure.

COMPLICATIONS

Intraoperative

Stage 1A Boston Kpro

1. Corneal perforation – could be pre- or intraoperative and requires tectonic penetrating keratoplasty.
2. Scleral rupture or thinning can be noted in eyes with chemical injury with or without preexisting glaucoma. A scleral patch graft provides the required tectonic support, especially if the area of thinning involves the perilimbal sclera where prosthesis-securing sutures need to be placed.

Stage 1B

1. Damage to parotid duct.

Stage 1C

1. Oronasal fistula,
2. Mandibular fracture,
3. Damage to adjacent teeth/oral structures.

These problems are treated in conjunction with the orofacial maxillary surgeon and may at times require further surgical intervention.

Postoperative

Immediate

Specific to MOOKP/Pintucci Kpro

1. Trophic mucosal alteration,
2. Infection of lamina in pocket,
3. Absorption of lamina in pocket.

General

1. Rise in IOP,
2. Expulsive haemorrhage,
3. Choroidal detachment,
4. Retinal detachment.

Late

1. Glaucoma,
2. Choroidal detachment,
3. Retinal detachment,
4. Endophthalmitis,
5. Retroprosthetic membrane,
6. Cylinder instability,
7. Expulsion of optic cylinder/prosthesis.

CONCLUSION

Keratoprosthesis has definitely changed the outcome in a select group of patients with corneal disorders and is here to stay. However, the success of these procedures depends on multiple factors with the need for coordination among specialists in all fields of ophthalmology, anesthetists, radiologists and dentists. The need to address comorbid conditions such as glaucoma cannot be overemphasized with alloplastic shunt forming the mainstay of managing glaucoma in patients with uncontrolled IOP. Retinal detachment prior to or following keratoprosthesis needs to be tackled efficiently and silicone oil may be needed to be retained in the eye for long periods. MOOKP can be associated with mucosa-related complications and issues of tooth resorption requiring mucus membrane revisions or at times tooth replacement. Despite this, since the visual results are good and with no other possible treatment for visual rehabilitation, keratoprosthesis surgery is worth the time and effort it takes. However, the characteristics of an ideal keratoprosthesis need to be woven into the keratoprosthesis design to allow especially better monitoring of intraocular pressure and drug penetration along with an enlarged field of vision and enhanced cosmesis.

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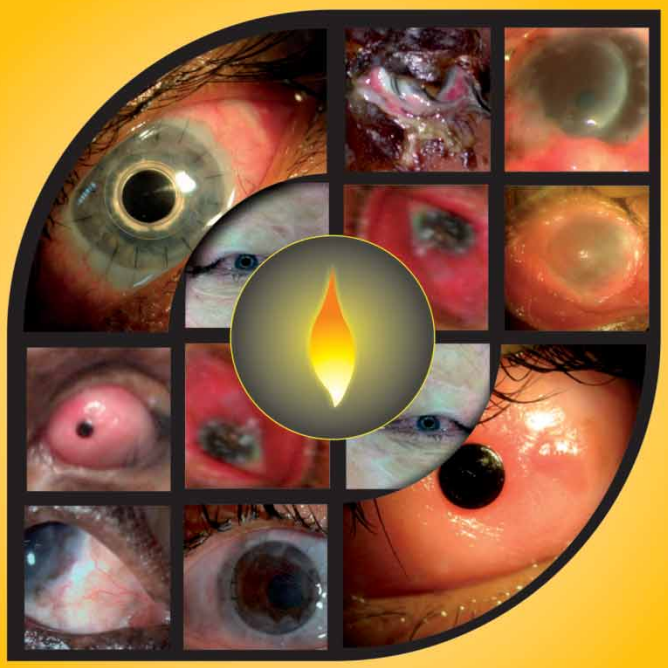

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