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Contents

Editorial
Eye Research in India
Sambuddha Ghosh

Review Article
Pathogenesis and Management of Diabetic Macular Edema
Atul Kumar, Sangeeta Roy, Subijay Sinha

Original Article
Xeno-Free Autologous Cultivated Oral Mucosal Epithelial Transplantation for Bilateral Ocular Surface Burns: Clinical Outcomes and Immunohistochemical Analysis
Subhash Gaddipati, Sayan Basu, Geeta K Vemuganti, Muralidhar Ramappa, Savitri Maddileti, Vrinder S Sangwan

Combination of Azathioprine and Corticosteroids in the Treatment of Serpiginous Choroiditis
Aneesha Lobo, Prachir Agarwal, Debmalya Das, Manmath Das, Rupak Roy, Jyotirmay Biswas

Community Based Intervention for Preventing Corneal Injury from Paddy Grain
Asim Kumar Sil

Retinal Vascular Caliber Measurement through Mahalanobis Distance Function based Segmentation
Subhamoy Chatterjee, Souvik Dasgupta, Jyotirmoy Chatterjee, Sambuddha Ghosh

Case Series
Multilayered Amniotic Membrane Transplantation for Mooren’s Ulcer – A Case Series of Ten Patients
Jayanta Dutta, Somnath Mukhopadhyay, Himadri Datta, Tamojit Chatterjee

Research Methodology
Multivariable Analysis
Bijay Prasad Mukhopadhyay

Commentary
Ophthalmology beyond Ophthalmologists and Also Including Ophthalmologists
Garga Chatterjee

History of Ophthalmology
Famous Ophthalmologists Who Suffered from Eye Disorders
Debmalya Das

Author Guideline

Cover photo: colourized scanning electron micrograph of rods and cones

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Ophthalmic Research Group

CURRENT INDIAN EYE RESEARCH
Eye Research in India

Ophthalmic research in India has a glorious past\textsuperscript{1,2} and a vibrant present\textsuperscript{3}. Sushruta, who practiced during the 5th century BC, classified eye diseases in the Uttar Tantram according to signs, symptoms, prognosis, and management. Sushruta’s description of cataract surgical method was probably the first account on extracapsular cataract surgery\textsuperscript{1}.

Indian ophthalmologists inherit the amazing contribution to ophthalmic research by scholars like King Serfoji II of Thaanjavur, who carried out methodical ophthalmic practices between 1798 and 1832 and kept detailed records with the help of charts and manuscripts\textsuperscript{2}.

In a bibliometric analysis of Indian ophthalmic papers published from 2001 to 2006 in peer-reviewed journals, a near doubling of the annual output of research articles was observed, two-thirds of these being published in international journals\textsuperscript{3}.

However the same study showed that 50% of the publications were contributed by only nine major eye hospitals in India and articles on basic science were the least common type in that series\textsuperscript{3}.

Ophthalmic literature from developed and developing countries were compared in a retrospective review of the five highest scoring impact factor journals in ophthalmology within the 3 year period 1998-2000. Contribution from the developing world was only 5.47% of the literature compared to 92.19% from the developed world; only 2.33% being the collaborative research from the two groups\textsuperscript{4}.

While developing countries account for the vast majority of world blindness, this inverse relationship between burden and research contribution demands urgent attention from Indian scientists engaged in ophthalmic research.

Only 16.5% of the free papers presented at the All India Ophthalmic Society Annual Conference 2000 were published over the next seven years in journals indexed with Pubmed\textsuperscript{5}.

Another study showed that only 30% of theses prepared by postgraduate students from a university medical college in India were later published\textsuperscript{6}.

Current Indian Eye Research plans to publish articles based on researches conducted in the Indian perspective with special thrust on inter disciplinary research. Emerging issues in ophthalmology will find special place in this journal.

We particularly look forward towards young researchers and trainees toiling hard on their research work. We hope that their work may find useful place and recognition in this journal.

I thank all contributors for their overwhelming response in our first issue.

References


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Diabetic macular edema (DME) is one of the major causes of vision loss in patients with diabetic retinopathy. It is defined as retinal thickening in the posterior pole essentially resulting from increased permeability of retinal vasculature leading to the disruption of the blood retinal barrier and other alterations in the retinal micro-environments.

Though chronic hyperglycemia is the primary factor leading to the development of diabetic retinopathy, the mechanisms by which elevated blood sugar levels lead to the development of DME and the histopathologically visible changes are still not clear.

DME may result from leakage of micro aneurysms or it may be due to diffuse leakage of hyper permeable capillaries. It may or may not be characterised by intraretinal cyst formation and also sub retinal fluid in the settings of severe cystic thickening involving the fovea. To characterize the severity of macular edema and for treatment guidelines, the term clinically significant macular edema (CSME) is used. Macular edema is clinically significant if one of the following conditions is present: retinal thickening at or within 500 um of the center of the macula; and/or hard exudates at or within 500 um of the center of the macula if associated with thickening of the adjacent retina; and/or a zone or zones of retinal thickening 1 disk area in size, at least part of which is within 1 disk diameter of the macular center.

**Pathogenesis of diabetic macular edema**

**Biomechanical mechanisms in pathogenesis of diabetic macular edema**

1. **The aldose reductase pathway**

Aldose reductase uses the reduced form of nicotinamide adenine dinucleotide phosphate as a cofactor to reduce many aldose sugars into their respective sugar alcohol. Glucose is reduced to sorbitol, which is then oxidised to fructose by sorbitol dehydrogenase. In normoglycemic conditions, the aldose reductase pathway is non-operative as glucose is a poor substrate for aldose reductase because of its high binding constant. Although, in the settings of hyperglycemia the aldose reductase pathways are activated which further lead to osmotic stress due to accumulation of sorbitol. Increase in the utilisation of aldose reductase in the hyperglycemic state of diabetes will result in a decline of intracellular NADPH that alters the cellular redox balance. These lead to oxidative stress and result in cellular damage.

2. **Advanced glycation protein endproduct theory**

Nonenzymatic glycation and cross linking of proteins have been proposed as a mechanism to explain the complications of diabetes. Chronic hyperglycemia leads to the formation and accumulation of AGEs that may be a primary contributor to diabetic microvasculopathy. AGEs form on the amino groups of proteins, lipids, and DNA with complex cross-links and lead to modification in the structure and function of proteins. Formation of AGEs may directly damage the cells by impairing the function of a variety of protein both extracellular and intracellular. The cellular effects of AGEs is also mediated by its binding to receptors, namely receptor for AGE (RAGE) are attached to the foot plates of Muller cells. When activated they can initiate a cascade of signal transduction involving at least p21, p44/p42 mitogen activated protein kinase(MAPK) Nuclear factor kappaB(NF-kB) and protein kinase C which further lead to cellular damage. Upregulation of VEGF is seen in Muller cells along with increased expression of glial fibrillary acidic protein (GFAP), which causes increased reactive gliosis, when RAGE is activated.
3. **Reactive oxygen intermediate theory**

The byproducts of oxidative phosphorylation includes free radicals such as superoxide anion, whose production is increased by high levels of glucose. Free radicals not only damage the cellular proteins, it also reduces nitric oxide levels, promotes leukocyte adhesions to the endothelium and decreases the barrier function of endothelial cell. Oxidative stress can also activate PKC by increasing the formation of diacylglycerol.

4. **Protein kinase C theory**

Activation of PKC by phorbol esters is associated with increased permeability in epithelial and endothelial culture cells. It seems that certain PKC isoforms may play an important role in VEGF induced vasopermeability. PKC inhibitors specific for the PKC-b isoform have been shown to significantly reduce VEGF-induced fluorescein leakage.

5. **Insulin receptors and glucose transporters**

There are at least 5 different types of facilitated cell membrane glucose transporters designated GLUT 1, GLUT 2, GLUT 3, GLUT 4 and GLUT 5 that appear to be the most impoetant for the intracellular transport of glucose in the tissues like retina that do not require insulin. Of these GLUT 1 appears to be most prevalent in the retina, occurring in the microvascular and macrovascular endothelial cells and on RPE cells as well as in the Muller cells. These up regulations of the cell membrane glucose transporters could be a mechanism that initiates glucose mediated damage by permitting a much greater influx of glucose into cells.

6. **Vascular endothelial growth factors:**

VEGF-A belongs to a gene family that includes placental growth factor (PGF), VEGF-B, VEGF-C, and VEGF-D. VEGF-A recently has come to be accepted as one of the most potent factors inducing angiogenesis. Six major isoforms exist: 121, 145,165, 183, 189, and 206. VEGF-A is a ligand for two receptor tyrosine kinases, VEGFR-1 and VEGFR-2,both of which act through downstream signalling cascades. VEGF-A,especially the VEGF-165 isoform, is emerging as an important factor in the pathophysiology of DME. VEGF is produced by RPE cells, ganglion cells, Muller cells, pericytes, endothelial cells, glial cells, neurons, and smooth muscle cells of the diabetic retina. Up regulation of VEGF by hypoxia occurs in all of these cell types. Muller cells are the most important source of VEGF in the retina due to their high rate of glycolysis. VEGF produces conformational changes in the tight junctions of retinal vascular endothelial cells. VEGF induces phosphorylation of the tight junction proteins, occludin and ZO-1, which leads to increased vascular permeability by phos-phorylation of adherent junction and cytoskeletal proteins of vascular endothelial cells and induction of fenestrations in endothelial cell membranes. VEGF also may be associated with the early inflammatory changes seen with diabetic retinopathy and DME. In early diabetes, vitreous levels of VEGF are elevated.

7. **Other vasoactive substances**

There are other vasoactive substances like histamine, Angiotensin II, Matrix meta-lloproteinase, Pigment epithelium derived growth factor, Platelet derived growth factor and basic fibroblast growth factor also plays an important role in the pathogenesis of diabetic macular edema.

**Anatomical and histological changes responsible for pathogenesis of diabetic macular edema**

1. **Loss of pericytes:**

Loss of pericytes is one of the earliest and most specific signs of diabetic retinopathy. Pericytes are contractile cells that play an important role in the microvascular autoregulation. Loss of pericytes leads to alteration of vascular intercellular contacts and impairment of blood retinal barrier. Loss of intercellular contact also appears to promote endothelial cell proliferation resulting in the development of microaneurysms. Two major theories that have been implicated for the loss of pericytes are aldose reductase pathway and the platelet derived growth factor.

2. **Capillary basement membrane thickening**

Electron microscopic findings in diabetics show thickening of the capillary basement membrane along with deposition of fibrillar collagen and swiss cheese vacuolisation of the otherwise homogenous pattern of the basement membrane collagen. Biochemical reactions like aldose reductase pathway, sorbitol pathway and enzymatic or nonenzymatic glycation of the basement membrane collagen have been play important role in the thickening of the basement membrane.

3. **Break down of the blood retinal barrier**

Break down of the blood retinal barrier is an important pathologic factor responsible for the development of diabetic macular edema. One mechanism by which there is a breakdown of the blood retinal barrier is opening of...
the tight junctions, also known as zonula occludens between the vascular endothelial cell processes. VEGF also plays an important role in the breakdown of the inner blood retinal barrier by altering the endothelial cell tight junctions. The other mechanism involved the increase in the vascular permeability is kallikrein kinin system through the production of bradykinin which in turn causes vasorelaxation of the retinal arterioles via nitric oxide.

4. Vitreoretinal interface

Clinical and anatomical evidence indicates that abnormalities in the structure of the vitreoretinal interface may play an important role in the pathogenesis of DME. DME may be exacerbated due to persistent vitreomacular traction by the residual cortical vitreous on the macula after PVD, thickened and taut posterior hyaloid that may or may not be adherent to ILM, macular traction due to tractional proliferative membranes, or loculation of cytokines in the pre-macular vitreous pocket. A diabetic retina compromised due to microvascular abnormalities may be vulnerable to increased exudation in the presence of any macular traction.

Classification of diabetic macular edema

The Early Treatment Diabetic Retinopathy Study (ETDRS) defined DME as retinal thickening or presence of hard exudates within 1 disk diameter of the center of the macula. To characterize the severity of macular edema and for treatment guidelines, the term clinically significant macular edema (CSME) is used. Macular edema is clinically significant if one of the following conditions is present: retinal thickening at or within 500 µm of the center of the macula and/or hard exudates at or within 500 µm of the center of the macula if associated with thickening of the adjacent retina; and/or a zone or zones of retinal thickening 1 disk area in size, at least part of which is within 1 disk diameter of the macular center. On optical coherence tomography the DME can be classified into central involving and noncentral involving macular edema based on the presence or absence of cystic spaces at the central fovea thereby increasing the central foveal thickness. It is important to differentiate DME into central involving or non central involving as the treatment protocol for each is different.

Extrafoveal foci of retinal thickening and hard exudates may not cause any symptoms or affect visual acuity but DME that involves or threaten the centre of the macula cause significant vision loss. In the ETDRS, the 3 year risk of moderate vision loss among untreated DME is around 32%. In focal CSME, discrete points of retinal hyperfluorescence are present on the FA due to focal leakage of micro-aneurysms. The discrete leaking microaneurysms are thought to cause retinal thickening. Commonly, these leaking microaneurysms are surrounded by circinate rings of hard exudates. The exudates are lipoprotein deposits in outer retinal layers. In diffuse DME, areas diffuse leakage are noted on the FA due intraretinal leakage from a dilated retinal capillary bed and/or intraretinal microvascular abnormalities (RMA), and/or (in severe cases) from arterioles and venules without discrete foci of leaking microaneurysms. There may be associated cystoid macular edema (CME). Cystoid macular edema results from a generalized breakdown of the inner BRB with fluid accumulation, primarily in the outer plexiform layer.

Management of diabetic macular edema

Diagnostic imaging techniques

1. Fundus fluorescein angiography

Fluorescein angiography is a standard method used to evaluate patients with DME that is sensitive for qualitative detection of fluid leakage. The DME can be classified as focal and diffuse with the help of fundus fluorescein angiography. It also helps us to diagnose macular ischemia. Fundus photography is an important tool to look for the progression of the retinopathy in individual patients.
2. **Optical coherence tomography**

OCT has been used for high-resolution imaging of the retina and detection of increased retinal thickness. OCT has several advantages as a retinal imaging technique: 1) it is non-invasive (no injected dye involved) and well tolerated (especially important in children); 2) it provides quantitative information regarding retinal thickness with a high degree of accuracy and reproducibility; 3) it clearly reveals the presence and extent of vitreomacular traction. As shown by Chan and Duker, central macular thickness on OCT is a highly useful method for evaluation and comparison of the different therapeutic modalities for DME.

**Treatment**

**Systemic therapy for DME**

The main aims of systemic therapy in DR/DME are to reduce the risk of diabetic patients developing these conditions in the first place and to reduce the risk of progression of existing retinopathy or maculopathy to more severe, sight-threatening forms.

**Modifying metabolic control**

Improving glycemic control and lowering the level of glycosylated hemoglobin (HbA1c) is, at present, the most effective medical treatment to slow the progression of DR. This was proven by the Diabetes control and complications trial (DCCT) in type 1 diabetics and the United Kingdom prospective diabetes study. As per DCCT there was a 35%-40% reduction in the risk of retinopathy progression for every 10% decrease in HbA1C. According to UKPDS for every percentage point decrease in HbA1C there was a 35% decrease in the risk of microvascular complications. Intensive glycemic control was found to have effects that persist well beyond the course of treatment. The DCCT and UKPDS established optimizing metabolic control as a priority and led to the suggestion that it should be implemented early and maintained for as long as is safely possible. Although, the intensive control arm of the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study was stopped because of increased all-cause mortality in people whose glucose was extremely tightly controlled with insulin and multiple oral agents.

**Modifying hypertension**

Hypertension is a major risk factor for DR and DME. The UKPDS demonstrated that control of blood pressure (systolic blood pressure <150 mmHg) led to a reduction in the progression of diabetic retinopathy and reduced need for laser treatment in the tight blood pressure control group compared with the less tight control group. More intensive blood pressure control resulted in a 37% reduction in the microvascular complications of DM.

**Lipid lowering agent**

Lipid lowering agents may decrease the risk of vision loss in patients with DR. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study on the effects of long-term fenofibrate on cardiovascular events in patients with type 2 diabetes found beneficial effects on microvascular complications that included DR. There were significant benefits in terms of the requirement for first laser and development of DME. The ACCORD-Eye study confirmed the results of the FIELD study. In ACCORD-Eye, the addition of fenofibrate to basal statin therapy resulted in a significant reduction in the progression of retinopathy, in a similar manner to that observed with intensifying blood glucose control, but with a good safety profile and without increasing the risk of hypoglycemia.

**Ocular therapy for diabetic macular edema**

**Laser therapy**

The ETDRS study was designed to evaluate the effects of argon laser photocoagulation for macular edema in a prospective, randomized, multicentre clinical trial. At 3 years, eyes with mild or moderate NPDR with macular edema at baseline treated with immediate focal/grid laser photocoagulation showed an approximately 50% decrease in the rate of moderate vision loss. Study done by DRCR.net compared the efficacy of modified ETDRS grid with mild macular grid in DME. In mild macular grid mild widely placed burns throughout the macula, both in the thickened and non thickened areas. At 12 months after treatment, the MMG technique is less effective at reducing OCT measured retinal thickening than the more extensively evaluated current modified ETDRS laser photocoagulation approach. However, the visual acuity outcome with both approaches is not substantially different. Since the advent of anti-VEGFs the role of focal laser in DME is decreasing although the efficacy of modified ETDRS grid in non central involving DME had been studied by DRCR.net. Focal/grid laser in these non-CI eyes was associated with relatively stable visual acuity and retinal thickness measurements, and decreased fluorescein leakage area at 1 year.

Focal or grid laser can cause a scotoma within 20° of the central fixation, choroidal neovascularisation and subsequent fibrosis.
Pharmacotherapy for DME

Although it has been the mainstay of DME treatment for decades, laser monotherapy has some important limitations. Intravitreal injections of anti-VEGF agents have recently replaced ETDRS-style macular laser as the choice for initial treatment of center-involving DME, likely based on the results of several well-controlled randomized clinical trials. In recent DRCR.net reports, treatment with modified ETDRS laser resulted in stable or improved vision in a majority of patients, but close to 20% of patients lost >10 letters of visual acuity. The efficacy of anti VEGF like ranibizumab, bevacizumab and aflibercept has been studied in different studies done by DRCR.net.

(a) Ranibizumab

Ranibizumab is an affinity-purified humanized monoclonal antibody fragment (Fab) that binds all VEGF A isoforms. It is a smaller molecule and has a molecular weight of 48 kilodaltons. The antiangiogenic effects of ranibizumab was first proven in wet age related macular degeneration by ANCHOR and MARINA studies. Following these, studies were done to prove the efficacy of ranibizumab in DME especially in central involving DME. DRCR.net evaluated intravitreal 0.5 mg ranibizumab or 4 mg triamcinolone combined with focal/grid laser compared with focal/grid laser alone for treatment of diabetic macular edema (DME). The 1-year mean change (±standard deviation) in the visual acuity letter score from baseline was significantly greater in the ranibizumab + prompt laser group (+9±11, P<0.001) and ranibizumab + deferred laser group (+9±12, P<0.001) but not in the triamcinolone + prompt laser group (+4±13, P=0.31) compared with the sham + prompt laser group (+3±13). Reduction in mean central subfield thickness in the triamcinolone + prompt laser group was similar to both ranibizumab groups and greater than in the sham + prompt laser group. In the subset of pseudophakic eyes at baseline (n=273), visual acuity improvement in the triamcinolone + prompt laser group was similar to both ranibizumab groups and greater than in the sham + prompt laser group. In the subset of pseudophakic eyes at baseline (n=273), visual acuity improvement in the triamcinolone + prompt laser group appeared comparable to that in the ranibizumab groups.

No systemic events attributable to study treatment were apparent. The expanded 2-year results reported herein are similar to results published previously and reinforce the conclusions originally reported, that ranibizumab should be considered for patients with DME. The RISE and RIDE study also the treatment protocol for use of intravitreal ranibizumab in central involving DME. The treatment protocol as described by DRCR.net is shown below in figure 2. Intravitreal ranibizumab (0.5 mg/0.05ml) treatment has to be started in patients with a central involving macular edema with increase in central foveal thickness (>250 µm) or visual acuity less than 20/32. The underlying rationale of the DRCR.net treatment algorithm for DME with intravitreal ranibizumab therapy requires monthly injections until an eye reaches “success” (the macular edema resolves or vision reached 20/20 or better); or until additional treatment is judged unlikely to be beneficial because of “no further improvement” compared with the previous visit(s) (edema improved after initiation of treatment, but eventually stabilized without reaching “success”); or an eye meets “failure” criteria (edema worsened or remained unaffected by treatment). Once ranibizumab is withheld, treatment could be resumed if macular edema recurs or worsens. If treatment is withheld and edema does not recur or worsen, the follow-up time could be doubled and if edema still does not recur or worsen, follow-up could be doubled again according to the study protocol. If macular edema persisted or was not improving despite anti-VEGF treatment additional focal/grid laser can be added as often as every 4 months.

The randomised study done by DCR.net for the efficacy of ranibizumab has also reported 3 cases on endophthalmitis and one case of progression of tractional retinal detachment. The most common side effects in clinical trials were conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation. There is a theoretical risk for arterial
thromboembolic events in patients receiving VEGF-inhibitors by intravitreal injection.

(b) Bevacizumab

Bevacizumab is a full length humanised monoclonal antibody that binds all VEGF-A isoforms and is FDA approved for colorectal carcinoma, but is used off label in the eye. Compared to ranibizumab it is a larger molecule and has a molecular weight of 149 kilodalton. A phase 2 trial done by DRCR.net to provide data on the short-term effect of intravitreal bevacizumab for diabetic macular edema (DME)52. The results demonstrate that intravitreal bevacizumab can reduce DME in some eyes, but the study was not designed to determine whether treatment is beneficial. A phase 3 trial would be needed for that purpose. The BOLT study demonstrates that at 12 months of follow up the patients treated with intravitreal bevacizumab has a significantly better mean change in visual acuity than in the laser group53.

The adverse affects are similar to ranibizumab although as per CATT trial some nonfatal adverse effects like GI bleeding were more in cases of bevacizumab54.

(c) Aflibercept

Vascular Endothelial Growth Factor Trap-Eye is a 115-kDA recombinant fusion protein comprising the key VEGF binding domains of human VEGF receptors 1 and 2 fused to the Fc domain of human immunoglobulin G155. Vascular Endothelial Growth Factor Trap-Eye is a panisoform VEGF-A inhibitor whose binding affinity to VEGF is substantially greater than that of either bevacizumab or ranibizumab55, leading to a mathematical model predicting it could have substantially longer duration of action in the eye 56. In addition, VEGF Trap-Eye binds placental growth factors 1 and 2, which have been shown to contribute to excessive vascular permeability and retinal neo-vascularization57.

The DA VINCI trial was done to show the efficacy, dosing and dosing schedule of aflibercept in DME58. In this phase 2 clinical trial, all VEGF Trap-Eye doses and dosing regimens were found to be superior to macular laser photocoagulation for the treatment of DME over the course of 52 weeks and produced similar results in terms of preserving and improving visual acuity. Patients who received VEGF Trap-Eye benefited from significantly greater increases in mean visual acuity at 1 year (9.7 to 13.1 letters of improvement) compared with laser treatment alone (1.3 letters change). The average number of laser treatments administered to eyes randomized to VEGF Trap-Eye was fewer than 1 (of a maximum of 2 possible lasers), with most patients not requiring laser photocoagulation, indicating that the visual acuity and anatomic benefits achieved were the result of VEGF Trap-Eye and not laser treatment. The 2 mg dose of VEGF Trap-Eye almost completely eliminated vision loss at all dosing intervals. A prolongation of the retreatment interval from 4 to 8 weeks based on this data and the rationale of the improved binding properties of VEGF Trap-Eye represents an opportunity to potentially reduce the treatment and monitoring burden in anti-angiogenic therapy for DME59.

(d) Combination therapy

As proven by the studies done by DRCR.net monotherapy with corticosteroids does not have much advantage over focal laser alone, several recent trials have provided good evidence to support combined treatment modalities for the treatment of DME. The RESTORE study was done to compare injection ranibizumab with laser treatment vs ranibizumab injection with sham laser vs laser alone60. Although the visual gain and the improvement of macular thickness was more in both the ranibizumab groups than the only laser group, it failed to show an outright advantage of combination therapy over ranibizumab monotherapy. The READ 2 study also had similar results like RESTORE61. At 2 years although both monotherapy and combination therapy with laser showed similar visual outcomes, the combination group required fewer treatments. Entry criteria for READ 2 were similar to the RESTORE study, but the READ 2 investigators chose to give injections every two months in the ranibizumab group. Importantly, in the combination group, patients received focal laser a week after injection, which differs from the RESTORE trial, which applied combined treatment on the same day. The DRCR.net's Protocol I study also investigated combination therapy involving ranibizumab or triamcinolone with focal laser62. By three years, VA scores had improved more in the ranibizumab plus deferred laser group compared to prompt laser (9.7 letters vs 6.8 letters)63.

(e) Intravitreal steroids

Corticosteroids have immune modulatory and antiangiogenic properties and have been utilised for the management of DME. The major side effects of using intravitreal steroids are IOP rise and cataract formation. DRCR.net evaluated the efficacy and safety of 1 mg and 4 mg doses of preservative-free intravitreal triamcinolone in comparison with focal/grid photocoagulation for the treatment of diabetic macular edema (DME)64. At 4 months,
a greater positive treatment response on visual acuity was seen in the 4 mg triamcinolone group compared with the other two groups. However, by 1 year, there was little difference in visual acuity between the groups, and at the time of the two-year primary outcome assessment, visual acuity and safety (with respect to intraocular pressure and cataract) were significantly better in the laser group than in either the 4 mg triamcinolone or 1 mg triamcinolone groups. There was no significant difference between the 1 mg triamcinolone and 4 mg triamcinolone groups in visual acuity at 2 years. Treatment group differences in the change in retinal thickening generally mirrored the effect on visual acuity, with initially a greater reduction in the 4 mg triamcinolone group, an eventual greater reduction in the laser group, and no difference between the two triamcinolone groups at 2 years. Results in a subset of randomized subjects who completed the 3-year follow-up are consistent with previously published 2-year results and do not indicate a long-term benefit of intravitreal triamcinolone relative to focal/grid photocoagulation in patients with diabetic macular edema similar to those studied in this clinical trial. Thus 1st line intravitreal triamcinolone is not recommended.

(f) **Posterior subtenon triamcinolone:**

DRCR.net also provided the pilot data on the safety and efficacy of anterior and posterior subtenon injections of triamcinolone either alone or in combination with focal photocoagulation in the treatment of mild diabetic macular edema (DME). No clinically important effects on central retinal thickness or visual acuity were found with peribulbar injections of triamcinolone with or without follow-up focal photocoagulation in comparison with focal photocoagulation alone.

(g) **Sustained release implants**

Steroids offer the potential advantage of longer duration of action. Rather than repeated bolus delivery of steroid, sustained-delivery devices have undergone development and testing for treatment of macular edema.

In the FAME trial, investigators used fluocinolone acetonide vitreous inserts (Iluvien, Alimera Sciences, Alpharetta, GA) to treat subjects with center-involving DME who had failed at least one prior macular laser. The investigators compared two doses of steroid (0.2 and 0.5 µg/day) to sham injections. Both treatment groups showed larger gains in VA compared to the sham group, with 4.4 and 5.4 letters gained at 2 years in the low and high-dose groups, respectively, compared to a gain of 1.7 letters in the controls. The differences were more pronounced in favor of the steroid group in patients with chronic DME of more than three years’ duration. Cataract progression was significant among steroid-treated patients.

The CHAMPLAIN and PLACID study demonstrates the efficacy of dexamethasone implant in refractory diabetic macular edema. In the CHAMPLAIN study, the investigators administered a single dexamethasone injectable implant (Ozurdex, Allergan, Irvine, CA) in a cohort of patients with refractory DME and prior vitrectomy. The PLACID study randomized eyes with central diffuse DME to treatment with dexamethasone implant combined with macular laser or to sham injection with macular laser.

### Poor responders and treatment failure

Definite worsening or treatment failure is defined as persistent edema along with 10 or more letter worsening from baseline at any visit, or, after at least 1 year of treatment, there was no improvement from baseline in central subfield thickness or visual acuity in the setting of “complete” laser. “Complete” laser was defined as direct treatment to all micro-aneurysms within areas of macular edema and grid treatment already applied to all other areas of macular edema. Once “failure” criteria were met, anti-VEGF treatment could be discontinued and any alternative treatment (such as intravitreal corticosteroids) along with focal laser could be performed.

In cases of treatment failure we obtain wide field angiography to assess peripheral and macular nonperfusion. Based on anecdotal evidence of peripheral ischemia driving persistent DME, we consider targeted PRP to areas of nonperfusion to reduce the ischemic burden. In those cases that remain refractory to treatment, the next step in the therapeutic algorithm is PPV with ILM removal. In eyes with, significant ERM, or vitreous adhesion and nonperfusion. Based on anecdotal evidence of peripheral ischemia driving persistent DME, we consider targeted PRP to areas of nonperfusion to reduce the ischemic burden. In those cases that remain refractory to treatment, the next step in the therapeutic algorithm is PPV with ILM removal. (figure-4), we often consider vitrectomy with peeling early in the course of treatment with adequate intravitreal pharmacotherapy in the perioperative period (Fig 3b). Study done by A. Kumar et al showed PPV with ILM peeling was beneficial by inducing a statistically significant reduction of macular thickness and macular volume. Visual acuity also demonstrated a trend towards improvement in both the ILM peel group and the grid laser group; however, the comparative VA outcome analysis between the two groups was not significantly different.

For poorly responsive patients, they should be evaluated...
about 2 weeks after intravitreal injection and if there is some improvement of the macular thickness in the OCT, then monthly intravitreal anti VEGF injections can be continued along with focal laser photocoagulation. In pseudophakic patients we can consider treatment with intraocular steroids, either in bolus dose or sustained release implants.

DME with vitreomacular traction

Structural modification of diabetic vitreous occurs secondary to enzymatic and non-enzymatic collagen glycation. Accumulation of AGEs in the vitreous of hyperglycemic patients promotes collagen crosslinking and may be the cause of VMT in diabetic eyes. AGE accumulates along the posterior vitreous cortex and the ILM, where it may cause structural alterations that promote vitreoretinal traction. Vitrectomy to remove the posterior hyaloid and ILM may be beneficial in two ways: (1) by removing AGE ligand-induced mechanical traction between the posterior cortical vitreous and the ILM of macula; and (2) removal of AGEs may also inhibit the activation of the RAGE axis and its proinflammatory effects. Muller cells lie between the ILM and ELM and in close apposition with capillaries. In diabetic eyes, upregulation of VEGF in Muller cells may increase the vasopermeability of the retinal endothelial cells. The DRCR.net examined the role of vitrectomy and membrane peeling in the treatment of DME with a tractional component in a small, prospective cohort study. At six months postoperatively, VA improved by more than 2 lines in 38% of eyes. The mean decrease in macular thickness on OCT was approximately 160 µm, with 43% of patients having macular thickness of less than 250 µm.

Conclusions:

A holistic approach should be taken for the management of the patients of DME. The baseline systemic status of the patients should be evaluated with respect to the control of diabetes (HbA1C levels), hypertension, serum lipids and nephrological status.

A good ocular examination should be done by slit lamp biomicroscopy with a 90D and 78 D lens. Fundus fluorescein angiography is done to classify the type of macular edema and to rule out macular ischemia. A wide field fundus fluorescein angiography also helps to detect peripheral ischemia. OCT is done to classify the type of edema, to look for any interface abnormalities and also to follow up the patients.

In patients with non centre involving macular edema without any tractional elements identified on OCT. We often consider initial laser photocoagulation. We direct treatment at microaneurysms and other treatable lesions within the area of as per the modified ETDRS guidelines.
If the edema is center-involving, either intravitreal bevacizumab or ranibizumab has to be used. We also initially treat center-threatening diffuse edema with anti-VEGF injections along with laser treatment. In recalcitrant cases we can also use intravitreal steroids or sustained release implants especially in pseudophakic patients. In cases with vitreomacular interface abnormalities pars plana vitrectomy with ILM peeling should be considered.

We repeat biomicroscopic examination with ancillary diagnostic testing monthly to assess the patient's response to therapy. We continue anti-VEGF therapy with possible additional focal laser until macular edema resolves, until it becomes clinically nonsignificant, or until futility criteria are reached as in the DRCR.net's Protocol I.

Summary of treatment

- A detailed systemic evaluation has to be done to look for diabetes control, control of hypertension, hyperlipidemia and renal status.
- A diabetic patient presenting with diminution of vision or referred for screening should be clinically examined thoroughly to diagnose nonproliferative or proliferative diabetic retinopathy with or without DME.
- Fundus fluorescein angiography and optical coherence tomography has to be done to look for macular perfusion status and quantitative as well as qualitative analysis of the DME.
- A holistic approach should be taken to control the patient systemically.
- In cases of noncentre involving DME laser treatment has to be done. Laser of the thickened retina and direct treatment over the microaneurysms has to be done.
- In cases of centre involving DME, anti VEGF therapy has to be started and should be continued till either success criteria or the failure criteria are fulfilled.
- In cases of failure, complete laser or alternative treatment like intravitreal triamcinolone or sustained release implant should be used. Steroids are not recommended as a first line therapy as triamcinolone has not been found to be superior to laser therapy.
- In cases of vitreomacular DME or recalcitrant DME, a pars plana vitrectomy with vitreomacular traction release with ILM peeling should be done.

References


61. Nguyen QD, Shah SM, Heier JS, et al. Primary endpoint (six months) results of the Ranibizumab for


ANNOUNCEMENT

ORG Award 2014

For Young Researchers (PG)

Most of the works by the post-graduate trainees in India end unpublished. In order to create an environment where the post-graduate trainees (MS/ MD/ DNB) would try to showcase their hard work in a proper peer-reviewed scientific forum, a scientific paper writing competition is announced.

8 articles (two from each group) will be selected

First Prize: A citation/certificate and a cash prize of Rs. 50,000/

Second Prize: A citation/certificate and a cash prize of Rs. 20,000/-.

The award-winning articles will be published free after peer review in Current Indian Eye Research, a peer reviewed open access journal.

Group A: Cornea, Refractive surgery, Ocular surface.

Group B: Retina-vitreous, Uvea.

Group C: Cataract, Glaucoma

Group D: Pediatric ophthalmology, Orbit, Lacrimal apparatus, Oculoplasty.

The last date for submission of the articles is 31st of October, 2014.

The panel of judges will consist of eminent post-graduate teachers from different teaching institutions and their decision regarding the awards will be final.

Eligibility: Post-graduate trainees in Ophthalmology (present trainees and also who have passed out in the last two years)

Article type: Unpublished original article

Word limit: 250 words structured abstract, 3-5 key words, Maximum 3000 words (excluding maximum 50 references), maximum three tables/figures (b/w).

For further details please visit www.ophthalmicresearch.in
Approved for use in

Age-related Macular Degeneration

Diabetic Macular Edema

Retinal Vein Occlusion

Myopic CNV

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**Visual impairment due to DRX**  **Visual impairment due to macular edema**  **Visual impairment due to CHD**  **Visual impairment due to CNV**

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Abstract

Purpose: To report the clinical and phenotypic findings following autologous cultivated oral mucosal epithelial transplantation in eyes with ocular surface burns in a retrospective case series. Methods: This study included 19 eyes of 18 patients with bilateral limbal stem cell deficiency following ocular burns treated between 2007 and 2010. All patients underwent an oral mucosal biopsy, following which the oral epithelium was cultivated on de-epithelized human amniotic membrane using a xeno-free explant culture technique. A monolayer of cultivated oral epithelium was transplanted onto the patient’s ocular surface. Post-operative ocular surface stability, corneal avascularity and visual improvement were assessed. From five eyes that subsequently underwent keratoplasty or keratoprosthesis surgery, the excised corneal tissue was subjected to histopathology and immunohistochemical analysis. Results: The mean follow-up was 22.3 months. All transplanted eyes showed superficial corneal vascularization by 3 months. A stable ocular surface was seen in 7 (37%) eyes at the end of one year. Vision did not improve in 12 (63%) eyes, vision improved from hand motions to counting fingers in 6 (32%) eyes and to 20/125 in one (5%) eye. Histopathology of excised corneal tissue showed six to eight layers of epithelial stratification and absence of goblet cells. Immunohistochemical analysis of the transplanted epithelium showed expression of p75, p63, suprabasal K19 and K3 and absence of K12, K14. Conclusions: Clinical outcomes of autologous cultivated oral mucosal epithelial transplantation in eyes with ocular surface burns were poor and the transplanted cells maintained the oral phenotype on the corneal surface.

Limbial stem cell deficiency (LSCD) is a rare cause of corneal blindness which results from physical, chemical or immunological damage to the corneal epithelial stem cells located at the limbus1,2. In unilateral cases LSCD can be treated by either conventional or cultivated autologous limbal transplantation from the unaffected fellow eye3,4. However, in bilateral cases there is no autologous source for limbal stem cells and either a living or a cadaveric allogeneic donor is required5. An alternative to allogeneic limbal grafting, which necessitates long-term systemic immuno-suppression, is transplantation of autologous epithelium from non-ocular sources.

The possibility of oral mucosa being used as a substitute for limbal epithelium was considered because of the phenotypic resemblance between the two epithelial lineages6,7. Animal trials and preliminary human trials also demonstrated that the ex-vivo cultivated oral mucosa could be a suitable therapeutic alternative to limbal epithelium in eyes with LSCD8-13. However the cell culture protocols described for cultivating oral mucosal cells for human transplantation utilized various animal derived or xeno-biotic materials9-19. Use of xeno-biotic materials in cell culture for clinical use is undesirable as it carries the risk of transmitting known or unknown infections to the transplant recipient20. To avoid xeno-biotic usage, we developed a xeno-free technique of culturing oral mucosal cells,6 adopted from our standardized protocol for limbal epithelial cultivation21, which has been used successfully to treat over 500 eyes with unilateral LSCD22-24. In this study we report the clinical outcomes and immunohistochemical findings in eyes with LSCD following ocular surface burns,
treated by xeno-free autologous cultivated oral mucosal epithelial transplantation (COMET).

Methods:

Patients: At the LV Prasad Eye Institute, Hyderabad, India autologous COMET was offered as an alternative to allogeneic cultivated limbal epithelial trans-plantation, between October 1, 2007 and November 1, 2010, to patients with bilateral and total LSCD (defined clinically as 360° superficial corneal vascularization, diffuse fluorescein staining of the corneal surface with or without persistent epithelial defects, conjunctivalization of the corneal surface and absence of limbal palisades of Vogt) following ocular surface burns. The Institutional Review Board approved of this pilot study for 20 eyes (LEC 06003) and recommended the following exclusion criteria to be applied before enrolment: (a) patients with LSCD due to unknown causes or causes other than ocular surface burns; (b) patients who had bilateral but partial LSCD; (c) patients with total LSCD, but with dry eye disease (Schirmer’s test without anesthesia of <10 mm at 5 minutes) or keratinisation of the ocular surface epithelium; (d) patients with no visual potential as determined by clinical examination and electrophysiological testing (flash visual evoked potential and flash electroretinogram); (e) patients with untreated concurrent ocular problems, such as glaucoma and infection.

Data Collection: The data retrieved from the medical records included age and sex of the patient, type and date of injury, details of prior ocular procedures, Snellen’s best spectacle corrected visual acuity (BCVA) and at each follow-up visit, presence or absence of lid abnormalities, dry eye disease, symblepharon, degree of limbal involvement, intra-operative surgical details, post-operative complications, duration of follow-up and status of ocular surface at each visit (slit-lamp findings including fluorescein staining).

Surgical Technique of Oral Mucosal Biopsy: All patients underwent an oral examination by a physician to rule out any contraindications to a mucosal biopsy. The patients were advised 5% povidone-iodine mouth wash twice daily for 3 consecutive days prior to the biopsy. After confirming satisfactory oral hygiene, an oral mucosal biopsy of 3 x 3 mm was obtained under local anesthesia (2% xylocaine sub-mucosal infiltration) from the inner surface of the patient’s lower lip. The biopsied area was left bare and the patient was advised to continue the mouth wash for one week following the biopsy. The oral biopsies were performed by one surgeon (MR).

Technique of Oral Mucosal Epithelial Cultivation: The tissue was transported to the laboratory in human corneal epithelium (HCE) medium, which has been described previously.21-24 Briefly, HCE medium composed of minimal essential Eagle’s medium (Sigma, cat. no. M0644) with alpha modification/Nutrient mixture (Sigma, cat. no. I2643), HAM’s F12 medium (1:1) containing 2 mM L-glutamine (Sigma, cat. no. G6392), 100 U/mL penicillin, 100 µg/mL streptomycin (Sigma, cat. no. P4333), 2.5 µg/mL amphotericin B (Sigma, cat. no. A2942), 10 ng/mL human recombinant epidermal growth factor (Sigma, cat. no. E9644) and 5 µg/mL human recombinant insulin (Sigma, cat. no. I2643) along with 10% (vol/vol) autologous serum. Under strict aseptic conditions, human amniotic membrane (hAM) was prepared and preserved by our eye bank, measuring 3 x 4 cm was de-epithelialised using TrypLE (Invitrogen, cat. no. 12604) and 0.25% EDTA (Sigma, cat. No. E5134) solution after incubating at 37°C for 30 minutes. The patient’s oral mucosal tissue was divided into small pieces after separation from the underlying connective and minor salivary glands. The tissue bits were explanted over the de-epithelialized hAM, epithelial side-up. A similar parallel culture was also prepared as a backup. A submerged explant culture system without a feeder cell layer was used. The culture was incubated at 37°C with 5% CO2 and 95% air in HCE media (Thermo Fisher Scientific, model: 371). The growth was monitored daily under an inverted phase contrast microscope (Olympus, CX40) and the HCE medium was changed every other day. The culture was transplanted when a monolayer of the cells growing from the explants became confluent, typically in 15 to 19 days. The laboratory cultures were performed by one experienced cell biologist (SG).

Technique of Cultured Oral Mucosal Epithelial Transplantation: Any symblepharon which prevented adequate separation of the lids was released to permit the insertion of a wire speculum (no additional surgery to treat the symblepharon was performed). A peritomy was performed and the corneal fibrovascular pannus was excised, fixed in 10% formaldehyde solution and sent for histopathological analysis. The hAM and monolayer of cultivated oral mucosal epithelial cells was spread over the cornea, epithelial side up. Using a sutureless technique the graft was secured to underlying ocular surface with fibrin glue (TISSEEL™ Kit from Baxter AG, Austria) and
the margins of the graft were tucked under the surrounding conjunctival edge. Bandage contact lenses were not applied at the end of surgery. The transplantations were performed by the one experienced ocular surface surgeon (VSS).

**Postoperative Treatment Regimen:** All the recipient eyes received topical prednisolone acetate 1% eye drops 8 times daily, tapered gradually based on the level of inflammation and ciprofloxacin 0.3% eye drops 4 times daily in the first post-operative week or until complete epithelization was noted.

**Follow-up Schedule:** All patients were seen on post-operative day one, at one week, at six weeks, and thereafter every six to eight weeks. Each examination included a complete history, including any new ocular or systemic symptoms, a complete ocular examination including fluorescein staining, and any signs of neovascularization or surface instability. The post-operative clinical assessment was performed by one ocular surface specialist (SB).

**Primary and Secondary Outcome Measures:** Based on the clinical appearance of the corneal surface an impression of success or failure of therapy was made. Success was defined as a totally epithelized, stable and avascular corneal surface. Failure was defined as appearance of any superficial corneal vascularization (even if the corneal surface was epithelized and stable), epithelial defects lasting more than two weeks and conjunctival overgrowth on the cornea (conjunctivalization). The secondary clinical outcomes were improvement in BCVA from baseline and ocular and oral complications.

**Additional Surgery:** Either penetrating keratoplasty (PK) or Boston Type 1 keratoprosthesis was performed in eyes with a stable ocular surface (irrespective of superficial vascularization) but poor visual improvement attributed to corneal stromal scarring. The corneal tissue excised during PK or keratoprosthesis surgery was fixed in 10% formaldehyde for histopathology and immuno-histochemical analysis as described below.

**Hematoxylin-Eosin, Periodic Acid Schiff (PAS) staining:** The pannus excised at the time of COMET, the unused back-up culture and the corneal button excised at the time of keratoplasty/keratoprosthesis were fixed in 10% buffered formalin, embedded in paraffin and serial sections of 5µm thickness were taken on silane-coated glass slides. Sections were deparaffinized, rehydrated with distilled water and stained with hematoxylin and eosin and Schiff's reagent and observed under light microscope.

**Immunohistochemistry:** The primary antibodies, anti-K3/12, anti-K14 and anti-K19 were procured from Chemicon, anti-p75 from Abcam, anti-p63 from Thermo Scientific, anti-Ki67 from Dako, while the anti-K19 and immunohistochemistry (IHC) developing reagents were purchased from BioGenex. The serial sections of the unused back-up culture and the excised corneal tissue were de-paraffinized and rehydrated, blocked for endogenous peroxidase using 3% H2O2 in methanol. Antigen retrieval was done using citrate buffer (pH-6.0) in a microwave for 15 minutes and allowed to cool to room temperature. Blocking was done using 2.5% bovine serum albumin in 1x phosphate buffered saline (PBS) before primary antibody incubation at room temperature for one hour. HRP-conjugated secondary antibody incubations and IHC developing was done as per manufacturer's instructions (BioGenex). The samples were counterstained with hematoxylin, mounted in a resinous mounting medium and observed under a light microscope. Cadaveric human conjunctival and corneal tissue obtained from the eye bank and oral mucosal tissue obtained from voluntary human donors (SG, SB, MR, GKV, VSS) were used as controls. The histopathology and immuno-histochemical analysis was performed and interpreted by one experienced ocular pathologist (GKV).

**Result:**

**Demographics:** During the entire study period 19 eyes of 18 patients with bilateral and total LSCD following ocular surface burns underwent autologous COMET. The mean age at the time of surgery was 23.7± (12.5) years with male to female ratio of 2.8:1. The median time period between the initial injury and autologous COMET was 34 months (range: 6 to 240) months. Other pre-operative clinical characteristics of the transplanted eyes are provided in Table 1.

**Biopsy, Ex-vivo Cultivation and Transplantation:** Three patients underwent biopsy and trans-plantation under general anaesthesia, whereas others were operated under local anaesthetics. No anaesthetic or intra-operative complications occurred during either biopsy or transplantation. Following the biopsy no donor site complications were noted. The mucosal defect created on the lower lip following the oral biopsy completely healed by one week. In the laboratory, a confluent monolayer of cells formed on the denuded-hAM in a mean duration of 19.3 days (range 15 to 27 days). No cultures showed microbial contamination or inadequate growth.
**Table 1: Preoperative clinical characteristics of the transplanted eyes**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Yrs)</th>
<th>Sex</th>
<th>Eye</th>
<th>Injury</th>
<th>Previous Ocular Surgery</th>
<th>VA Pre-COMET</th>
<th>Lid Abnormalities</th>
<th>Symbrophorin</th>
<th>PED</th>
<th>VA@3 months</th>
<th>VA@6 months</th>
<th>Surface Stability at 6 mnts</th>
<th>Outcome at 12 mnts (pk/kpro/time gap in months)</th>
<th>Subsequent surgeries (COMET-Sx)</th>
<th>Total follow up (COMET to last visit)</th>
<th>Outcome at last follow up</th>
<th>VA@Final FU</th>
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</table>

M=Male; F=Female; Allo-LT= Allogeneic Limbal Transplantation; PK= Penetrating Keratoplasty; AMG= Amniotic Membrane Grafting; VA=Best Corrected Visual Acuity; HM= Hand Movements; PL= Perception Of Light; CF= Counting Fingers; Kpro= Boston Type 1 Keratoprosthesis; TA+BCL= Tissue Adhesive And Bandage Contact Lens Application; Tarso= Tarsorrhaphy; COMET= Autologous Cultivated Oral Mucosal Epithelial Transplantation
**Primary Outcome:** The mean follow-up was 22.3 (range: 7 to 48) months. Post-operatively on day one and at one week, fluorescein staining was negative over the grafted area and no folding or loosening of the hAM was noted. At six weeks all the grafted eyes had a completely epithelialized and stable corneal surface but absence of peripheral superficial corneal vascularization noted in 16 (84%) of 19 eyes. However, peripheral superficial corneal vascularization was seen in all eyes by three months. Therefore none of eyes met the clinical criteria of success at 3 months and thereafter. In 7 (36.8%) eyes the peripheral vascularization did not progress and the corneal surface was completely epithelialized and stable at 12 months after COMET. In the remaining 12 (63.2%) eyes the central cornea became progressively vascularized or developed persistent epithelial defects with recurrence or worsening of symblepharon between 3 and 9 months of COMET.

**Visual Outcomes:** Prior to COMET the BCVA ranged from hand movements to perception of light in all eyes. On the last date of follow-up or before undergoing keratoplasty or keratoprosthesis surgery the BCVA had not improved in 12 (63%) eyes; had improved to counting fingers in 6 (32%) eyes and to 20/125 (5%) in one eye.

**Additional Surgery:** Of the 7 eyes with a stable ocular surface, one eye underwent PK and four eyes underwent Boston type 1 keratoprosthesis surgery for visual improvement. Following PK the corneal graft developed repeated epithelial defects and a permanent tarsorrhaphy had to be performed three months later. Three years after PK the BCVA with an intact tarsorrhaphy was hand movements. The final BCVA in the four eyes that underwent Boston type 1 keratoprosthesis ranged from 20/20 to 20/30 with a maximum follow-up of 26 months.

**Histopathology:** (a) hematoxylin and eosin and PAS staining of the pannus excised during COMET showed eight to ten layer thick stratified columnar epithelium with presence of goblet cells and underlying loose fibrovascular stromal tissue. These findings were consistent the clinical impression of LSCD. (b) hematoxylin and eosin and PAS staining of the unused back-up oral mucosal cultures showed a monolayer of epithelium on a thick eosinophilic membrane. (c) hematoxylin and eosin staining of the corneal buttons excised during keratoplasty or keratoprosthesis surgery following COMET showed a six to eight cell stratified epithelium with basement membrane. No remnants of the hAM were seen. Goblet cells were not observed in PAS staining. A few sub-epithelial blood vessels were also seen in close proximity to the basement membrane both at the periphery and at the centre. Bowman’s membrane was absent and variable stromal scarring was noted. The Descemet’s and endothelial complex was noted to be normal in all eyes.

**Immunohistochemistry:** (a) Immunohistochemical examination of the unused back-up culture showed: (1) Cytoplasmic K3/K12 expression was seen in all cells; (2) p63 expression was seen in all cells; (b) Immunohistochemical examination of the post-COMET corneal tissues and control corneal and conjunctival specimens showed: (1) K19 being expressed in the basal layer of the epithelial cells of post-COMET corneas, in the basal layer of the limbal epithelium in control corneas, in all layers of the conjunctiva and in the basal cells of the oral mucosa; (2) expression of K14 was absent in post-COMET corneas, absent in control corneas, present in the basal cells of conjunctiva, absent in oral mucosa; (3) Cytoplasmic K3/K12 expression was seen in all epithelial layers of post-COMET corneas, control corneas and oral mucosa but absent in conjunctiva; (4) Cytoplasmic K12 staining was seen only in the control corneal epithelium and was absent in oral, conjunctival and post-COMET epithelium; (5) Ki-67 expression was seen in the supra-basal layer of all specimens; (6) p63 expression was seen in basal and supra-basal layers of the post-COMET corneas, control corneas, conjunctiva and oral mucosa; (7) p75 expression was seen in basal epithelial cells of post-COMET corneas, basal epithelial cells of the limbus in control corneas, basal cells of conjunctiva as well as oral mucosa; (8) CD31 and CD34 expression was seen in sub-epithelial layers of the central and peripheral post-COMET corneas.

**Discussion:** COMET emerged with the promise of being an autologous alternative to allogeneic cell based therapy in eyes with bilateral and total LSCD. Based on the experience with ex-vivo cultivation of limbal epithelial cells\(^{21-24}\), we developed an efficient xeno-free explant culture technique of expanding the oral mucosal cells into a transplantable epithelial sheet on denuded hAM\(^8\). We also noted that the cultivated oral mucosal epithelium had certain phenotypic similarities to limbal as opposed to conjunctival epithelium\(^6\). These results along with the promising clinical outcomes of COMET reported by other groups\(^9-14\) encouraged us to attempt this present clinical study in 2007. However, as...
elucidated in the results, the clinical experience with autologous COMET for the treatment of chronic ocular burns was extremely disappointing in terms of achieving ocular surface stability, corneal clarity, avascularity and visual improvement.

When we compare this study with the indications, laboratory techniques and clinical outcomes of previous studies on autologous COMET with a sample size of 9 or more eyes\textsuperscript{12-14, 17-19}, it is noteworthy that: a) none of the previous studies used a xeno-free culture technique; b) the indications for COMET varied widely among different studies; c) all studies used clinical criteria for assessing the outcome of therapy; d) success rates with regards to ocular surface stability ranged from 28.5\% to 100\% with mean follow-up durations ranging from 12 months to 55 months; and e) all studies reported appearance of peripheral superficial corneal vascularization after COMET.

In the context of this heterogeneous data, ocular surface stability achieved in our study (37\% in 19 eyes) compares well with that reported by Satake and associates (36\% in 11 eyes)\textsuperscript{18} and Burillon and associates (44\% in 9 eyes)\textsuperscript{19} in eyes with ocular surface burns.

A comparison between this study and previous studies on COMET with those on allogeneic limbal transplantation is again difficult, because the indications and sample sizes vary among different studies. Indeed, there are no comparable published studies (with a sample size of five eyes or more) of allogeneic cultivated limbal transplantation in eyes with ocular burns\textsuperscript{25, 26}. With regards to keratolimbal allografts, in two series of 16 and 17 eyes with ocular burns among other indications, Solomon and associates\textsuperscript{27} and Maruyama-Hosoi and associates\textsuperscript{28} reported long-term corneal epithelial stability in 71.3\% and 58.8\% eyes respectively. Similar to ocular surface stability, the proportion of patients who gained 20/200 or better vision, after keratolimbal allografting (43.5\% to 44.6\%)\textsuperscript{27, 28} was also greater as compared to that after COMET (7\% to 30\%, Table 2)\textsuperscript{12-14, 17-19}. This limitation of COMET is particularly significant because unlike patients with unilateral LSCD, who usually have good vision in the unaffected eye and may be satisfied with a stable and symptom free ocular surface in the affected eye, the primary need of a patient with bilateral blindness is improvement in vision. Therefore the benefit of COMET of being an autologous therapy not requiring immunosuppression does not outweigh its poor clinical outcomes. In view of these results, currently we do not offer COMET to patients with bilateral LSCD.

Other findings of our study were similar to Chen and associates and Nakamura and associates who performed histopathology and immuno-histochemical analysis in four and six post-COMET eyes, respectively\textsuperscript{15, 29}. On histopathology, they found the transplanted epithelium to be five to twelve layers thick without goblet cells or apical microvilli. On immunohistochemistry, they also found that K3 was present in all epithelial layers, K12 was present occasionally at the peripheral portion of corneal tissue, p63 and p75 was present in the basal epithelial layers. These findings along with ours suggest that the transplanted oral mucosal epithelium maintains its original phenotype without any trans-differentiation to the corneal phenotype. Additionally we showed expression of vascular endothelial markers CD31 and CD34 in the sub-epithelial region of the post-COMET corneas to corroborate with the clinical findings of superficial vascularization.

This is the first report on transplantation of oral mucosal cells cultivated using a xeno-free technique of cell culture. Another strength of this study is the homogeneity of the patient cohort; being the largest such study in cases with bilateral ocular burns. Unlike others we used an explant culture technique and transplanted at a monolayer stage, like we do for cultivated limbal epithelial transplantation\textsuperscript{21-24}. It may be argued that these unconventional techniques of cultivation and transplantation may have affected the results. But similar poor outcomes of COMET in ocular burns have been reported with conventional cell culture protocols as well\textsuperscript{18, 19}. We also found that the oral epithelium does not convert to a corneal phenotype when transplanted onto the ocular surface and because of the associated vascularization, which is probably essential to its survival, a conjunctivalized ocular surface and one reconstructed after COMET are virtually indistinguishable.

In summary, the findings of our study suggest that transplantation of autologous oral mucosal epithelium cultivated using a xeno-free explant culture system, is unsuccessful in restoring a stable ocular surface and improving vision in eyes with bilateral LSCD following ocular burns. However, our results do not apply to other causes of LSCD or other cell-culture protocols.

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Original Article

Combination of Azathioprine and Corticosteroids in the Treatment of Serpiginous Choroiditis

Aneesha Lobo1, Prachir Agarwal1, Debmalya Das1, Manmath Das1, Rupak Roy1, Jyotirmay Biswas2

Abstract

Purpose: To evaluate the role and efficacy of combination of azathioprine and corticosteroids in the treatment of serpiginous choroiditis. Methods: Medical records of all patients with serpiginous choroiditis those were treated with a combination regimen of azathioprine 2.5 mg/kg/body weight in 3 divided doses and prednisolone 1mg/kg body weight for at least 6 months were reviewed. The patients were followed up every month for atleast 6 months or till the lesions showed clinical and angiographic signs of inactivity. Doses of both azathioprine (50 mg/month) and prednisolone (10 mg/week) were tapered as and when the lesions became inactive. On subsequent follow-up visits, any episode of relapse was treated by resuming the therapy with both agents in the doses described above with a more prolonged tapering. Blood counts were monitored every 15 days and liver function tests every month. Sight threatening serpiginous choroiditis was initially treated with 1 gram/day of intravenous methyl prednisolone for 3 days, followed by the above mentioned regimen. Wilcoxon sign rank test was done to obtain the p value for comparison of mass of treatment and post treatment visual acuity. Results: Medical records of 30 eyes of 19 patients were evaluated. All patients showed disease regression having inactive lesion at the time of last follow-up. Only four out of the thirty eyes showed deterioration of visual acuity. Thirteen (43%) out of the thirty eyes maintained their pretreatment visual acuity and another thirteen (43%) eyes had improvement in visual acuity. (p = 0.023). Conclusion: Combination therapy of azathioprine and corticosteroids is an effective regimen for treatment of serpiginous choroiditis.

Key words: azathioprine, corticosteroids, immunosuppressive agents, intravenous methyl prednisolone, serpiginous choroiditis.

Serpiginous choroiditis is a chronic, visually debilitating, progressive condition of undetermined etiology. The disease shows recurrent episodes of choroiditis involving both eyes primarily affecting the retinal pigment epithelium (RPE), the choriocapillaris and the choroid. Apart from the typical peripapillary form of the disease, many different forms have been described like macular serpiginous choroiditis (predominantly involving the macular area) and ampigenous choroiditis (which combines the signs of serpiginous choroiditis and APMPE) wherein the fundus lesions involve the disc and the macula in a multifocal pattern.

Many systemic diseases are reported to be associated with serpiginous choroiditis, but they are, merely an association rather than the cause of the disease. Infections such as tuberculosis and herpes have been associated with serpiginous choroiditis in India. Approximately 25% of the patients may develop choroidal neovascular membrane (CNVM) that leads to loss of central vision late in the course of disease. The visual implications of serpiginous choroiditis are, therefore, quite serious and about 75% of patients have a final visual acuity of less than or equal to 6/60. The therapeutic options available for treatment are systemic corticosteroids, antimetabolites and immunosuppressive agents. A number of studies have been done to find the optimum therapeutic regimen for serpiginous choroiditis. Hooper and Kaplan have described triple agent therapy for serpiginous choroiditis consisting of corticosteroids, azathioprine and cyclosporine. However this therapeutic regimen exposes the patient to serious side effects of two immunosuppressive agents and corticosteroids. Therefore, in the present study, we have tried to explore the efficacy of treatment using a single immunosuppressive agent (azathioprine) along with systemic corticosteroids.

Materials and Methods:
The medical records of all patients diagnosed as...
serpiginous choroiditis and treated with combination therapy of Azathioprine and Corticosteroids, in a tertiary care centre in southern India between January 2000 to December 2008 were reviewed. Patients on any other immunosuppressive regimen were excluded from the study. The study was approved by the Institutional Review Board and a written consent taken as per Helsinki Declaration. All patients received oral azathioprine 2.5 mg/kg body weight/day in three divided doses. Systemic corticosteroids were given in a dose of 1 mg/ kg body weight/day orally with 10 mg/ week tapering for a minimum period of 6 months. Sight-threatening disease was treated initially with intravenous methyl prednisolone (IVMP) 1 gm/day for 3 consecutive days followed by azathioprine and oral corticosteroids10. Azathioprine was continued for at least 6 months along with corticosteroids and the patients were reviewed every month for first 6 months.

A detailed ophthalmic examination including best-corrected visual acuity (BCVA), applanation tonometry, slit-lamp biomicroscopy, fundus examination by noncontact +78D lens and indirect ophthalmoscopy were performed at each visit. Fundus fluorescein angiogram was done as and when felt necessary. During each review, activity of the lesion, any drop in vision from the previous visit and side effects of azathioprine and corticosteroids were noted. Azathioprine and corticosteroids were continued till the lesions showed clinical signs of regression. If required, fundus fluorescein angio-graphy was performed during follow up and the absence of late phase leakage from the margins of active lesions was considered as the evidence of regression. If the lesions were found to be active, therapy was further continued till they became inactive. Corticosteroids were tapered at the first sign of inactivity at a rate of 10 mg per week and azathioprine in a dose of 50 mg per month.

During follow up visits, development of new lesions and / or appearance of lesions adjacent to the previously inactive lesions were taken as signs of relapse, that were treated by resuming the therapy with a more prolonged tapering. Total leukocyte count and total platelet count were monitored every fortnight and liver function test was repeated at monthly interval.

Statistical analysis was performed using SPSS for Windows (version 14; SPSS, Inc., Chicago, IL). Disease activity at final follow-up, visual acuity outcome, duration of azathioprine therapy and number of relapses were recorded. Vision was recorded in Snellens and converted into logMAR for statistical analysis. Pre and post treatment visual acuity were compared by Wilcoxon sign rank test. Significance was assessed at p 0.05 level for all parameters.

Results:

Thirty eyes of 19 patients were analyzed. Six (31.5%) were females and 13 (68.5%) were males. The mean age was 36.16 years (range 16-63 years). Eleven (58%) patients had bilateral disease. Mean duration of follow-up was 32.9 months (range 6 to 79 months, S.D. 18.15) and mean duration of azathioprine therapy was 6.5 months (range 3 to 20 months, S.D. 5.7). All patients showed disease regression in the form of inactive lesion at the time of last follow-up. But three patients (5 eyes, 16.6 %) developed recurrence once and one (2 eyes, 6.6 %) had two episodes of recurrence which were managed by increasing the doses of oral corticosteroids and azathioprine. The mean initial BCVA was 0.33 LogMAR units. The mean final BCVA was 0.15 LogMAR units (Mean visual acuity cannot be calculated in snellens as it is a non continuous parameter hence we have provided the mean logMAR value- Please mention snellen’s VA) The improvement in the visual acuity before and after institution of combination therapy was statistically significant (p = 0.023, Wilcoxon sign rank test). Thirteen (43.3%) eyes had improvement in visual acuity, 13 (43.3%) maintained the pretreatment visual acuity. Four (13.4%) eyes showed deterioration of visual acuity in spite of disease remission clinically as a result of involvement of the center of fovea.

Discussion:

The pathogenesis of serpiginous choroiditis remains unknown despite various studies done in the past to identify the infectious, the immunological, and the vascular etiology of the disease. Various infections such as tuberculosis and herpes virus have also been implicated in the pathogenesis2,10. The inflammatory nature of the disease is supported by histopathological findings such as lymphocytic infiltrates in the choroid and the vessel wall11.

A large number of immunosuppressive agents have been tried either alone or in combination to treat serpiginous choroiditis (Table 1). Corticosteroids have both anti-inflammatory and immuno-suppressive actions. The difficulty with corticosteroid therapy alone is that it has no effect on prevention of recurrences. Recurrences are common while tapering the dose or shortly after discontinuation of the treatment. Gupta et al treated
Patients of serpiginous choroiditis with corticosteroids. Sixty-five percent of their patients achieved visual acuity of 20/40 or better, while only one patient developed CNVM. However, the study reported very high rates of recurrence in their cohort (92%).

Azathioprine is a prodrug that gets metabolized to the active agent, 6-mercaptopurine. It affects the DNA and RNA metabolism on getting converted to 6-thioinosine-5-phosphate (T-IMP) by the enzyme hypoxanthine guanine phosphoribosyltransferase. T-IMP is probably incorporated into the nucleic acids leading to false codes being generated. The adverse effects of azathioprine are leucopenia, thrombocytopenia, and gastrointestinal disturbances. The long-term concern is the increased risk of neoplasia on chronic immunosuppression. Andrasch and colleagues have used the combination of azathioprine with low dose corticosteroids in the treatment of uveitis. Half of their patients showed a positive therapeutic response and the other half had either no response or developed severe adverse effects. Raul et al were the first to describe the efficacy of this regimen (azathioprine + corticosteroids) in 5 eyes of 4 patients of serpiginous choroiditis. They were able to achieve disease inactivity in all the eyes within the first month of therapy, but two patients had relapses which were treated by adding methotrexate and mycophenolate mofetil. In our study the relapses could be managed only with increasing the dose of immunosuppressives without adding another medication.

Akpek et al treated 9 patients with alkylating agents (Cyclophosphamide or Chlorambucil) for triple therapy-resistant cases. Six patients (9 eyes) had improvement in visual acuity by 1 to 5 Snellen's lines and seven patients had long-term drug-free remission. Only one patient developed CNVM. Unfortunately, all patients had bone marrow suppression as a short-term side effect and one patient developed carcinoma bladder as a long-term complication. Alkylating agents are relatively toxic drugs. In addition to reversible bone marrow suppression, cyclophosphamide causes hemorrhagic cystitis, alopecia, and sterility. Chlorambucil causes bone marrow suppression and sterility. Moreover, increased risk of malignancies with alkylating agent therapy is a matter of great concern. In our opinion, this regimen should be reserved for those rare cases of serpiginous choroiditis that are resistant to all other available therapeutic options.

Cyclosporine is an immunomodulatory agent which acts by inhibiting activation of T-lymphocytes. Although cyclosporine theoretically would affect any cell with its binding protein, its major clinical effect is directed against the factors that promote T-cell activation and proliferation.
recruitment. Its common side effects are nephrotoxicity, hypertension, and myelosuppression. Hooper and Kaplan have reported triple agent immunosuppression9 (combination of azathioprine, corticosteroids and cyclosporine) in the treatment of serpiginous choroiditis. They treated five patients of bilateral disease where three patients had remission while being maintained on low dose triple therapy and two had relapses. Christmas et al treated 34 eyes of 17 patients with corticosteroids and various immunosuppressive agents10. Only 23-26% of their patients had a final visual acuity of less than 20/200, but they had a higher rate of CNVM development (35%). Akpek et al treated 6 patients with different combinations of immunosuppressive agents, cyclosporine and azathioprine in 3 patients, cyclophosphamide in 2 patients and cyclosporine in 1 patient15. They reported visual improvement in 10 eyes and recurrences in two. Araujo et al treated 14 eyes of 7 patients with corticosteroids and cyclosporine17. In addition, two patients received azathioprine and one had mycophenolate mofetil. Vision was maintained in 10 eyes, 3 had improvement and one had deterioration due to the formation of cataract. Laatikainen et al treated 15 patients with antitubercular drugs or corticosteroids18. They reported development of CNVM in 2 eyes and their series did not report any favorable outcome at all. All these studies employing different regimens did not show convincing evidence to prove one's superiority over another. Till date, ours is the largest case series evaluating the efficacy of combination therapy of azathioprine and oral corticosteroids in the treatment of serpiginous choroiditis.

In the present series, the patients in whom the final BCVA in the affected eye showed deterioration had involvement of fovea by the disease process. In fact the lesions became inactive with the treatment and all the patients showed resolution of the disease. None of the patients reported major side effects of either azathioprine or oral corticosteroids. Two patients had one episode of relapse and one had two episodes of relapse that were managed by increasing the dose of immunosuppressive and by more prolonged tapering of corticosteroids. The outcome of this study as determined on analysis of 19 eyes of 30 patients was that an improvement in visual acuity does occur with treatment with azathioprine and corticosteroids. We report stabilization or improvement in around 86% of the eyes and minimal recurrence. Limitations of this study are inherent to its retrospective, cross-sectional design and that it lacked a comparator group. As a retrospective review, the sample size and selection bias are likely significant factors. Furthermore, the patients in this study were not a homogenous group, and represent serpiginous choroiditis in various stages of disease. We recognize that there are subjective limitations to fundus picture and angiogram interpretation. We suggest further prospective studies to optimize treatment protocols.

We conclude that combination therapy employing azathioprine and corticosteroids is an equally effective alternative to triple agent therapy in the treatment of serpiginous choroiditis.

References:

Community Based Intervention for Preventing Corneal Injury from Paddy Grain
Asim Kumar Sil

Abstract

Purpose: To assess community based intervention in preventing general injury from paddy grain. Method: Six villages were selected. Educative materials were produced to propagate use of protective glass during the time of threshing. Interactive meetings were organised. Plastic dark goggles and white goggles were promoted. Hospital records were studied and compared with previous year. Result: Not a single case of paddy grain injury was reported from these area. Conclusion: Campaign and availibity of protective goggles is helpful in preventing such injury.

The prevalence of ocular injury in agriculture-workers is unknown in India; but data from few studies suggest that this is quite common. It varies from region to region according to the nature of the crop. Injury from sugarcane leaf is quite common in northern and western India, grape vine injury is common in central and south India. Paddy grain injury of cornea is very common in coastal India where rice is grown as main crop. In recent years the incidence of paddy grain injury has gone up because mechanical threshers have replaced the practice of manual separation of grains by beating the plant against a hard platform. This article depicts the results of a community based intervention for preventing corneal injury in a rural area of West Bengal, India.

Paddy grain and the eye: The age old practice of separation of paddy grains from the plant used to be hitting the tip of the plant against a bamboo platform. This process takes longer time and involves more manpower. Since the speed of the grain during separation is less there is less chance of eye injury by paddy. Now the process has been replaced by the use of mechanical threshers to do the same work in much shorter time and save manpower.

Rice is the main crop in major part of India. In some fertile areas of the country it is grown thrice a year. Naturally farmers engaged in paddy cultivation in those areas are more exposed to the risk of corneal injury at the time of harvesting. Plants grown in different seasons are not of same length. In Southern part of Bengal one high yielding variety of rice is harvested during April-May. This plant is shortest in length and has much more number of grains than the other ones. That is why cases of corneal abrasion are much more reported during April-May.

During harvesting the entire family of a farmer is involved in the work. Mechanical threshers are used usually by two gentlemen who operate the machine by feet and place the tip of the plant over the spin. Another person, usually a lady constantly sweeps the ground to collect the grains at one place. Her face is usually closer to the machine and more prone to injury. Anybody, even a child moving close to the thresher may get injured.

Farmers have a habit of covering the head and face with a piece of cloth to avoid dust but leave the eyes open while threshing. This practice keeps the eyes unprotected. The commonest mode of injury is abrasion of cornea by rapidly moving seed. The most unfortunate sequel of this injury is development of fungal keratitis. Paddy grain has fine hair like structures over the outer coating. That is why the grains get anchored to conjunctiva firmly. Sometimes the grain lodges inside the upper fornix and remain unnoticed. Even the plant may start growing inside the eye. Treatment of fungal keratitis is difficult in any peripheral location. The cases report late and are often complicated by the use of unknown eye drops and native medication. Fungal culture facility is usually not available in the periphery and antifungal medications are used empirically. Application of too many drops often reduces the efficacy of antibiotics.
All these issues contribute to unilateral corneal blindness after paddy grain injury and most of them belong to active working age. Many factors like huge cost of medication, loss of wages and ultimately loss of vision make paddy grain injury a public health issue.

**Method:**

The most obvious way of preventing this corneal injury is protecting the eyes at the time of threshing. Wearing plastic goggles was considered to be the cheapest and easiest option. Education materials were produced to propagate the use of protective glass. Posters were displayed in places that farmers visit usually. Eye health talks were organized in different occasions and festivals. One public education video was developed in local language to motivate people to wear glasses. This video was shown in different places and in local cable network. CD of this 6 min. film was distributed among volunteers who used it locally.

The most effective way of communication was interactive meeting with the farmers. Farmers’ Co-operatives were selected for holding the meetings (fig 1). Every large village in this part of Bengal has one co-operative where farmers get agricultural assistance and evening is the suitable time to get them there. Interactive meetings were organized. It started with the thought provoking video and followed by discussions. It came out through discussion that many farmers do the job of threshing in the evening using electric lights. Sometimes it is overtime work and sometimes it is done to avoid daytime heat. Initially we tried to promote plastic dark goggles usually used after cataract surgery (Fig 2). But we had only thought of day time use. We made change in the goggles; dark glass was replaced by a white one (Fig 3) without increasing the cost. This white goggle got more acceptances. The price could be kept under INR 30. The message was conveyed that any kind of spectacle available at home is good enough to protect eyes.

**Result:**

Six villages in Mahishadal Block of East Medinipur district in west Bengal were selected for intensive campaigning few weeks before the harvesting time. These villages were selected because of the proximity to the hospital and for the villagers this is the closest eye care facility. The population of these villages was approximately 15,000. We looked at the hospital records after the harvesting season and compared it with the previous years. Till 2010 about 3 to 4 cases of paddy injury used to be reported from this population. After this intervention not a single case has been reported from this area.
Discussion:

There are always barriers in the usage of safety eyewear amongst workers. In one study from central India about three-fourth of the workers reported using it all or most of the time during work. Despite knowing that protective eyewear devices offer safety from work-related injuries workers do not tend to use them for multiple reasons. These include some blurring of vision, discomfort, fogging, unusual appearance, people making fun of them, slipping of the goggles due to sweat and slowing work pace.

Prevention of ocular injuries in agriculture workers will indirectly reduce the incidence of microbial keratitis amongst them. Srinivasan et al had demonstrated that treating corneal abrasions with antibiotic ointment by health workers at the village level significantly reduced the incidence of bacterial and fungal corneal ulcers, but primary prevention of injury is always the best. It is all about developing the attitude of adopting safety measures. Constant effort of educating the community would result in consciousness about eye safety and develop peer pressure to wear protective goggles. Providing protective goggles at an affordable cost should complement this effort. The manufactures of the threshers have great responsibility in ensuring safety by modifying the design.

The current experience with a small defined population encourages us to scale up the campaign involving all stake holders and making the goggles available locally.

Acknowledgement: Dr. Samar K. Basak, Disha Eye Hospitals, Barrackpore.

References:

Retinal Vascular Caliber Measurement through Mahalanobis Distance Function based Segmentation

Subhamoy Chatterjee¹, Sowik Dasgupta², Jyotirmoy Chatterjee³, Sambuddha Ghosh²

Abstract

Purpose: To measure retinal vascular caliber by image segmentation with training based on color image segmentation using Mahalanobis distance function. Method: cross-sectional observational study with 20 eyes of 10 normal individuals. Fundus photographs were captured and processed for target feature segmentation using MATLAB R2008a. Data matrices for all the classes were built followed by calculation of mean and covariance matrix for respective classes. Subsequently, to segment out different target classes from the fundus images, each pixel in the image was mapped to one of the classes giving minimum value of Mahalanobis distance. Optic disc was segmented out. Two circles were drawn at radial distances 0.5D (disc diameter) and 1D from the boundary of the disc. Edge detection was performed through gradient calculation and vascular caliber was determined using two points marked on the walls of a vessel where they intersected the two concentric circles. Result: Average arterial diameter was 147 ± 16µm and average venous diameter was 168 ± 22µm in our study population. Conclusion: Method used in the present work relies on segmentation by manual marking of the pixels over different study classes to calculate their respective covariance matrices used in Mahalanobis distance function taking care of the correlation of different variables in the feature set. Thus the resulting segmentation determines the edges of the vessels properly which results in accurate computation of vessel width.

Computational image analysis involves segmentation in order to detect objects to divide an image into regions which can be considered homogeneous according to a given criterion, such as shape, color, texture etc. Image is not just a random compilation of pixels; it is a consequential arrangement of regions and objects. Image segmentation involves dividing an image into different regions where each region is homogeneous. In retinal image processing for vasculature segmentation, there are algorithms for extraction of image ridges, which coincide approximately with vessel centerlines. Every line element comprises a local coordinate frame for its corresponding patch and feature vectors, for each pixel are calculated by properties of patches and line elements.

There are reports about other methods of image processing like either 1-D or 2-D matched filters¹, applied strategies for locating bifurcation, branches, estimating vessel diameter and calculating angle geometry², mathematical morphology and linear processing for vessel recognition in noisy retinal images³, identification of bifurcation and retinal vasculature by both edges and regions⁴. Mahalanobis Distance is a very useful way of determining the similarity of a set of data points to an unknown. Its advantage over Euclidean distance is that it takes

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distribution of the points (Fig. 1(a-b)) into account. It takes into account not only the average value but also its variance and the covariance of the variables measured. It compensates for interactions (covariance) between variables. Also, it is dimensionless.

The aim of this study was to measure retinal vascular caliber by image segmentation with training based on color image segmentation using Mahalanobis distance function.

**Materials and Methods:**

In this cross-sectional observational study between January 2011 and June 2012, we studied for 20 eyes of 10 normal individuals. Patients with refractive errors (> +2 D or < -2 D), hazy media, previous history of glaucoma medication and surgery, diabetes, hypertension, ischaemic heart disease, cerebrovascular accidents, peripheral vascular diseases, collagen vascular diseases, hyperlipidaemia, smoking habit and alcohol addiction were excluded from study. Fundus photographs were captured by TRC 50 DX TOPCON camera at three angulations- 200, 350 and 500. Fundus photographs were processed for target feature segmentation using MATLAB R2008a.

**Image segmentation:** The preprocessed (histogram equalized) digital retinal fundus image was taken as input. The input image pixel RGB triplet was considered to be the feature set for discrimination of four target classes in the image viz. class 1 = optic disc(OD), class 2 = artery, class 3 = vein, class 4 = background. Using medical ground truth, data matrices for all the classes were built followed by calculation of mean and covariance matrix for respective classes. Subsequently, to segment out different target classes from the fundus images, each pixel in the image was mapped to one of the classes giving minimum value of Mahalanobis distance (a statistical distance function: Di representing distance of data point x from ith class in 3-D RGB space) given by

$$D_i = (x - \mu_i)^T S_i^{-1} (x - \mu_i),$$

where $\mu_i$ and $S_i$ were the mean vector and covariance matrix for ith class; $S_i^{-1}$ was the inverse of covariance matrix $S_i$ (a matrix consisting of covariance of different pairs of variables along off diagonal positions and variances along diagonal).

OD was segmented out and its diameter (D) was measured in terms of number of pixels using Euclidean distance (a mathematical distance function which is used to calculate distance between two points having n-dimensions in Euclidean space) between the OD centroid pixel coordinates and boundary pixel coordinates i.e. optic disc radius (D/2). Two circles were drawn at radial distances 0.5D and 1D from the boundary of the disc. Segmentation of the background (i.e. class 4) was done and merged with OD segmented images. Edge detection was performed through gradient calculation and vascular caliber was determined using two points marked on the walls of a vessel where they intersected the two concentric circles. Pixel to Pixel Euclidean Distance in mm = (D in mm/D no. of pixels) * Distance in no. of pixels, where D ~ 1.5mm. Using artery and vein segmented images vessels of both kinds were identified on the OD and background merged images. Thereafter artery to vein caliber ratio was determined on the mentioned circles.

**Flow chart for image processing of retina**

Segmentation of all four classes → Segmentation of class1 and centre of optic disc was detected on that and disc diameter was measured → Two circles were drawn at 0.5 D and 1 D, radial distances from the boundary of the disc → Processing of the background (class 4) was completed and merged with optic disc images → Edge detection done through gradient calculation → Processed image is ready for interpretation → The image result was opened with MATLAB R2008a Software for determination of vascular caliber → Two points were marked on two walls of a vessel, where they intersected by concentric circles → Distance is measured in unit of pixel → Pixel value is transformed in to true diameter (in mm), by multiplying with converting factor.

**Results:**

Retinal images were segmented out using the above mentioned steps (Fig. 2a-c).

Average arterial diameter was $147 \pm 16\mu m$ and average venous diameter was $168 \pm 22\mu m$ in our study population.
Fig. 2b: Segmentation of class 1 (Optic Disc) with circles

Fig. 2C: Final processed image result

Discussion:
In the existing method (unsupervised) Gaussian curve fitting is done over the actual intensity line profile of the vessel which may not be an accurate representation of the actual scenario and thus may give some erroneous vessel width. But the method used in the present work (supervised) relies on segmentation by manual marking of the pixels over different study classes to calculate their respective covariance matrices used in Mahalanobis distance function taking care of the correlation of different variables in the feature set. Thus the resulting segmentation determines the edges of the vessels properly which results in an accurate computation of vessel width which corroborates other findings.

Reference:
Case Series

Multilayered Amniotic Membrane Transplantation for Mooren's Ulcer – A Case Series of Ten Patients

Jayanta Dutta¹, Somnath Mukhopadhyay², Himadri Datta¹, Tamojit Chatterjee¹

Abstract

Purpose: To examine the efficacy of amniotic membrane transplantation as a primary procedure in the treatment of Mooren’s ulcer. Method: A total of 10 patients with primary Mooren’s ulcer were treated by this method: none of the patients had undergone any previous treatment for their ulcer. The ulcer in each of the 10 patients (10 eyes) was treated with amniotic membrane transplantation. Separate amniotic membranes were transplanted (in lay) as material to fill the gap in the stromal layer (amniotic membrane filling), as a basement membrane (amniotic membrane graft), and as a wound cover (amniotic membrane patch). In the postoperative period all the patients were treated with topical antibiotics, topical steroids and artificial tear drops. Result: 7 eyes (70%) healed with epithelialization in (range, 7 to 18 days) with 4 eyes showing corneal epithelialization and 3 eyes showing conjunctival epithelialization. 3 eyes showed persistent defect with no epithelialization and showed recurrence of ulcer on follow up. Conclusion: Multilayered amniotic membrane transplantation is effective for treatment of deep ulceration of the cornea as occurs in Mooren’s ulcer.

Key words: Mooren’s ulcer, amniotic membrane, cyclosporine, keratoplasty

Mooren’s ulcer is a chronic, painful, peripheral ulcerative keratitis that was described as a clinical entity by Mooren in 1867. It is characterised by progressive, crescentic, peripheral corneal ulceration that is slightly central to the corneoscleral limbus. It is associated with a characteristic extensive, undermined overhanging edge. It typically progresses with an anterior stromal yellowish infiltrate at the advancing margin. An overlying epithelial defect then develops followed by progressive stromal melting. The ulcer progresses circumferentially and centrally. The pathogenesis remains unknown but appears to involve an autoimmune reaction against a specific target molecule in the corneal stroma which may occur in genetically susceptible individuals. It is usually treated as a stepladder approach by local, systemic and surgical therapy. Local treatment includes topical steroids or topical cyclosporin. Subconjunctival heparin injections and topical collagenase inhibitors have also been used.

Systemic immunosuppressives are initiated if treatment with topical therapy and conjunctival resection fails.

Surgical therapy include lamellar keratoplasty, epikeratoplasty, delimiting keratotomy, patch grafts of periosteum, fascia lata and amniotic membrane. Surgical management for visual rehabilitation is a challenge; penetrating keratoplasty is usually associated with disease recurrence, graft rejection and melting. In the present series we have used amniotic membrane transplantation as primary modality of treatment in all the 10 cases.

Amniotic membrane has long been used as a surgical material in this surgery. Amniotic membrane has a number of indications, both as a graft to replace damaged ocular surface stromal matrix and as a patch to decrease inflammation.

Methods:

10 Patients (7 males and 3 females) with severe ulceration of the cornea from Mooren’s ulcer were treated with amniotic membrane transplantation. All the operations were performed by a single surgeon after obtaining informed consent from the patient.

Amniotic membrane was obtained during caesarian section in donors who were sero-negative for hepatitis B, hepatitis C, syphilis and HIV. The amniotic membrane with underlying chorion was washed in phosphate buffered saline containing 1mg/ml of dibekacin sulphate and bluntly
separated from placenta. The membrane was then cut into 3×3 cm pieces and rinsed in 0.5mol/l dimethyl sulfoxide dissolved in phosphate buffered saline and preserved in -80 degree centigrade. All procedures were performed under sterile conditions. Preoperatively the container with amniotic membrane was thawed at room temperature and the membrane was rinsed three times in saline and then once in saline containing 1mg/ml of dibekacin sulphate. The amniotic membrane was separated bluntly from the underlying choroin with forceps during surgery.

Surgery was performed under subconjunctival anaesthesia with 2% lidocaine and1:8×10 noradrenaline. First the bottom of the ulcer was debrided and poorly attached epithelium at the edge of the ulcer was removed as bluntly as possible. After the ulcer surface was treated and healthy corneal stroma exposed, the first segment of amniotic membrane was transplanted as filling material (in lay) in the stromal layer. The amniotic membrane was cut into small pieces and stuffed into the ulcer. The second amniotic membrane was transplanted as a basement membrane (amniotic membrane graft). Amniotic membrane was placed on the ulcer with epithelial side up and secured with 10-0 nylon sutures. The third amniotic membrane was given as a cover (amniotic membrane patch) with 10-0 nylon. The amniotic membrane patch was placed on the entire wound and corneal limbus with epithelial side up to protect the area of re-epithelialization. Post-operatively antibiotic and corticosteroid drops were instilled.

Results:
7 eyes (70%) healed with epithelialization (range, 7 to 18 days) with 4 eyes showing corneal epithelialization and 3 eyes showing conjunctival epithelialization (Fig 1a & 1b). 3 eyes showed persistent defect with no epithelialization and showed recurrence of ulcer on follow up of 6 months. Improvement in visual acuity was recorded in 5 eyes. In other 2 cases the visual acuity remained unchanged in the post-operative period.

Discussion:
Several studies have demonstrated that the amniotic membrane has unique properties including antibacterial, wound protecting, pain reducing, epithelialization promoting and fibrosis suppressing effects. These properties are considered suitable for treatment of impaired epithelialization of ocular surface. Improvement in epithelialization may be attributed to inhibition of collagenase by amniotic membrane and supplementation of the basement membrane and growth factors. The present study utilized these properties. A combination of collagen layer supplementation, basement membrane reconstruction and promotion of epithelialization and wound healing is required to treat severe ulceration. We used multilayered amniotic membrane to achieve these goals. Amniotic membrane filling provides a substitute for collagen, the amniotic membrane graft provides a basement membrane for proper epithelialization and the amniotic membrane patch protects the wound.

In summary, we found that multilayered amniotic membrane transplantation is highly effective for treatment of Mooren's ulcer of cornea. The unique properties of amniotic membrane appear to offer a better surgical outcome for the treatment of this disease. The exact mechanism of the healing effect of amniotic membrane is still unknown. One
shortcoming of the present series is the lesser number of patients. Further studies with a larger number of patients are needed to fully understand the mechanism of beneficial effects of amniotic membrane in the treatment of Mooren's ulcer.

Reference:

is it absolutely essential to include multivariable analysis in one and all studies? The answer is "probably not". Here I have consciously excluded the term "certainly not" because there are many instances where multivariable analysis helps a lot in analysis.

What is multivariable analysis? Multivariable analysis is a tool to determine the relative contribution of different causes to a single cause. This, when I put in statistical term, I mention it as "simultaneously predicting a single outcome (dependant or response variable) from multiple factors (independent or predictor variables)". This is important because we live in multivariable world and most events, whether political, social or personal have multiple causes. Diseases also, as we know, are associated with multiple factors. In fact any (pretty or large) event can be fit in this model of multiple factors versus single or multiple effect or outcome (or response variable in statistical term). Though many researchers use multivariable and multivariate analysis interchangeably, some has reservation for using single outcome as multivariable and multiple outcomes as multivariate analysis¹.

Why multi variable analysis? Multi variable analysis is probably a non absolute requirement in any analysis. It is so because we use this tool for addressing the third factor (which is also familiar by name “confounding”). There are other methods of tackling the third factors like stratification, randomization, matching and restriction beside multivariable analysis. But every method has its own advantage and disadvantage and these choices are also no exception. Stratification can not be done for a large number of variables (it gives huge number of permutation and combination tables which is difficult to digest, also to publish in journals). It is very useful to assess interaction (also known as effect modification). Randomization is an excellent process for addressing the unknown confounders (in fact even in multivariable models also we can not address unknown confounders; but these are particularly useful in randomized controlled trials). In cases of serious issues like use of anti-retroviral in post exposure prophylaxis, nobody would like to be randomized in non anti retroviral group. Unless specially called for, unmatched analysis is easier and preferred; there may be some crypto matching or over matching in some cases. Restriction is again restricted to some individual consideration and there is very little scope of including multiple variables at one time.

One great advantage of multivariable analysis is that it unmasks the suppressive effects of any interaction and indicates an apparent insignificant association to significant. This was very clear in table provided by Hasdai, D. et al in table for comparison of bivariate and multivariable association between status and risk of death. Here a bivariate analysis of smoking status and relative risk of death² were as follows (figures in the parenthesis 95% confidence interval): non smokers 1.0 (ref), former smokers 1.08 (0.92 – 1.26), recent quitters 0.56 (0.4 – 0.77) and persistent smokers 0.74 (0.50 – 0.94). This apparently may indicate that non smokers were at greater risk for death than other groups and smoking might had played a protective role in prevention of death. But the actual fact was that, young age low BMI, low triglyceride levels, normal blood pressure etc all played the protective roles in the smokers (suppressive interaction, in persistent smokers or in recent quitters where as non smokers and former smokers were already at higher risk and probably left smoking because of those higher risks). When those factors were adjusted for known variables, the effect of smoking on risk of death was prominent: non smoker 1 (ref), former smoker 1.34 (1.14 – 1.57), recent quitters 1.21 (0.87 – 1.7) and persistent smoker 1.76 (1.37 – 2.26). In recent quitters risk included 1 in the confidence intervals but this could be due to wide confidence interval of a small sample number in that sub strata. Similar effect could be seen in a study of Zidovudine use and sero conversion of HIV infection in post exposure prophylaxis study where the interaction variable was the severity of injury. More

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What are the common uses of multivariable analysis? There may be variety of uses not only in the field of clinical research but in other areas like marketing, sociology also. In clinical research there are four uses namely: (1) identification of prognostic factors while adjusting for potential confounders like effect of low density lipoprotein on coronary artery diseases adjusting confounding effects of other known risk factors of the same disease, (2) in adjusting base line characteristics variables in observational studies (which is main type of study for most of the public health post graduate students), (3) use in prognostic models which is particularly useful in estimating survival periods of different lethal diseases like malignancy. There are some limitations and these models give valid estimates of risk factors only in similar group of patients which in practice may not be same (biological variation). Similarly it may not be accurate in heterogeneous genetic marker of cancer detections and (4) diagnostic model though scientifically sound, this is not favoured by clinicians mainly because of uncertainty. The mathematical explanation is that all these models are based on probability calculation and they are true only for a reasonably large number of trials. But in practice, we do not see that many single disease patients at a time. Moreover, the clinician may not like to accept even five percent failure in diagnosis for a living human being, particularly in emergency.

What are the common types of multivariable analysis and where are they used? Are there any assumptions for a particular type of multivariable analysis model to be true? Well, this is a vast matter to explain. In brief, there are three types of analysis we commonly use: (1) Multiple linear regressions, (2) multiple logistic regressions and (3) proportional hazard analysis. In multiple linear regression, the outcome variable increases or decreases in linear fashion (first order equation in index mathematics or arithmetic progression) where as in multiple logistic regression, the logarithm of odds of outcome (odds means probability of an occurrence to the probability of non occurrence), also called 'logit', changes in linear fashion with the independent variable. Like in any bacterial count study, the bacteriological count increase or decreases in geometrical series pattern and it is then logarithmically treated to make the relationship linear. The proportional hazard model has the similar logic like multiple regressions where the logarithmic value of relative hazard (ratio of time to outcome given a particular risk factor to the time to outcome without the risk factor) changes in linear fashion with unit change of interval independent variable. Here one important consideration is tackling the censor cases (it is similar to tackling non responders in any study). There are several examples of studies which fit in these three models like diffuse linear association model showing association between Vitamin B12 level and pneumococcal antibody titer after pneumococcal vaccination, Z curves or S curve (or a simple Sine curve pattern and ultimately linear) association between skeletal muscle strength and presence of cardiomyopathy among alcoholics. But one has to remember, these are still models and the direction of the model at a particular point of time may not match with the ultimate direction of the model. Still these models have remarkable utility. It can be explained with one very relevant example. In a very lethal malignancy, ninety percent patient may die within first two years with or without treatment. But after adjustment with multivariable model if it is found that eighty percent die between one and half and two years in the treatment group than only six months in the non treatment group it becomes very vital so far the individual patients are concerned. Because one patient may find very valuable time to arrange his/her daughter's marriage or within that precious time gained; one may see the smiling face of new born grand children. There are so many patterns of association found in these models like logarithmic, anti logarithmic, U shaped, upside down U shaped, J shaped, step ladder shaped etc. and the interested readers are requested to look for reference books in the library or internet for that.

Some of the important issues which are to be taken into consideration for setting up multivariable model are estimation of sample size (it again depends on several considerations like alpha error, beta error, dispersion within population or homogeneity of the population etc), selection of the outcomes (preferably ten to twenty in some models), number of study subjects per each independent variable (preferably twenty or more), consideration of inclusion of variables (forward method), consideration of elimination of variables (backwards method), omission of variables (depending on theory, measurement and experimental methods), combining multiple variables to single variables or scale, consideration of the problems of factor analysis in the perspective of original variables (for example several income groups are lost to below median and above median group in multiple logistic regression: some sorts of data
loss). There are several methods of handling the missing values which are again beyond scope of this brief topic. Then there are considerations for calculation of cumulative survivals based on current survival or event rate (number of outcome divided by the subjects at risk), conditional probability (1-event rate), loss to follow up, alternative outcome, withdraw etc.

Performing the analysis was critical in earlier days when a bunch of formulae of “correlation, regression” were used. Now a days specially designed statistical or epidemiological softwares perform the analysis for the researcher. Nevertheless, when the researcher becomes familiar with the concepts of multivariable analysis, not only that s/he understands the method in the computer but also possesses a grip to interpret the results so obtained through the software. The pleasure is like matching the correct answer with a long struggling difficult problem solving answer in a puzzle. For these softwares, manuals are also available with the special softwares.

Reference:

Ophthalmology beyond Ophthalmologists and Also Including Ophthalmologists

Garga Chatterjee

Abstract

Ophthalmology stands in close relationship with other branches of medical science, but the linkages of ophthalmology have long been with disciplines beyond conventional medicine. Project Prakash in the department of Brain and Cognitive Sciences of the Massachusetts Institute of Technology identifies congenitally but curably blind children in India who have so far remained untreated due to poor medical access. The Prakash studies have yielded evidence of significant adverse effects of congenital blindness on some aspects of post-operative vision, but also impressive visual skill acquisition by those who gain their sight even after several years of blindness.

Keywords: ophthalmology ; vision science ; blindness

Clinical ophthalmology is a discipline with a long and glorious past that has served mankind admirably. The implications of ophthalmological research are enormous; its translation potential has been life changing for much of humanity. Imagine life today in the absence of intra-ocular lenses (IOL) and we will appreciate what I am talking about.

However, ophthalmology today does not stand in isolation to other bodies of knowledge. I would argue, it never has been in the case in the past either. It is obvious that ophthalmology stands in close relationship with other branches of medical science, but the linkages of ophthalmology have long been with disciplines beyond conventional medicine. Consider the IOL example provided above. It could not have been developed without the close interaction between material science and ophthalmology. The demonstration of its efficacy needed epidemiological knowledge. Note that I did not mention specialists like ophthalmologists, material scientists, epidemiologists, etc but disciplines like ophthalmology, material science, epidemiology and so on. I will return to the significance of this distinction.

Ophthalmology today closely interacts with vision science which cuts through various disciplines like “psychology (more specifically sensation and perception psychology, cognitive psychology and psychophysics), neuroscience, physics (specifically optics) and computer science (more specifically computer vision, artificial intelligence, and computer graphics), as well as practical applications such as data visualization, user interface design, and human factors and ergonomics.” With these kinds of interactions, certain kinds of collaborations and research have taken place whose success bear testimony to the value of such interdisciplinary knowledge and methods. Let me illustrate this point using the example of a research initiative I have been personally involved with.

Project Prakash was born in the department of Brain and Cognitive Sciences of the Massachusetts Institute of Technology and was conceived by a scientist (Pawan Sinha) whose original training was in Computer Science and Artificial Intelligence.

“The Prakash initiative identifies congenitally but curably blind children in India who have so far remained untreated due to poor medical access.” The two major kinds of treatable childhood blindness in India are cataracts and corneal opacities. “By providing such children corrective surgery, Project Prakash is creating a population of children across a wide age range who are just learning how to see. Longitudinal studies of these unique children offer insights into how they develop visual skills. This approach combines the best of both worlds: it directly addresses the pressing humanitarian need to treat curably blind children and, in the process, illuminates fundamental questions regarding brain plasticity and learning.” Till date, Project Prakash has been able to screen over 40,000 children (primarily in rural
North India) and has provided surgical treatment to over 400 of them as well as non-surgical care to over 1,400 individuals.

What has been the research impact of this project? To quote a recent paper summarizing its results - ‘The Prakash studies have yielded evidence of significant adverse effects of congenital blindness on some aspects of post-operative vision, but also impressive visual skill acquisition by those who gain their sight even after several years of blindness. We have investigated how patients acquire many aspects of visual function, including low-level attributes such as acuity and contrast sensitivity as well as high-level functions such as object and face recognition and spatial imagery. This work has also demonstrated how motion information plays a crucial role in parsing the world into distinct objects and, more importantly, in helping the visual system learn heuristics that can be used even with static images. The results have guided our studies of normally sighted individuals, as well as our computational modeling efforts. Complementing our behavioral studies, we have begun to employ non-invasive brain imaging technology, specifically functional magnetic resonance imaging (fMRI), to examine the kinds of cortical changes that accompany the very initial stages of human sensory development. These neuroimaging studies so far have revealed that the onset of patterned visual information results in rapid modification of the visual cortex even in individuals who have been congenitally blind for the first two decades of their lives.’

The Project Prakash team consists of a variety of individuals which include ophthalmic surgeons specializing in paediatric ophthalmic surgery, optometrists with experience in rural field testing, social workers with good public relation skills, sensitive nursing staff, computer scientist who now is a world-acclaimed neuroscientist, low vision researcher, medical doctor turned vision scientist, biomedical engineer turned vision scientist, and others. Being one of the above-mentioned people, I can now understand how each member is integral to the execution of the project. It is also important how ophthalmologists in the project are simply not ‘surgery providers’ or ‘diagnosis providers’ to the scientists but important part of the scientific conceptualization of the project. In medical science, clinicians are often ‘service providers’ or even worse, ‘sample’ or ‘patient’ providers. Clinical scientist is quite a different idea. Project Prakash and various other such projects bring this idea to life.

Now let me return to the point that I had promised I would take up. I had mentioned interacting disciplines and not people because the essence of collaborative science lies in people coming together with their core competencies but also learning what needs to be learned so that each person becomes a repository of interdisciplinary knowledge. Thus, the ophthalmologist strives to understand in some depth the material science or the visual neuroscience or epidemiology. The idea is to become a part master of multiple trades with a core competency. This also points to the direction of future ophthalmology where the advances will come not only from ophthalmologists but also from people trained originally in other disciplines and from enthusiastic ophthalmologists who have the courage and zeal to wear multiple hats.

References:
Famous Ophthalmologists Who Suffered from Eye Disorders

Debmalya Das

Abstract

Ophthalmologists with eye disorders were many but they seldom came out in open or reported about their diseases to maintain privacy and to maintain his/her practice in the society. However, these ocular conditions prompted them to experiment with various medicines or surgeries to counter the ill effects of the diseases often leading to discoveries of newer modes of management. We review three such famous ophthalmologists whose eye disorders were reported and were subjected intense debate in the ophthalmology fraternity.

Key-words: Ophthalmologist, eye disease, glaucoma.

Ophthalmologists with eye disorders were many but they seldom came out in open or reported about their diseases. Most of the time an Ophthalmologist hid his/her systemic and ocular problem to maintain privacy and to maintain his/her practice in the society. However, these ocular conditions prompted them to experiment with various medicines or surgeries to counter the ill effects of the diseases often leading to discoveries of newer modes of management. Cataract, strabismus and refractive errors were common amongst ophthalmologists but were seldom reported. Glaucoma, often angle closure, was the most commonly reported eye disease amongst ophthalmologists as it forced an ophthalmologist to seek another’s opinion, required a long term follow up and often surgical intervention was necessary. We review three such famous ophthalmologists whose eye disorders were reported and were subjected intense debate in the ophthalmology fraternity at that time.

One such was Alexander Pagenstecher (1828-1879), famous for introducing yellow mercury oxide ointment for managing external eye diseases, which bears his name. His papers were mainly concerned with sympathetic ophthalmia, the indications for enucleation, iridodesis, glaucoma and cataract. He was instrumental in introducing intracapsular cataract extraction as the primary treatment of cataract having personally performed more than 2000 cataract operations. In 1878, he had an acute glaucomatous attack in the right eye and was immediately operated on by his brother, Hermann, who performed a complete iridectomy. No visual deficit remained. The long term effect, however couldn't be ascertained as Pagenstecher died shortly thereafter from an unusual hunting accident.

Ludwig Laqueur (1837-1909) introduced physostigmine as a medical treatment for glaucoma. Since childhood he was red-green color blind, was photophobic and suffered from chronic recurrent conjunctivitis for which he used a 1.5% silver nitrate solution. At the age of 30, prodromal signs of an angle closure glaucomatous attack started. In his paper, he vividly described these attacks and stated that the attacks were triggered by emotions or when in the theatre and resulted in cloudy visions. He also noticed that with Physostigmine, a calabar bean extract, he was able to repeal the attack every time but recurrences were common and became very frequent. Hence, total iridectomy were performed in both the eyes by Horner. Although, the surgery prevented any further attack or progression of visual loss, the large surgical colobomas, which were visible to the naked eye made Laqueur photophobic and extremely self-conscious.

Louis-Emile Javal (1839-1907) is often called ‘the Father of Orthoptics’. His contribution to strabismus was immense. He was also instrumental in designing the Javal-Schiøtz ophthalmometer. He was earlier an engineer but became interested in strabismus because his father and sister both had squint. Since childhood, he had a high degree of astigmatism, chronic conjunctivitis and heterochromia. In 1881, he noticed the first attack of an
acute glaucoma in his right eye. However, treatment started late and he became legally blind in right eye in 1886. The first prodromal signs in the left eye appeared 1885 and was not operated till 1900. But the surgeries couldn't arrest the disease progression and he became blind in left eye in 1901. Right eye was enucleated in 1901. Too frequent changing of ophthalmologists, non-compliance on part of Javal, delaying necessary operations and too many surgeries in a short span had been attributed to the loss of vision in the left eye. The blind Javal invented an armrest with a cogwheel device advancing the writing paper by 1 cm at the end of each line. This 'planchette scotographique' could be used by blind persons to write ordinary script.

References:
5. Jacobson J. Briefe an Fachgenossen (Margarethe Quidde, editor), Königsberg 1899, p.10.
AUTHOR GUIDELINES

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journals" developed by the International Committee of Medical Journal Editors (October 2004).

The manuscripts will be reviewed for possible publication with the understanding that they are being submitted to one journal at a time and have not been published, simultaneously submitted, or already accepted for publication elsewhere.

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- Case report: The limit is up to 1000 words excluding references and abstract with a maximum of 10 references.
- Letter to the Editor: up to 500 words and 5 references.
- History of Ophthalmology: Up to 1000 words and 10 references

Authorship credit should be based only on substantial contributions

1. Conception and design or acquisition of data or analysis and interpretation of data;
2. Drafting the article or revising it critically for important intellectual content;
3. Final approval of the version to be published.

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References should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Identify references in text, tables, and legends by Arabic numerals in superscript before the punctuation marks. The titles of journals should be abbreviated according to the style used in Index Medicus.

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