SPECIAL TALKS

29th July, 2018

KERATOCONUS INTERNATIONAL CONSORTIUM TO COMBAT THE NEED FOR CORNEAL TRANSPLANTATION

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Keratoconus is a devastating and potentially blinding eye disease affecting 1 in 375 people. It is typically diagnosed during early teenage years and has a significant lifetime burden on an individual. While corneal cross-linking treatment is now available to slow disease progression, poor outcomes still persist due to delay in diagnosis or treatment, or other unknown factors that can ultimately lead to the need for corneal transplantation. Our research team has conducted a pilot study wherein we have recruited 400 subjects from public and private clinics in Melbourne to begin to address these issues. As a result, we have obtained extensive knowledge regarding a range of biomarkers from clinical features, imaging and genetic through to studying its impact on quality of life, economic burden, risk factors and treatment modalities. To take this research further, we established Keratoconus international consortium (KIC), with the main aim to produce a more streamlined and concerted effort for better understanding the condition and ultimately curing keratoconus. This global collaborative initiative is a game-changer- with already on board 15 national and international corneal specialists, willing to collect and share data and thus contribute to expanding our knowledge about keratoconus. KIC will be a first worldwide study in KC that will allow us to undertake a unified analysis to enable better diagnosis of individuals at early risk of disease, explore a therapeutic algorithm, identify biomarkers to be targeted for future therapy and thus potentially avoiding graft surgery.

ANTIMICROBIAL PEPTIDES MEDIATED HOST IMMUNE RESPONSE IN BACTERIAL KERATITIS

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Bacterial keratitis is a painful, sight threatening, progressive and difficult to treat corneal infection. In India, *S. pneumoniae* and *P. aeruginosa* are major causes of bacterial keratitis, resulting in severe corneal opacity, ocular pain and visual impairment. The common risk factors are corneal injury, trauma and contact lens wear. Currently, antibiotics are primarily used for the treatment. The emergence of bacterial resistance to conventional antibiotics makes it urgent to develop new class of alternative therapeutic agents that will be helpful against these pathogens. Our laboratory works on innate immune response of host cells towards bacteria responsible for causing corneal infections. Our study showed how bacteria manipulate host cells and subverts the immune responses. We are presently working on the biology of antimicrobial peptides (AMPs) as an alternate mode of therapeutic interventions. AMPs are key components of the host's innate immune system, providing the first line of defense against microbes. We are looking into the expression of endogenous host defense peptides that are expressed during bacterial keratitis and the immunomodulatory role of these peptides. Presently we are studying the antimicrobial property of human cathelicidin and exploring the various other roles of this peptide. We are also studying small molecules and chemicals that might act as inducers for expression of antimicrobial peptides to boost host immune responses against pathogens.

THE ENIGMA OF CULTURE NEGATIVE ENDOPHTHALMITIS

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Infectious endophthalmitis is a vision threatening and potentially devastating intraocular inflammatory process. The ability to identify the causative pathogen(s) has wide implications in management of these patients. Culturing ocular fluids underestimates the actual rate of endophthalmitis, and the etiologic agent is therefore unknown in a majority of patients. Among many other causes, culture-negativity could be attributed to fastidious nature of the inciting organism that may be difficult to grow or may be unculturable. With the advent of massively parallel DNA sequencing platforms, and increasing computational capacities, it is becoming possible to sequence all DNA in a biopsy sample and identify all nonhuman DNA present to detect potential occult or novel pathogens. We tested the feasibility of the application of deep DNA sequencing to vitreous biopsies from patients with endophthalmitis to understand the etiologic basis of culture-negative endophthalmitis, which would help in guiding antimicrobial selection and / or further surgical management. We also hypothesize that regulators of the host immune response, especially the cytokine and chemokine levels could give us leads that can be used as prognostic/diagnostic markers for infectious endophthalmitis especially culture negative endophthalmitis. As fungal and gram negative bacterial endophthalmitis are more commonly reported in our country compared to developed countries, understanding the basis for the inherent differences in the immune response to these virulent infections will provide unique insights into the pathogenesis of endophthalmitis and advance the goal of developing new therapeutic strategies for these diseases.

TUBERCULAR UVEITIS: A DIAGNOSTIC CHALLENGE

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Tubercular uveitis is a diagnostic challenge. A series of case of tubercular uveitis will be presented to highlight diagnostic challenges in tubercular uveitis. Ophthalmic findings, systemic associations, microbiologic and molecular biologic study of a series of tubercular uveitis cases will be presented. Patients had undergone anterior chamber tap, vitreous biopsy. Fine needle aspiration biopsy as indicated. The cases include anterior uveitis with pigmented hypopyon (1 case), granulomatous anterior uveitis with broad posterior synechiae (1 case), intermediate uveitis (1 case), ampiginous choroiditis(1 case), multifocal serpginoid choroiditis (2 cases), military tuberculosis of the choroid (1 case), choroidal granuloma with spinal tuberculosis(1 case), tubercular granuloma with cervical lymphadenopathy (1 case), subretinal abscess (2 cases), frosted branch angiitis with abdominal tuberculosis(1 case), sclerouveitis (1 case) and sclerochoroidal mass (1 case). Tubercular uveitis can have varied presentations. Histopathologic, microbiologic and molecular biologic study of intraocular fluid can resolve the diagnostic dilemma.

FREE PAPERS

28th July, 2018

INVOLVEMENT OF CRYSTALLINS IN PRIMARY CONGENITAL GLAUCOMA

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Purpose: Primary congenital glaucoma (PCG) is largely attributed to homozygous mutations in three candidate genes (*CYP1B1, LTBP2 and TEK)* along with heterozygous interactions across other genes. Crystallins are structural proteins in the lens that are also present in other ocular structures. Based on their functional implications in other ocular phenotypes, we aimed to understand their involvement in PCG.

Methods: A gene panel with eleven crystalline genes known to be associated with congenital cataract was designed. PCG cases that did not harbor homozygous mutations in the known candidate genes (n=323) along with ethnically matched normal controls (n=1157) were screened by deep sequencing on an Ion Proton platform using the Ion Ampliseq chemistry. Bioinformatic analysis (SIFT, PolyPhen and Grantham) for individual variations was done and the potential mutations were further validated by Sanger sequencing (ABI 3130 XL) using BigDye chemistry.

Results: Screening of crystallin genes led to the identification of a 10 novel heterozygous mutations in PCG patients with an overall frequency of 3.71%, of which, eight of them were nonsynonymous changes (p.Gly28Ser [CRYAA], p.Arg22Cys [CRYAB], p.Arg25Trp [CRYBA4], p.Arg214Gln [CRYBB1], p.Ala149Val and p. Glu177Lys [CRYBB3], p.Q13H [CRYGD], p.Pro68Ser [CRYGS] and the rest were potentially abrogating the splice sites (c.40-1G>C [CRYBA4] and c.253-1G>C [CRYGB]). These variations were absent in the controls and rarely found in the ExAC and 1000G databases. Interestingly, 6/10 heterozygous crystallin mutations co-occurred with heterozygous mutations in CYP1B1 (3), MYOC (1), LTBP2 (1) and TIE2 (1).

Conclusions: The present study implicates the involvement of crystallin genes in PCG, which needs to be functionally characterized.

GENOME-WIDE ASSOCIATION ANALYSES OF PHENOTYPIC EXTREMES IDENTIFIES A NEW LOCUS FOR PRIMARY ANGLE CLOSURE GLAUCOMA

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Purpose: Primary angle closure glaucoma (PACG) is one of the major causes of blindness worldwide. In India, ~30% people show narrow-angle but 0.5-1% people actually develop PACG.

Methods: Based on an age-dependent model of progressive angle closure, we conducted an extreme phenotype GWAS where we took older (age ≥60 years) anatomically suspect controls (PACS) showing narrow-angle <15° and PACG individuals having age ≤50 years in order to identify genomic risk factors involved in the progression of angle closure and consequent glaucomatous neurodegeneration.

Results: A total of 713,599 markers were included for genome-wide genotyping of 101 PACG and 43 PACS subjects recruited in AIIMS, New Delhi. 641,895 markers conformed to HWE and standard quality checks. We performed GWAS using a chi-squared association test under additive model. Subsequent Manhattan plot showed that four SNPs (rs2828460, rs2013016, rs984557, rs12037713) were found above the genome-wide suggestive significance threshold (P < 1e-05). Among these, rs12037713 (P-value: 9.11e-06; Odds Ratio- 0.30; 95% CI: 0.18-0.52) mapped to a protein-coding gene Formin-2 (*FMN2*). GTEx-portal indicates that *FMN2* is expressed primarily in the neural tissues. We checked the epigenomic profile for rs12037713. Further, LocusZoom plot suggests rs12037713 has the highest statistical strength of an association among surrounding markers of *FMN2* gene.

Conclusion: The associated A allele in the *FMN2* gene potentially protects individuals from progression to PACG despite having a narrow-angle. Previous reports in a rat model of glaucoma indicated a potential neuroprotective role of *FMN*2 could be suggestive of its protective role for PACG progression.

MMP'S REGULATES OPTICIN DEGRADATION IN MICROGLIA UNDER HYPOXIA

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Purpose: Retinopathy of prematurity (ROP) is a vasoproliferative eye disease, characterized by incomplete retinal vascularization in premature infants. Recently, we reported the presence of activated microglia/macrophages in the vitreous of ROP patients that further activated the microglial cells leading to increased activation of C3 and MMPs. Further, our mass-spectrometry analysis of ROP vitreous revealed a lower expression of opticin in ROP patients. Thus, we hypothesize that activated MMPs under hypoxia degrades Opticin in microglia thereby contributing to ROP pathogenesis.

Methods: The vitreous samples from ROP patients and controls were subjected to zymography and western blot analysis for MMPs and opticin respectively. To assess the opticin expression mediated by MMPs under hypoxia, microglia cells were treated with $CoCl_2$ to induce hypoxiaand further treated with EDTA and/or doxycycline to inhibit MMP activity. Expressions of MMP9, Opticin, VEGF, and TIMP2 were assessed by qPCR and immunofluorescence. IHC was performed in retinal membrane to further validate the the interaction between Opticin and MMP in ROP.

Results: Elevated levels of the MMP's and downregulation of opticin was observed in ROP vitreous compared to no ROP vitreous samples. *In vitro* analysis revealed that high level of MMPs in microglia cells downregulate the opticin expression under hypoxia. Further, the inhibition of MMPs activation checked the degradation of opticin in microglia. Interestingly, the IHC analysis of MMP and opticin in ROP retinal membrane corroborated the findings from the *in vitro* experiments.

Conclusion: The present study confirms the regulation of opticin expression by activated MMPs in microglial cells under hypoxia.

HYPOXIA AND HYPERGLYCEMIA REPROGRAM THE PATTERN OF INTRACELLULAR CALCIUM SIGNALING AND GENE EXPRESSION IN HUMAN MIXED RETINAL CULTURE

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Purpose: Diabetic retinopathy (DR) is a retinal neurovascular complication of diabetes. Studies on individual retinal cells have shown the dysregulation of Ca^{2+} transients under diabetes. The pattern of Ca^{2+} transients of neurons and glia, can provide a valuable understanding of neurodegenerative events in DR, however these have to be looked for in mixed retinal cell (MRC) cultures to mimic the condition in retina. The aim of present study is to study the Ca2+ transients in human MRC under diabetic stress and correlate these with the changes in gene and protein expression.

Methods: MRCs were established from human cadaveric retina and characterized by immunofluorescence and PCR. Hypoxia and hyperglycemia were induced chemically and viability was tested. Ca2+imaging was done using Flu4Am dye and time-lapse movies were acquired every 10s, for 10min and data was analyzed using MATLAB. The cytosolic Ca2+ levels under stress conditions were evaluated. qRT PCR was performed for selected candidate genes. IBA1 and GFAP proteins expressions were measured by immunocytochemistry.

Results: IF and PCR data confirmed all types of glia and neurons in MRC. Cell Viability assay indicated a significant ($P<0.05$) cell loss in treated MRC. A significant ($P<0.05$) increase in maximum Ca2+ amplitude in MRC were identified. qRT PCR of treated MRC revealed significantly increased expression of genes such as HIF1α, VEGF, CXCR4, C3, BAX, IL-8 and IL1-β.

Conclusions: Diabetic stress modulates Ca2+ flux level in MRC along with significant changes in gene expression.

PROMOTER METHYLATION STATUS OF CIS EQTL GENES IN PRIMARY GLAUCOMAS

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Purpose: Intraocular pressure (IOP), optic nerve parameters, Central Corneal Thickness (CCT) etc. are measurable and highly heritable clinical determinants (Quantitative traits (QT)) of glaucoma. In animal models, high throughput system level approaches have mapped candidate genes / loci (cis-eQTLs) for the various QTs of glaucoma. The current study analyses the promoter methylation status in the eQTLs mapped for varying IOP levels (*CACNA2D1*) and optic disc parameters (*ALDH7A1*).

Methodology: The CpG islands flanking 5' upstream of promoter regions of the candidate genes were predicted in Eukaryotic promoter database (EPD) and analysed by PCR based direct sequencing. Initially bisulfite modification of genomic DNA was performed followed by PCR amplification using specific primers for the methylated and unmethylated regions. Bisulfite sequencing of the corresponding CpG islands was done in ABI Prism 3500 Genetic analyser followed by statistical analysis for significance and functional correlation.

Results: Promoter analysis predicted CpG islands upstream to the transcription start site of *CACNA2D1* (-1to -600bp) and *ALDH7A1 (*-59 to -800bp) genes. The deferentially methylated regions between glaucoma cases and controls were clinically correlated with IOP levels / optic disc parameters levels for statistical significance and the results are discussed.

Conclusion: *CACNA2D1and ALDH7A1* genes were identified as potential IOP modifiers and modulators of RGC loss respectively. Differential expression of these genes were correlated with varied IOP levels and axon loss in experimental models of glaucoma. The methylation pattern of *CACNA2D1* and *ALDH7A1* genes would explain the possible genetic attributes to the variation in expression of these genes, association with the variable QTs and their role in glaucoma.

FIBULIN-5, A MATRICELLULAR PROTEIN IN THE PROGRESSION OF PSEUDOEXFOLIATION

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Purpose: Pseudoexfoliation (PEX) is an age-related ocular disorder characterized by deposition of extracellular fibrillar proteins on the anterior segment of eye. Being a multifactorial disorder the actual cause and progression of the disorder remain unknown. This study aims to evaluate the candidature of fibulin-5 (FBLN5), an extracellular scaffold protein in the pathogenesis of PEX.

Methods: Fluorescence based capillary electrophoresis was employed to scan the exons and exon-intron boundaries in search of putative polymorphisms within the gene as risk factors for PEX in Indian population. *In vitro* functional analysis of the risk variants was done through luciferase reporter assays. Quantitative RT-PCR, western blotting and immunohistochemistry were used to study the expression and localization of fibulin-5 in lens capsule of study subjects.

Results: Two single nucleotide variants within *FBLN5* were found to be significantly associated with PEX as risk factors in the study subjects comprising of 338 control and 375 PEX affected individuals. We observed a decreased expression of FBLN5 at both mRNA and protein levels in the PEX affected subjects compared to control.

Conclusions: Deregulation of fibulin-5 expression in cases implies a link between extracellular matrix maintenance and onset of PEX and our data suggests that *FBLN5* is a candidate gene involved in the pathogenesis of PEX.

IL-17 PRODUCTION BY $CD14^{++}CD16^{+}$ INTERMEDIATE MONOCYTES MAY DRIVE STEROID REFRACTORY UVEITIS

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Purpose: Human peripheral monocytes are crucial for orchestrating the adaptive immune response. The monocytes are divided into 3 phenotypes – classical, intermediate and non-classical, depending on relative expression of CD14 and CD16. Among these, intermediate monocytes are typically associated with various autoimmune diseases. The aim of this study was to determine the predominant monocyte phenotype, and its intracellular cytokine response, in patients with steroid-resistant uveitis.

Methods: We included patients with non-infectious uveitis, who were defined as steroid refractory, when persistent/recurrent intraocular inflammation was noted with oral prednisolone ≥10 mg/day, and steroid responsive if dose was <10 mg/day. Patients using non-steroidal immunosuppressive therapy were excluded. Following written, informed consent, peripheral blood was collected from steroid refractory or steroid responsive patients. Monocytes were isolated by magnetic bead isolation kits and stimulated with LPS (100 ng/mL) for 12 hours. These were stained with anti-CD14, anti-CD16, IFNγ, IL-17, TNFα and IL-10 antibodies and analysed by flow cytometry.

Results: Three patients each of steroid-refractory and steroid-responsive non-infectious uveitis, were included. Steroid-refractory patients had significantly higher proportion of $CD14^{++}CD16^{+}$ intermediate monocytes, compared to steroid-responsive patients. These monocytes produced IFNg, IL-17 and TNFa; however, the intermediate subset produced significantly higher IL-17, than the classical ($CD14^{++}CD16^{-}$) and non-classical ($CD14^{+}CD16^{++}$) subsets.

Conclusions: Intermediate monocytes are predominant in steroid-refractory uveitis and are characterised by significantly higher IL-17 production. This is the first report of differential IL-17 production by monocyte subsets in any human inflammatory disease.

PARENT-CHILD AGREEMENT ON HEALTH-RELATED QUALITY OF LIFE IN CONGENITAL GLAUCOMA

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Purpose: To assess parent-child agreement regarding child's health-related quality of life (HRQoL) in children operated for primary congenital glaucoma (PCG).

Methods: One hundred and twenty-one children aged 8-18 years (mean age, 11.8 years) operated for PCG (mean duration since surgery, 10.2 years) by a single surgeon at a tertiary eye care centre, South India, and their parents (mean age, 36.5 years) completed the child and parent versions of Kidscreen-27 questionnaire, respectively. Psychometric properties of Kidscreen-27 were assessed using Rasch analysis, and child-parent agreement regarding child's HRQoL was investigated using the Bland-Altman limits of agreement (LoA) method.

Results: Minor modifications resulted in a psychometrically robust Kidscreen-23 questionnaire. Average parental HRQoL score was higher than child's own ratings, with a significant difference between their scores (mean ± SD difference= 0.53 ± 2.58 logits, p=0.02; lower LOA [95% CI] -4.52 [-5.31 to -3.72] and upper LOA [95% CI] 5.58 [4.79 to 6.38]). The range of child-parent agreement was wide and was bidirectional, with parents tending to both underestimate and overestimate their child's HRQoL. Younger children and girls showed greater discordance in their HRQoL with parental reports than adolescents and boys, respectively.

Conclusions: Discordance between congenital glaucoma child's self-report of HRQoL and parent report indicate that both groups perceive the broader impact of living with PCG very differently. Since they represent perspectives of different sources of information, the reports should be interpreted independently and both assessments should be taken into account in clinical practice and in research studies involving patients with PCG.

SPECTACLES OR REFRACTIVE SURGERY FOR MYOPIA: THE PATIENTS' PERSPECTIVE

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Purpose: To evaluate different aspects of vision-related quality of life (VRQoL) using the Quality of Life Impact of Refractive Correction (QIRC) in myopes corrected with spectacles and different refractive treatments including laser-assisted in situ keratomileusis (LASIK), photorefractive keratectomy (PRK), and small incision lenticule extraction (SMILE).

Methods: The 20-item QIRC was completed by 1425 spectacle wearers (mean age, 24.9 years) and 217 patients who had undergone refractive surgery (mean ± SD age, 25.4 years) at a tertiary eye care centre, South India (N=1642). Both groups were comparable for age and gender. Translation of QIRC questionnaire from English to Indian language versions followed standard international guidelines. Following Rasch analysis, only "Functional" dimension (13 items) of QIRC was found to possess adequate measurement properties. The main outcome measure was Functional QIRC score (VRQoL) and groups were compared to functional QIRC score and on each of the 13 items by Independent t-test.

Results: Patients who had undergone refractive surgery reported significantly better VRQoL (mean ± SD score, 1.66 ± 1.46 logits) than spectacle wearers (0.83 ± 1.09, $p<0.0001$). Significant differences between groups were found regarding convenience, functioning, symptoms and health concerns (all $p<0.05$). There was no significant difference in the VRQoL among spectacle wearers with low/moderate versus high myopia, and also among the three sub-groups of refractive surgery.

Conclusions: Quality of life was significantly lower among spectacle wearers compared to those who had undergone refractive surgery. The Indian versions of QIRC are reliable and valid outcome measures of QoL impact of refractive correction.

DELINEATING THE PATHOPHYSIOLOGY OF ELASTOSIS IN PSEUDOEXFOLIATION SYNDROME: *EX VIVO* AND *IN VITRO* STUDIES IN LENS EPITHELIUM

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Purpose: Pseudoexfoliation syndrome (PXF) characterized by amyloid-like material aggregates in the eye is an elastogenesis-associated disorder. The study evaluated the pro-elastogenic effect of various PXF-associated pathogenic factors in human Lens epithelial cells *in vitro.* Elastin expression and turnover was analyzed in human lens of PXF cases.

Methods: Elastin expression (qPCR) and Desmosine [Elastin cross-linker] (HPLC) were checked in Lens capsules from Cataract, PXF and PXF-Glaucoma (PXF-G) cases (age/sex-matched). Human lens epithelial cells (hLEC) were exposed to TGFβ1, CTGF, Homocysteine (Hcy) and IL-6 for 24 h to evaluate Elastin expression (qPCR/Immunofluorescence) *in vitro*. The Elastin transcription factor *Sp1* (qPCR), Elastin cross-linker Lysyl Oxidase [LOX] (wb) and the pro-fibrotic factor CTGF (ELISA) were assessed.

Results: Elastin mRNA in lens capsule was up-regulated significantly in PXF (2-fold) and PXF-G (12-fold; p=0.0007), respectively. The Desmosine content was 4-fold elevated in PXF (12.93±2.63 nmole; p=0.03) compared to Cataract-alone (3.20±6.32 nmole) indicating increased Elastin turnover. hLEC exposure to TGFβ1, CTGF, Hcy and IL-6 showed increased Elastin mRNA by 6-folds (p=0.05), 4-folds (p=0.04), 23-folds (p=0.03) and 1.5-folds (p=0.04), respectively along with concomitant increase in Elastin protein in IF. *Sp1* expression was up-regulated on exposure to TGFβ1, Hcy and IL-6. LOX and LOXL1 protein were up-regulated by CTGF (p=0.02) and Hcy (p=0.01), respectively. CTGF protein secreted was elevated on exposure to TGFβ1 (p =0.05). Further studies are on to delineate the mechanism.

Conclusion: The PXF-associated pathogenic factors TGFβ1, CTGF, Hcy and IL6 were found to promote elastosis in lens epithelium cell line.

A NOVEL NONENZYMATIC APPROACH TOWARDS COMPLETE POSTERIOR VITREOUS DETACHMENT

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Purpose: With age, spontaneous molecular reorganization of the vitreous weakens vitreoretinal adhesions, resulting in posterior vitreous detachment (PVD). Incomplete PVD exerts tractional forces leading to retinal detachment. Pharmacologic vitreolysis by Ochriplasmin (FDA approved) is nonspecific and toxic, affecting proteins of the retina, choroid and lens. Recent studies have questioned its safety. Therefore, nonenzymatic approaches are preferred for treatment. Here, we evaluate the efficacy and safety of intravitreal injection of collagen-binding domain (CBD) based non-enzymatic reagents in facilitating PVD. We have used a CBD derived from *Vibrio mimicus* collagenase (VMC) and a variant of this CBD containing an RGD motif (RCBD). The rationale of using RCBD was to loosen up the vitreal collagen fibril network, and compete with cellular interactions at the vitreoretinal interface. CBDs from human MMP2 and MMP9 (hCBDs) and recombinant antibodies directed to vitreous componentswere also explored.

Methods: Constructs were cloned and expressed in *E. coli*, purified and injected (intravitreally) into fresh cadaver goat eyes.Post-treatment, PI staining and histology retina assessed reagent toxicity. *Ex-vivo* evaluation of vitreolysis was assessed by Optical Coherence Tomography (OCT) and Scanning Electron Microscopy (SEM). Cell viability assays were also done using ARPE19 cells.

Results: CBD reagents solubilized the gel-like collagen and released monomeric collagen chains, with no retinal damage. OCT and SEM suggest that RCBD/CBD are effective at vitreoretinal detachment, with dose-dependence.

Conclusion: CBD/RCBD are non-toxic, nonenzymatic, specific vitreolytic reagents and an effective alternative to the toxic, non-specific, enzymatic reagents. Fusion of these with vitreous-specific antibodies may further aid vitreolysis.

Cytotoxicity of lactoferrin loaded Carboplatin and Etoposide drug delivery in Y79 Rb cell line and cancer stem cells

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Purpose: Therapeutic resistance is one of the hallmarks of Cancer stem cells (CSCs) in all tumors including Retinoblastoma (Rb). Our previous studies identified $CD133^{lo}$ subset of cells as CSCs in Rb Y79 cell line with evidence of chemoresistance to standard drugs. In this study, we aimed to evaluate the cytotoxicity of nanoformulated cancer drugs (Carboplatin and Etoposide) using lactoferrin protein nanoparticles in Y79 Rb cell line and CD133 sorted populations.

Methods: Carboplatin and Etoposide loaded nanoparticles were prepared using the solution-oil chemistry based method. The drug and lactoferrin in a ratio of 1:4 was dissolved in PBS, incubated on ice with 25ml of olive oil, centrifuged and resuspended in PBS. SEM characterization and drug release efficiency was evaluated by HPLC. Cytotoxicity of the standard and nanoformulated drugs was evaluated by MTT assay using total Y79 cells and CD133 sorted subsets following a 48h exposure.

Results: Carboplatin and Etoposide loaded lactoferrin protein nanoparticles size measured 60±10 nm, 45±10nm and 25±5nm respectively as compared to the Standard drugs. Drug loading efficiency of Carboplatin and Etoposide release in pH-5.2 is 60±5 and 48±5 respectively. $CD133^{lo}$ cells, which constituted 15±0.6% of total population, exhibited lower drug cytotoxicity as compared to total and non-CSCs ($CD133^{hi}$) cells ($p<0.05$). The Carboplatin and Etoposide loaded lactoferrin nanoparticles lowered the IC50 values in all the three subsets when compared to the standard drugs ($p<0.05$) with lowering of chemoresistance of $CD133^{lo}$ CSCs by 32-77% ($p<0.05$).

Conclusion: The efficacy of nanoformulated carboplatin and etoposide was higher than the standard formulations with reduction of chemoresistance of $CD133^{lo}$ CSCs in Rb Y79 cell line by more than 50%. Further validation using combination and *in-vivo* testing are warranted to pave way for potential clinical application.

EXTRACELLULAR MATRIX-MIMETIC HYDROGEL FOR CORNEAL TISSUE REGENERATION

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Purpose: Corneal wounds are a major reason for blindness in developing countries, and there is an acute shortage of donated corneas and trained surgeons available for transplantation. The utility of currently used ophthalmic tissue adhesives in corneal tissue repair, such as cyanoacrylate and fibrin glue, is limited due to opaque scar formation and neo-vascular response. One of the most demanding challenges in corneal wound healing is in finding methods of tissue repair via regeneration as opposed to fibrosis. To address this issue, Pandorum Technologies Pvt. Ltd. (PTPL) in collaboration with LV Prasad Eye Institute (LVPEI) has developed an extracellular matrix (ECM)-mimetic hydrogel formulation that shows potential to regenerate wounded corneal tissue.

Methods: The ECM-mimetic hydrogel was developed at PTPL by entrapping ECM- derived proteins and glycosaminoglycans within a semi-interpenetrating hydrogel network. Corneal stromal stem cells (CSSCs) were encapsulated and cultured inside this hydrogel for 72 hours to demonstrate viability and monitor phenotypic changes using immunohistochemistry.

Results: CSSCs encapsulated in the ECM-mimetic 3D hydrogel showed >90% cell viability relative to 2D CSSC culture. The encapsulated CSSCs were able to maintain their phenotype in the presence of ECM-derived proteins within the hydrogel matrix; whereas the absence of ECM-derived components causes the cells to differentiate towards fibrocytic lineage.

Conclusions: The novel ECM-mimetic hydrogel has the ability to maintain viability and phenotype of encapsulated CSSCs and has immense potential to aid scarless regeneration of wounded cornea. A more comprehensive picture is expected to emerge after the completion of ongoing pre-clinical animal trials/studies.

DIFFERENTIAL EXPRESSION OF MICRO-RNA IN HIV POSITIVE PATIENTS WITH OCULAR MANIFESTATION OF IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

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Purpose: Small noncoding miRNAs bind with mRNA to regulate gene expression. miRNAs play important roles in immune cells, such as T cells, natural killer (NK) cells, B cells, and dendritic cells (DCs) and glial cells of the brain.Seventy percent of HIV-positive patients go onto develop HIV associated retinopathy and up to twenty percent of HIV positive individuals progress to Immune Reconstitution Inflammatory Syndrome (IRIS).Retinopathy is largely due to chronic neuroinflammation and neurodegeneration.

Methods: In this pilot study we compared miRNA profiles with real time PCR between isolated PBMCs of HIV-positive patients with and without ocular complications. We have built up a patient cohort of HIV patients who develop, ocular complications at the retina and a subset which show manifestations of IRIS at the retina.

Results: We have identified known miRNAs that were significantly differentially expressed in these cohorts. A subset of the upregulated miRNAs hsa miR-132-5p, hsa miR-192-5p, hsa miR-216a, and hsa miR-543 were validated with real time PCR in patient PBMCs. Many of the potential target genes of these miRNAs are involved in inflammation associated pathways, such as JAK/STAT, MAPKs, and PI3K and several were also previously reported to be involved in age associated changes.

Conclusions: In our data we observe hsa miR-192-5pdownregulationin the patient cohort with ocular manifestations. Similarly, hsa miR-543 shows downregulation in cohort with ocular manifestations.This implies that they have an important role in chronic overinflammation. ThesemiRNAs target mechanism by which HIV-1 may subvert innate immune responses.

TRANSPORTER TARGETED OCULAR DELIVERY SYSTEM TO IMPROVE OCULAR BIOAVAILABILITY OF DRUGS

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Purpose: The objective of the present study is to develop P-Glycoprotein (P-gp)/Multiple Resistance Protein (MRP) transporter targeted ocular delivery system to improve ocular bioavailability of substrate drug, erythromycin.

Method: The pharmaceutical excipients were screened for the potential to modulate the Pgp and MRP transporters in transporting erythromycin across cornea using exvivo studies. The excipients modulated Pgp/MRP transporters significantly were chosen to make micelles delivery system for erythromycin. The resulting micelles were then characterized and analyzed for their particle size and surface morphology. *Invitro* permeability study were performed using excised goat cornea and *Invivo* ocular pharmacokinetics studies were performed in rabbits for the developed micelles.

Results: Among the studied excipients, peceol and pluronics have showed significant modulation of P-gp and MRP-2 transporter in cornea. The mean particle size of micelle developed using peceol and pluronics was 148.0 nm. The encapsulation efficiency of erythromycin in the micelle was above 98%. *In vivo* studies show eight-fold higher ocular bioavailability for erythromycin micelles (3959.4± 245.8 $ng.h.mL^{-1}$) as compared to erythromycin solution (503.5±67.8 $ng.h.mL^{-1}$).Treatment of corneas with erythromycin micelles has not produced any toxicity.

Conclusion: The present findings suggest that excipients can play an active role in modulating P-gp and MRP-2 mediated drug transport across cornea. The micelles formulated using the excipients Peceol and Pluronics improved the ocular bioavailability of erythromycin by inhibiting both P-gp and MRP-2 mediated drug efflux.

METABOLOMIC PROFILING OF GLAUCOMATOUS PATIENTS

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Purpose: Intraocular pressure is primarily regulated by the endogenous metabolites using the targets often utilized for the pharmacological management. Therefore, the aim of the present study is to understand the adrenergic and seratonergic metabolic profile of ocular fluids and plasma which are expected to explain pathophysiology involved in glaucoma.

Method: LC-MS/MS method was optimised for the selected metabolites (nor-epinephrine, epinephrine, levodopa, serotonin, N-acetyl serotonin, dopamine, and 5-hydroxy tryptophan) on ZIC-cHILIC column using gradient mobile phase conditions. Quantification was done using multiple reaction monitoring mode. A prospective case controlled study was designed in human subjects involving 3 groups (primary open angle glaucoma, POAG; primary angle closure glaucoma, PACG and cataract, control group). An aliquot of 70 to 100µL aqueous humor was collected by paracentesis during the cataract (control group) and glaucoma filtration surgery (study group). Blood samples were collected before surgery on the same day and plasma was separated and stored at -80°C until analysis.

Results: LCMS/MS method was successfully developed and relative quantification of selected metabolites (n=7) was performed. This study revealed 1.5-19.2 fold increase in the metabolite levels in aqueous humor of glaucomatous patients in comparison to the control cataract group. Fold change in the metabolite levels was found in the range of 1.3-2.1 in plasma.

Conclusion: Variation in the levels of the metabolites found to have a significant correlation in different degrees of glalucomatous conditions. Further, validation of the method is needed for the analysis of metabolites in large number of samples.

COMPARISON OF TEAR PHARMACOKINETICS OF CALCIUM CHANNEL BLOCKERS FOLLOWING SYSTEMIC ADMINISTRATION IN RABBITS

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Purpose: The proposed study was designed to evaluate precorneal tear penetration of 8 calcium channel blockers after systemic administration in rabbits. The objective of the present study was to derive and compare tear pharmacokinetic (PK) parameters of calcium channel blockers (CCBs) in rabbits.

Methods: Under sterile condition, cassette dose of 8 CCBs (Amlodipine, Azelnidipine, Lercanidipine, Nicardipine, Nifedipine, Nimodipine, Nisoldipine) was formulated in rabbit's serum. Twelve New Zealand albino rabbits of either sex weighing (1.8-2kg) received intravenous dose of 0.1µM/kg body weight. Blood samples were collected at predetermined time points (30, 60 and 120min) along with tear samples by using a calibrated Schirmer's strips. Plasma and tear concentration were quantified using validated LC-MS/MS method. PK parameters were calculated using PK Solver excel add-in software (ver 2). Percentage tear penetration and log tear-to-plasma (Log_{10} (t/p)) were calculated and a correlation was found between Log(t/p) and plasma absolute concentration.

Results: Mean percentage tear penetration after cassette dosing in rabbits of felodipine was found to be highest (0.84±0.48%) followed by nicardipine (0.5±0.1%) > nisoldipine (0.4±0.21%)> lercanidipine (0.31±0.07%) > azelnidipine (0.3±0.4%) > nifedipine (0.15±0.04%) > nimodipine (0.13±0.05%) > amlodipine (0.08±0.05%). An overall negative correlation (r = -0.7) was found between Log(t/p) and plasma absolute concentration.

Conclusion: Felodipine in comparison to other CCBs showed maximum tear penetration with respect to plasma. This data would be further correlated with the physicochemical and molecular dynamic properties for QSPR algorithm development for modelling tear penetration**.**

DEVELOPMENT OF POLYMERIC CONTACTS FOR THE OCULAR DELIVERY OF MOXIFLOXACIN

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Purpose: This work aims to study the corneal penetration and efficacy of the polymeric contacts loaded with moxifloxacin.

Methods: The composition of polymeric contacts was chitosan, polyethylene glycol (PEG) 400 and glycerol. 0.4% moxifloxacin was added in to the drug loaded contacts. The shape of the contacts was made to mimic the marketed lens, which was casted using pre-designed mold. Contacts were characterized using FTIR, X-ray diffraction (XRD), scanning electron microscope (SEM) and analyzed for mucoadhesion, tensile strength and drug loading. Drug release kinetics was performed using franz's diffusion cells. Corneal penetration was studied using human cornea.

Results: The diameter of the lens was 14 mm with the radius of curvature of 8.6 mm from the center. FTIR and XRD spectra shows incorporation of moxifloxacin into the blank contacts. SEM images support the results of XRD. Mucoadhesion was approx. 4.0 N/cm^2. Drug loading was >95% and the release from the contacts was >80% within 24h compared to the free drug (100% in 10h). Corneal penetration of moxifloxacin released from contacts was comparable to the free drug solution. Tissue concentration was two-fold higher with the contacts compared to the free drug solution.

Conclusions: Moxifloxacin loaded polymeric contacts with sustained release effect can replace the use of topical drops after ocular surgeries.

INTRAVITREAL (IVT) PHARMACOKINETICS OF SELECTED ANTIANGIOGENIC AGENTS IN OCULAR FLUIDS AND TISSUES USING ESI-LC-MS/MS

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Purpose: For ocular angiogenesis small molecules such as curcumin, emodin, thalidomide and valdecoxib targeting other than VEGF have been attempted to rationalize for their intravitreal disposition. Therefore, the present study deals with the evaluation of IVT pharmacokinetics in rabbits. To study IVT pharmacokinetics of curcumin, emodin, thalidomide and valdecoxib formulation in comparison with bevacizumab as standard in ocular fluids and tissues in rabbits.

Methods: For IVT pharmacokinetics, New Zealand albino rabbits of either sex weighing 1.3-2kg were used after obtaining ethics permission from institutional ethics committee (961/IAEC/16). Cassette IVT formulations of all 4 drugs were reconstituted with 1mL of normal saline in sterile conditions. An aliquot of 0.1mL at a dose of 10µg each drug and 1.25mg of bevacizumab were intravitreally injected aseptically in left eye of rabbits (n=4). Rabbits were euthanized at 1, 4 and 8hr using carbon dioxide. Ocular fluids, tissues, and plasma were collected, stored at -80°C and subjected for analysis using LC-MS/MS.

Results: IVT cassette dosing pharmacokinetics showed a rapid clearance of curcumin from vitreous cavity followed by thalidomide > valdecoxib > emodin. Intra-vitreal half-lives of emodin, valdecoxib and thalidomide were found to be 4.37, 2.49, 2.04h, respectively, in comparison to bevacizumab (43.18h). The reported IC50 value of all the agents was not been achieved at the end of 8 hrs due to the rapid clearance.

Conclusion: None of the agent showed longer median residence time when injected intravitreally in comparison with bevacizumab. Therefore, they are not been taken forward for further experimental studies in animal models of ocular neovascularization.

ROLE OF MYBL2 TRANSCRIPTION FACTOR IN THE PROGRESSION OF RETINOBLASTOMA

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Purpose: To investigate the role of MYBL2 in Retinoblastoma tumorigenesis.

Methods: The expression of MYBL2 was determined in retinoblastoma cell lines and patient samples using gene specific primers and compared with normal retina. Lentiviral mediated shRNA approach was used to selectively silence MYBL2 in Y79 retinoblastoma cell line. The expression of MYBL2 was determined in knockdown cells by real-time PCR and compared to scrambled control. Cell viability assay was performed for knockdown cells and scrambled control cells.

Results: The expression analysis confirmed that MYBL2 was overexpressed in retinoblastoma cell lines and patient samples compared to control retina. Further, shRNA-mediated knockdown of MYBL2 led to decreased expression of MYBL2 mRNA. In addition, knockdown cells showed decreased cell viability compared to scrambled control cells.

Conclusions: Expression analyses and knockdown experiments indicate a role for MYBL2 in retinoblastoma progression.

FREE PAPERS

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OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY BASED MANAGEMENT OF ACUTE CHEMICAL BURNS

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Purpose: The degree of ocular surface ischemia has immense prognostic value in the management of acute chemical injuries. However, there is no objective way of identifying or quantifying limbal ischemia. This study evaluated the role of using optical coherence tomography angiography (OCTA) as a guide to the management of severe grades of acute chemical injuries.

Methods: This was a prospective imaging study. Patients with severe ocular burns classified as Dua's grade 4 or higher were eligible for the study. All patients received standard medical therapy in the affected eye/s and underwent serial quadrantic OCTA scans until complete epithelization. The main outcome measure was the association between the extent of ocular surface ischemia detected on OCTA and the rate of epithelization.

Results: The study included 28 eyes of 24 patients. The extent of ocular surface ischemia on OCTA and re-epithelization time were significantly correlated (r^2=0.8, P<0.0001), unlike clinical grading which correlated poorly with both extent of ischemia and speed of healing (r^2=0.49, P=0.2). Those eyes with 75% or greater ischemia at presentation had significantly worse visual outcomes, irrespective of the clinical grading (p=0.003).

Conclusion: The findings of this study suggest that OCTA-based assessment of ocular surface ischemia has greater predictive and prognostic value than clinical grading in acute chemical injuries.Optical coherence tomography angiography (OCTA) based determination of the extent of ocular surface ischemia is more useful than clinical grading in the management of acute chemical injuries.

OCULAR SURFACE ANGIOGRAPHY (OSA): VALIDATION OF A STANDARDIZED OPTICAL COHERENCE TOMOGRAPHY-BASED ALGORITHM USING TOPICAL VASOCONSTRICTORS

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Purpose: To test the reliability and accuracy of ocular surface angiography (OSA), based on a novel non-invasive imaging algorithm using optical coherence tomography angiography (OCTA).

Methods: This prospective imaging study included 15 normal eyes of healthy volunteers and 10 eyes of 10 patients with nasal pterygiums. A novel imaging and post-processing algorithm was developed using image-J software.The reliability of the algorithm was tested by evaluating the intra- and inter-observer variability amongst two amateur technicians. Serial quadrantic OCTA scans were then performed before and after instillation of vasoconstricting eye drops. The accuracy of the model was tested by comparing the temporal change in amount of vasoconstriction in healthy eyes as compared to eyes with primary nasal pterygiums.

Results: The intra-class correlation coefficient for intra and inter-observer agreement was noted to be 0.91 and 0.88 and respectively. Significant vasoconstriction was noted at 5 mins (13-15%) from baseline, which peaked at 10 minutes (14-17%) and was sustained until 20 minutes followed by gradual recovery. The degree of vasoconstriction was greatest with a combination of 5% phenylephrine and 0.15% brimonidine tartrate (BT) eye drops than either 1 or 2 drops of BT alone, both at 10 ($p=0.0058$) and 20-minute ($p=0.0375$) time-points. This temporal trend was replicated in eyes with primary nasal pterygium ($p=0.31$).

Conclusions: The findings suggest that OSA using OCTA is a reliable and accurate technique of objectively quantifying the relative change in ocular surface vasculature. This may have significant implications in management of ocular surface diseases particularly ocular burns.

SURGICAL REMOVAL OF IMPLANTATION IRIS CYSTS: INDICATIONS, SURGICAL CHALLENGES AND LONG-TERM OUTCOMES

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Purpose: To describe the clinical spectrum and clinico pathological correlation of patients with epithelial iris implantation cysts.

Methods: Clinical profiles, surgical details and treatment outcomes of 31 cases of iris implantation cysts from 1989-2015, were evaluated retrospectively. Histopathology records were reviewed.

Results: Median age at presentation was 5 years. Average follow-up was 14.37 months after surgery. Iris cysts were due to trauma (17), unknown (13), prior surgery (1). All underwent cyst excision with additional surgical procedures done where necessary, followed up for 1 month to 6 years after surgery. Mean preoperative and final log MAR visual acuity was 1.3 and 1.15 respectively. Complications were corneal decompensation (4), secondary glaucoma (4), and severe uveitis (1). Histopathological confirmation was available in 23 cases.

Conclusion: Epithelial iris implantation cysts are associated with significant ocular morbidity. Complete excision of cyst with sector iridectomy can result in an excellent functional outcome with minimal complications.

A NEW TECHNIQUE FOR FITTING OF TRICURVE RIGID GAS PERMEABLE CONTACT LENS IN PENETRATING KERATOPLASTY EYES USING SCHEIMPFLUG IMAGING

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Purpose: Rigid gas permeable (RGP) contact lens fitting after penetrating keratoplasty (PK) is challenging due to significant irregular astigmatism. The aim of the study was to determine a guideline for selecting the initial base curve (BC) of the RGP contact lens fitting in post PK eyes.

Methods: The data of patients who had tricurve RGP contact lens fitting post PK were collected retrospectively. Following data were collected: best corrected visual acuity (BCVA) with glasses and contact lenses; contact lens parameters which included the BC and diameter; corneal topography parameters which included steep keratometry value (K), flat-K, mean-K; and anterior Best Fit Sphere (BFS) measured using Scheimpflug imaging.

Results: The median age of the 40 subjects (46 eyes) who met the inclusion criteria was 37.5 years (IQR 26.7-45.5). The spherical equivalent was –3.00 diopter (D) (–8.31 to –1.56). The median steep-K, flat-K and mean-K in them were 6.76 mm (6.28-7.07), 7.78 mm (7.37-8.14) and 7.26 mm (6.93-7.46) respectively. The median anterior BFS value of the transplanted cornea was 6.96 mm (6.6-7.37). The median BC of final RGP lens was 7.0 mm (6.7-7.23) and the median diameter was 9.8 mm (9.4-10.4). Among all the Scheimpflug imaging parameters, the BFS correlated well with final BC of the RGP contact lens dispensed (R 0.742, $p<0.0001$).

Conclusion: The anterior corneal surface BFS value can be used as a reference in selecting the initial base curve of tricurve RGP contact lens to achieve the best fitting and reducing the chair time of patients after PK.

INTRACAMERAL ANTIBIOTIC PROPHYLAXIS FOR PREVENTION OF ENDOPHTHALMITIS AT SECONDARY CENTERS (ENDOPHTHALMITIS PROPHYLAXIS STUDY – EPS)

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Purpose: To study role of intracameral (IC) antibiotic in prevention of endophthalmitis at Secondary Centers (SCs).

Methods: Prospective, comparative, interventional, multicenter study. Patients >18 years who underwent cataract surgery (SICS or Phacoemulsification with/without IOLs) at SCs received IC-cefuroxime or IC-moxifloxacin at the end of surgeries were included. Patients with known allergies were excluded. Acute endophthalmitis and SSI (within 6 weeks postoperatively) are reported. All patients underwent comprehensive eye examination pre-and-post-IC injection. Chi square test was done to compare the results. IRB approved amendment of not giving topical antibiotics in the postoperative period except in patients with complications, poor hygiene or felt need by surgeons.

Results: 21120 (47% Males) received IC injections (SICS-October2016-May2017, Phacoemulsification-October 2016-November 2017). Mean age was 59.13±10.18 years. 17/15838 (0.107%) patients who did not receive IC antibiotics in 2015 developed endophthalmitis (infection rate- 1/932). 8/21120 (0.0368%) patients (infection rate 1/2640) developed endophthalmitis who received IC antibiotics. (p-value<0.01 - statistically significant). Two in IC- Cefuroxime (n= 8873, infection rate =1/4436) and four patients in IC-Moxofloxacin (n=12247, infection rate=1/2041) developed endophthalmitis. Infection rate was 1/3500 with IC antibiotic and postoperative antibiotic (n=13998).The p-value was statistically significant when compared to 2015 data. One /1781 developed endophthalmitis when only IC antibiotic without topical antibiotic (n=7122).

Conclusion: IC antibiotics resulted in statistical significant reduction in rate of endophthalmitis from 0.107% to 0.038%. No statistical significant difference was noted between the IC antibiotics. IC with topical antibiotics was better than no Intracameral. IC antibiotic when compared to no postoperative antibiotics was not statistically significant.

CAN CONTRAST RIVALRY BE USED AS A PSYCHOPHYSICAL MARKER FOR QUANTIFYING BINOCULARITY OF THE VISUAL SYSTEM?

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Purpose: To determine the utility of binocular contrast rivalry as a psychophysical marker for quantifying each eye's contribution to binocular single vision in the presence of interocular differences in image quality.

Methods: Ten subjects (mean age±1SD: 23.8±1.2yrs) with normal binocularity and full refractive correction viewed orthogonally-oriented (45°and 135°) Gabor grating patterns (3cpd) presented dichoptically on a luminance-calibrated LED monitor at 50cm for 120sec. Subjects indicated every instance of a switch in grating-orientation during the stimulus presentation period using a key press, from which the percentage time duration spent in a given orientation was calculated. This task was repeated from no interocular difference in stimulus contrast (baseline condition) to 98% interocular contrast difference.

Results: The mean±1SD dwell time of a given orientation with no interocular contrast difference was 2.6±0.9sec (range: 1.5 to 3.6sec) and the mean contribution to the binocular percept was 49.8±3.2% and 50.2±3.2% for right and left eye, respectively. The mean contribution of each eye remained similar to baseline up to 80% interocular contrast difference ($p>0.05$). Beyond this value, contribution of the eye with lesser contrast decreased steadily from 60±6.2% with 80% interocular contrast difference to 93.11±10% with 98% interocular contrast difference ($p<0.05$).

Conclusion: The contribution of each eye to binocular single percept depends on the magnitude of interocular contrast difference, with the eye with greater contrast dominating the binocular percept as determined here using the contrast rivalry paradigm. Contrast rivalry may therefore be a putative psychophysical marker for quantifying the contribution of each eye to binocular single vision.

FACTORS AFFECTING PARTICIPATION OF CHILDREN WITH LOW VISION

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Purpose: To identify various types of contextual factors that may influence participation of children with low vision (LV) in daily life and assess the relative importance and relevance of contextual factors in affecting participation.

Methods: Children with LV (n=19), their parents (n=13), peers with normal vision (n =13), teachers (n=13) and low vision rehabilitation (LVR) experts (n =21) reviewed a list of 123 statements related to contextual factors and sorted the statements based on the similarity and rated each statement based on their relevance, importance and perceived barriers in participation of children with LV. Data was analysed using the Concept System software.

Results: Cluster analyses revealed 10 domains: product and technology, light, family support, teacher's support, society, teasing, services, systems and policies, child's confidence, enjoyment with activities and emotional status. Fifty statements out of 123 were rated highly important and relevant. Services, systems and policies was rated the most important facilitator. Relative importance rating assigned to contextual factors by children with LV and their parents had moderate correlation (r = 0.55). However relative importance rating assigned by their peers and LVR experts were poorly correlated (r=0.01 and r=0.21 respectively) with the importance rating of children with LV.

Conclusions: Participants identified 7 environmental factors such as society, teacher's support, and 3 personal factors such as child's confidence, emotional status that affect participation of children with LV. Our study demonstrates the differences in perspectives of various key stakeholders regarding contextual factors affecting participation of children with LV.

LENS MAGNIFICATION AFFECTS THE ESTIMATES OF REFRACTIVE ERROR OBTAINED USING ECCENTRIC INFRARED PHOTOREFRACTION

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Purpose: Positive and negative powered ophthalmic lenses are used in eccentric infrared photorefraction to calibrate the device, correct the subject's baseline refractive error before an experiment or stimulate blur-driven accommodation. Using theoretical modelling and empirical measurements, this study systematically addressed the impact of image magnification/minification induced by these on the refractive power estimates obtained using a commercially available photorefractor (PlusOptix PowerRef 3®, Nuremberg, Germany).

Methods: Luminance gradients of photorefraction formed across the pupil were computationally simulated using Matlab® for three magnitudes of myopia and then magnified or minified from 1% to 20% to simulate the effect of 0D to ±7D ophthalmic lenses. The resultant change in luminance gradients were then calculated and translated to percentage errors of refractive power estimates obtained using photorefraction. Empirical measures of refractive power were also obtained from 40 subjects [22–30yrs; 0D to -5D spherical equivalent myopia] using afocal "size" lenses of magnitude ±1%, ±5%, ±8% and ±12%.

Results: Both theoretical and empirical data indicated that myopia was underestimated with image magnification and overestimated with image minification ($p<0.001$). The magnitude of this over- or under-estimation also scaled with the baseline refractive error and it was greater for minification than for equivalent values of magnification ($p<0.001$). Image magnification/minification did not impact the refractive error estimates of emmetropic eyes obtained using this technique.

Conclusion: Image magnification/minification by ophthalmic lenses produces an artifactual change in the refractive error estimates of photorefraction. This effect appears non-trivial in experimental paradigms involving ophthalmic lenses to manipulate the eye's optics during photorefraction.

STUDY OF REACTION TIME USING PEDIATRIC PERIMETER IN INFANTS WITH NORMAL MILESTONES

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Purpose: The time interval between light stimulus presentation and time taken to look at the stimulus is called reaction time (RT). We studied the RT of typically developing infants (age: 3-10 months) using Pediatric Perimeter (PP) device.

Methods: PP has light emitting diodes (LEDs) embedded inside the dome. The LEDs were controlled using a computer program to light up the hemispherical field or a quadrant in the dome. This presentation was termed as Gross visual fields (GVF). Infant's eye or head movement response towards GVF was monitored with an infrared (IR) camera by an examiner who would register such a response (RT) with a button press. A total of 160 infants in age group bins: 3-5, 5-7, 7-10 months were tested.

Results: Data of 120 infants was eligible for analysis. Median RT for 3-5 months is 822ms (IQR=600-1101ms) and 580ms (IQR=462-857ms) in the superior field (SF) inferior field (IF) respectively. RT was greater in SF when compared to 5-7 months (SF: 606ms, IQR=460-740ms and IF: 557ms, IQR=466-638ms) and 7-10months (SF: 540ms, IQR=450-640ms and IF: 501ms, IQR=398-595ms). Right hemifield 544ms (IQR=482-754ms) and left hemifield 659ms (IQR=471-866ms) RT followed the same trend.

Conclusion: A decrease in variability and median RT to GVF with increase in age was observed in our study. This observation could indicate the maturing visual system in both sensory and motor domain. This data also provides a normative database for saccadic eye movements in typically developing infants.

INVESTIGATING MICROSACCADES IN ORIENTING REFLEXIVE EYE MOVEMENTS

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Purpose: Microsaccades are small rapid and involuntary fixational eye movements. Microsaccades could indicate orientation of attention by having a directional bias. We studied microsaccades in a reflexive eye movement task with the aim of understanding a participant's visual processing in the absence of any verbal instructions.

Methods: We enrolled 30 adult participants who were shown a priming target followed by two targets one of which was the primed target (familiar) for a brief exposure duration (200,400 and 600ms), and three different sizes (LogMAR 0.5,0.6 and 0.7). No instructions were given to the participant besides asking them to look at the monitor. The direction of the first and majority of the microsaccades to these briefly exposed stimuli was analyzed. The proportion towards the familiar and unfamiliar targets were compared.

Results: Twelve of the 30 participant's data was analyzed. For the first microsaccade 36% (±7) orientation was toward the familiar target and 39% (±9) to the unfamiliar target. For the majority of microsaccades 39% (±6) and 37% (±7) were oriented to the familiar and unfamiliar targets respectively. No directional bias was observed towards the familiar targets in comparison with the unfamiliar target (paired t-test, $p=0.611$).

Conclusions: When no verbal instructions are given, the tendency of microsaccadic orientation to the familiar and unfamiliar target is equivocal. This result contradicts the saccadic eye movement orientation, in which a greater predisposition was found for the familiar target. Larger sample size is required to further analyze the microsaccades.

POSTERS

28th July, 2018

DYSBIOSIS IN THE GUT BACTERIAL MICROBIOME OF PATIENTS WITH UVEITIS, AN INFLAMMATORY DISEASE OF THE EYE

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Purpose: Uveitis (UVT), an inflammatory disease of the eye significantly contributes to vision impairment and blindness. UVT is associated with systemic infectious and autoimmune diseases, but in most cases, the aetiology remains unidentified. Dysbiosis in the gut microbiome has been implicated in autoimmune, inflammatory diseases, cancers and mental disorders. In a mice model of autoimmune UVT, it was observed that manipulating the gut microbiome reduces the inflammation and disease severity. Further, alterations in the bacterial gut microbiome and their metabolites were reported in UVT patients from a Chinese cohort. Hence, it is worth comparing the bacterial gut microbiome of UVT patients with that of healthy controls (HC) to ascertain whether dysbiosis of the gut microbiome has implications in UVT.

Methods: In the present study, gut bacterial microbiomes were analysed in the fecal samples of HC (n= 13) and UVT patients (n= 13) using high-throughput Illumina sequencing of V3-V4 region of 16S rRNA gene.

Results: Our analyses showed reduced diversity of several anti-inflammatory organisms including *Faecalibacterium*, *Bacteroides*, *Lachnospira*, *Ruminococcus* and members of Lachnospiraceae and Ruminococcaceae families, and enrichment of *Prevotella* (proinflammatory) and *Streptococcus* (pathogenic) OTUs in UVT microbiomes compared to HC. In addition, decrease in probiotic and antibacterial organisms was observed in UVT compared to HC microbiomes. Heatmap and PCoA plots also indicated significant variations in the microbiomes of UVT versus HC.

Conclusions: This is the first study demonstrating dysbiosis in the gut bacterial communities of UVT patients in an Indian cohort and suggests a role of the gut microbiome in the pathophysiology of UVT.

MYCN PROMOTES RETINOBLASTOMA CELL GROWTH AND MIGRATION THROUGH MODULATION OF METABOLIC REPROGRAMMING

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Purpose: To understand the role of MYCN in cell growth, migration and metabolic reprogramming in retinoblastoma.

Methods: The mRNA expression of MYCN was analyzed using gene-specific primers and real-time PCR. Next, we determined MYCN protein expression by immunoblotting and immunohistochemistry. The RB cell lines were treated with MYC inhibitors and cell viability was determined using trypan blue dye exclusion assay. Cell migration was measured by wound healing assay. Additionally, shRNA mediated knockdown was employed to understand the role of MYCN in retinoblastoma. The changes in MYCN protein levels upon inhibition or knockdown were determined by immunoblotting. The relative changes in various metabolic parameters such as glucose uptake and lactate production were measured in response to MYCN inhibition. Further, we determined the changes in expression of genes involved in glucose metabolism using specific primers upon MYC inhibition or knockdown.

Results: MYCN was found to be over expressed in retinoblastoma tumor specimens and cell lines compared to control retina. Inhibition of retinoblastoma cells with MYC inhibitors resulted in decreased cell growth as well as migration. Further, shRNA knockdown of MYCN led to similar decrease in cell growth and migration. Also, MYC inhibitor treated and knockdown cells showed decreased glucose uptake and lactate production. In addition, inhibition or knockdown of MYCN led to changes in expression of genes involved in glucose metabolism.

Conclusions: MYCN is an important oncogene that regulates cell growth, migration and metabolism in retinoblastoma. Targeting MYCN to modulate metabolic reprogramming could be a potential therapeutic strategy in retinoblastoma.

GENERATION OF LIMBAL STEM CELL DEFICIENT (LSCD) RABBIT MODEL AND COMPARISION WITH THE CASES OF HUMAN LSCD

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Purpose: Simple limbal epithelial transplantation or the cultivated limbal epithelial transplantation serves as an appropriate redressal for treating the condition of limbal stem cell deficiency (LSCD). Studying the mechanism of action pre/post transplantationis ethically limited for patients and requires an animal model. The present study aims to generate the LSCD in animal model and compare its features to that of human LSCD.

Methods: Rabbits (n=15) were topically administered with 1N NaOH for 30 seconds to generate alkali burn. Photographs/Slit lamp imaging of the injured eye was obtained at regular intervals for six months. Clinical and histopathological features of rabbit LSCD were compared with human LSCD cases (n=12).Immunofluorescence (Cytokeratin3,Cytokeratin19, Cytokeratin10) was performed to understand the extent of conjunctivalization of the cornea.

Result: Corneal haze with the extensive progression of blood vessels from the peripheral cornea was seen in a month with complete pannus formation in 3-4 months (n=12). 20% of the rabbits (n=3/15) had remarked regression in blood vessel density with decrease in the haze and had shown corneal phenotype after six months.Histopathological features of epithelial hyperplasia, goblet cells, vascularization andkeratinization were seen in both human and rabbit LSCD. Expression of corneal epithelium (CK3) and conjunctival epithelium marker (CK19) was also similar.

Conclusion: Generation of LSCD using alkali burn in rabbits serves as a reliable method mimicking the conditions of human LSCD that could serve as good experimental model. It is also to be noted that in few cases there is notable self-restoration of corneal phenotype without any intervention/medication.

EXPERIMENTAL *PYTHIUM INSIDIOSUM* KERATITIS–PART II: PRELIMINARY RESULTS OF CYTOKINE GENE EXPRESSION IN RABBIT CORNEA

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Purpose: This preliminary report is the second part of an experimental *Pythium insidiosum* keratitis study that developed a rabbit model and reported clinical and histopathological characteristics of the disease. The aim of this study is to investigate the transcriptional cytokine response in rabbit cornea infected with *P. insidiosum.*

Methods: At the time of harvesting of cornea from the rabbits (Right eye-infected, Left eye-phosphate buffered saline, pH 7.4- uninfected control) one fourth of the cornea from both eyes was preserved in RNA *later*. ™RNA was extracted (Trizol method) and comparative real time reverse transcriptase PCR (RT-qPCR) was done (QuantStudio 3, Applied Biosystems) for four cytokines in one infected and three uninfected tissues. The primers and PCR conditions were basis earlier publication. GAPDH gene was used as the housekeeping gene.

Results: This pilot study included one infected cornea at late time point of 9 days postinfection. Infection was achieved in this eye without use of steroid and inoculation of *P. insidiosum* zoospores by intrastromal route. In this tissue, compared to contol eyes, proinflammatory cytokine IL-17A was upregulated by 41.7 fold, followed by IL-10 (12.8 fold). There was marginal upregulation of TNF-α (1.9 fold) while TGF- β showed down regulation (-13.8 fold).

Conclusions: These preliminary results show successful infection of rabbit cornea with *P. insidiosum* associated with inflammatory cytokine response. Further profiling of the immune response in the rabbit cornea is being done.

ISOLATION AND CHARACTERIZATION OF STROMAL STEM CELLS IN A cGMP FACILITY FOR THE TREATMENT OF DIFFERENT CORNEAL PATHOLOGIES

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Purpose: Limbal derived corneal stromal stem cells have the potential to cure blinding corneal diseases. This study aimed to describe a standardized protocol of stem cell expansion for clinical transplantations in a cGMP facility.

Methods: Therapeutically accepted and serologically tested cadaveric donor corneas were obtained from a certified eye bank. The annular limbal rims were excised, minced and subjected to collagenase digestion. After digestion, the tissues were cultured with 2% human serum-fortified media which was replaced every 3 days. Primary cultures grew epithelial cells while serial passaging resulted in the populations of stromal stem cells. Quality control for sterility, mycoplasma and immunostaining for stem cell markers were performed as the cells were being used for clinical purposes. All the documentation of donor tissue data and records of cultures were maintained and reviewed.

Results: Between August 2016 and May 2018, about 93% of 89 donor corneas were successfully cultivated and 84% of them were utilized for clinical transplantation. 2% tissues did not show any growth. Mycoplasma was not detected in the cultures; early microbial contamination was seen in ~4% cultures and not in any later passages. Cultured human limbal stromal stem cells hLMSCs expressed both mesenchymal ($CD105^+$,$CD90^+$, $CD45^-$, $CD73^+$, VIM^+, $CK3+12^+$, $COL\text{-}III^+$, $\alpha\text{-}SMA^-$ and $HLA\text{-}DR^-$) and stem cell markers ($P63\text{-}\alpha^+$, $ABCG2^+$, $PAX\text{-}6^+$).

Conclusion: The results indicate that this standardized isolation and expansion method of the hLMSCs in cGMP, was safe, adequate and of optimal quality for various clinical applications.

NEXT GENERATION SEQUENCING IN PREDICTIVE DIAGNOSIS OF SYNDROMIC LCA/ EORP

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Purpose: Early onset retinal dystrophy (EORD) and Leber congenital amaurosis (LCA) are heterogeneous degenerative diseases with characteristic features of early infancy / congenital visual loss, markedly reduced or absent electroretinogram, nystagmus and photophobia. In the current study, we discuss the implication of Next Generation Sequencing (NGS) technology in diagnosis, risk prediction and management of associated syndromic features in EORD/LCA.

Methods: Targeted re-sequencing of LCA candidate genes followed by whole exome sequencing in Illumina Hiseq 2500 were performed with LCA/ EORD cases. The data were analysed using standard bioinformatics pipeline and the identified variants were validated by Sanger sequencing, segregation analysis and control screening. *Insilico* analyses were used to predict the pathogenicity of the identified mutation.

Results: Of the 93 cases analysed, we identified mutation in *IQCB1* (n=5), *SLC19A2* and *CNNM4* (n=1 each) which were validated for pathogenicity of the identified variant. On re-examination for other clinical features associated with the identified genes, we observed kidney abnormalities in 3 patients with *IQCB1* mutation, anaemia, diabetes and sensorineural deafness in *SLC19A2* and tooth abnormality in *CNNM4* mutation.

Conclusions: The ocular phenotype is the first characteristic feature diagnosed early in infancy with the associated clinical features being asymptomatic; NGS has helped in redefining the diagnosis of isolated LCA/ EORP as syndromic forms- Senior-Loken syndrome in *IQCB1*, TRMA, Jalili syndrome in *SLC19A2* and *CNNM4* mutation positive cases respectively. This aided in genetic counselling and further management of other systemic complications.

EXPLORATION OF A NOVEL CHEMICAL CROSS-LINKER FOR THE TREATMENT OF KERATOCONUS

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Purpose: This study developed a chemical cross-linker containing EDCI, NHS and Suberic acid to cause corneal cross-linking without removing the epithelium or the use of UV-A irradiation, in turn avoiding the risks associated with the conventional treatment for keratoconus.

Methods: We analyzed the cell death and the phenotype of cells in the corneal layers after novel cross-linker treatment following a pseudo-clinical approach. Extent of penetration of the cross-linker into the corneal tissues, tensile strength of the cornea and morphometrical analysis of cadaver corneal and keratoconus tissue sections by H&E staining were analyzed. Peptides cross-linked were analyzed by mass spectrometry of separated corneal layers.

Results: The novel chemical cross-linker at the full or $1/8^{th}$ concentration did not induce apoptosis in the corneal layers as analyzed by TUNEL assay. Cells from the corneal layers of the cross-linker treated cadaver globes and stromal cells from keratoconus cornea maintained their phenotype intact. The cross-linker at either concentration also did not induce any gross morphological changes in the corneal layers of the cadaver as well as keratoconus cornea. Tensile test analysis of the cornea from the cross-linker treated cadaver globes indicated that the $1/8^{th}$ cross-linker treatment induced a better 2.12 fold increase in stiffness by penetrating upto 200 mm into the stroma. Proteomic analysis of the layers of the cadaver cornea treated with the cross-linker has revealed subtle changes in the protein profile.

Conclusions: Hence novel cross-linker at $1/8^{th}$ concentration is good to use for making a formulation in the form of eye-drops for further trials.

HUMAN *PAX6* AND ITS TARGET GENE REGULATION IN OCULAR CELL TYPES

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Purpose: To evaluate the regulation of human *Pax6, K3, K12, p63* gene promoters in different ocular cells.

Methods: The ocular surface ectoderm (OSE) enhancer and the promoter regions of human *PAX6* (PA, Pc), *p63* (TAp63, ΔNp63), *K3* and *K12* genes were PCR amplified, sequenced and cloned into pGL3-Basic vector. Also, the Wt and 5a variants of *PAX6* were cloned into a mammalian expression vector. These constructs were transfected into different ocular cells for luciferase reporter activation assays.

Results: The PA is the dominant promoter of the human *Pax6* gene in ocular tissues and is highly active in corneal and retinal pigmented epithelium and shows about 429 and 456 folds activation in HCE and ARPE-19 cells respectively. The PC is a week promoter and is more active in neuro-retinal and lens epithelial cells and shows about 16 and 37 folds activation in 661W and HLE-3B cells respectively. The OSE enhancer activates P_A promoter mainly in the lens epithelium and shows about 1.6, 1.8 and 3 folds activation in 661W, ARPE-19 and HLE-3B cells respectively and has no significant effect in HCE cells. Co-expression of *PAX6* variants (Pax6-Wt and Pax6-5a) repressed the activity of Pax6-PA, K3, K12, TA-p63 and ΔN-p63 gene promoters and the repressive effect of Pax6-5a was higher than the Pax6-Wt.

Conclusion: The PA is the dominant promoter for the human *Pax6* gene in ocular tissues. The OSE enhancer activates P_A promoter in retina and lens epithelium and has no effect in corneal epithelium. Pax6 or p63 genes are mutually repressive and regulate each other.

CHARACTERIZATION AND ESTIMATION OF VIRULENCE FACTORS OF PATHOGENIC *FUSARIUM* SPECIES CAUSING KERATITIS

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Purpose: *Fusarium spp.* cause many infections. The present study was done to characterize and understand the role of virulence factors in its pathogenesis.

Methods: *F. Solani* (n=14), *F.delphenoides* (n=3), *F. sacchari* (n=4) were isolated from corneal ulcers and identified by ITS and TEF sequencing. Protease specific activity was determined using azocasein assay and characterization was done using different pH and inhibitors for azocasin assay and by gelatinzymography using inhibitors. Beta glucan quantification was done using congo red (total glucan) and ELISA assay (1,3 beta glucan) and characterization was done using FTIR (fourier transform infrared spectroscopy) Toxin characterization was done using TLC.

Results: Specific activity of protease was ranging from 95.3±8.0 units/mg to 223.6±18.8 units/mg of protein. Optimum pH for all proteases was 7.4. Studies with inhibitors shows that most of the proteases are serine protease and metallo protease and few are aspartyl proteases. The concentration of total β-glucan ranges from 0.850±0.03 g/100g to 36.13±0.83 g/100g. Concentration of β-(1,3)-glucan ranging from 0.0036±0.0004 to 6.62±0.83 g/100g. FTIR showed presence of beta polysaccharides. Some of the isolates showed presence of T2 toxin, zearalenone and aflatoxin when compared with standard toxins on TLC.

Conclusion: The difference in production of toxins, proteases, pigments between isolates of *Fusarium* could indicate the difference in pathogenic potential.

DETECTING THE SECOND HETEROZYGOUS ALLELE IN AUTOSOMAL RECESSIVE PRIMARY CONGENITAL GLAUCOMA

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Purpose: Primary congenital glaucoma (PCG) is largely attributed to homozygous mutations in the *CYP1B1* gene. In the present PCG cohort (n=485), homozygous *CYP1B1* alleles accounted for 33% cases, while a large majority (13%) were heterozygous (n=64). In order to determine the second contributing allele, 30 genes previously implicated in glaucoma, anterior segment dysgenesis and optic atrophy were screened in these cases by targeted sequencing.

Methods: Screening was accomplished through a customized gene panel on a next generation sequencing platform (Ion Proton) using Ampliseq chemistry at an average depth of 500x. The raw data were analyzed using the GATK, imported to the Ion Reporter software (version 5.2). Pathogenic variants based on SIFT, PolyPhen-2 and Mutation Taster were further validated by Sanger sequencing using BigDye chemistry.

Results: Heterozygous mutations in glaucoma-associated [(*TEK* (p.E103D, p.I148T, p.Q214P, p.G743A), *FBLN5* (p.R54Q), *WDR36* (p.K93N, p.P291R)] and optic atrophy [*ACO2* (p.T256M) and *OPA1* (p.A988G)] genes were observed in 11/485 cases (2.3%), which co-occurred with heterozygous *CYP1B1* alleles in the proband. The combinations of these alleles were not observed in ethnically matched controls (n=1303). PCG patients harboring heterozygous alleles of *CYP1B1* and *TEK* had poor prognosis post treatment.

Conclusions: The present study provided a snapshot of the possible genetic interactions seen in PCG. Candidate genes (*CYP1B1*, *LTBP2* and *TEK*) accounted for 54% while the other genes contributed to an additional 10.7%, including co-occurring alleles that resulted in 1.03% of PCG cases. Further validations of these genetic interactions will help in understanding the underlying molecular mechanism in PCG.

EXPERIMENTAL *PYTHIUM INSIDIOSUM* KERATITIS–PART I: DEVELOPMENT OF A RABBIT MODEL AND CLINICO-HISTOPATHOLOGICAL STUDY

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Purpose: To develop an animal model of *Pythium insidiosum* keratitis and to characterize clinical and histological features.

Methods: Right cornea of 48 rabbits were inoculated with *P. Insidiosum* zoospores by either intrastromal injection of high dose (HD) inoculum -1 X 10^8 zoospores /ml (N=24), or intrastromal injection of low dose (LD) inoculum - 1 X 10^4 zoospores /ml (N=8) or topical application of HD inoculum after abrading corneal epithelium (N= 16). Left eye of all rabbits received equal amount of normal saline, via route similar to the right eye. Half of the animals in each group received subconjunctival injection of corticosteroids in both eyes. The animals were examined daily using a scale for clinical score (CS). They were sacrificed at various time points to retrieve both corneas. One fourth of each cornea was immediately preserved in 10% formaldehyde and examined later by histopathological studies.

Results: Irrespective of subconjunctival corticosteroid, HD inoculum produced severe infection by day 2 (mean CS: 9.6±2.4) while LD inoculum reached similar score by day 4 (mean CS: 9.7±0.5). At day 7 the CS was lower in non-steroid compared to steroid group indicating role of host immune response. Keratitis failed to develop in eyes with topical administration of zoospores on abraded cornea. Histopathological examination showed that the load of the organism in the corneal tissue was not related to the inoculum size.

Conclusion: This study recommends use of intrastromal injection of LD inoculum of zoospores without corticosteroid for successful creation of rabbit model of *Pythium insidious* keratitis.

IN VITRO ASSESSMENT OF THE ANTIMICROBIAL ACTIVITY OF TWO TYPES OF SILICONE OILS USED IN OPHTHALMIC SURGERY AGAINST ENDOPHTHALMITIS CAUSING AGENTS

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Purpose: To test the antimicrobial properties of silicon oil (Aurosil 1000 cSt, Aurosil Plus 5000 cSt) on *in vitro* growth of different microorganisms related to endophthalmitis.

Methods: *S.aureus, S.epidermidis, P. aeruginosa,* Multi-Drug Resistant (MDR) strain of *K.pneumoniae, C. Albicans* and *A.flavus* were prepared to 0.5 Mc Farland turbidity. The bacteria and fungi were inoculated into the silicone oils, BHI broth for bacteria and Sabouraud broth for fungi, respectively and cultured aerobically for 30 days. From each sample, 10 µl was plated onto nutrient agar and Potato dextrose agar for testing growth of bacteria and fungi respectively. After overnight incubation, colony-forming units (CFUs) were enumerated. Cultures from specimens, overnight incubation and CFU counting were repeated periodically at given intervals (0, 1, 3, 5, 7, 14, 21, 24 and 30).

Results: All bacteria including the MDR strain showed an apparent decrease in CFUs, with elimination between 21 and 30 days in silicone oil. Both the silicon oils however did not have any effect on fungal growth of any of the studied microorganisms even upto 30 days. Colony-forming units of microorganisms remained stable in physiologic saline during the study. In BHI and Sabouraud broth, both bacteria and fungi showed a growth pattern that was compatible with the growth curve of microorganisms.

Conclusion: Silicone oil seems to have a significant antimicrobial activity against bacteria *in vitro* after 30 days but was ineffective against fungi. The application of silicone oil into the vitreous cavity during vitrectomy may contribute to the elimination of microorganisms from the intraocular space in patienst with endophthalmitis but clinical trials are needed to assess its safety.

MicroRNA IN OCULAR VASCULOPATHIES

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Purpose: We have attempted to catalogue the miRNAs in ocular vasculopathies- age related macular degeneration (AMD), diabetic retinopathy/diabetic macular edema (DR/DME) and Polypoidal choroidal vasculopathy (PCV) followed with identification of the common miRNAs involved in these disease. The other aim was to map the target genes and gene regulatory network modulating ocular vasculopathy thus comprehending the role of miRNA in vasculopathies.

Methods: Publications were collected and the number of miRNAs implicated were identified in major ocular vasculopathies. Role of the common miRNAs in ocular disease and their effect on the target genes have been identified. Ingenuity Pathway analysis (IPA) was used to map the gene network for inflammation, vascularisation and angiogenesis. Target prediction tools and circos plots were used to build the miRNA predicted target gene networks.

Results: Our analysis revealed that 91miRNAs were implicated in ocular vasculopathies, of which 48 miRNA were involved in AMD, 42 miRNA in DME and 1 miRNA in PCV, respectively. Further analysis showed that 19 miRNA are commonly involved in all the vasculopathies. The target gene identification and miRNA network analysis has revealed the gene regulatory network modulating ocular vasculopathy. Target prediction tools and circos plots indicates predicted miRNA target genes and their network.

Conclusion: The network analysis data generated in this review has identified putative candidate genes for biopharmaceutical industry providing a lead for possible drug development. This review presents comprehensive information on miRNA and ocular vasculopaties and has identified putative target genes for further research.

DELIVERING CORNEAL EPITHELIAL CELL PENETRATING PEPTIDE WITH ANTIFUNGAL ACTIVITY USING HYDROGEL

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Purpose: Corneal fungal infections are one of the major infectious eye disease which could lead to potential blindness. India being one the warm temperate region, the abundance of corneal fungal infections is high. The major challenge in the treatment is the poor penetration of antifungal drugs through corneal tight junction barrier and stromal layer. Hence, in this study we designed a corneal specific peptide with antifungal activity and delivered through hydrogel.

Methods: The cell penetrating peptides were designed insilico through subtractive proteomic approach. The peptides were modelled and simulated for membrane penetration. Further characterised for biophysical properties through vibrational spectroscopy. The hydrogels were made encapsulated with peptides and delivered to corneal epithelial cells. The antifungal activity of the peptides were tested against Fusorium spp and Candida Albicans.

Results: The designed peptides were insilico modelled and simulated with the standard parameters. The secondary structures of peptide were helix and stable when encapsulated hydrogel. The release kinetics of the peptides for the 72hrs showed sustained release. The antifungal activity of peptide encapsulated hydrogel against *Fusorium sp*. and *Candida albicans* was sustained for 4hrs.

Conclusion: We have designed and delivered cell penetrating peptide for corneal epithelial cells through hydrogel. The peptide showed antifungal activity against *Fusorium sp.* and *C.Albicans* proving that hydrogel could be a system to deliver antifungal peptides for corneal epithelial cells.

IN VITRO ANTIFUNGAL SUSCEPTIBILITY PROFILE OF FUNGAL ISOLATES FROM PATIENTS WITH KERATITIS

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Purpose: To report antifungal susceptibility of 294 fungal isolates from patients with keratitis.

Methods: All patients who met with a laboratory diagnosis of fungal keratitis and were seen between 2012-13 at L. V. Prasad Eye Institute were included in the study. Corneal scrapings from these patients were processed as per the institutional protocol and fungal isolates were identified based on morphology (colony characteristics and microscopic features) and were subjected to antifungal susceptibility testing. Minimum inhibitory concentration (MIC in µg/ml) was determined for the drugs amphotericin B, natamycin, caspofungin, posaconazole, voriconazole, and ketoconazole; using micro broth dilution method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: *Aspergillus flavus*(n=116), *Fusarium* species (n=97), *Curvularia* species (n=14) and other fungal species (n=67) were tested. All *A. flavus* isolates showed resistance to amphotericin B (MIC_{90}=16, MIC_{50}=8) and susceptibility to posaconazole (MIC_{90}=0.25, MIC_{50}=0.125) while 61.2% were susceptible to natamycin (MIC_{90}=32, MIC_{50}=16). Resistance to amphotericin B and posaconazole was high in *Fusarium* species (96.9%, 95% respectively) but 86.7% isolates were susceptible to natamycin (MIC_{90}=32, MIC_{50}=8). At 8 and 1 respectively the MIC_{90} of voriconazole was higher for *Fusarium* species compared to *Aspergillus flavus*. Susceptibility to ketoconazole was in general low for most of the isolates.

Conclusions: *Fusarium* isolates were resistant to most antifungals except natamycin. Natamycin continues to be a good drug for the treatment of *Fusarium* and *Aspergillus* keratitis the two most common type of keratitis across India. Search for better antifungals for the treatment of fungal keratitis must continue.

MICROBIOME OF VITREOUS IN PATIENTS SUFFERING WITH POST FEVER RETINITIS

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Purpose: Post-fever retinitis (PFR), usually manifests between 2 to 4 weeks after the fever in immunocompetent individuals. A variety of infectious agents viz. bacteria, fungi, viruses, protozoa may cause a febrile illness with or without various ocular manifestations. One of the major challenges of post-fever retinitis is that since it occurs 2 to 4 weeks after the fever many a times the causative organism is not visible and not cultivable. Therefore it is considered very important to use DNA based methods to identify the causative agent. Thus the purpose of the study is to identify the causative agent of post fever retinitis by metagenomic approach.

Methods: In the present study, metagenome was generated for DNA/cDNA extracted from vitreous samples of individuals suffering from PFR (n=6) by using high throughput Illumina sequencing. Non-infectious vitreous sample served as control (n=5).

Results: Viral BLAST search analysis of the metagenome in all the PFR samples revealed presence of viral hits for the species of family *Herpesviridiae*. Further the general BLAST search for other microorganisms revealed presence of bacteria such as *Staphylococcus aureus, Streptococcus agalactiae, Alcanivoraxhongdengensis, Enterobacter cloacae, Enterobacter aerogenes, Peptoclostridium difficile,Streptococcus pneumonia, Klebsiella pneumonia* and *Chlamydia psittaci* and the yeast, *Saccharomyces cerevisiae*.

Conclusion: This study reveals the occurrence of multiple organisms such as virus, bacteria and yeast in the vitreous samples of individuals with post fever retinitis.

LOCALIZATION OF MCTS IN HUMAN CORNEAL ENDOTHELIUM AND THEIR DEREGULATED EXPRESSION IN FUCHS' CORNEAL ENDOTHELIAL DYSTROPHY

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Purpose: Fuchs' Endothelial Corneal Dystrophy (FECD) is a complex, multigenic disorder characterized by decreased visual acuity with aging and progressive deterioration of cornealendothelial cells. It is one of the most frequent (16.6%) reasons for Penetrating Keratoplastyin India. Many genes have so far been attributed to cause this disease, thereby revealing itsgenetic complexity. Advanced stages of FECD are characterized by corneal edema, loss of transparency and apoptosis of corneal endothelial cells. It has been reported that mutations inSLC4A11 (Sodium-borate transporter) cause late onset FECD in Caucasian families. One ofthe SLC family transporter MCT1, a monocarboxylate transporter is one of the key regulatorfor lactate concentration throughout the cornea and it helps to maintain corneal transparency and deturgescence. However, localization studies for MCTs in human corneal endothelium has not been reported.

Method: Non-transplantation grade, Descemet membrane-endothelial sections post trephining of whole corneal tissues were used to study the localization of MCT1, 2, 3 and 4,using Immunohistochemistry (IHC) analysis. Real-time PCR was performed with penetrating keratoplasty (PK) tissues of control and FECD to detect the expression pattern.

Result: We have observed expression of MCT1, MCT2 and MCT4 in Z-sections of theDescemet membrane-endothelium and their differential localization pattern respectively. Alsoit was observed that MCT1 and MCT4 were upregulated in penetrating keratoplasty (PK) -derived FECD tissue samples, whereas MCT1, MCT4, and MCT2 were downregulated inDSEK -derived FECD specimens when compared with respective control tissues.

Conclusion: This study indicates a differential expression of MCTs across the corneal layersto actively channelize the carboxylate-build-up due to the disease condition, into the aqueoushumour. It also helps to understand the spatial distribution of MCTs in corneal endotheliumas well as their heterogeneous expression in FECD.

ENCAPSULATION OF HUMAN LIMBAL-DERIVED MESENCHYMAL /STROMAL STEM CELLS FOR PRESERVATION AND TRANSPORTATION AT ROOM TEMPERATURE

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Purpose: The hurdles of transporting cells intended for cell therapies from site of manufacturing to the clinic, are still an unmet challenge. This study aims to optimize the processes for preserving and transporting the human Limbal-derived Mesenchymal/Stromal stem Cells (hLMSCs) at room temperature by encapsulating them in alginate beads.

Methods: Cell suspension of trypsinizedh LMSCs is mixed in equal volumes with sodium alginate solution, at a final density of $2.5x10^6$/mL. One half of the encapsulated hLMSCs with complete media were kept in transit at room temperature (RT) or 4°C in a specialised container pre-conditioned to maintain RT between 15°C-30.6°C for 3 days. Post-storage, hLMSCs were released using Trisodium-citrate, quantified for viability and plated. The cells were analysed for the phenotypic characteristics and proliferation abilities post 48 and 96-hours of culture via MTT, LIVE-DEAD assays and immunostaining.

Results: Encapsulated hLMSCs under transit at RT were recovered with high viability (86.77±2.63%) compared to those stored at 4°C (68.59±3.79%) and have a better proliferation rate (77.65±3.05%) than 4°C-stored cells (64.54±12.92%) with respect to the control cells. Cells under transit have their characteristic phenotype retained, showing the expression of mesenchymal ($CD105^+$, $CD45^-$, $CD73^+$, VIM^+, $COL\text{-}III^+$, $HLA\text{-}DR^-$) and stem cell markers ($P63\text{-}\alpha^+$, $ABCG2^+$, $PAX\text{-}6^+$), whereas the 4°C-stored cells had a deviation in their phenotype ($P63\text{-}\alpha^-$, $ABCG2^-$, $HLA\text{-}DR^+$.

Conclusion: Alginate encapsulation serves as a potential and low-cost alternative for the current storage methods, offering high viability of the cells, over a longer duration. It helps in maintaining the cell phenotype intact even at extreme Indian temperatures.

IS TORQUE TENO VIRUS ASSOCIATED WITH INFECTIOUS ENDOPHTHALMITIS?

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Purpose: Torque teno virus (TTV) and TTV-like mini virus (TMLV), of the genus *Anellovirus*, being reported to be present at high levels in culture-negative endophthalmitis, raises the possibility that it may have some pathogenic role. The aim of the current study was to determine whether these DNA viruses are involved in our patients diagnosed with infectious endophthalmitis.

Methods: Vitreous biopsies from 44 patients presenting with presumed infectious endophthalmitis and 32 vitreous samples from patients with non-infectious retinal disorders were included. Along with traditional bacterial and fungal culture, DNA was extracted using QIAamp DNA mini kit and quantitative real time PCR was performed to verify the presence of TTV and TMLV in all samples, on an ABI PRISM 7900. The primers were chosen to amplify a distinct conserved region of in TLMV located just upstream of the ORF 2, while in TTV located within the ORF 2.

Results: Twenty-three of the 44 infectious samples (52.3%) were positive for the TTV and / or TMLV including 17 out of 34 (50%) of culture-negative samples. In contrast, 9 out of 32 (28%) of the control samples were positive for TTV and / or TMLV. There was no correlation between TTV infection, clinical manifestations and the clinical course, of the patients evaluated.

Conclusion: This study cannot distinguish whether TTV is a direct intraocular pathogen, a general marker of inflammation, or a commensal virus but provides a testable hypothesis for a pathogenic mechanism in culture-negative endophthalmitis. The detection of anellovirus, profoundly so in vitreous of patients with presumed infectious endophthalmitis is imperative and warrants elucidation of its clinical significance.

ASSOCIATION OF hsa-miR-143-3p IN THE REGULATION OF CORNEALEPITHELIAL STEM CELLS

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Purpose: This study aims to understand the molecular regulatory function of hsa-miR-143-3p in the maintenance of stemness in corneal epithelial stem cells (CESCs).

Methods: The CESCs were enriched by isolating the basal limbal epithelial cells fromdonor tissues (n=33) by differential enzymatic treatment (Arpitha*et al.,* 2008) followedby laser capture micro-dissection of cells having nuclear to cytoplasmic ratio > 0.7(Jhansi *et al.,* 2016).The expression of hsa-miR-143-3p in enriched CESCs and isolatednative central corneal epithelial cells (CCECs) were analyzed by quantitative real timePCR (Q-RT PCR) using miScript SYBR Green PCR Kit (Qiagen). MicroRNA lockednucleic acid *in-situ* hybridization (miRNA-LNA ISH) was used to identify the locationspecific expression of has-miR-143-3p in cryosections of limbal and corneal epithelium.miRNA transfection of primary limbal epithelial cells with pre-miR-143-3p, AntagomiR-143-3p and Scrambled sequence (25 nM) was carried out using lipofectamine 3000(Invitrogen). The transfected cells were seeded on to mitomycin treated 3T3 feederlayer for analyzing the colony forming efficiency (CFE).

Results: Higher expression of hsa-miR-143-3p was observed in CESCs in comparisonto CCECs with fold change of 74±3.3 by Q-RT PCR analysis. The high expression of hsamiR143-3p was observed exclusively in cluster of cells in limbal basal epithelium bymiRNA-LNA ISH. Cells transfected with pre-miR-143-3p had high CFE (2.18±0.39%)compared to untreated cells (1.83 ±0.17%) while transfection with antagomiR-143-3preduced the CFE to 0.68±0.33%. In addition the pre-miR transfected cells produced,significantly more holoclones, which are formed by the stem cells (20.88 ± 5.38 %)compared to antago-miR (1.66 ± 2.88 %) and untreated cells (12.75±1.77%).

Conclusion: High expression of hsa-miR-143-3p in CESCs and their ability to positively regulate holoclone formation indicated its significance in the maintenance of stemness. Further studies are essential to elucidate its regulatory role at transcriptional level.

EXPLORING THE ROLE OF CONNECTIVE TISSUE GROWTH FACTOR (CTGF) DOMAINS IN CORNEAL WOUND HEALING

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Purpose: Corneal wound healing helps in maintenance and restoration of corneal integrity. However, unchecked healing can result in remodelling of ECM with formation of permanent scars (fibrosis). Connective tissue growth factor (CTGF), a secreted matricellular protein, appears to be the central mediator of the corneal healing process, as CTGF expression is correlated with the onset of tissue repair and fibrotic conditions. CTGF has four domains: the N-terminaI section (N-CTGF) includes GFBP, and VWFC domains, with role(s) in myofibroblast differentiation; the C-terminal section (C-CTGF) has TSP-1, and CK domains with pro-proliferative role(s). The presence of different fragments of CTGF in varied pathologies including corneal healing, suggests domain-specific roles. Without domain-specific anti-CTGF antibodies, it is difficult to delineate roles of individual domains. The present study is about (a) development of constructs of individual domains, (b) development of antibodies, based on phage display library technology, and (c) understanding roles of individual domains.

Methods: Domain constructs and antibodies were made, and *in-vitro* assays of cell viability, proliferation and wound closure of corneal cells were conducted, with and without antibodies present.

Results: The CTGF constructs were made and showed neither any cytotoxicity, nor any effects on non-corneal cell proliferation up to the concentration of 2μM. Experiments with corneal cells are ongoing. CTGF domain-specific antibodies were screened. Wound closure assays may offer a mechanistic explanation of healing/fibrotic roles played by CTGF domain constructs, singly and in combinations.

Conclusion: CTGF domains and their specific antibodies might hold promise in the context of corneal wound healing.

IN SILICO ANALYSIS OF INTERACTIONS BETWEEN OPTICIN AND MATRIX METALLOPROTEINASES (MMPS): IMPLICATIONS FOR RETINOPATHY OF PREMATURITY

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Purpose: Retinopathy of prematurity (ROP) is a disease exclusive to premature babies characterized by an abnormal retina vascularization leading to vision loss. MMPs arezinc and calcium dependent proteolytic enzyme found in brain and eye that upregulatesangiogenesis. Whereas Opticin is a glycoprotein synthesized by non-pigmentary epithelium and possess the anti-angiogenic activity. Our recent finding from *in vitro*studies showed that MMPs regulates Opticin. To further understand the MMP regulated Opticin expression in the vitreous, an *in silico* analysis was performed. We hypothesized that inhibiting MMP activation impairs its binding with Opticin thereby preventing its degradation and thus may upheld the Opticin expression and its anti angiogenic function.

Methods: Threading/Fold recognition was first applied to build 3D model for Opticin protein. Molecular docking scoring was used to examine the potential protein binding sites (protein-protein docking) on MMPs and Opticin. The molecular docking scoring for proand active form of MMPs were also then used to examine potential ligand binding pockets (protein-ligand docking) for Doxycycline and EDTA.

Results: At first, the predicted 3D structures for Opticin were obtained by threading/fold recognition modeling and the best structure was selected based on C-score, RMSDvalue and TM -align score provided by I-TASSER.The protein ligand docking showedthat the Doxycycline or EDTA interact with zinc ion in catalytic domain hence inhibiting the MMPs activity thereby preventing the degradation of Opticin by MMPs.

Conclusion: Our *in silico* analysis confirmed that Doxycycline/EDTA inhibits the MMP9 activity in its active form only by interacting with Zinc and thereby preventing Opticin degradation.

HOST DEFENSE MEDIATED BY ANTIMICROBIAL PEPTIDES IN *STREPTOCOCCUS PNEUMONIA* KERATITIS

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Purpose: Bacterial keratitis is an inflammatory disease of the cornea that causes corneal opacity and severe pain that may lead to blindness. One of the most common causative agents is *Streptococcus pneumoniae.* Currently, antibiotics are primarily used for treatment. However, with advent of antibiotic resistance in *Streptococcus* sp., role of alternate therapeutic agents, like antimicrobial peptides (AMPs), needs to be studied.

Methods: Expression levels of AMPs were examined in patients' samples and *in vitro* model of infection using human corneal epithelial (HCECs) by qPCR. Protein expression of various signaling molecules was determined by western blotting. Reactive oxygen species (ROS) generation was determined by fluorescence microscopy using 2'7'-dichlorodihydrofluorescein diacetate (CFDA) dye.

Results: Differential expressions of several AMPs were determined in the patients' samples and immortalized human corneal epithelial cells infected with *S. pneumoniae.* Increased ROS generation was seen in *S. pneumoniae*infected HCECs. Human cathelicidin was found to be effective against *S. pneumoniae.*

Conclusions: Increased AMPs expression indicates that they play a vital role in innate immunity of the cells. Understanding the role of various signalling pathways involved in AMPs expression may provide an insight into the mechanisms to enhance its expression level and hence effectiveness. Further, increased ROS production in host has been implicated as a defense mechanism against infection, which needs to be studied further.

ESTIMATION OF OPTIMUM TIME SINCE DEATH FOR OBTAINING A GOOD QUALITY TOTAL RNA FROM RETINAL TISSUE OBTAINED FROM CADAVER (DONOR) EYES

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Purpose: Human RNA is a major prerequisite for molecular investigations of different eye diseases. Lack of retinal tissues from a healthy eye is a scarcity that forced us to look for donor eyes. However, the integrity of RNA from the cadaveric donor eyes remains controversial for several factors like time since death, time of RNA extraction and storage procedure. The present study aims to evaluate the quality and quantity of RNA extracted from cadaveric retina and macula subjected to different time since death and storage hours.

Methods: Donor Eye balls received at The Ramayamma International Eye Bank, Hyderabad were provided to us after quality testing. Retinal imaging was performed for the eyes. Macular tissue and the peripheral retina were preceded for RNA extraction separately using Trizol method on the same day and a week after storage in RNA later. Quantity of RNA was assessed on Nanodrop and quality by agarose gel electrophoresis. RNA was converted to cDNA and PCR was done to assess the expression of retinal specific genes to test the quality of RNA.

Results: Quality and quantity of RNA from fresh tissues were better than the stored tissues. The expression of retinal specific genes such as *pax6, rhodopsin, beta 3 tubulin*,*gfap*, *aldh1L1*, and *beta actin* was morein fresh tissue RNA extracted within 24 hrs than the stored tissues.

Conclusions: RNA extracted within 24 hrs of eye retrieval provides good quality and quantity to be used for further experiments.

UNDERSTANDING THE ROLE OF RD3 IN LCA DISEASE PATHOGENESIS

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Purpose: To study the sub-cellular localization and retina-specific functions of RD3 protein.

Methods: The wild type and mutant gene constructs of human *RD3* was cloned into a retroviral vector as N-terminal HA-tag fusions and sequence confirmed. The constructs were transfected into HEK-293T cells and sub-cellular localization of RD3 wild type and mutant proteins were studied by immunecytochemistry.

Results: RD3-wt protein was observed to organize into punctate structures in the nucleus as well as in the cytoplasm. But the expression level of the C-terminal truncated mutant was significantly reduced and showed a diffused pattern of localization. In dual staining experiments, the punctate nuclear structures of RD3-wt protein were either co-localized or organized in the near vicinity of sub nuclear structures such as the splicing compartments, nucleoli and coilin bodies. The cytosolic RD3 protein was found to co-localize with a Golgi marker.

Conclusions: The cytosolic function of RD3 is well known and it mediates the intracellular transport of retinal guanylate cyclase via the golgi network. However, its localization in the nucleus and its association with sub-nuclear compartments suggests a novel role for RD3 in diverse gene regulatory processes and warrants further evaluation.

COMPARATIVE ANALYSIS OF THE EXOPROTEOME OF *ASPERGILLUS FLAVUS* SPORES DURING THE EARLY STAGES OF GROWTH: SAPROPHYTE VERSUS CORNEAL ISOLATE

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Purpose: Fungal infection accounts for up to one-third cases of all keratitis. Almost 80% of corneal *Aspergillus* infection is due to *A. flavus*. The proteins secreted by the fugal conidia are the earliest factors that interact with the cornea during infection. They are important determinants in establishing an infection. In this study, we identified and compared the secreted proteins of *A. flavus* during the early stages of growth between a saprophyte (ATCC 26) and two corneal isolates (CI 1123 and CI 1698) to identify proteins that might play a crucial role during corneal infection.

Methods: Dormant spores were grown in CZB at 30 °C for 6 hours until the spores start to germinate. The culture supernatant was precipitated and a shot gun proteomics approach was used for MS identification of proteins. The data was analysed using Proteome Discoverer followed by label-free quantification using Scaffold software.

Results: The total proteins identified in ATCC 26, CI 1123 and CI 1698 are 146, 227, 212, respectively. Nearly 65% of the identified proteins had N-terminal signal peptides indicating that the others are secreted through non-canonical pathway. Proteins involved in carbohydrate metabolism, particularly enzymes acting on the cell wall polysaccharide were the major class. Among the proteases, next abundant category, serine and metalloproteases are overrepresented in the CI1123 and CI1698. In addition, Phytase, a phosphate acquisition enzyme was found to be secreted exclusively by the corneal isolates.

Conclusions: The common core proteins among all the three strains are involved in the germination process. The unique proteins of the corneal isolates might reflect their requirement for infecting the cornea and are potential virulence factors.

PRESENCE OF STEM CELLS IN THE CENTRAL REGION OF THE HUMAN ANTERIOR LENS EPITHELIUM

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Purpose: The anterior lens epithelium has the ability to differentiate into lens fibres throughout life. Maintenance of the lens homeostasis depends on the presence of adult stem cells in the anterior lens epithelium. Therefore it is required to elucidate the location, characteristics and niche of these stem cells along with their role in maintenance of tissue homeostasis. This study aims to identify the presence of stem cells in human lens epithelium using donor tissues.

Methods: Human lens were obtained from excised donor globes (n=3) after removal of the cornea for transplantation from Rotary Aravind International Eye Bank, Madurai. Paraffin sections (5µ) of human lens were stained with haematoxylin - eosin and immunostained for the universal stem cell marker ABCG2 and differentiation marker connexin-43 (cx-43). Images were acquired and analyzed in Leica SP8 confocal microscope.

Results: Confocal analysis of the immunostained lens sections (n=3/donor) revealed that the cells in the central region of anterior lens epithelium to be strongly positive for ABCG2 but negative for the differentiation marker cx-43. In contrast, the epithelial cells in the germinative, transitional and equator region were positive for cx-43 but were low positive/negative for ABCG2.

Conclusions: The above findings indicate the presence of stem cells in the central region of the human anterior lens epithelium. Further studies are essential to elucidate their niche, and to understand their role in tissue homeostasis.

RB1 PROMOTER METHYLATION ANALYSIS IN RETINOBLASTOMA PATIENTS

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Purpose: In addition to the genetic events, retinoblastoma might also be caused by epigenetic factors. It is hence important to elucidate the epigenetic mechanisms like promoter methylation and miRNA regulation underlying the tumor progression of retinoblastoma. One of the epigenetic mechanisms that operate to shut off the *RB1* expression is the promoter methylation. In many of the cancers, promoter methylation of various genes account for about 50% of the total mutational spectrum. Here, newer methods were developed to study the promoter methylation of *RB1*.

Methods: The Samples (tumor) were collected from the patients and DNA was isolated using Shrimpex-tumor DNA isolation kit. For screening *RB1* mutations, the multistep approach was carried out using Sanger sequencing, MLPA and NGS. The samples which do not show variations or deletions in *RB1* gene were analysed for promoter methylation analysis by MS-MLPA and Bisulfite sequencing.

Result: Inactivation of *RB1* by its promoter methylation was analysed by two methods by MS-MLPA and Bisulfite sequencing. Out of 100 samples, promoter methylation was identified in 5 cases including 3 samples with one allele and 2 with both alleles methylated. From MS-MLPA, one monoallelic and one biallelic methylated sample were taken and optimized for the bisulfite sequencing and also found concordant result with further single base resolution shows the evidence for the *RB1* inactivation by the promoter methylation.

Conclusions: MS-MLPA has shown to be an efficient method of analysis of the *RB1* promoter methylation. *RB1* Promoter methylation was observed in 5% of the total cases and it has expanded the spectrum of alterations in *RB1* gene. Further confirmation of the methylation sites by bisulphite sequencing helped to distinguish partial and complete methylation.

AGE RELATED REDUCTION OF STEM CELLS IN NATIVE HUMAN TRABECULAR MESHWORK CELLS

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Purpose: We have earlier demonstrated the presence of stem cells in the Schwalbe's line region of human Trabecular Meshwork (TM). The purpose of the present study is to determine whether there is a reduction of TM stem cells during ageing.

Methods: Native TM cells were isolated from corneoscleral rings (age group <30 years, 30-60 years and >60 years, n=5 each) by collagenase treatment. The cytosmears of TM cells were double immunostained for ATP- Binding Cassette protein G2 (ABCG2) and neurotrophin receptor p75. Images of 100 consecutive cells were acquired using Leica SP8 confocal microscope. The stem cells were quantified by two parameters- high ABCG2 expression (mean pixel intensity 182± 46) and high Nucleus to cytoplasmic (N/C) ratio (>0.7). Spearman rank order correlation analysis was carried out to determine the association between age and the stem cell content using Stata 14.0. P-value less than 0.005 was considered statistically significant.

Results: Analysis of native TM cells revealed 21.5±1% (Mean±SD) of TM stem cells in younger donors (<30 years) to have high ABCG2 expression and high N/C ratio. This percentage decreased significantly to 10.75 ± 6% in middle (30-60 years) and 4±3.5% in older age group (>60 years) (rho=-0.92 and p=<0.001). The cells with high ABCG2 positivity and high N/C ratio were also positive for p75.

Conclusion: A significant reduction in the native TM stem cell content was evident upon ageing. Further studies are essential to understand the nature of these stem cells in glaucomatous condition.

COMPARATIVE ANALYSIS OF HUMAN CORNEAL ENDOTHELIAL CELLS CULTURED FROM YOUNG AND OLD DONORS

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Purpose: Human corneal endothelial cells are known to undergo endothelial to mesenchymal transition (EnMT) when cultured *in vitro*. This study aimed to determine when the cells undergo EnMT in culture by systematically assessing their growth characteristics and functional properties at every passage (P) or sub- culture. This information is critical for us to assess the safety of the cells before taking them for human transplantation.

Methods: Corneas from donors 5 days to 45 years old were cultured from P1-P8. Growth characteristics such as population doubling time, cell proliferation and total cell density (at confluence) were quantified at every passage. Similarly, changes in the putative EnMT and endothelium gene and protein expression was quantified using real time PCR and immunostaining at different passages.. Finally, barrier function of the cultured cells was measured as permeability to FITC dextran (10kDa). Statistical analysis was performed using graphpad prism ($p \leq 0.5$ was considered significant).

Results: Between passages 1-3 the following were noted a) young donors (≤ 10 years) showed a population doubling time of 41.4±3.9 hours which was comparable to adult donors (11-30years) averaging 46.2±13.6 hours and significantly lesser than older donors of 40-50 years (90.2±8.6 hrs) b) 60±23% of the cells were proliferating in cultures from young donors when compared to 42±11% in older donors c) the average cell density obtained from young donors was significantly more (2000±353 cells/mm^2) than older donors (754±287 cells/mm^2).Interestingly, in all the cultures, irrespective of age of donor, there was a sudden increase in the doubling time at P4 (to 121.1±35.7hrs) when compared to P3 (31.08±8.6) and reduced to 53.69±13.3 hrs at P5. Concomitant with this, we noticed that the morphology of cells altered from polygonal and homogeneous to more fibroblastic confirmed by the increased expression of a-SMA in P6 cells compared to P2 cells. Further, late passage (P6, 7) cells were more permeable to FITC-dextran than early passage cells (P1-3).

Conclusion: Our data is in agreement with earlier studies that the yield of cells from young donors is better than from older donors. However, we have shown here that at passage 4 the cells start undergoing a transition from endothelium to more fibroblastic phenotype. This suggests that at present only early passage (1-3) cells are safe to take to the clinic for transplantation.

REPLICATION OF GWAS LOCI ASSOCIATED WITH DIABETIC RETINOPATHY IN SOUTH INDIAN COHORT

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Purpose: Diabetic Retinopathy (DR) is a sight-threatening complication of diabetes. It is an epidemic of the current century and affects the majority of the population. It is the most common microvascular complication of type 2 diabetes. Previous studies on DR have identified VEGF, ALR2 and RAGE as some of the specific candidates that increase the risk of DR development. The current study is a replication study for the SNPs that were significantly associated with DR performed cohort of cases and controls.

Methods: The replication study was done by a case-control study design, defining type 2 diabetes patients >40 years of age with Proliferative Diabetic Retinopathy (PDR) and Non-Proliferative Diabetic Retinopathy (NPDR) as cases and with >8 years duration of diabetes without any retinopathy changes as controls. These patients were of south Indian origin recruited partly from (i) SNDREAMS project and (ii) Indo-US collaborative project. Genomic DNA was isolated from whole blood followed by PCR based direct sequencing. PLINK analysis with adjustment for confounding factors, including age, gender, duration of diabetes, and presence of hypertension was done for statistical significance and genetic association.

Results and conclusions: Comparison of the demographic details between DR cases (N=81) and controls (N=88) showed significant association ($p<0.0001$) for age of diabetes onset, insulin user status, HbA1c, diastolic blood pressure and microalbuminuria with DR. Here, we discuss the result of the replication study for the SNPs (rs17115362, rs1241597, rs1241600, rs1241599, rs7096907, rs4818681) in those genes implicated in angiogenesis and neural pathway with p<10-6.

AGE RELATED MACULAR DEGENERATION (AMD): CLINICAL AND GENETIC PERSPECTIVES FROM WESTERN INDIA

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Purpose: AMD is the leading cause of irreversible blindness. Genetic screening allows assessment of genetic variations of AMD cases and could play a role in the management of disease. The purpose of this study was to find out association between *ARMS2* gene variation and clinical presentations in AMD subjects across western India, as there is a dearth of such data.

Methods: This study included subjects aged 50 years or above, having AMD, who visited our institution from 2008 to 2018. Demographic details, smoking history and systemic diseases' status were collected; followed by detailed ophthalmic evaluation including genetic counselling. Blood and plasma samples were collected from sixty patients after obtaining their consent and IRB approval; of which; five DNA samples were subjected to targeted exome sequencing (TES), for preliminary analysis.

Result: Preliminary analysis has shown that subjects with a history of diabetes, hypertension and cholesterol are more likely to have AMD. Smoking was an important modifiable risk factor associated with the progression of AMD. Out of five patients, four were presented with dry AMD which evolved into wet AMD. The remaining patient presented with intermediate stage of AMD. TES revealed a genetic variation in *ARMS2* gene in all AMD cases.

Conclusion: The AMD subjects from western India demonstrated association between *ARMS2* profiling and their clinical presentation.Based on presumptive findings, it would be promising to perform further analysis for an improved understanding of environmental, genetic and lifestyle related risk factors associated with AMD.

THE EFFECT OF MATRIX ON PCL MELPHALAN NANOPARTICLES FOR RETINOBLASTOMA TUMOR CELLS.

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Purpose: Retinoblastoma is a pediatric intraocular malignant tumor. Chemotherapy is the most prominent form of treatment for the disease. Melphalan is the most commonly used chemotherapeutic drug for the intravitreal treatment of retinoblastoma. However, in certain stages of retinoblastoma, the cancer cells attached to their respective extracellular matrices migrate to the vitreous humor. In order to investigate the effect of ECM on RB cancer cells after the Melphalan treatment, we developed a drug encapsulated nanoparticle to study its effect both in cells grown in suspension and in a 3D matrix.

Methods: The PCL Melphalan nanoparticles were prepared by solvent displacement method. The size and the zeta potential of the nanoparticles were measured by Zetasizer. The morphology of the nanoparticle was characterized by Scanning electron microscopy. Fourier Transform Infrared spectroscopy was performed to characterize the nano particles with and without the drug. Y79 cell line was grown in matrigel and treated with the nano particles to check the efficacy of the Melphalan nanoparticles.

Results: The size and zeta potential of the Melphalan nanoparticles were in the range of 300-400nm and -15mV. The morphology of the nanoparticles were found to the spherical in shape. The Efficacy of the nanoparticles containing the drug was found to be more than the pure drug.

Conclusion: Thus, by encapsulating Melphalan within the PCL nanoparticles, the bioavailability of the drug can be enhanced, for retinoblastoma tumor cells.

TRANSCRIPTOMIC STUDY TO DEFINE LIMBAL STROMAL STEM CELLS – A UNIQUE CELL POPULATION FOR CORNEAL HEMOSTASIS

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Purpose: The aim of the present study is to characterize the limbal epithelial and stromal cells in comparison to the native limbal tissue *in vitro*.

Methods: Limbal epithelial and stromal cells were cultured from the cadaveric tissues. Transcriptome analysis for the limbal cultures and tissues was performed by Next Generation Sequencing (RNA-Seq). The obtained data was further validated by quantitative PCR (q-PCR) analysis and immunofluorescence to identify the biomarkers.

Result: NGS revealed the whole transcript expression levels of the limbal tissue and cultures and is corroborated by q-PCR analysis. Isolated population of limbal stromal cultures were established by extending the culture passages and limbal epithelial cultures were obtained in the initial primary culture. The q-PCR results had shown the decreased expression of CK3, CK12, CK19, P63 and E-Cadherin with increased expression of N-Cadherin, Vimentin and HIF1α in stromal culture indicating the mesenchymal properties. Limbal stromal stem cells in the culture expressed ABCB5, ABCG2, CD90, CD105, CD34, COL3A, COL5A1 and TIMP1 identified by NGS, q-PCR and immunofluorescence.

Conclusion: Limbal epithelial progenitor cells and the limbal stromal cells that acts as a niche could be isolated from the limbal tissue. These cultured cells show the stem cell properties similar to that of the native tissue and are shown to have hematopoietic stem cell characteristics that could aid in corneal homeostasis.

MOLECULAR GENETICS OF ABCA4 GENE IN STARGARDT DISEASE

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Purpose: The ABCA4 gene involved in the transport and clearance of all-transretinal aldehyde in visual cycle. It's belongs to ATP-binding cassette (ABC) transporter family and composed of 50 exons located in chromosome 1p13. Rate of Stargardt disease progression depends on clinical presentations and *ABCA4* mutations. Genetic heterogeneous of *ABCA4* were showed in large cohort of ethnic population. So far, there are very limited reports of this gene from India. Therefore, our current study is focusing on the mutation analysis of ABCA4 gene in more number of families to further prove its involvement in Indian population.

Methods: A total of 15 probands were diagnosed with STGD1; recruited for genetic analysis of ABCA4 gene. All probands underwent ophthalmic examinations. Initially we screened 9 exon–intron boundaries (ex – 5, 17, 39, 40, 42, 44, 46, 47 and 48) of the ABCA4 gene (out of 50 exons) to identify the mutations by PCR-based bidirectional DNA sequencing.

Results: We have screened only nine exons including the intron exon junctions of ABCA4 gene and identified 10 different mutations in 15 unrelated probands. Among these mutations included frameshift (ins/del), novel synonymous intronic variants and rest of the mutations are already reported in other ethnic populations. There were no sequence variations in 5 probands out of 15 probands in the nine exons screened.

Conclusion: Further, we will be increasing the sample size and the entire coding regions of ABCA4 gene will be screened for all the samples to identify the frequency of gene mutations in south Indian population.

DIFFERENTIAL EXPRESSION OF GENES RELATED TO STEROID RESPONSIVENESS IN HUMAN TRABECULAR MESHWORK CELLS

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Purpose: Steroid-induced ocular hypertension (SIOHT) is a serious side effect associated with steroid therapy. The elevated intraocular pressure (IOP) is observed in only 40% of the general population who are on steroid therapy (steroid responders) and others are not (steroid non-responders). Among 40%, 6% of them are likely to develop glaucoma (SIG). The molecular mechanism for such responsiveness is not well understood. Therefore, the purpose of the present study is to investigate the genes related to steroid responsiveness in human trabecular meshwork (HTM) cells.

Methods: The characterized primary HTM cells (n=3 cell strains from 3 donors) were used for the present study. HTM cells were treated with dexamethasone (DEX) or 0.1% Ethanol (ETH) for 10 days. The conditioned media collected before and after drug treatment was assayed for fibronectin by sandwich ELISA. Eighteen genes related to steroid responsiveness were quantified by qPCR after respective treatment in HTM cells.

Results: The levels of fibronectin were high in DEX treated cells as compared to vehicle treated cells. Among 18 genes analyzed, 5 genes (MT2A, ALOX12, CCL5, S100A12, and PTX3) were up regulated in DEX treated cells as compared to vehicle treated cells. In addition, the mRNA expression of glucocorticoid receptor alpha (GR-α) was found to be high in all cell strains studied.

Conclusion: Pathway analysis showed that genes related to inflammation and apoptosis pathways were up-regulated in steroid responsive cells. This study will help us to understand the steroid responsiveness and its associated mechanism in the pathogenesis of SIOHT and SIG.

METADATA ANALYSIS TOWARDS THE IDENTIFICATION OF DYSREGULATED METABOLIC PATHWAYS IN NEOVASCULAR AGE RELATED MACULAR DEGENERATION

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Purpose: Neovascular age related macular degeneration (wet AMD) is a common cause of severe, irreversible vision loss in people of 55 years and older. It involves retinal angiogenesis, and the molecular mechanism remains poorly understand. We aim to identify dysregulated metabolic pathways through gene expression metadata analysis.

Methods: Microarray data for retinal, RPE/C samples of AMD phenotypes and controls were retrieved from NCBI-GEO. Data were merged, imputed missing values using Bayesian PCA, quantile normalized and outlier samples were removed based on PCA and clustering. The differentially expressed genes (DEGs) were identified by fold change and p-value of moderated t-test. The pathway aberrance score was calculated using GSEA.

Results: The 5 different analysis based on tissue type insight the common profile of wet AMD phenotype. Top DEGs and notable altered pathways were common in all the analysis, some were unique to retina and RPE/C samples. The 34 dysregulated pathways common to all the analysis are associated with angiogenesis, which is the major pathogenicity of the disease. From the gene-pathway network analysis, 18 unique DEGs in all analysis showed higher closeness centrality, which may the potential targets. As well the tissue specific target genes were identified.

Conclusions: This meta-analysis unifies various results of previous studies and highlighting the potential targets and altered metabolic pathways.

PREVELANCE OF ESBL PRODUCING *PSEUDOMONAS AERUGINOSA* IN HUMAN OCULAR SAMPLES

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Purpose: Extended-spectrum β lactamases (ESBLs) mediated resistance are more prevalent worldwide, especially among Gram-negative bacteria. The data available on the prevalence of ESBLs in this area is very limited. Hence, the present study was envisaged for the isolation and identification of *Pseudomonas aeruginosa* to explore its antibiotic susceptibility patterns in ocular infections with special reference to detect ESBL.

Methods: All the samples were subjected to molecular studies towards confirmation through 16S rRNA gene sequencing. Susceptibility of isolates to 17 different antibiotics were tested. The isolates showed resistance to β - lactam antibiotics were further subjected to ESBL confirmatory test and performed PCR assays targeting CTX-M and VEB gene that encode ESBL.

Results: Among 30 isolates, 12 were screened as ESBLs producers based on their resistance to β-lactam antibiotics such as Cefotaxime and Ceftazidime, were further conformed through double disc diffusion method with Clavulinic acid (CA). The results demonstrated CA effect only with Cefotaxime by means of zone diameter differences. PCR results depict the occurrence of two genes in four different samples *suggests the possibility of diverse range of resistant mechanism among the ESBL positive samples.*

Conclusions: Altogether, our data indicated a high prevalence of ESBL among *P. aeruginosa* and their diverse enzyme types. Dissemination of ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients. Hence, proper infection control practices and barriers are essential to prevent outbreaks of ESBL-producing bacteria.

TRANSPORTERS AFFECTING THERAPEUTIC EFFICIENCY IN RETINOBLASTOMA

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Purpose: Resistance to various anti-cancer drugs has been observed in the Y-79 retinoblastoma cell line *in – vitro*. The objective of this study was to analyze the gene expression profile of various drug transporters in cultured Y–79 cell lines, resistant to chemotherapy. Resistance was induced in Y-79 cell line against the conventional drugs used in therapy, and the expression of MATE, PePT, OAT, OATP3, OATP4, OCTN1, OCTN2 and PgP transporters was subsequently studied on the resistant cells.

Methods: The Y – 79 cell line obtained from NCCS-Pune was cultured in RPMI – 1640 media supplemented with FCS. Resistance was induced by individually co- culturing the cells with the chemotherapeutic drugs (Etoposide, Carboplatin and Vincristine). The dosage was increased from IC_{10} to IC_{50} across subsequent passages. Trypan blue assay was performed at each passage. The resistant cells (to each drug) were used further for transporter expression analysis using RT-PCR.

Results: Carboplatin co-cultured Y-79 cell line was highly resistant to treatment, whereas etoposide & vincristine caused the cells to go into stationary phase. Higher expression of influx transporters like OAT, OATP4 and OCTs were observed in the carboplatin-treated group. All the transporters were overexpressed in the vincristine-treated group except the OAT transporters, whereas etoposide treated group showed unaltered levels of all transporters.

Conclusion: Contrary to efflux transporters, overexpression of influx transporters, leading to excessive uptake of the drug, could be attributed as a probable cause for the increasing resistance observed with carboplatin. Pharmacological intervention of such drug transporters could serve as important treatment modalities in resistant cases of retinoblastoma.

PH10

TEMPORAL TRENDS IN THE PREVALENCE OF SPECTACLES USE AND SPECTACLES COVERAGE IN STATE OF TELANGANA, INDIA

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Purpose: To assess the temporal trends in the prevalence of spectacles use and spectacles coverage for refractive errors and presbyopia in population based studies conducted during 2011-12 and 2017 among those aged ≥ 40 years in the state of Telangana, India

Methods: Two population-based cross-sectional study was conducted in Khammam and Warangal districts during 2011-12 and in 2017. In both the studies, cluster random sampling methodology was used to select the participants for the study. In addition to the clinical examination, a questionnaire was used to collect information on current and previous use of spectacles, type of spectacles and details of the spectacles provider. Same questionnaire was used both the surveys.

Results: In total, 2485 participants were examined in 2011-12 and 2711 participants were examined in Khammam district. Similarly, 2438 and 2646 participants were examined in Warangal. The prevalence of spectacles use increased from 28.0% (95% CI: 26.2 – 29.8) to 34.9% (95% CI: 33.1 – 36.7) in Khammam during 2011-12 to 2017 while in Warangal, the prevalence has declined from 32.5% (95% CI: 30.6 – 34.3) to 29.1% (95%: 27.4 - 30.8). The spectacle coverage changed from 35% to 37.4% in Khammam and from 40.3% to 36.4% in Warangal.

Conclusions: We found an increase spectacle use and coverage in Khammam compared to Warangal in five last years. As spectacles use is a surrogate measure for the availability and uptake of primary eye care in a region, the results are suggestive of an improvement in primary eye care services in Khammam.

LIFETIME OCULAR ULTRAVIOLET RADIATION EXPOSURE LEVELS AND ITS RISK FOR PTERYGIUM, PINGUECULA AND SPHEROIDAL DEGENERATION - A REPORT FROM THE CHENNAI EYE DISEASE INCIDENCE STUDY

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Purpose: To study lifetime ocular UV exposure levels and its association with the Pterygium, Pinguecula and Spheroidal Degeneration.

Methods: The Chennai Glaucoma study (CGS) was a population-based study which was followed by the Chennai eye disease incidence study (CEDIS) after six years. Subjects above 40 years of age, from 27 villages of rural Tamil Nadu and 5 urban cities of Chennai, of both studies were included. Written informed consent was obtained. A detailed ocular examination, was done for all subjects including documentation of of Pterygium, Pinguecula and Spheroidal Degeneration. Lifetime ocular ultraviolet exposure was calculated for each individual and fitted in the Melbourne Visual Impairment model. The prevalence, incidence, association with age and gender, risk factors and lifetime ocular UV exposure were analysed.

Results: We have studied 2091 subjects (1080 rural and 1011 urban). The prevalence of pterygium, pinguecula and spheroidal degeneration were 6.7% (95% CI: 5.6 to 7.8), 10.8% (95% CI: 9.4 to 12.1) and 6.7% (95% CI: 5.6 to 7.8) respectively. The risk factors for pterygium and pinguecula were rural residence, smokeless tobacco use and increased lifetime ocular UV exposure. The other risk factors for spheroidal degeneration were increasing age, rural residence, smokeless tobacco use, non-use of spectacles, presence of diabetes mellitus and increased lifetime ocular UV exposure. The risk showed an increase from 4th highest quintile of lifetime ocular UV exposure.

Conclusion: Higher UV radiation exposure was found to be associated with the presence of Pterygium, Pinguecula and Spheroidal Degeneration.

ARE VISUAL HALLUCINATIONS COMMON AMONG THE VISUALLY IMPAIRED? : RESULTS FROM A POPULATION BASED SURVEY

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Purpose: Prevalence of Charles Bonnet Syndrome (CBS), a condition in which individuals with visual impairment (VI) experience visual hallucinations (VH) is unknown in India. In an earlier study done by our group, the prevalence of patients consulting in a tertiary eye care centre was found to be ranging from 7.29% to 9.48%. As part of a larger epidemiological study, we aimed to determine the prevalence of individuals experiencing VH, which is a key indicator for CBS.

Methods: A large cross-sectional study was conducted to determine the prevalence of VI using rapid assessment techniques in two districts of Telangana (Warangal and Khammam). A total of 4936 individuals (mean age: 55.5±0.16 years, 56% females) had undergone eye examinations and interviews. One of the interview questions included if the participants had ever experienced seeing imaginary animals, people or objects. Responses for this question were analyzed using logistic regression controlling for multiple factors.

Results: A total of 774 (15.6%) participants were noted to have VI as per the WHO criteria. Out of this, 107 individuals (13.8%) reported experiencing VH (mean age: 67.2±10.8 years, 54% females). Age (OR:1.42, p=0.02, CI:1.05-1.93), education status (OR:0.76, p=0.03, CI:0.59-0.97) and vision status (OR:1.52, p<0.01,CI:1.17-1.97) were found to have higher odds for reporting VH.

Conclusion: Individuals having VI were more likely to report experiencing visual hallucinations, which could be indicative of CBS. A complete screening assessment with appropriate preliminary cognitive screener would be useful to identify patients with CBS and manage them appropriately to improve their quality of life.

POPULATION BASED ASSESSMENT OF PREVALENCE OF DISABILITIES IN ELDERLY POPULATION IN THE STATE OF TELANGANA, INDIA

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Purpose: To assess the burden of disabilities (seeing, hearing, mobility, cognition, self-care and communication) among elderly population in Khammam and Warangal districts in Telangana, India.

Methods: A total of 5347subjects were examined for a study on visual impairment. As a part of this study, Washington Disability Questionnaire (WDQ) was administered. The questionnaire was administered in local language by trained field investigators. Disability is defined as a physical, mental, or psychological condition that limits a person's activities.

Results: The data was analyzed for elderly participants (aged ≥60 years). Of the total, 1821 participants, 54.5% are women, and 73.3 % had no education. The prevalence of self-reported disabilities were: seeing (5.8%; 95% CI 4.7 to 6.9), hearing (3.7%; 95% CI 2.9 to 4.7), mobility (13.2%; 95% CI 11.7 to14.9), cognition (5.0%; 95% CI 4.0 to 6.1), self-care (3.5%; 95% CI 2.6 to 4.4) and communication (1.8%; 95% CI 1.2 to 2.5). At least one disability was reported by 21.6% (95% CI: 18.8 – 22.6) of the participants. On multivariable analysis, seeing (OR 2.3; 95% CI 1.6 to 3.5), hearing (OR2.2; 95% CI 1.3 to 3.7), communication (OR 2.3 95% CI 1.1 to 4.8) were associated with older age. None of the disabilities were associated with gender.

Conclusion: Every fifth person aged 60 years and older in districts of Khammam and Warangal in Telangana has at least one self-reported disability. Comprehensive public health strategies are needed to address this burden.

TRANSLAMINAR- CRIBROSA PRESSURE DIFFERENCE (TLCPD) AND CEREBROSPINAL FLUID PRESSURE (CSFP) PROFILE IN NORMALS & GLAUCOMA: A POPULATION BASED STUDY

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Purpose: The two independent pressurized regions are intra ocular pressure anteriorly and supra-choroidal space posteriorly are separated by the lamina cribrosa. The purpose of this study is to assess the TLCPD and CSFP in normals and glaucoma.

Methods: The Intra Ocular Pressure and CSFP data of 4289 subjects were collected retrospectively from the Chennai glaucoma Study (CGS). The TLCPD is the difference between IOP and CSFP. CSFP was calculated based on an equation provided by Jonas Jost B *et al.* 2011 which includes body mass index and diastolic pressure.

Results: A total of 4289 subjects of mean (SD) age 54 (10.5) years were included, out of which 4121 were normal subjects and 168 were diagnosed with Primary glaucoma. For CSFP, significant ($p<0.001$) difference was found between normal [12.2(3.6) mmHg] & glaucoma [11.4(4) mmHg]. Mean IOP was 15.7 (4) mmHg for normals and 19(7.8) mmHg for glaucoma subjects ($p<0.001$). TLCPD was found to be higher ($p<0.001$) in glaucoma patients [7.6(9.3) mmHg] than in normal [3.4(4.7) mmHg].

Conclusions: In our study lower CSFP and higher TLCPD was found in glaucomatous subjects which is consistent with the literature. The lamina cribrosa of the Optic nerve head is the primary site of glaucomatous damage, TLCPD may be more important factor than IOP for the glaucomatous optic neuropathy. Further studies need to be performed including different types of glaucoma to investigate the association of TLCPD in prevalence of glaucoma.

SPECTACLE PRESCRIPTION PATTERNS IN L V PRASAD EYE INSTITUTE VISION CENTRE NETWORK

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Purpose: L V Prasad Eye Institute has established vision centres (primary eye care centres) as an integral part of the pyramidal model of eye care service delivery. Refraction and dispensing of spectacles is one of the core functions of a vision centre. The purpose of this study is to review the spectacle prescription patterns across the vision centre network and suggest strategies for faster delivery of spectacles.

Methods: In total, 300,072 patients were examined in 153 vision centres during April 2017 and March 2018. The refractive error data of patients who underwent refraction and were either prescribed with spectacles or advised to continue their current spectacles were included for analysis. The prescriptions were divided into eight different categories based on spherical, cylindrical, spherocylindrical and near addition in both eyes.

Results: In total, 116,581 patients had refraction values in both eyes. Among these, 18.9% had spherical prescription in both eyes, 4.2% had cylindrical prescription, 3.9% had spherocylindrical prescription. In total, 23% had spherical with near addition in both eyes, 3.6% had cylindrical with near addition and 3.6% had spherocylindrical with near addition. Only near addition prescription was required for 26.9%. About 16% had different combination of spherical, cylindrical and near prescription in both eyes.

Conclusion: A large proportion of the prescriptions were simple spherical or spherical with near addition and only near correction. These lenses can be stocked and dispensed faster and reduce delivery time. Data can be used to plan effective dispensing services across the vision centre network.

AWARENESS AND KNOWLEDGE OF EYE CONDITIONS AMONG PATIENTS ATTENDING URBAN AND RURAL EYE CENTRES IN SOUTH INDIA

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Purpose: To assess awareness and knowledge of eye conditions among patients attending urban and rural eye care centres in South India

Methods: A prospective cross-sectional study was carried out among patients visiting an urban and a rural eye care centre of L V Prasad Eye Institute, India. A validated questionnaire was administered to assess the knowledge on common eye conditions such as cataract and glaucoma and awareness of rare eye conditions such as retinoblastoma, squint, retinopathy of prematurity (RoP). Awareness is defined as having heard about the specific eye condition. Knowledge is defined awareness of a specific condition and know the cause of that condition.

Results: Among the 535 participants enrolled, the mean age of the participants was 46 years (standard deviation-14.6 years), 285 (53.3%) were women and 267 (50%) were from the rural centre. The knowledge of cataract and glaucoma was higher among patients presenting in urban centre when compared to rural centre (70.1% versus 49.4%; $p<0.01$) and (22.3% versus 4.1%; $p<0.01$) respectively. The awareness of other eye conditions was as follows: retinoblastoma (5.42%), RoP (1.49%), squint (78.87%) and refractive error (50.85%). Awareness of eye diseases was poor both in urban and rural centre.

Conclusion: Except for cataract, squint and refractive errors, knowledge and awareness of eye conditions was low among patients visiting urban and rural eye centres. Extensive mass media campaigns are needed to improve the awareness of eye conditions.

USE OF POLYMERIC MATERIALS IN PREVENTING BIOFILM FORMATION ON CONTACT LENS CASES

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Purpose: Development of biofilm on contact lens case as well as on contact lens plays a critical role in the pathogenesis of corneal infection. The purpose of the study was to test the efficacy of two polymeric materials in preventing the growth of biofilm on lens cases. These are material A (Nottingham) and material B (ARCI).

Methods: The polymeric materials were developed by the University of Nottingham (Material A) and the ARCI (Material B) The coatings of lens case with the test materials was achieved by treating the lens case with air plasma followed by dip coating it in polymer solution. Coating was assessed by SEM. Finally we tested the efficacy of the coatings by MTT and SEM studies. Log reduction was determined by determining colony forming units (C.F.U/ml) in the culture containing uncoated and coated lens case coupons.

Results: MTT analysis showed 80-90% reduction in growth of gram positive cocci, 50-70% reduction in gram negative bacilli for material A. The material B showed ~ 95 % reduction in metabolic activity of bacterial growth both for gram positive and gram negative bacteria. SEM analysis showed coating resulted in significant reduction of biofilm formation compared to uncoated lens cases. Material B also resulted in 99.9% log reduction in growth of gram negative bacteria. Both materials were found to be non-toxic on corneal epithelial cell line studies.

Conclusion: A uniform and firm coating of lens cases can be achieved with both polymeric materials. The coatings are effective in preventing the attachment and growth of biofilm on contact lens cases. Furthermore, material B also has potential to kill the gram negative bacilli. Both the materials do not show any cytotoxic effect.

ALTERATIONS IN THE GUT BACTERIAL MICROBIOME IN FUNGAL KERATITIS PATIENTS

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Purpose: Dysbiosis in the gut microbiome has been implicated in several diseases including auto-immune diseases, inflammatory diseases and cancers. Keratitis is an inflammatory disease of the eye significantly contributing to corneal blindness in the developing world. It would be worthwhile to investigate the possibility of dysbiosis in the gut microbiome being associated with Keratitis.

Methods: Bacterial populations in stool samples were analyzed using Illumina sequencing of V3-V4 region of 16S rRNA gene in healthy controls (HC, n=31) and fungal keratitis (FK, n=32) patients.

Results: The gut bacterial richness and diversity in FK patients was significantly decreased when compared to HC. The OTUs prominently enriched in HC were identified as *Faecalibacterium prausnitzii, Bifidobacterium adolescentis, Lachnospira*, *Mitsuokella multacida*, *Bacteroides plebeius* and *Megasphaera*. In FK samples, 5 OTUs affiliated to *Bacteroides fragilis, Dorea*, *Treponema*, Fusobacteriaceae, and Acidimicrobiales were significantly enriched. The functional implications are that *Faecalibacterium prausnitzii,* an anti-inflammatory bacterium and *Megasphaera*, *Mitsuokella multacida* and *Lachnospira* which are butyrate producers were enriched in HC, whereas *Treponema* and *Bacteroides fragilis*, which are pathogenic were abundant in FK patients, playing a potential pro-inflammatory role. Heatmap, PCoA plots and functional profiles further confirm the distinct patterns of bacterial composition in FK and HC samples.

Conclusions: Our study demonstrates dysbiosis in the gut bacterial microbiomes of FK patients. Further, based on inferred functions, it appears that dysbiosis in the gut of FK subjects is strongly associated with disease phenotype with decrease in abundance of beneficial bacteria and increase in abundance of pro-inflammatory and pathogenic bacteria.

EXPRESSION PROFILING OF CELLULAR MIR-20B TARGETING VEGF IN VITREOUS HUMOR OF DIABETIC RETINOPATHY CASES

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Purpose: Diabetic retinopathy is as one of the most common causes of blindness spreading worldwide affecting the adults of age between 35-70 yrs. MicroRNA has become an emerging biomolecule to be used as biomarker in several complex diseases. Several miRNA has been reported in regulating the genes involved in DR pathogenesis. Vascular endothelial growth factor (VEGF) is a prime angiogenic factor regulating angiogenesis in DR. The aim of the present study is to assess the expression of miR-20b that targets VEGF via Akt signaling pathway.

Methods: MiRNA were isolated from equal volume (100µl) of vitreous humor and serum from PDR patients (n=25) and macular holes patients as controls (n=25) using miRCURY kit. Further, cDNA conversion was done using TaqMan primer as per the kit protocol. The cDNA were used for qPCR (TaqMan assay) and fold change was calculated by $2^{-\Delta\Delta Ct}$ method and significance ($p \leq 0.05$) was tested by Student's t-test. Sybr green assay was performed for total RNA obtained from PDR and NPDR patients and No DM/No DR controls (n=50 in each category) to measure VEGF expression. Western blotting was performed to measure VEGF level in vitreous. VEGF levels in blood were then correlated with the miR-20b levels in serum and vitreous.

Result: Our preliminary analysis indicated higher expression of VEGF in vitreous of PDR cases as compared to controls while regulatory molecule miR-20b expression was expected to be down-regulated in PDR cases when compared with control samples. However, VEGF expression was not found statistically significant in total RNA in compared to controls.

Conclusion: MiR-20b could serve as a potential biomarker and therapeutic target for the early treatment of PDR cases.

GENERATING RETINAL DYSTROPHY MODELS IN ZEBRAFISH

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Purpose: To knockout zebra fish genes linked to retinal dystrophies in humans and to study their effects on retinal development and function.

Methods: The guide RNAs specific to target *Rd3* and *Abca4b* were designed using the CHOPCHOP tool (http://chopchop.cbu.uib.no/). A donor construct with *GFP* reporter was created for easy screening of target gene knock outs. For gene editing, an injection mix consisting of specific guide RNAs and CAS9 protein was prepared and injected into single cell stage fertilized embryos. For gene knock in, the linearized donor DNA construct was included in the injection mix. Genomic DNA was isolated from tail clips of 7 days post fertilization (dpf) larvae and analyzed for site specific edits. The larval heads were fixed in formalin for further histological analysis.

Results: At 7 dpf, the eyeballs of *Rd3* gRNA-Cas9 injected fish appeared smaller when compared to that of wild type fishes. H&E staining of F0 animals revealed a gross reduction in cell densities in the retinal ONL layer. Sequence analysis of the targeted genomic region revealed site-specific edits and introduction of NHEJ-mediated indels. Also, in case of Abca4b-GFP knock in, the injected embryos expressed GFP in the yolk sac and head region. Sequence analysis revealed site-specific edits and indels. Also, the GFP transgene was found to be integrated at the targeted site in some animals. The heads of these fishes were further processed for IHC to examine GFP expression and photoreceptor degeneration phenotypes.

Conclusion: Zebra fish mutants carrying R*d3* and *Abca4b* gene edits were created successfully by CRISPR-based editing.

FUNCTIONAL IMPORTANCE OF DRUG TRANSPORTER MODULATION IN BLOOD OCULAR BARRIERS DURING INFLAMMATION

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Purpose: The aim of this study was to evaluate the role of various drug transporters on Blood Ocular Barriers in the experimental model of ocular inflammation.

Methods: Wistar rats of either sex (n=6) were used for this study. Endotoxin (LPS) was administered at the dose of 200 μg into the hind paw to establish an experimental model of ocular inflammation and saline was injected in control animals.Expression analysis of 15 transporters of SLC and ABC superfamily was done in the ocular barriers (Cornea, Iris Ciliary body, Retina-Choroid) by qRT-PCR at 24 hrs post LPS challenge. Fold change in the gene expression was calculated by $2^{-\Delta\Delta}$Ct method. For functional evaluation studies, at 22nd hr both control and the rats challenged with LPS were injected with drug transporter substrates. Aqueous humor was collected at 24 hrs post LPS challenge for the quantitation of substrates using LC-MS/MS.

Results: In the blood-ocular barriers, P-gp was upregulated, Cnt1, Cnt3, and Ent1 were downregulated in the cornea; Cnt1, Ent1, P-gp were upregulated, Cnt2, Cnt3, Oat1, Oat2, Oat3, Oct1, Oct3, and Pept2 were downregulated in the Iris ciliary body; Cnt3, Ent2, Oat3, Oct2, P-gp1, and P-gp2 were upregulated in the Retina-Choroid, significantly. The functional evaluation studies revealed higher levels of substrates in aqueous humor of inflammatory model. All the values were found significantly different ($p \leq 0.05$) in comparison with respective control.

Conclusion: This study reveals that inflammation dysregulated the expression levels of drug transporters present on ocular barriers. Further studies on functional importance of the altered expression levels of the drug transporters in ocular inflammation suggested the need for earlier intervention in the inflammatory disease conditions.

HWP1 GENE IS REQUIRED FOR BIOFILM FORMATION IN OCULAR *CANDIDA ALBICANS*

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Purpose: To establish the functionality of *HWP1* gene in biofilm formation in *Candida albicans*.

Methods: Expression of 27 different genes was monitored during adhesion, expansion, maturation and dispersal phase of biofilm formation in ocular *C. albicans*. The results indicated that *HWP1* gene involved in adhesion was up regulated during biofilm formation in *Candida albicans*. To establish whether *HWP1* gene is required for biofilm formation attempts were made to mutate the genes by CRISPR-Cas9 method (Vyas et.al. 2015). The methods involved:

1. A guide RNA targeting the (51-73)thbp *HWP1* gene was cloned into pv1093 plasmid containing *CAS9* coding region (pV1093-*HWP1*-gRNA).
2. A donor template carrying the stop codon with an unique restriction site for quick screening was introduced in to the 51-73 thbp *HWP1* gene.

Linearized pV1093-*HWP1*-gRNA vector was co-transformed with the donor template in to *Candida albicans*.

Results: Transformation of *Candida albicans* with pv1093-*HWP1*-gRNA yielded 45 colonies. Ten of the 45 transformants showed no amplification of *HWP1* gene indicating that the gene was mutated. Restriction digestion by *EcoRI* resulted in two bands in the mutants and only one in the control indicating integration of restriction site from donor template in to the *HWP1* gene. XTT assay showed no biofilm formation in the mutants. Mutants appeared as isolated cells with clear morphology where as in the wild type individual cells were not visible and they formed a luxuriant biofilm of 17.01 μm thickness.

Conclusion: CRISPR –Cas9 protocol for mutation generation was standardised for ocular *Candida albicans*.*HWP1*gene is required for biofilm formation.

CANCER STEM CELLS IN RETINOBLASTOMA

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Purpose: To evaluate the Cancer Stem Cells (CSCs) in primary Rbtumors and RbY79 cell line through integrated *in vitro* and *in vivo* approach.

Methods: Rb Y79 cells were evaluated for the CSC properties using flow cytometry, self-renewal, dormancy, proliferation/differentiation, invasiveness, cytotoxicity assays and gene expression signature with specific emphasis on $CD133^{lo}$ cells. *In vivo* studies were carried out on Chorio-Allantoic Membrane of Chick embryo (CE-CAM) using fluorescent labelled Y79 cells, CD133 enriched cells and evaluated for tumor growth on CAM and spontaneous metastasis by confocal, histopathology and *in vivo* imaging. CSC markers for stemness (Nanog, Prox1, Smad3) and metastasis (Macc1, c-MET), were evaluated in the primary tumors (n=18) with and without histologic risk factors.

Results: CSC specific properties of small size (FSClo/SSClo), dormant phase (G0/G1:83.3±4.1%), higher colony forming efficiency ($p<0.05$), increased invasive ability($p<0.05$), resistance to Carboplatin treatment($p<0.05$); expression of stem cell/progenitor genes (Bmi1, Nanog, Oct4, Prox1), and metastasis related gene(Macc1) were noted in the $CD133^{lo}$ cells. Transplantation of Y79 cell line on the CE-CAM demonstrated pinkish-white raised, wet, perivascular nodules with feeder vessels, and spontaneous metastasis to the embryo as confirmed by confocal microscopy, in-vivo imaging and histopathology. $CD133^{lo}$ showed increased tumornodules($p<0.05$) and higher metastatic potential. In primary Rbtumors, stem cell related gene expression (Prox1, Smad3) was observed to be higher in cases with HRF(n=9). MACC1, upstream regulator c-MET were increased in HRF cases.

Conclusion: This integrative approach documents the CSC properties in the $CD133^{lo}$Y79 cells. Chick embryo Rb CSC-specific xenograft animal model and MACC1/c-MET pathway are the novel aspects that could pave way for new strategies in therapy and prognostication.

OPTIMISING THE METHOD FOR DECELLULARIZATION OF CORNEA FOR VARIOUS CLINICAL APPLICATIONS

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Purpose: Cornea is a spherical, avascular and transparent layer which controls the entry of light into the eye. The major part of the cornea consists of stroma which is made up of keratocytes, collagen and proteoglycans. According to 'National Programme for Control of Blindness, India', there is an addition of 25,000-30,000 corneal blindness cases to existing 1.2 million every year. This study aims to optimize the process of decellularizing stroma by using mild chemical and/or detergent treatment, maintaining the integrity of corneal stroma.

Methods: Cadaveric corneas were washed with saline buffer and epithelium, endothelium, iris muscles were scrapped out. The corneal stromal buttons were obtained using trephine followed by Povidone-iodine wash to remove the disinfectants. Further, corneal buttons were treated with hypertonic solution of NaCl or SDS followed by digestion of nuclear material using DNase. These decellularized buttons were further frequently washed with penicillin-streptomycin saline and preserved in saline at 4^0C.

Results: Decellularized corneas were crosschecked further by Haematoxylin & Eosin staining which showed the cells are completely absent after process. Endotoxin levels were monitored to check the sterility of the process. Our results shows that using both SDS and NaCl gives similar results of decellularization with no cell debris. However, NaCl could be a better and mild decellularizing agent.

Conclusion: The decellularized corneal stroma or extracellular matrix obtained from this process has wide applications, mainly for corneal scars, regenerating the damaged tissue in cornea, DALK replacement and can also be used as natural scaffold.

POSTERS

29th July, 2018

USE OF PEDIATRIC PERIMETER IN OLDER CHILDREN: 3 CASE REPORTS

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Purpose: Perimeters are diagnostic tools to detect disorders of the visual pathway. There are no commercially available devices to measure visual fields in infants (0-12 months). We developed a device, "Pediatric Perimeter," (PP) that has the potential of quantifying the visual field extent in infants. Although PP was built primarily to test infants, we were able to use this device to test older children as well. Here, we report 3 such cases where PP was used.

Methods: Three patients (9/f, 13/m, 16/f) diagnosed with refractory occipital lobe epilepsy by a neurosurgeon were referred to L V Prasad Eye Institute for visual field assessment as they were unable to perform the standard perimetry testing. One of the patient (16/f) had cognitive impairment and the other two had autistic spectrum disorder. PP was attempted for these three children.

Results: In all the patients perimetry testing could be performed safely. Both binocular and monocular testing was done. Estimates for gross visual field and visual field extent was performed. Visual field extent was within normal limits for two patients (9/f, 13/m). Right side visual field loss was detected in the 3rd patient on testing binocularly. On monocular testing similar results were found indicating right hemianopia.

Conclusions: PP has the potential to be used in older age group and in patients where conventional perimetry testing is not possible. A prospective study can be planned to validate the use of Pediatric Perimeter in patients with neurological impairment.

THE THRESHOLD INTEROCULAR DIFFERENCE IN RETINAL ILLUMINANCE REQUIRED TO EXPERIENCE THE PULFRICH-ILLUSION

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Purpose: In the Pulfrich illusion, subjects perceive the lateral motion of an object to follow an elliptical path in depth with interocular differences in retinal illuminance. This study determined the threshold interocular retinal illuminance difference required to perceive this illusion in visually healthy adults.

Methods: Seventeen subjects [Mean±1SD: 22±1yrs] judged the relative depth of two fields of black dots (dot density=106dots/cm^2) moving in opposite directions at 3pixels/sec speed in the upper and lower halves of an LCD monitor viewed from 80cm at three different monitor luminance levels (10, 30 and 80cd/m^2). Interocular luminance differences were induced by placing neutral density filters (NDF's) of 0 to 0.9log units intensity in 0.1log unit steps before their left eye. Threshold NDF was calculated as the 63% correct response of the resultant psychometric function. Pupil diameters of both eyes were measured for each trial using an infrared sensitive camera.

Results: The threshold NDF ranged from 0.02 to 0.21log units and the corresponding pupil diameters of the two eyes ranged between 3.41 to 5.45mm (anisocoria: ≤0.32mm) across subjects tested. These values translated into interocular retinal illuminance differences ranging from 130 to 200Trolands across subjects for experiencing the Pulfrich illusion. The threshold value did not vary significantly with the different monitor luminance tested ($p=0.04$).

Conclusion: Pulfrich illusion can be experienced with as small as 130Trolands of interocular difference in retinal illuminance. Anisocoria was in this cohort, suggesting that the Pulfrich illusion is largely driven by the interocular luminance differences induced by the NDF.

DISTRIBUTION OF OCULAR BIOMETRIC PARAMETERS IN URBAN SOUTH INDIAN POPULATION

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Purpose: To describe the distribution of spherical equivalent refraction and ocular biometric parameters of adults in urban south India.

Methods: A subset of subjects who participated in the urban division of the Chennai Glaucoma Study (CGS) underwent a comprehensive ophthalmic evaluation including the Spherical Equivalent of refraction (SE), corneal curvature and ocular biometry parameters such as Axial Length (AL), Anterior Chamber Depth (ACD) and Lens Thickness (LT) readings.

Results: Of the 256 eligible subjects, right eye of 245 and left eye of 244 phakic subjects were included for the analysis whose mean age was 50 (SD: 8) years and 44.4 % were men. The mean SE of refraction was 0.37 (SD: 1.78) D. A statistically significant difference in the average keratometry ((K1+K2)/2) was found with respect to age (ANOVA p=0.016). in which a mean steep corneal curvature was noted in the 50 to 59 years age group which became flatter in subjects above 60 years of age. The mean AL was found to be 22.93 mm (SD: 1.10) which showed an undulating trend, but with a weak statistically significant (ANOVA, p=0.04) difference with increasing age. Women had shorter AL and a steeper average Keratometry value compared to men in both the eyes.

Conclusion: Our study provided the distribution of ocular biometry parameters in adult urban population of southern India. The AL and corneal curvature were normally distributed with statistically significant gender differences.

INVESTIGATION OF ANTERIOR AND POSTERIOR SCLERAL THICKNESS IN HIGH MYOPES WITH SWEPT SOURCE OPTICAL COHERENCE TOMOGRAPHY

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Purpose: To investigate the anterior and posterior peripheral scleral thickness in high myopes.

Methods: A total of 15 eyes of 15 subjects (mean age 23.27±5.40 years) were included in the study. Anterior and posterior scleral thickness were measured with SS-OCT (DRI OCT triton plus, Topcon) using 12 mm and 16 mm long vertical and horizontal single line scan protocol, respectively. Images were analyzed using semi-automated programme designed for measuring scleral thickness. Anterior and posterior scleral thickness (AST and PST) were measured at an interval of 1 mm distance from scleral spur located in limbal region (AST) and fovea (PST) till 5 mm periphery on either side.

Results: Median spherical equivalent refractive (SER) error of 15 eyes was -16.28 D (Range -10.56 to -26.25 D) and median axial length was 30.59 mm (Range 27.11 to 35.65 mm). Mean sub foveal PST was found to be 248.83±63.81 μ. Temporal posterior sclera was thinnest (mean±SD: 224.39±50.90 μ) and the thickest one was nasal sclera (246.56±65.41 μ). A significant difference was seen in anterior scleral thickness (AST) across different meridians. Superior AST was thinnest among all the meridians both at scleral spur and towards periphery (mean thickness across the meridian- 395.46±61.77 μ) while inferior AST was thickest (555.81±11.98 μ).

Conclusion: Posterior sclera showed similar amount of thinning irrespective of different meridians supporting global posterior coat extension theory in myopia progression. AST and PST didn't show any correlation in terms of pattern of thinning, suggesting scleral thinning might restrict only posterior to equator.

EFFECT OF CENTRAL LENS CLEARANCE AND LENS CENTRATION ON REFRACTION IN MINISCLERAL LENS DESIGN

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Purpose: The purpose of our study was to understand the potential change in effective lower and higher order aberrations with change in thickness of post lens fluid reservoir and decentration of mini scleral contact lens.

Methods: Sixteen eyes of 16 subjects with mild refractive error (±3.00 D) were fit with mini scleral contact lenses for 4hours. Baseline auto refraction, aberrometry and oculyzer were recorded followed by fitting of mini scleral contact lens. Anterior segment optical coherence tomography was performed to measure the post lens tear fluid reservoir thickness. The same measurements were repeated immediately, 2 hours and after 4 hours of lens insertion.

Results: Central thickness of post lens fluid reservoir reduced significantly from baseline 461.2 µ(IQR428.8 to 513µ) to 4 hours of lens wear 412.16µ(IQR 389.08 to 490.54 µ). Maximum reduction in lens vault was observed within the first two hours of lens wear. The magnitude of lens decentration remained constant throughout the lens wearing period. There were no significant changes observed in lower ($p=0.86$) and higher order aberrations ($p=0.46$) values across the lens wearing period.

Conclusion: Change in lower and higher order aberration remains insignificant throughout the lens wearing period. A non-uniform post lens fluid reservoir does not induce higher order aberrations in mini scleral contact lens, and a decentered lens would still be considered for prescription if overall corneal touch is avoided.

OVERLAPPING OF CENTRAL VISUAL FIELD TEST POINTS IN DIFFERENT GRADES OF GLAUCOMA

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Purpose: To compare the central visual field test points in 24-2 and 10-2 in patients with glaucoma.

Methods: Subjects aged 40 years and above were recruited. Glaucoma patients were categorized in two groups based on the severity of glaucoma as mild (30) and moderate (30), according to HAPs criteria. All patients underwent Visual fields with SITA standard strategy, 24-2 and 10-2. Overlapping co-ordinates of these test points were identified and plotted using Matlab R2014a. The mean threshold values and the mean total deviation values of central 8 points in 24-2 and adjacent 16 points in 10-2 were plotted among normal, mild and moderate glaucoma.

Results: Thirty normal subjects and 60 glaucoma subjects were included and their mean age are 48.8 (5.9) and 61.4 (10.3) years respectively. The mean threshold values (dB) in 24-2 and in 10-2 among normal were 30.41(1.76) and 30.78(1.20), among mild glaucoma were 27.35(2.29) and 26.36(3.64), and among moderate glaucoma were 22.94(0.56) and 22.90(3.57) respectively. Similarly, with the total deviation values (dB) in 24-2 and 10-2 among normal were -1.77(1.76) and -1.23(1.18), among mild were -4.37(2.31) and -5.22(3.76) and among moderate glaucoma were -8.50(0.57) and 8.55(3.64). There was no statistically significant difference noted within the group in normal and moderate glaucoma whereas significant difference was noted in mild glaucoma for mean threshold (p value 0.03) alone.

Conclusion: Increased resolution for measuring sensitivity in paracentral region is important, as even a mild visual field loss can affect the quality of life.

LOW VISION INTERVENTION IN PATIENTS WITH CENTRAL AND PERIPHERAL FIELD LOSS

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Purpose: To evaluate the clinical profile and prescribing patterns of low vision devices in patients with central and peripheral visual field loss.

Methods: The medical records of patients with central visual field loss (CVFL) and peripheral visual field loss (PVFL) referred to the low vision care department in a tertiary eye care hospital during 2009 to 2011 were extracted. The details regarding the patient history, ocular examination including visual function assessment, prescribing patterns of low vision devices (LVD), patient preference, and the compliance of patients with the prescribed low vision devices during different follow up visits and reasons for non compliance was retrospectively analysed.

Results: The medical records of a total of 85 patients were included and the report of 42 CVFL (mean age: 45±20 years) and 43 PVFL patients (mean age: 36±14 years) was analysed. The most common low vision devices preferred by patients included half eyes spectacles, cutaway magnifier and CCTV.The use of prescribed devices was reported to be comfortable by 33.8% with CVFL and 56.5% with PVFL who were compliant. The reasons for non-compliance to prescribed device included worsening nature of disease condition, handling difficulty, cosmesis, working distance (causing back pain).

Conclusion: The preference pattern and the compliance of low vision device usage in patients with central and peripheral field loss are useful for better management. The comparison of clinical visual function information and patient preference of low vision devices can aid better clinical care.

SHORT TERM EFFECT OF OPTICAL DEFOCUS AND FORM DEPRIVATION ON HUMAN EYES

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Purpose: To investigate which structure (retina/choroid) and region (central/peripheral) of the eye undergoes early changes during myopiogenesis via an experimental study.

Methods: Right eyes of 27 participants with mean age of 17.9±0.7 years were imposed with either myopic defocus (MD; n=18) or the hyperopic defocus (HD; n=9) and the fellow eye with a Bangerter diffuser/plano lens. After inducing the defocus, participants watched a video (placed at 5 meters) for 60 min. Biometry and posterior segment imaging were performed at 0 mins (baseline), 60 mins (post-defocus) and 90 mins (rebound effect). Retinal-thickness (RT), choroidal-thickness (CT), choroidal-vascular and choroidal-stromal area were determined at fovea, nasal (3mm) and temporal (4mm) regions using a custom-built segmentation software.

Results: Defocus caused small but significant bi-directional changes in axial length (AL) i.e. AL decreased with MD (-12.7±1.5 μm; $p<0.001$) and increased with HD (+14.4±1.7 μm; $p=0.001$). With decrease in AL, there was significant increase in the CT in the temporal region (15.4±4.9μm; $p<0.001$) and vascular area (0.01±0.003 mm^2; $p=0.004$) at the fovea. However, with increase in AL, measured parameters i.e. RT, CT, stromal area and vascular area did not change significantly.

Conclusion: Greater changes with MD than in HD in different parameters suggest that signals with MD are stronger than HD. This results indicate that early changes due to defocus (during myopiogenesis) may start at the level of choroid and more in temporal part of the eye.

MYOPIA PROGRESSION IN INDIAN CHILDREN AND ADULTS

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Purpose: To investigate myopia progression pattern in Indian children and young adults and its association with age of onset of myopia and type of astigmatism.

Methods: The required data of individuals who visited at least twice to L V Prasad Eye Institute were retrospectively extracted from the medical records data base. The age criteria for inclusion of patients data in the study was set to 1 to 30 years and only those with diagnosis of myopic refractive error in their first visit (taken as baseline) was included. There were 7069 participants who came for “one year follow-up visit” and 2745 participants who came for “two year follow-up visit”.

Results: Overall one year and two year myopia progression in children was -0.50±0.73D and -0.80±0.97D, respectively ($p<0.001$). In adults, there was minimal change in spherical equivalent in both 1 and 2 years follow up visits i.e. -0.16±0.55D, -0.20±0.60D respectively. Earlier age of onset and higher degree of myopia lead to significant increase in myopia progression in both children and adults for 1 and 2 years follow up visits. Gender, location, types of astigmatism did not show significant difference in change in spherical equivalent power.

Conclusion: Myopia progression in Indian children seems to be slighter greater than that of Caucasians and lesser than Chinese children and warrants implementation of various strategies for myopia control in Indians. Early age of onset of myopia might trigger for high myopia at later life and emphasis on anti-myopia strategies should be made appropriately to control the progression of myopia.

LOW HYPEROPIA CAN TRIGGER ACCOMMODATIVE SPASM: 3 CASE REPORTS

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Purpose: Children have high accommodative amplitude therefore low hyperopia (:51.50) is usually not corrected. While refractive errors are estimated for distance, its effect gets compounded for sustained near activities, particularly for hyperopia. Symptoms of uncorrected hyperopia manifest as head/eye ache. In some rare cases uncorrected hyperopia can trigger accommodative spasm. We are reporting three such cases.

Methods: Case 1: A 12 year old girl reported with blur vision. Visual acuity was 20/100 and 20/80. Retinoscopy showed -2.00DS and -1.50OS in right and left eye. Cycloplegic refraction showed piano (both eyes).
Case 2: A 10 year old girl reported a history of double and blurred vision. Visual acuity was counting fingers close to face in both eyes. Retinoscopy was -6.25OS in both eyes with orthotropia. Cycloplegic refraction showed +1.50OS and +0.50OS in the right and left eye.
Case 3: A 13 year old girl presented with visual acuity of counting finger (1 meter). Retinoscopy was -6.75OS and -7.00DS. Right esotropia (16PD) was observed. Cycloplegic refraction showed +1.50OS (both eyes).

Results: All the cases showed accommodative spasm with pseudomyopia and case 3 showed esotropia thus exhibiting spasm of near reflex. To relieve that spasm we adopted a modified Berish delayed subjective test, prescribed the hyperopic correction and vision therapy exercises. With this visual acuity improved to 20/20 for all. The condition resolved and no recurrence noticed over 6 months.

Conclusion: Accommodative spasm can occur due to uncorrected hyperopia. This can be treated with full refractive error correction and vision therapy exercises.

CHALLENGES IN CLINICAL REFRACTION AFTER IMPLANTATION OF EXTENDED DEPTH-OFFOCUS IOL LENSES: A CASE REPORT

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Purpose: The Closed-field Auto-Refractometer (CFAF) is the commonly used instrument for estimating the objective refraction, that helps clinicians determine the final prescription. The extended depth-of focus IOL (EDF-IOL) manufacturer has cautioned that the auto-refractors and aberrometer might give an erroneous reading due to the compensation for chromatic aberration made in the EDF-IOL. To the best of our knowledge no published report is available on the difference between auto-refraction and Retinoscopy in EDF-IOL implanted eyes.

Methods: A 58-year-old man underwent a phacoemulsification for bilateral age-related cataract. On the third week of post-operative visit the objective refraction was measured by three different methods using Auto-Kerato-Refractometer (KR-8900; Topcon Co, Tokyo, Japan), Streak Retinoscopy (Welch-Allyn) and the iTrace aberrometer (Tracey Technologies, Inc.).

Results: The uncorrected visual acuity (UCVA) was OD 6/7.5 and OS 6/9; OU N6 at 30-35 cms. The binocular UCVA 6/5; N6 25-35 cms. The objective refraction with CFAF showed OD -1.12DS/-0.50DC X92° and OS -1.37DS/-0.50DCX102°. Retinoscopy showed OD 0.00DS/-0.50DCX90° and OS -0.25DS/-0.50DCX105°. Refraction by iTrace showed OD -0.75DS/-0.12DCX165° and OS -1.00DS/-0.37DCX 159°.The subjective refraction was OD -0.25DS/-0.50DCX90° and OS -0.25DS/-0.25DCX105°, with which the distance visual acuity improved to 6/4.5. With +1.25DS, the near acuity and working range becameN6; 20-50 cms. No refractive correction was prescribed since the patient had a binocular UCVA of 6/5;N6.

Conclusion: Both CFAF and Abberrormeter overestimated close to -1.00DS in EDF-IOL implanted eyes. The errors in prescription can be minimized by knowing the design of the IOL and correlating the UCVA with objective refraction before prescribing.

STRUCTURAL ABNORMALITIES OBSERVED WITH ADAPTIVE OPTICS SCANNING LASER OPHTHALMOSCOPE CAN EXPLAIN UNEXPLAINED SYMPTOMS

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Purpose: The bounds of making a diagnosis is tied up with the technology limitations. Tools like adaptive optics scanning laser ophthalmoscope (AOSLO)pushes such limits. We demonstrate the use of AOSLO to better understand the unexplained symptoms of two patients.

Methods: Case 1: A 25 year old male had acuity 20/25p and 20/20 in the right and left eye respectively. He complained of letters appearing up & down in the right eye. All his clinical tests were normal, except 4-prism-BO test. Microtropia was diagnosed in the right eye. Case 2: A 34 year old male followed up for 10 years always reported of seeing wavy lines and reduced vision. His acuity was 20/20 in both eyes. His OCT, aberrometry tests were all normal. Both these patients were imaged on AOSLO their fovea while looking at a fixation target (blue LEDs) 240 images were scanned at a sampling rate of 23 Hz.

Results: Both cases showed tiny empty pockets amidst tightly packed photoreceptors. This was observed in the microtropic right eye of Case 1 and in both eyes of Case 2. The empty pockets (50μm for Case 1 and about 60μm on an average for Case 2) indicates photoreceptors absence.

Conclusion: AOSLO can be used as a diagnostic tool to explain visual symptoms such as minute visual distortions. This is the first report to document a subclinical anatomical defect in a case of microtropia. More studies are needed to investigate the anatomy in other macrotropia conditions.

NORMATIVE DATABASE FOR COMPUTER DISPLAY-BASED COLOUR VISION TESTING

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Purpose: To establish normative database for Color Assessment and Diagnosis (CAD) test in Indian population.

Methods: Thirty-three (11/M, 22/F) healthy young adults with a mean (± SD) age of 22.2 (± 2.1) years, who had normal trichromatic vision (assessed using Ishihara pseudoisochromatic plates) participated in the study. Color vision was tested using a computer display-based CAD test, which assesses chromatic thresholds by varying the saturation along 16 hue directions (12 for red/green (RG) and 4 for yellow/blue (YB)). The participant indicated the location of chromatic target using buttons on the game pad in a 4 - Alternative Forced Choice test. The chromatic sensitivity, expressed as Standard Normal Units (SNU), was computed separately for red/green and yellow/blue colour. Higher SNU indicates the larger magnitude of color vision impairment.

Results: The (mean ± SD) red/green chromatic discrimination (1.28 SNU ± 0.30) was better than yellow/blue discrimination (1.36 SNU ± 0.28). Females had a slightly better chromatic discrimination compared to males for R-G (1.24 Vs 1.36 SNU) as well as Y-B (1.29 Vs 1.50 SNU). The mean (± SD) time taken for completion of test was 16.95 minutes ± 2.1.

Conclusions: Preliminary results in this study indicate that the chromatic thresholds of an average trichromat are similar to the normative database obtained in the Caucasian population.

OCULAR PHOTOSENSITIVITY IN HEALTHY EYES

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Purpose: To determine the visual photosensitivity threshold (VPT) in healthy Indian eyes using the ocular photosensitivity analyzer (OPA) specially built for this purpose.

Methods: Sixty Indian subjects with no ocular pathology were included in the study. OPA, a non-invasive technique, varied the intensity of light stimuli and the subject was instructed to indicate when the light intensity is uncomfortable. After the initial light stimulus and dependent on subject sensation response, each succeeding stimulus was adjusted with the Garcia-Perez staircase technique and yielded a VPT measurement from the mean of 10 response reversals.

Results: The median age of the subjects was 34.2 years (inter-quartile range IQR, 22.5 to 48.3 years). Twenty-nine were males and 31 were females. The median binocular VPT was 2963.5 lux (IQR, 637.3 to 5593 lux). The median monocular VPTs were 2160.4 lux (IQR, 553.7 to 5703.8 lux) and 2023 lux (373.1 to 5546.2 lux) respectively. There were no significant differences among binocular and monocular VPTs ($p = 1.00$, Friedman test).

Conclusions: This is the first study to characterize the visual photosensitivity thresholds of the healthy Indian eyes. The VPTs appear to be higher than those obtained in a smaller series of Western population whose median binocular, monocular (right eye) and monocular (left eye) VPTs are 106 lux, 170 lux and 171.1 lux respectively. The difference in VPTs can be attributed to ethnicity and iris color. This information from healthy eyes can be used as a reference in assessment of photosensitivity in diseased states and outcome analysis of various ocular surgical procedures.

IS ASOCT- AN USEFUL TOOL IN CASES OF AGV IMPLANTATION?

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Purpose: The current study reports the series of cases with implantation of Ahmed Glaucoma Valve (AGV) and the utility of Anterior Segment Optical Coherence Tomography (ASOCT).

Methods: Series of cases which was advised for ASOCT from 2014 to 2018 were retrospectively reviewed. Cases which were advised for Ahmed Glaucoma Valve implantation were included for the current study. We have collected the information on indications for AGV, baseline intraocular pressure (IOP), corneal status, corneal thickness, past ocular surgeries, status of AGV and ASOCT findings pre and post surgery.

Results: We have 17 cases (53% females) satisfying the inclusion criteria. The median age (IQR) was 31 (40) years. The median IOP (IQR) at baseline, before and after AGV implantation was 20(18), 26(15) and 14(5) mmHg respectively. The baseline and post AGV corneal status in 3 eyes were normal whereas 14 eyes showed corneal abnormalities such as corneal haze, decompensated cornea, corneal edema, perforated corneal ulcer, graft failure post penetrating keratoplasty, corneal edema. ASOCT images showed that 5 eyes had patent AGV with no valve corneal touch, 5 eyes showed presence of valve but unable to comment about valve corneal touch and 7 eyes showed retracted AGV either temporally, near sulcus, in posterior chamber or behind haptic of IOL .

Conclusion: ASOCT is an useful tool to view the structural orientation, position and complications of AGV implantation and anterior chamber to decide about repositioning, corneal surgeries and to ensure the absence of valve corneal touch to avoid further corneal complications.

HEAD AND FACIAL ANTHROPOMETRY OF INDIAN POPULATION FORDESIGNING A SPECTACLE FRAME

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Purpose: To provide normative data of the head and face measurements needed to design an appropriate spectacle frame for Indian population.

Methods: Indian subjects between 20-40 years visiting L V Prasad Eye Institute, Hyderabad were included. Thirteen parameters were measured, three with *direct* methods using appropriate tools and ten in *indirect* method using *Image J* software. Photographs of subjects were captured with a camera positioned on a tripod stand at a two meter distance from the subject's position. Pictures were clicked, one, in primary gaze position, second and third with head turn towards subject's right and left by 90˚ from the primary gaze.

Results: Mean±SD age was 27.6±5.7 years consists of 55.38 % males. An *independent t-test* showed a significant difference in *nose width* (*P*=0.001), *Interpupillary distance* (*P*=0.032), and *body mass index* (*P*=0.012) between males and females. Whereas, *Inner intercanthal distance* (*P*=0.265), *outer intercanthal distance* (*P*=0.509) and *frontal angles* (*P*=0.536) showed no significant difference. Mean *head width* of males (154.17±9.12) was wider than females (145.43±8.92). This suggests for a smaller distance between the temples of a spectacle frame for females.

Conclusion: Considering the above factors, the need for a customized spectacle frame design is important in providing better optics, improved cosmesis and comfort to the wearer.

VARIATION OF DEPTH OF FOCUS WITH CONTRAST MEASURED USING NORMAL POLARITY AND REVERSED POLARITY VISUAL ACUITY CHARTS

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Purpose: To study the changes in depth of focus (DOF) with contrast using normal and reversed polarity visual acuity charts.

Methods: Ten normal subjects participated in the study following a comprehensive eye examination. Visual acuity in a randomly chosen eye cyclopleged with 1 drop of 1% cyclopentolate was measured in all subjects by displaying logMAR charts on a gamma corrected CRT monitor using a custom written MATLAB program. The charts were of either normal polarity (NP: background luminance greater than letter luminance) or reversed polarity (RP: background luminance less than letter luminance). For each polarity, three contrast values (100%, 20% and 10%) were used. For each chart, visual acuity was measured for nine levels of induced blur between +2.00 D and -2.00 D. Experiments were carried out in a dark room. Participants wore an artificial pupil of 3 mm diameter. Data were analyzed using MATLAB.

Results: Mean age of participants was 22.7 ± 1.4 years and spherical equivalent refractive error was +0.45 ± 0.35 D. DOF measured using NP chart at 100%, 20% and 10% contrast were 0.72±0.08 D, 0.91±0.15 D, and 1.21±0.10,D respectively. Corresponding DOF values for RP charts were 0.85±0.09 D, 1.70±0.11 D, 1.87±0.10 D. Statistical analysis revealed that DOF with NP chart at 100% did not differ significantly with NP chart at 20% and RP chart at 100% but not with the other three charts. DOF from RP charts differed significantly from each other and increased linearly with decreasing contrast.

Conclusion: At each tested contrast, the DOF is wider for the RP chart than the NP chart. As the contrast is reduced, the DOF widened for both types of charts. For the NP charts, there was not much of a change in DOF for contrast reduction up to 20%.

3D PRINTING ASSISTED CUSTOMISED CONFORMERS FOR THE MANAGEMENT OF CONGENITAL ANOPHTHALMIA.

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Purpose: The aim of this study was to construct customized conformers of graduating sizes for socket expansion in a child with congenital anophthalmia assisted by 3D printing technique. Primary outcome measure was ability to configure customised conformers with a good fit into the socket. The secondary objective is to reduce the number of visits and frequent sessions of examination under anaesthesia for the patient.

Methods: One patient with unilateral congenital anophthalmia was included in the study. Impression of the socket was taken using a silicone gun and converted into a wax model. A ridge was added to the anterior part of the wax. This ridge would help in expansion of the horizontal fissure length. The wax was then converted into an acrylic conformer following the usual steps that are included in making a customized ocular prosthesis. The final conformer was fit into the patients eye socket to check for optimum fit. A 3D Computed Tomography scan of the conformer was done. The dicom images from the CT were exported into an open source online software, Meshlab. A 2mm increase in all dimensions of the conformer was made and the data transferred to a 3D printer. The printer used was Ultimaker 2^{+}.The 3D printed replicas were converted into clear PMMA conformers using the same method mentioned above. Two such conformers of graduating sizes were made. These were given to the parents of the patient and they were advised to change it every three weeks. Insertion and removal was taught to the parents.

Results: A difference of 5mm was seen in Horizontal fissure length when the patient reported back after nine weeks (15mm at the beginning to 20mm by the end). The parents were able to insert and remove the conformers with ease and the patient had no adverse effects.

Conclusion: 3D printing assisted customized conformers can be a useful technique in children with congenital anophthalmia. It helps in faster and effective method of socket expansion and also reduces the number of hospital and operating room visits for the patient.

MEASURING EYE MOVEMENTS WITH AND WITHOUTHEAD STABILIZATION USING EYE TRACKER

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Purpose: Studying eye movements are helpful to understand visual functions of nonverbal patients and children. However eye movements require a stable head position, which may not be possible in children. Hence we investigated the effect of head stability on saccadic eye movements.

Methods: Emmetropic (spherical equivalent 0.75DS to -0.50DS) participants (n=21)with no ocular pathology were included in the study with informed consent. We used Eye Link 1000 plus eye tracker. A Landolt's C target (7.5') was presented in one of the 4orientations and the participant should press the corresponding arrow key. A MATLAB program presented the target, registered the participant's response and recorded the eye movements. A total of 128 trials (64 with and 64 without head stabilization) were shown. Out of the 64 trials, 32 trials had small target amplitude (10-20 deg) and the remaining 32 trials had large target amplitude (30-40 deg).

Results: No difference in peak velocity was observed for smaller target amplitude with and without head stabilization (p=0.381). Peak velocity reduced without head stabilization for larger target amplitude (p=0.061).

Conclusion: Reduction in peak velocity could be due to the vestibulo-ocular reflex (VOR) that is dampening the saccadic eye movement. For adult participants, smaller amplitude saccadic eye movements will be comparable with and without head stabilization and this may not be the case for larger saccadic eye movements.

EVALUATION OF CHOROIDAL THICKNESS IN MILD DIABETICRETINOPATHY USING SWEPT SOURCE OPTICAL COHERENCETOMOGRAPHY

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Purpose: To investigate the choroidal thickness in mild diabetic retinopathy.

Methods: We have included all patients diagnosed as mild diabetic retinopathy with preserved foveal contour, macular thickness within 250 micron and both intact and distorted foveal vascular zone. All patients enlisted underwent comprehensive eye examination including thorough history of diabetic age, current sugar level and control measures. OCTA was performed to rule out the ischemic and non ischemic changes at the level of FAZ. Swept source OCT 6mm radial lines over the macular region was scanned to evaluate overall macular thickness and to identify the thickness of choroidal scleral interface (CSI).

Results: Twenty eyes were evaluated (10 eyes were non ischemic and 10 eyes were ischemic FAZ). Mean age was 49.29 (SD + 2.62 years). Mean macular thickness was235.56 (SD + 15.88 μm). There was no significant difference between ischemic and non ischemic macular thickness (P=0.13). Statistically significant difference was noted while analyzing GCL distribution between ischemic (75.55 + 3.77 μm) and non ischemic (86.66 + 3.17 μm)category (P<0.0001). Mean CSI were 317.83 (+ 5.08 μm) irrespective of all two groups. However we did not find any significant change of CSI thickness while comparing ischemic and non-ischemic groups separately (P= 0.17).

Conclusion: Non invasive OCTA would be an important marker to detect early structural damage at the foveal level. This study was able to pick up the subclinical structural loss in mild diabetic retinopathy. However choroidal circulation plays an important role to maintain an ecosystem in the retina, still early stage might to reflect the significant change.

OVS5

DOES ANSIOMETROPIA INFLUENCE MYOPIA PROGRESSION IN CHILDREN WITH BILATERAL APHAKIA?

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Purpose: Emmetropization is known to get altered based on the cataract involvement in one or both eyes. This study aimed to evaluate the refraction trend in bilateral aphakic children with isometropia and anisometropia.

Method: Refraction data from thirty-two eyes of 16 aphakic children who underwent cataract surgery at the age <1 year (mean±SD: 0.37±0.31 years), and who came for follow-up at age ≥5 years (5.62 ± 1.20 years), was retrospectively reviewed. There were 8 isometropes (difference of <1D between eyes) and 8 anisometropes (difference of ≥1D between eyes) after excluding patients with any ocular condition that influences the refractive error. The main outcome measure was the change in myopia shift after five years.

Results: Overall, median (IQR) spherical equivalent refractive (SER) error at baseline and final refraction was +18.75 D [+17.00 D; +20.25 D] and +12.68 D [10.44 D; 14.78 D]. Myopic shift of 6.80 D was observed in a period of 5 years (P<0.001).Myopic shift in iso-hyperopic group was 6.70±3.70 D and in aniso-hyperopic group was 6.90±4.30 D (P=0.896). Change in refraction (myopic shift) was not significantly different (P=0.287) between high hyperopic and the fellow eye in both iso and aniso-hyperopic group.

Conclusion: Similar trend of myopic shift was seen in both iso and aniso-hyperopic group. However, the change of refractive error in bilateral aphakes child after 5 years was higher than a natural phenomenon.

CHANGES OF CORNEAL ENDOTHELIAL CELLS ANDCONTRAST IN PAN RETINAL PHOTOCOAGULATION

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Aim: To compare the endothelial cell changes and loss in Pan Retinal Photocoagulation lasers used in treatment of different retinal pathologies.

Methods: All the patients diagnosed as diabetic retinopathy underwent comprehensive eye examination and recruited in the study if PRP is required based on the clinical findings. Endothelial cell density and corneal thickness were measured following PRP till three months. Contrast sensitivity was also assessed pre and post laser simultaneously to look for the change.

Results: Total 34 eyes of 34 patients were included in the study. All the eyes were grouped into diabetic (19 eyes) and non diabetic (15 eyes) category. Notable change was observed between corneal endothelial cell density pre and post laser overall ($p= 0.0231$, One way ANOVA). Corneal thickness also showed a significant change after post laser three months ($p=0.0210$, One way ANOVA). However mean corneal thickness pre laser and post laser did not vary much. Diabetic group showed significant reduction of overall cell density after laser in all the follow ups but non diabetic group showed reduction only after one and three months. Corneal thickness also reduced in diabetic group but not in non diabetic group. There was no change in overall contrast in any of the groups.

Conclusion: Corneal endothelial cell density reduced more in the diabetic group that could act as a marker. The cell loss in non diabetic group can only be evident if the laser burns get saturated.

CORRELATION BETWEEN VISUAL ACUITY AND BLUR THRESHOLDS

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Purpose: The purpose of this study is to find if blur threshold can be used as an alternative to assess changes in the visual system.

Methods: Ten adults, aged 18 to 23 years, participated in this study. Blur thresholds and visual acuity were measured at different levels of induced defocus between +2.00 D and -2.00 D in a randomly chosen eye. A 3x3 matrix of Landolt C was used to measure visual acuity where the participant had to judge the orientation of the central C. Natural images at different levels of blur were presented to measure blur threshold, where the participant had to judge whether the image presented was blur or sharp. A modified staircase procedure was used to measure thresholds. Data analysis was done using Statistical Package for the Social Sciences (SPSS) software and Microsoft Excel.

Results: The mean best corrected visual acuity was -0.08±0.07 logMAR and mean blur threshold was -1.02 in the tested eye. In general, there was no correlation between visual acuity and blur thresholds (Spearman's coefficient $r=0.23$, $p=0.029$). However, there was a significant correlation between visual acuity and blur threshold for three participants ($\rho \geq 0.7$; $p<0.05$).

Conclusion: Visual acuity and blur thresholds are not correlated in individuals with emmetropia or mild ametropia.

FIELD OF INTEGRATION FOR COHERENT MOTION DETECTION

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Purpose: To find the field of integration for coherent motion detection in normal individuals

Methods: We studied coherent motion in ten normal individuals using random dot kinematograms and calculated the threshold for signal-to-noise ratio (SNR) using an adaptive, one up one down staircase for six stimulus areas, presented randomly. Dots moving in the same direction constituted signal dots and dots moving in random direction formed the noise dots. We defined SNR as the ratio of the number of signal dots to the total number of dots. The experiment was done using a program written in MATLAB. The stimuli were displayed on a calibrated cathode ray tube (CRT) monitor. The dots were moving either towards the 2 o'clock position or the 10 o'clock position. The task of the participant was to detect the direction of signal dots and respond using the arrow keys on the keyboard. Every response was followed by an auditory feedback. The experiment was done in a dimly illuminated room. We kept other parameters like dot size, dot density, dot speed, luminance and contrast constant.

Result: SNR threshold decreased with increasing stimulus area up to 4 square degrees, beyond which it remained constant.

Conclusion: The estimated field of integration for coherent motion detection in normal individuals is up to 12.56 degree^2.

CONSTRUCTION OF 3D PROFILE FOR TEAR LIPID LAYER USING MATLAB

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Purpose: Tear film lipid layer thickness (TLLT) plays a major role in evaluating evaporative dry eye. Lipiview® II is the only instrument which measures TLLT accurately, however is very expensive. The purpose is to develop feasible, accessible and portable software to assess TLLT.

Methods: This is a pilot study in which a program code was developed using Matlab® to read the given interferometric image of tear lipid layer in sRGB color space and convert into HSV color space at each pixel position. This generates 3D coloured plot and provides corresponding TLLT at each pixel position which is represented on a scale of 0 to 1 (least to highest thickness respectively).

Results: Interferometric images of 29 open meshwork, 29 closed meshwork, 25wave, 22 color fringes tear lipid layer patterns captured using Tearscope plus collected from open source. All the 3D plots of same lipid layer patterns were put together for correlation. A Weak correlation was observed between the plots of similar patterns ($p>0.05$).

Conclusion: A new program is developed for measuring the TLLT in 3D profile using Matlab®. However, this program has to be fine-tuned for better correlation between the plots of same interferometric patterns.

CHANGES IN CONTRAST SENSITIVITY FUNCTION WITH SIZE OF GABOR STIMULUS

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Purpose: The purpose of the study was to find the changes in the contrast sensitivity function in individuals with normal vision using Gabor stimuli of various sizes.

Methods: Contrast sensitivity function was measured for 6 adult individuals with visual acuity of 0.1 log MAR or better in both eyes. Gabor stimuli of spatial frequencies from 0.50 to 16 cpd taken in geometric progression were used; measurements were made for three different sizes of the target. Stimuli generated using software written in MATLAB were presented on a calibrated CRT monitor. Temporal two-alternative forced-choice paradigm was used to measure the target contrast threshold. Threshold was determined using a modified staircase procedure. Six measurements of thresholds were made for each subject.

Results: Contrast sensitivi:y functions averaged across the six subjects at the three different sizes were compared using ANOVA ($p = 0.9383$).

Conclusion: Contrast sensitivity function does not change with increase in size of Gabor stimulus. Therefore, charts such as FACT that have same size stimuli of different spatial frequencies can be used in the clinics for measuring the contrast sensitivity function.

DOES TEMPORAL CORNEAL THICKNESS HAVE AN EFFECT ON VAN HERICK GRADING IN PRIMARY ANGLE CLOSURE SUBJECTS?

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Purpose: To understand the influence of temporal corneal thickness (TCT) and van Herick's (VH) grading in subjects with normal and occludable angles.

Methods: A prospective, case-control study was conducted. Subjects above 40 years age underwent a comprehensive eye examination. Central and temporal corneal thickness was measured using ultrasound pachymetry. Anterior chamber depth was graded using van Herick method.

Results: A total of 159 subjects were examined (81 - occludables; 78 – normals). Mean ± SD age (years) of occludables and normals was 57.7 ± 8.4 and 55.8 ± 9 respectively. The difference in the mean age among the normals and occludables was not statistically significant (p=0.178). Mean ± SD TCT (microns) between occludables and normals was 625.7± 41.4 and 628.1 ± 38.1 respectively. Similarly, mean ± SD central corneal thickness (CCT in microns) of occludables and normals was 520.8 ± 32.5 and 517.1 ± 27.5 respectively. There was no significant difference in CCT and TCT between the two groups (p=0.24). The maximum TCT in VH grade II and grade I was 180 microns. The minimum TCT in VH grade III and grade IV was 263.5 microns with a thickness difference of 83.5 microns. On comparison of maximum TCT in grade II and minimum TCT in grade III, it was found that there was no overlap between the two grades.

Conclusion: van Herick grading is not significantly influenced by TCT. There is no overlap between Grade II and III in VH technique used to classify an angle as open or narrow.

SCLERAL SCHIOTZ TONOMETRY ACCURATELY PREDICTED CORNEAL GOLDMANN APPLANATION TONOMETRY READINGS IN EYES WITH CLEAR CORNEAS

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Purpose: To evaluate the predictability of corneal Goldmann applanation tonometer (GAT) intraocular pressure (IOP) from scleral IOP measurements using Schiotz and ICare tonometers.

Methods: Prospective study including one eye of 245 subjects with clear corneas. In Experiment I, IOP was measured on central cornea and temporal sclera in 116 eyes with 3 different tonometers; the Schiotz, ICare and ICare PRO and compared to corneal GAT. Mean scleral and corneal IOPs measurements were compared. Based on conclusion from experiment 1, the study was extended to 245 eyes with IOP measurements by only Schiotz and GAT. Formulae were derived using Support Vector Regression to predict GAT IOP from scleral Schiotz IOP, the model performance was tested on a new dataset. Statistical analyses were performed using R software (version 3.3.2).

Results: In experiment I, mean scleral Schiotz IOP differed from GAT IOP by 1.52mmHg ($p=0.7$), however mean ICare and ICare PRO IOP differed by 25.9 and 22.7mmHg respectively ($p<0.001$). In experiment II, mean difference between GAT and scleral Schiotz IOP by Bland-Altmann plots was 1.45mmHg with narrow limits of agreement (LoA: -3.24 to +6.4mmHg. The predicted GAT IOP from regression formulae showed a mean difference of -0.22mmHg with LoA from +4.23 to -4.67mmHg.

Conclusion: The mean difference between corneal GAT and scleral Schiotz IOP was <2mm Hg and predicted GAT IOP was even better. Hence, predicted GAT from scleral Schiotz IOP can be used as an alternative to GAT IOP in conditions with unreliable/acquirable corneal IOP measurements.

THREAT TO LIFE DURING RETINOPATHY OF PREMATURITY (ROP) MANAGEMENT

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Purpose: To report the root cause analysis and protocol changes after sentinel events that were encountered during management of ROP over last 20 years.

Methods: Our prospective Indian Twin Cities ROP database of 18,560 babies was evaluated for death/near death, sentinel events. Analysis of events by various experts and protocol changes done were tabulated.

Results: There were 8 cases, 5 were deaths and 3 had successful resuscitations after sudden cardiac arrest. Deaths occurred before eye examination (2), few hours after laser (2) and after surgery (1). Successful resuscitation happened one each after ROP screening, laser and surgery. After complete root cause analysis involving ophthalmologists, neonatologists, anaesthetists and Quality Improvement teams, protocols of care were modified and will be presented. Protocols changed included in areas of anaesthesia, communications, handling baby and feeding.

Conclusions: ROP management though safe, deals with high risk babies. Ophthalmologists should be aware of threat to life and how to prevent, analyse events and implement changes.

DELAY IN PRESENTATION FOR SURGERY IN INDIAN PATIENTS WITH CONGENITAL CATARACT

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Purpose: To determine the extent and reasons for delay in presentation, and investigate factors associated with delay in presentation for congenital cataract surgery in South India.

Methods: Parents of children presenting with congenital cataract (n=167) to a tertiary eye care centre, South India, between January 2016 and September 2017 were asked when they first detected the condition, and reasons for delay between detection and surgery were studied. Presentation delay was defined as duration of > 1 year from recognition. Logistic regression was employed to determine factors associated with delay.

Results: The mean ages at recognition and at surgery were 9.6 months and 20.8 months, respectively. Thirty-three children (20%) had delayed presentation (median, 2.6 months; range, [0-177.8 months]).Congenital cataract was recognized ≤ 6 months in 101 (73%) children with bilateral cataract, and in 24 (86%) children with unilateral cataract. Of these, 58 (42%) children with bilateral cataract and 17 (61%) with unilateral cataract underwent surgery between 3 and 6 months of age. Common reasons for delay were ignorance (33.3%), economic (27.3%), and lack of awareness about eye hospital (12.1%). Logistic regression showed that lower child's age at surgery (OR 0.95 [95% CI, 0.93-0.97), P<0.0001) was significantly associated with delayed presentation.

Conclusions: Diagnosis was delayed in 20% of children with congenital cataract and reasons such as lack of awareness and socioeconomic constraints were responsible for the delay. There is a need to develop an efficient referral system and improve public awareness for early diagnosis and management of children with congenital cataract.

CLINICAL PRESENTATIONS AND COMPARATIVE OUTCOMES OF PRIMARY VERSUS DEFERRED INTRAOCULAR LENS EXPLANTATION IN DELAYED-ONSET ENDOPHTHALMITIS

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Purpose: To describe clinical presentations and comparative outcomes of primary versus deferred intraocular lens explantation in delayed-onset endophthalmitis.

Methods: Retrospective, interventional case series. Seventy-seven eyes of 77 patients that were diagnosed as delayed-onset endophthalmitis and underwent intraocular lens explantation were included from January 1990 to January 2018. Undiluted vitreous biopsy and IOL were subjected to microbiologic evaluation. Duration of symptoms, presenting visual acuity, organisms isolated, time to IOL explantation, time to endophthalmitis resolution after explantation, number of repeat intravitreal injections and final visual acuity were compared in the primary IOL explantation and the deferred IOL explantation groups.

Results: Interval between inciting event and endophthalmitis, between onset of symptoms to presentation, total follow up, complication rate and final visual acuity were comparable between the two groups. Median time to IOL explantation in the deferred group was 70 days. Number of repeat intravitreal injections were 0.58±0.86 and 2.62±1.78 respectively, ($p<0.0001$, 95% C.I. 2.00 to 2.22). The number of days to resolution after IOL explantation were 35.16±14.26 and 55.5±8.24 respectively, ($p<0.0001$, 95% C.I. 15.22 to 25.45)

Conclusion: Early IOL explantation in delayed-onset endophthalmitis causes quicker clinical resolution and reduces the quantum of repeated intravitreal injections. Final visual improvement however is unaffected.

CHARACTERISTICS OF MICROBIAL KERATITIS IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTED PATIENTS

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Purpose: To review the epidemiological characteristics, microbiological profile and treatment outcomes of keratitis in patients with Human Immunodeficiency virus (HIV) infection.

Methods: A retrospective analysis was done for32patients who presented between 2004 and 2012 to LV Prasad Eye Institutewith microbial keratitis and HIV

Results: Of the 32 patients, 27 were males and 5 were females. The mean age at presentation was 36.2 years. Unilateral involvement was the most common presentation (n=31). Only 3 patients had history of significant trauma to the eye. Underlying ocular predisposing factors were recognized in 16 cases, the most common being prior Herpes zoster ophthalmicus(n=10).Fungus was the most common microbiological agent implicated (n=10). The various fungi implicated *were Aspergillus, Candida, Penicillium*. Other micro-organisms implicatedwere *Herpes zoster, Herpes simplex virus,Acanthamoeba,Pseudomonas, Proteus mirabilus, Moraxella*. Mixed infection was noted in 4 cases. Therapeutic penetrating keratoplasty was done in 4 patients and evisceration in 2 patients.9 patients had perforation of corneal ulcer and 3 patients had recurrence.

Conclusion: Old HZO is the most important predisposing factor for microbial keratitis in HIV. Multifactorial approach targeting the micro-organisms as well as the ocular surface determines the success in these patients.

TRANSIENT CHANGES IN CORNEA WITH PROSTHETIC REPLACEMENT OF OCULAR SURFACE ECOSYSTEM (PROSE) LENS FOLLOWING CORNEAL TRANSPLANT

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Purpose: Primary objective was to assess the short-term changes in corneal thickness after PROSE lens wear in cases with corneal transplant and evaluate the rate of recovery after lens removal. Secondary objective was to determine if the corneal changes observed in the first objective are related to changes in corneal endothelial cell density.

Methods: Ten eyes of 8 patients [Mean±1SD: 37.1±7.9yrs] with clear corneal graft were included for measurements. Baseline High contrast visual acuity (HCVA), corneal topography, endothelial cell density and global pachymetry were performed at baseline, immediately post lens removal and 1 hour after PROSE lens removal.

Results: Mean±1SD uncorrected HCVA (0.92±0.31 logMAR units), improved up to 0.11± 0.20 logMAR units ($p<0.01$). The change in pachymetry in the central 0-2 mm area (21.3±8.6 μm) and peripheral 7-10 mm area (38.7± 10.1 μm) reverted back to the baseline measurements after 1 hour of no lens wear state ($p>0.05$). However, the changes in the 2-5 mm area (24.8±7.9 μm) and 5-7 mm area (32.6±9.8 μm) did not recover to baseline measurements after the same period ($p=0.03$). Endothelial cell density (1365.25± 237.4 cells/mm^2) had no correlation with the changes in the pachymetry values.

Conclusion: Corneal pachymetry changes at the centre and at the extreme periphery reverted back to its previous state after an hour of no lens wear, whereas, the changes still remained significant after that period in the mid peripheral region of the graft. Corneal endothelial cell density had no correlation with the change in the graft thickness after lens wear.

CLINICOPATHOLOGICAL CORRELATION OF MORPHOLOGICAL CHANGES IN OCULAR SURFACE SQUAMOUS NEOPLASIA TO TREATMENT WITH CHEMOTHERAPEUTIC AGENTS.

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Purpose: To understand morphological changes in ocular surface squamous neoplastic lesions after treatment with Mitomycin C and/ or Interferon.

Methods: Retrospective chart analysis (June 2014- June 2018) of patients of Ocular surface squamous neoplasia (OSSN) that received chemotherapy with Interferon-alpha2b and/or Mitomycin-C. Out of 38 cases, 24 underwent incision or excision biopsy, before and/or after treatment. The morphology of neoplastic lesions that had received chemotherapy and had subsequently undergone excision (12/24) was studied by histopathology and immunohistochemistry. Seven of these 12 cases had undergone incision biopsy as well as excision subsequent to treatment and 5/12 had excision only after therapy. Morphology of treated OSSN was also compared with biopsy specimens of OSSN patients (12/24) that had undergone biopsy only prior to chemotherapy, but not after therapy.

Results: The actual size of neoplastic lesion on histopathology was significantly lesser than the clinical size of treated lesion. Dysplasia was patchy, full thickness, to diffuse. The cells demonstrated a decrease in cell volume with chromatin condensation and an increased expression of caspase 3. Stroma showed increased vascularity and inflammation. Inflammation involved deeper stroma, scleral tissue, and even intraocularly in enucleated cases with predominance of plasma cells and CD 8 positive T cells.

Conclusions: Chemotherapeutic agents provoked an intense inflammatory reaction, which may clinically mimic unresponsiveness or worsening to therapy. Interferon alpha-2b induces T cell suppressor activity and apoptosis as an antitumor agent, further work is needed to understand if anti- inflammatory agents can help patients of OSSN while on chemotherapy.

EVALUATING VISUAL FIELD DEFECTS USING HIGHER DENSITY VISUAL FIELD SAMPLING GRIDS IN PRE-PERIMETRIC GLAUCOMA

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Purpose: To study the visual field (VF) defects in preperimetric glaucoma using a densely sampled grid in the central 10°

Methods: Eight eyes of 7 subjects with preperimetric glaucoma were included in the study. Preperimetric were diagnosed with visible retinal nerve fiber layer (RNFL) defect on clinical examination and normal central 24° VF. All underwent routine eye examination follows by VFs examination using the Humphrey VF Analyzer (HFA) with the 24-2 grid and SITA Standard strategy. Subjects have also undergone Microperimetry using the MAIA perimetry. Central 10° was tested using a high-density grid. The high density grid was created by manually adding points in between the regular 10-2 grid. Localized VF defects were identified using Anderson's criteria; three contiguous points on the pattern deviation plot have a probability of <5%, one of which has a probability of < 1%. We also calculated the mean deviation (MD) and pattern standard deviations (PSD) for the high-density grid and the 10-2 grid.

Results: The global indices parameters did not significantly differ between the high density and the 10-2 grid. The MD for the high density grid was -5.417dB ± 3.68dB and the 10-2 grid was -5.50dB ± 3.73dB (p=0.965). The PSD for the high density grid was 3.70dB ± 2.85dB and the 10-2 grid was 3.88dB ± 2.84dB (p=0.896). However, 6 out of 8 eyes showed localized visual field defects in high-density sampling grid and only 3 out of 8 eyes showed VF defects in the 10-2 grid. None of the eight eyes showed visual field defects in central 10° in the 24-2 grid.

Conclusion: The high-density grid showed localized VF defects in greater number of eyes compared to the 10-2 grid. Adding extra points in the central 10 degrees will improve scotoma detection in preperimetric glaucoma.

PROSPECTIVE STUDY OF VITRECTOMY WITH INTRAVITREAL DEXAMETHASONE IMPLANT FOR IDIOPATHIC EPIRETINAL MEMBRANE (ERM)

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Purpose: To study rapidity of visual gain after vitrectomy and intravitreal dexamethasone implant in idiopathic ERM.

Methods: Non-randomized, comparative, interventional study. Thirty eyes of thirty patients with idiopathic ERM enrolled. Fifteen eyes underwent 25-G pars plana vitrectomy (PPV) and ERM peeling (Control group). Fifteen eyes underwent 25-G PPV and ERM peeling with dexamethasone implant (Study group). Primary outcome measure was time taken to gain maximum final visual acuity. Central retinal thickness (CRT) analyzed by swept source optical coherence tomography.

Results: Mean age of patients was 67.46 years (+/- 8.24) in control group and 66.4 years (+/- 6.46) in study group with Male: Female ratio 7:8 in both groups. At 1 month post-operatively, there was significant difference between change in BCVA from baseline in two groups (baseline log MAR 0.5 (+/- 0.2)(Snellen equivalent 20/60) to 0.5 (+/- 0.36) (Snellen equivalent 20/60) in control grou, and baseline log MAR 0.5 (+/- 0.21) (Snellen equivalent 20/60) to 0.2 (+/- 0.35) (Snellen equivalent 20/30) in study group (P= 0.04). At 6 months follow up, there was no significant difference (P= 0.91). Change in CRT from baseline at 1 month and 6 months follow-up was not significant (P=0.3, P=0.8) respectively.

Conclusion: Dexamethasone implant can achieve faster visual recovery after PPV with membrane peeling in ERM.

CONJUNCTIVAL MELANOMA IN ASIAN INDIAN PATIENTS: A STUDY OF 40 CASES

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Purpose: To describe the clinical features, histopathology, treatment, and outcomes of conjunctival melanoma at a tertiary centre in South India.

Methods: Retrospective study of 40 patients.

Results: The mean age at presentation of conjunctival melanoma was 44 years (median, 45 years; range, 9 years to 78 years). There were 19 males and 21 females. Nineteen patients (48%) had past history of a pigmented or pre-existing lesion, nevus (n=14, 35%), primary acquired melanosis (PAM) (n=5, 13%). The mean basal diameter of the lesion was 12 mm (median, 10 mm; range, 4 to 30 mm). Majority of the patients had pigmented lesion (80%). As primary treatment, 26(65%) patients underwent excision biopsy, 11 (28%) underwent exenteration, and 3 were lost to follow-up. On histopathology, out of 33 patients whose HPE was available, 7 (21%) had an associated nevus, 10 (30%) had PAM, and 1 (3%) patient had both PAM and nevus. Four (13%) patients had tumor recurrence, 3 (10%) developed lymph node metastasis, and 1 (3%) had brain metastasis during a mean follow-up period of 11 months (median, 5 months; range, <1 to 67 months).

Conclusion: Conjunctival melanoma is rare in Asian Indians and has a high rate of tumor recurrence and regional lymph node metastasis.

MACULAR PIGMENT OPTICAL DENSITY-NORMATIVE DATA

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Purpose: To report the normative data of macular pigment optical density (MPOD).

Methods: This was a prospective study conducted at L V Prasad Eye Institute, Hyderabad, India. All subjects underwent comprehensive eye examination including Best Corrected Visual Acuity (BCVA), slit lamp examination and dilated fundus examination. The eligibility criteria were: age between 20-60 years, no ocular pathology which conformed by comprehensive examination. The exclusion criteria were; presence of with any systemic diseases such as diabetes, hypertension, excluded eyes with known ocular diseases such as diabetic retinopathy, glaucoma and any other retinal pathology. Further, all subjects underwent fundus photography using VISUCAM 500. MPOD was calculated using the fundus photographs, and the following four parameters were automatically calculated Maximum optical density, Mean optical density, Area and Volume. Mean and maximum MPOD were used for analysis. Subjects were divided into 4 age groups for comparison (20-30, 31-40, 41-50 and 51-50). Mean and maximum MPOD was compared using Kruskal Wallis test among the age groups. For comparison among gender, Mann Whitney test was used.

Results: Sixty three eyes of 33 subjects were included with mean age of 35.65±10.44 years (range 20-60 years). The mean BCVA was 20/20. The mean refractive error was -0.14±0.66 D, and mean axial length was 23.09±0.66 mm. The mean central macular thickness was 234.88±18.46 μm. The mean and maximum optical density was 0.155±0.068 and 0.054±0.06 respectively. There was no statistical significance in both maximum and mean MPOD p=0.99 and 0.92 respectively between the age groups. No statistical significance was found between genders (p=0.28 and 0.65).

Conclusion: MPOD remains stable with age and there is no difference between the genders. This data would be useful in evaluating MPOD in various macular pathologies.

SUBRETINAL HYPERREFLECTIVE MATERIAL IN CENTRAL SEROUS CHORIORETINOPATHY

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Purpose: To describe the the appearance and behaivior of subretinal hyperreflective material (SHRM) in eyes with central serous chorioretinopathy (CSCR).

Methods: This retrospective study included 13 eyes of 13 patients with CSCR presenting with SHRM. The eyes underwent either laser (11 eyes) or observation (2 eyes). Optical coherence tomography (OCT) and fundus fluorescein angiography (FFA) charcterisctics were analyzed both at baseline and at resolution of neurosensory detachment, which were then co-related with the best corrected visual acuity (BCVA) at resolution using linear regression analysis.

Results: An improvement in vision was seen in 10 eyes while the remaining three maintained vision. Two eyes developed subretinal scarring secondary to SHRM not involving the fovea. SHRM characteristics such as reflectivity and location, Inner Segment/Outer Segment damage and diffuse retinal pigment epithelium (RPE) abnormalities on FFA did not co-relate with the final visual acuity. External limiting membrane damage ($p=0.000$) at resolution; and the presence of scar ($p=0.021$) were significantly associated with a poorer BCVA at resolution in univariate analysis. Both ELM damage at resolution ($p=0.000$) and presence of scar ($p=0.004$) were statistically significant in multivariate analysis.

Conclusion: CSCR with SHRM have a good visual prognosis. ELM damage at resolution and presence of scar are are associated with a poor visual acuity at resolution. Diffuse RPE abnormality on FFA might predispose to scar formation.

OCULAR BIOMETRY IN EYES WITH ANGLE CLOSURE DISEASE: RESULTS FROM LV PRASAD EYE INSTITUTE GLAUCOMA EPIDEMIOLOGY AND MOLECULAR GENETIC STUDY (LVPEI- GLEAMS)

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Aim: To compare ocular biometric values in eyes with angle closure disease and normal subjects.

Method: 8000 subjects from a population based glaucoma prevalence study underwent complete ocular examination including ocular biometry. The subjects were classified according to international classification as primary angle closure suspects (PACS, n=148), primary angle closure (PAC, n=137) and primary angle closure glaucoma (PACG, n=11) and were grouped as angle closure disease. 592 age and gender matched normal eyes with no prior history of intraocular surgery, a spherical equivalent of <5D were randomly selected.

Results: The age ranged from 40-75 years in normal and the angle closure disease group. Mean Axial length was shorter in the angle closure group (Mean ± standard deviation) (22.19 ±0.07 vs. 22.64± 0.05 mm in normal; $p<0.001$). Mean Anterior chamber depth (ACD) was shallower ($p<0.001$) among subjects with ACD (2.86 ± 0.05 vs. 3.19± 0.03mm; $p<0.001$). Mean Lens thickness (LT) was significantly greater ($p<0.001$) in people with angle closure disease (4.64 ± 0.05 vs. 4.41± 0.03 mm). On comparing the eyes with PAC and PACG with the PACS eyes, no significant difference was noted in the axial length ($p=0.04$) and LT ($p = 0.9$). However, Mean Anterior chamber depth was significantly shallower in eyes with PACG and PAC (2.79±0.08 vs 2.94±0.07; $p<0.05$).

Conclusion: Eyes with angle closure disease have significantly shorter axial lengths, shallower anterior chambers and greater lens thickness compared to the normal group. Eyes with PAC and PACG had significantly shallower anterior chamber depths compared to eyes with PACS.

LONG-TERM OUTCOMES OF DESCEMET'S STRIPPING ENDOTHELIAL KERATOPLASTY (DSEK) AFTER REBUBBLING FOR GRAFT DETACHMENT

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Purpose: To compare long-term outcomes after rebubbling for graft detachment with eyes which did not have graft detachment after Descemet's stripping endothelial keratoplasty(DSEK).

Methods: From 2260 eyes that underwent DSEK from July 2008 to June 2015, 160 eyes (80 control eyes without graft detachment and 80 eyes with graft detachment after DSEK) were retrospectively reviewed. Host-related, surgery-related and donor-related factors that have a bearing on graft adhesion were studied retrospectively. Clinical outcomes after rebubbling procedure were also studied and compared with control patients.

Results: The demographics were comparable between the two groups. There were more number of patients with aphakia or anterior chamber IOL in the study group (p=0.003) and more patients with > 2 surgeries prior to DSEK in the control group (p=0.01). In the study group successful graft attachment was observed in 77 (96.25%) eyes, and clear grafts in 55 (68.75%) eyes while graft attachment and clear graft was observed in 80 (100%) eyes of control group. The logMAR BCVA was 0.65 in study group and 0.35 in control group which shows statistically significant (p=0.02) difference in two groups. Lower donor endothelial cell density was found to be a significant risk factor (p = 0.03) for graft failure. The median graft survival following rebubbling was 30 months.

Conclusion: Rebubbling in detached grafts after DSEK can reattach the lenticule in 96% of eyes in immediate post-operative period with results comparable to control patients and majority of grafts remained clear on long-term follow-up with median graft survival period of 2.5 years.

NEW APPROACH OF LASER CAPSULO-HYALOIDOTOMY FOR THE MANAGEMENT OF PSEUDOPHAKIC MALIGNANT GLAUCOMA

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Purpose: To describe the novel approach of Laser Capsulo-hyaloidotomy for the management of pseudophakic malignant glaucoma.

Methods: We present a series of 2 cases of Pseudophakic Malignant Glaucoma which developed spontaneously few years after an uneventful cataract surgery. We managed by cycloplegia and laser Capsulo-hyaloidotomy with a novel approach through the dialing hole of the Intra-Ocular Lens (IOL) in the first case and through the optic-haptic junction of the IOL in the second case. Post laser anterior chamber was well formed and intra ocular pressure was under control with two anti glaucoma medications. However, there was recurrence after 1 week in both cases due to vitreous.

Results: Both cases were managed without the need for core vitrectomy and one week later there was complete resolution of malignant glaucoma.

Conclusions: Novel laser approach through the dialing hole of the IOL and Optic-haptic junction helps in resolution of malignant glaucoma in pseudophakic eyes.

CHOROIDAL VASCULARITY IN NON-ARTERITIC ANTERIOR ISCHEMIC OPTIC NEUROPATHY

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Aims: To evaluate the peripapillary choroidal vascularity in eyes with non-arteritic anterior ischemic optic neuropathy (NAION) and compare with healthy fellow eyes and age-matched healthy subjects.

Methods: The peripapillary choroidal vascularity index (CVI) was calculated using horizontal swept-source optical coherence tomography (SS-OCT) scans. CVI was calculated using previously validated automated algorithm. CVI in NAION, and fellow eyes of NAION patients, were compared with age-matched eyes of healthy individuals using Kruskal-Wallis Test.

Results: A total of 20 eyes of 20 patients with acute unilateral NAION with healthy fellow eyes (20 eyes) and 40 eyes of 40 healthy patients were included in the study. Average age of patients with NAION was 56±8 years and 55±7 years in age-matched healthy controls. NAION eyes had a significantly lower CVI than age matched controls in both nasal and temporal areas. NAION nasal CVI was 0.47 ± 0.47 compared to 0.62 ± 0.04 in controls ($p<0.001$). NAION temporal CVI was 0.45 ± 0.48 compared to 0.58 ± 0.04 in controls ($p<0.001$). Temporal CVI was 0.45 ± 0.48 in NAION eyes and was significantly lower than counterpart healthy fellow eyes 0.48 ± 0.02 ($p = 0.007$).

Conclusion: NAION eyes have significantly reduced vascularity in the peripapillary area. CVI is lower in the nasal and temporal of the optic disc compared to healthy individuals. This may suggest those with smaller CVI are more prone to ischemia from reduced vascularity resulting in NAION.

RHIZOBIUM RADIOBACTER KERATITIS - A RARE AND CHALLENGING ENTITY

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Purpose: To describe clinical features and treatment outcomes of *Rhizobium radiobacter* keratitis.

Methods: Retrospective review of medical records of culture proven *Rhizobium radiobacter* keratitis presenting in between 2008-2018 to a tertiary centre in South India.

Results: A total of five cases was reviewed with mean duration of infection at presentation being 49.6 days (8-150 days). Presenting Visual acuity was less than 20/200 in all cases. Mean size of the infiltrate was 8.16 mm (6.3-10mm) vertically and 8.74 mm (6.7-11 days) horizontally. Smear was positive in only one case (20%) and two cases (40%) had mixed infection in culture fungus (20%) and pseudomonas (20%). All cases of *Rhizobium radiobacter* was susceptible to ciprofloxacin except in one (20%). Four cases (80%) underwent therapeutic penetrating keratoplasty. Final Visual outcome was better than 20/200 in 2 cases (40%) with 1 (20%) case phthisical. Mean duration of follow up was 52.4 days (24-90 days).

Conclusions: *Rhizobium radiobacter* is an opportunistic pathogen, being a newly recognised entity causing keratitis, is difficult to detect and treat. It has a long protracted course with most of the cases requiring multiple scraping and surgical intervention.

OUTCOME OF INADVERTENT SURFACE DISRUPTION WHILE TREATING RETINAL CAPILLARY HEMANGIOBLASTOMA

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Purpose: To report the effect of an inadvertent complication of laser and cryo-ablation while treating retinal capillary hemangioblastoma.

Methods: Fundus images of all the retinal capillary hemangioblastomas treated at a tertiary eye care center were retrospectively reviewed for hemorrhagic complications of lasers or cryotherapy. The outcomes of the complication were recorded.

Results: Three out of 12 eyes treated with lasers/ cryotherapy had documented evidence of tumor disruption and hemorrhage. These lesions to our surprise showed signs of complete resolution. The mean follow up was 12months, average reduction in tumor size was 29% and the feeder vessel caliber was reduced by 47% with appreciable decrease in tortuosity.

Conclusions: While ablating retinal hemangioblastoma with laser photocoagulation or cryotherapy a hemorrhagic treatment endpoint may prove to be a boon not bane.

COMPARATIVE STUDY ON VISUAL OUTCOMES, COMPLICATIONS AND LEARNING CURVE OF GLUED INTRA OCULAR LENS BY TRAINEE SURGEON AND CONSULTANT VR SURGEON

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Purpose: A comparative study of surgical outcomes, complications and learning curve of glued intra ocular lens of a vitreo retinal (VR) fellow in training.

Methods: Retrospective analysis of 50 eyes requiring glued IOL surgery for various indications was done. Both the consultant VR surgeon (DBK) and VR fellow in training (OLI) operated 25 eyes each. The primary outcome measures were visual acuity at 3 months, and time taken for completion of surgery. Secondary outcome measures were refractive correction, intra ocular pressure and intra operative or postoperative complications.

Results: All patients in both the groups showed improvement in unaided visual acuity from 1.593 +/- 0.54 (Snellen 20/800) to 0.50 +/- 0.37 (Snellen 20/63). The best-corrected visual acuity (BCVA) improved from 0.79 +/- 0.75 (Snellen 20/123) to .37+/- 0.39 (Snellen 20/46) which was clinically and statistically significant ($p = 0.00$). The complication rates in both the groups were comparable. The time taken by consultant VR surgeon (64.26 min) as compared to VR fellow (107.16 min) was significantly lower ($p< 0.05$). Transient hypotony, (IOP < 11) was seen in 56% (14/25) of eyes in OLI group and 44% (11/25) in DBK group ($p = 0.39$).

Conclusion: The study results are encouraging for a VR fellow with good short term visual outcomes and comparable surgical complications. The procedure seems to give promising results and has a learning curve, which can be overcome by supervision and desire to learn.

INFECTIOUS ETIOLOGY IN OCULAR ADNEXAL LYMPHOMA

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Purpose: To ascertain an association between possible infectious agents such as Hepatitis B virus, Hepatitis C virus, *Chlamydia psittaci*, *Helicobacter pylori* and ocular adnexal lymphoma.

Methods: Histopathologically proven OAL cases were included in the study. DNA was extracted from orbital tissue samples. The presence of microbial agents such as Hepatitis B Virus, Hepatitis C Virus, *C. psittaci*and *H. pylori* was determined by using specific primers and polymerase chain reaction (PCR). The existence of *H. pylori* was also studied in controls. The amplified PCR products were confirmed by Sanger sequencing.

Results: A total of 15 patients of ocular adnexal lymphoma were included. Eleven patients had extranodal marginal zone lymphoma (EMZL), high-grade B cell lymphoma was present in 2, low-grade B cell lymphoma and B cell lymphoma in one patient each. Mean age of this group was 53.2±16 years (range 11-80) and males (n=11) predominated the group. PCR for *H. pylori*was positive in 46% of orbital tissues (7/15) and out of which, 6 patients belonged to EMZL group. Hepatitis B Virus was positive in one patient. PCR for *C. psittaci* and Hepatitis C Virus was negative in all patients. PCR for *H.pylori* was positive in 1/10 controls including 6 pterygia, 3 lymphoproliferative disease showing polyclonality and one idiopathic orbital inflammation.

Conclusion: PCR for *H. pylori* was positive in 54% patients with EMZL. This may hold promise in formulating future therapeutic strategies in EMZL.

OUTCOMES OF INTRAVITREAL BEVACIZUMAB (IVB) AS AN ADJUVANT THERAPY IN COATS DISEASE

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Purpose: To evaluate the treatment outcomes of adjuvant IVB in Coats disease and also evaluate any tractional retinal worsening as reported in some cases in literature**.**

Methods: A retrospective study was done of medical records of past 18 years of patients diagnosed with Coats disease who underwent adjuvant IVB in a tertiary eye care centre in Southern India.

Results: A total of 13 patients, 7 males and 5 adults, underwent IVB adjuvant injections. They received injections either during initial management (10 eyes) or in subsequent sessions (3 eyes). All eyes had additional therapy including Cryopexy (5 eyes), laser (8 eyes), intravitreal triamcinolone (1 eye), and Vitreo retinal surgery (5 eyes). Disease severity included Stage 2B (7 eyes), Stage 5 (4 eyes) and Stages 3A and 3B one eye each. At a mean follow up of 4.9 years, the condition resolved in 7, became stable in 2 and progressed despite all therapies, in 4 eyes. Final visual acuity ranged from 20/30 to 20/400. Eyes with progressive coats developed phthisis (1), neovascular glaucoma (3). None developed tractional retinal detachment.

Conclusions: Anti-VEGF injections as adjuvants seem to help in overall treatment strategy of this difficult to treat chronic retinal vascular pathology. Individual surgeons chose the drug based on their experience and understanding of pathology of this rare condition. We observed no cases of increased traction in our small series having nearly five years of followup. Well known complication related to disease severity, were noted. Further evaluation of exact indications is needed.

TYPICAL OF ATYPICAL MYCOBACTERIAL KERATITIS

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Purpose: To describe 2 cases of presumptive *Atypical mycobacteria* keratitis successfully treated with Amikacin 2.5% and oral Trimethoprim and Sulfamethoxazole.

Methods: Diagnosis of Atypical Mycobacteria was made on the basis of highly suspicious clinical presentation and history. Presence of a long-protracted course, unresponsiveness to routine medical therapy, waxing and waning episodes, history of fall of foreign body, cracked glass wind shield appearance were taken into consideration.

Results: After an initial worsening with empirical therapy, regression of lesions and cracked wind shield like was noted by as early as 2 weeks of intense medical therapy. Resolution of the with scarring and by the end of 6 weeks.

Conclusions: The patients described here with presumptive Atypical Mycobacterial keratitis completely resolved with Topical Amikacin 2.5% and oral Trimethoprim and Sulfamethoxazole.

NOVEL CPP CONJUGATED NATAMYCIN IN AN EXPERIMETAL MICE MODEL OF *FUSARIUM* KERATITIS: AN IN-VIVO ANIMAL STUDY

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Purpose: To evaluate the antifungal efficacy of a novel conjugate, CPP-Natamycin in an experimental mice model of Fusarium spp. keratitis.

Methods: Experimental *Fusarium spp.* keratitis was established in the right eye of Balb/c mice. The study animals were evaluated with slit-lamp and keratitis was graded and animals were divided into three groups (Day0). Each group received hourly topical instillation of the CPP-Natamycin conjugate (Group 1), 5% Natamycin (Group 2) and Phosphate buffer saline (Group 3) for three days (Day 1-3). The animals were evaluated for resolution of keratitis (Day 4). The animals were sacrificed and the right eye was enucleated and evaluated for colony forming units (CFU) using broth dilution technique. Outcome measure – 1.Clinical resolution of keratitis 2.CFU/ml of *Fusarium*.

Results: Five animals were included in each group. Complete resolution of keratitis was noted in 4 and 3 eyes respectively in Group 1 and Group 2 while Group 3 showed no clinical resolution of keratitis. CFU/ml was comparable between Group 1 and Group 2. Group 3 demonstrated the highest CFU/ml.

Conclusion: Compared to Natamycin alone, currently the drug of choice in *Fusarium* keratitis, the novel conjugate CPP – Natamycin demonstrated comparable antifungal efficacy both clinically and by CFU/ml estimation.

OUTCOMES OF THERAPEUTIC PENETRATING KERATOPLASTY IN A TERTIARY EYE CARE CENTRE IN SOUTHERN INDIA

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Purpose: To assess the demographic spectrum, microbiological profile and outcomes of therapeutic penetrating keratoplasty (TPK) performed for active microbial keratitis in a tertiary eye care centre.

Methods: The electronic medical records of 400 patients who underwent Therapeutic penetrating keratoplasty from April 2016 to March 2017 were reviewed retrospectively. The sociodemographic details, ulcer characteristics, the donor graft characteristics and intra-op complications were co-related with the outcomes.

Results: The average age of the patient who underwent TPK was 47.45 years. The most common organism implicated was fungus. Mean follow up post surgery was 277.9 days. Therapeutic success was seen in 369/388 eyes (95.1%).

Conclusion: It was concluded that the most common organism implicated in microbial keratitis undergoing therapeutic penetrating keratoplasty was fungus (Fusariumspp). Average success rate after TPK was found to be 95.1% with recurrence rate of 5.1%.

OUTCOMES OF THERAPEUTIC KERATOPLASTY IN FUNGAL KERATITIS: A RETROSPECTIVE ANALYSIS OF 198 CASES

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Purpose: The purpose of this study was to analyse the outcomes of therapeutic penetrating keratoplasty for fungal keratitis.

Methods: A retrospective audit of 198 consecutive eyes that underwent therapeutic penetrating keratoplasty (ThPK) for fungal keratitis at L V Prasad eye institute between January 2008 to December 2010 was performed. The data on demographics, clinical characteristics, intraoperative and late postoperative complications were noted. The primary outcome measure was eradication of infection and postoperative anatomical success. Secondary outcome measures were graft survival, risk factors, clinical features and management of recurrent fungal keratitis post ThPK.

Results: Mean age was 45 ± 14.3 years. A total of 189 (95.45%) eyes were positive for organisms. The commonest fungal isolate was Aspergillusflavus in 64 eyes (32.3%). Mean follow-up after ThPKwas 24±17months. 178 (89.9%)eyes had complete eradication of fungal infection whereas 20 (10.1%) eyes developed recurrence. Anatomical restoration was achieved in majority of cases (192 eyes; 97%). Larger infiltrate size and graft size were associated with a higher risk of recurrence of infection. The median graft survival rate was 5.9 months. The graft survival was better for grafts <8mm versus those with > 8mm (P = 0.026) and not found significantly related to the species of fungus.

Conclusion: As larger infiltrate and size of grafts had much higher risk of recurrences; early surgical intervention should be considered in cases not responding to medical therapy.

ENUCLEATION IN ASIAN INDIAN PATIENTS: A HISTOPATHOLOGICAL REVIEW OF 2009 CASES

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Purpose: To review the indications of enucleation in Asian Indian patients.

Methods: Retrospective study of 2009 patients.

Results: The mean age at presentation of patients who underwent enucleation was 155 months (range, 0.3 to 1020 months). There were 1191 (59%) males and 818 (41%) females. The histopathology diagnosis included a benign tumor (n=22, 1%), malignant tumor (n=1472, 73%), acute trauma (n=93, 5%), retinal vascular disease (n=50, 3%), inflammatory/infective pathology (n=33, 2%), or other miscellaneous/non-specific diagnosis (n=460, 23%). There was a good correlation between the clinical and histopathology diagnoses at 96%. The most common indication for enucleation in young patients (< 20 years) was retinoblastoma (n=1257, 82%), atrophic bulbi or phthisis bulbi (n=163, 39%) in middle-age adults, and uveal melanoma (n=25, 42%) in older adults. A decreasing trend of enucleations for atrophic bulbi/phthisis bulbi/painful blind eye (33% from the years 1996 through 2000 to 7% from 2010 to 2018) and acute trauma (3% from the years 1996 through 2000 to <1% from 2010 to 2018) and an increasing trend for intraocular tumors including retinoblastoma (56% from the years 1996 through 2000 to 73% from 2010 to 2018) and uveal melanoma (3% from the years 1996 through 2000 to 11 % from 2010 to 2018) was noted.

Conclusion: In Asian Indian population, malignant tumors remain the most common indication for enucleation in young and older patients, while desire for better cosmesis with customized ocular prosthesis is the main indication for enucleation in middle-age adults.

360 DEGREE INVOLVEMENT OF EYE WITH OCULAR SURFACE SQUAMOUS NEOPLASIA - A RARE CASE REPORT

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Purpose: To report a rare case of limbal mass with intraocular extension.

Method: A 42 Years old male patient presented with left eye limbal growth 8-10 'O' clock position for 4 months and diagnosed elsewhere as limbal papilloma and filtering surgery was done. After 2 months patient develop increased intraocular pressure and referred to LV Prasad Eye Institute for further management. On examination conjunctiva showed congestion with pigmented mass. In cornea, edema and pigment dispersion over the endothelium noted. Anterior chamber was flat due to a mass growing from the temporal site. Extended enucleation was done and specimen was sent for histopahological examination.

Result: Gross examination showed a mass measuring (28x7) mm and extending from 5 'O' clock position to 12 'O' position in limbal region with intra ocular extension. Histopathology report showed Ocular surface squamous neoplasia arising from the limbus and invading into the intra ocular tissue.

Conclusion: Ocular surface squamous neoplasia is most common tumor of conjunctiva arising from the limbus and intraocular extension may occur from the previous surgical tract.

QUALITATIVE COMPARISON OF CHOROIDAL VASCULARITY MEASUREMENT ALGORITHMS

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Purpose: To compare the accuracy of manual and automated binarization technique for the analysis of choroidal vasculature.

Methods: This was a retrospective study performed on 98 eyes of 60 healthy subjects. Fovea-centered swept source optical coherence tomography (SS-OCT) scans were obtained and choroidal area was binarized using manual and automated image binarization technique separately. Choroidal vessel visualization in the binarized scans were subjectively graded (grades 0-100) by comparing them with the original OCT scan images by two masked graders. The subjective variability and repeatability was compared between two binarization method groups.

Results: The mean accuracy grades of the automatically binarized images were significantly ($P<0.00$) higher (93.38%±1.70%) than that of manually binarized images (78.06%±2.92%). There was a statistically significant variability and poor agreement between the mean inter-observer grades in the manual binarization arm.

Conclusion: Automated image binarization technique is faster, more accurate in comparison to the manual method.

STUDY OF MORPHOLOGICAL,REINAL NERVE FIBRE LAYER(RNFL) AND BLOOD FLOW CHANGES OF OPTIC NERVE HEAD ON FOURIER DOMAIN OCT(FD-OCT) ANGIO ™ IN NON ARTERITIC ANTERIOR ISCHEMIC OPTIC NEUROPATHY(NAION) AT TERTIARY EYE CARE CENTRE IN EASTERN INDIA.

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Purpose: Analysis of optic disc morphology, blood flow changes (microvasculature) at optic nerve head in first three months of of follow up of patients of NAION.

Method: Prospective observational study of 33 eyes of 33 patients with newly diagnosed NA-AION and who underwent FD-OCT-Angio™examination between July-2016 and March 2017.Systematic demographic, ocular and OCT examination findings recorded.OCT examination of the following parameters were analyzed -1) RNFL thickness 2) Rim area of optic disc 3) Optic disc area 4) CDR (Cup: Disc ratio) 5) GCL (Ganglion cell layer) thickness 6) Angio Changes (Blood flow changes).

Results: 33 eyes of 33 patients (mean age= 53.3 years, SD ± 9.7), 20 males (60.6%) and 13 females (39.3%) underwent screening of NA-AION at presentationand follow up visit at 1 and 3 month. Total RNFL changes at presentation was 227.39+/-74.84 µm, which reduced to 100.9+/- 35.76 um and 80.53+/-23.91 µm at follow up visit 1 and 3 month respectively. It was found to be statistically significant (<0.0001) when compared with superior RNFL at 1 month and 3 month visit .The affected eye mean GCL thickness was 104.24 +/-38.55 µm, 74+/-19.19 µm and 64.17+/-13.6 µm at presentation,1month and 3month respectively. The blood flow changes revealed that diminished blood supply (Hypo intense images) of the optic nerve head in affected eye at all visit with predominant drop out at presentation.

Conclusion: FD OCT Angio showed gradual decrease in RNFL and GCL thickness over time with no recovery. It also depicts diminished blood flow of the optic nerve head in early stage of NA-AION.

QUANTIFICATION AND COMPARISION OF MACULAR VASCULAR DENSITY IN BRANCH RETINAL VEIN OCCLUSSION AND NORMAL: AN OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY – FRACTAL ANALYSIS BASED STUDY

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Purpose: To compare the macular vascular density in BRVO versus normals on OCTA by fractal analysis and describe factors bearing on final visual outcome.

Methods: OCTA images of cases and control eyes were analyzed using fractal dimension on the MATLAB platform. Vascular density fraction was calculated for each eye for both the retinal vascular layers. Images were cleaned of artifacts before processing.

Results: 16 eyes were normal and 98 had BRVO. Mean age among normals was 56.63±12.5 years and among BRVO was 58.41±10.7 years (p=0.6). Superficial vascular density fraction was noted as 0.29±0.08 (95% C.I. 0.27 to 0.31) and 0.25±0.1 (95% C.I. 0.23 to 0.27) respectively (p=0.003). Deep vascular density was noted as 0.39±0.11 (95% C.I. 0.32 to 0.41) and 0.14±0.09 (95% C.I. 0.12 to 0.19) (p<0.0001). Pearson's co relation coefficient for the effect of age on vascularity as -0.4 (p=0.04), for the effect of vascularity on final vision was -0.53 (p=0.001) and for the effect of duration of symptoms on final vision was 0.35 (p=0.09).

Conclusion: In BRVO vascular density decreases significantly in the deeper retinal layer. Vision deficit correlates to increased age, duration of symptoms and greater vascularity loss.

ENDOSCOPIC VITRECTOMY IN ENDOPHTHALMITIS: INITIAL EXPERIENCE OF 33 CASES AT A TERTIARY EYE CARE CENTER

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Purpose: To report the presentations and management outcomes of endophthalmitis with endoscopic vitrectomy.

Methods: Retrospective interventional case series at a tertiary eye care center in south India. Thirty three eyes of 33 patients were included. The medical records of the patients who underwent endoscopic vitrectomy for endophthalmitis from April 2014 to March 2018 were reviewed. Data was collected including age, gender, etiology of endophthalmitis, corneal and retinal examination, type of intervention, final anatomic and visual outcome and the total follow up. The main outcome measures were the final visual acuity and evisceration rates.

Results: The mean age at presentation was 46.84±19.89 years, median age 50 years. Etiology-wise, 13 eyes(39.4%) were post trauma, 11 eyes (33.33%) were post cataract surgery, 3 eyes (9.09%) were endogenous, 3 eyes (9.09%) were post perforated corneal ulcer, 2 eyes (6.06%) post retinal surgery and 1 eye (3.03%) was post combined cataract and corneal surgery. Twenty four eyes (72.72%) had a favorable anatomic outcome at the last visit, 5 eyes (15.15%) had a favorable visual outcome. Of those with unfavorable visual outcome, 10 eyes had further visual potential. 16 eyes (48.48%) showed a positive culture on microbiologic evaluation. The predominant organism isolated was *Pseudomonasaeruginosa*. Evisceration was required only in one eye (3.03%).

Conclusion: Endoscopic vitrectomy allows early management of endophthalmitis in spite of hazy media. This ensures a reasonable visual outcome, contains the infection and reduces the incidence of evisceration in these eyes.

EVALUATION OF DRY EYE FOLLOWING VITREO-RETINAL SURGERY

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Purpose: Dry eye is a highly prevalent inflammatory disease of the lacrimal functional unit caused by multifactorial disorders that affects the quality of life. Surgical intervention may influence the dry eye status. The effect of vitreoretinal surgery on dry eye is not known. The study explores objectively the dry eye status before and after vitreoretinal surgery.

Methods: Patients undergoing vitreo-retinal surgery (n=44, Mean age: 56±10y; M/F:31/13) were recruited after consent. Ophthalmic evaluation for dry eye included Schirmer's test, TMH, TBUT, Fluorescein stain, impression cytology (IC) and McMonnies Questionnaire. Tear was collected using schirmers strip. All the tests were repeated 8 weeks after vitreoretinal surgery (2 weeks after stopping the topical treatment). Goblet cells (GC) were stained in IC using Periodic acid and Schiff's reagents. Cytokines were profiled by Bead array; Imprints were analyzed for MUC5AC, MUC4, MUC16, AQP4 and AQP5 mRNA expression by qPCR.

Results: None had symptomatic dry eye developed after surgery. But 5 patients had mild increase in tear osmolarity. GC was significantly reduced post-surgery (65%,p=0.031). Gene expression profile for mucins in the conjunctival imprint revealed significant increase in MUC4 (20 fold, p=0.025) and MUC16 (16 fold, p=0.05) but not in Muc5AC (p=0.86,NS). The cytokines namely, IL-4 ($p<0.015$), IL-5 ($p<0.05$), IL-6 ($p<0.05$), IL-9 ($p<0.03$) and IL-15 ($p<0.021$) were significantly increased post surgery. The type of surgery and the surgical approach did not significantly alter the observations. The changes were more prominent in diabetic patients than non-diabetic.

Conclusion: Though symptomatic dry eye was not observed after VR surgery**,** conjunctival changes in form of reduced goblet cell density, altered MUC4, MUC16, aquaporins and altered cytokines were seen.

DIAGNOSIS, CLINICAL PRESENTATIONS AND OUTCOMES OF NOCARDIA ENDOPHTHALMITIS

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Purpose: To describe the clinical presentations, diagnosis and management outcomes of Nocardia endophthalmitis.

Methods: This was a retrospective, interventional, multi-centric case series study conducted in a multi-centric institutional practice setup including 25 eyes of 25 patients with culture-proven Nocardia endophthalmitis. AC fluid and/or vitreous and/or intraocular lens were submitted for microbiological evaluation is all cases. Patients with non-Nocardia etiology and those that were culture-negative were excluded. Case records were studied and data regarding demography, clinical presentations, interventions received and final visual and anatomic outcomes were noted. The main outcome measures were final visual outcomes and factors determining them.

Results: The mean age of the patients was 54.7±22.9 years. By the etiology of infection, 18 (75%) eyes were post cataract surgery (operated elsewhere), 3 (12.5%) eyes were post-trauma and 3 (12.5%) eyes were endogenous. The final follow up in months was a mean of 14.25±30.35 months, median 2 months. The odds of a favorable outcome were 42.5 (95% C.I. 3.15 to 571.84, p=0.0047) when the vision was more than hand motions at presentation, 9.42 (95% C.I. 0.92 to 95.89, p=0.05) in male gender, 21 (95% C.I. 0.9 to 489.7, p=0.05) when presentation was within 48 hours and 2.5 (95% C.I. 0.23 to 26.48, p=0.44) with primary vitrectomy instead of a biopsy. The in vitro susceptibility was poor for vancomycin and was best for amikacin.

Conclusions: The visual outcome in Nocardia endophthalmitis is very guarded when presenting vision is poor. On diagnostic confirmation or high index of suspicion intravitreal amikacin is preferred.

INFECTIOUS ENDOPHTHALMITIS LEADING TO EVISCERATION: SPECTRUM OF BACTERIAL AND FUNGAL PATHOGENS AND ANTIBACTERIAL SUSCEPTIBILITY PROFILE

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Purpose: To describe the spectrum of bacterial and fungal pathogens in cases of endophthalmitis requiring evisceration and report their antimicrobial susceptibilities.

Methods: Retrospective, consecutive, descriptive case series of endophthalmitis that underwent evisceration from January 2004 to December 2017. Vitreous samples from all patients had been investigated for bacteria and fungus using institutional protocol.Bacterial isolates were identified using Analytical Profile Index (API) system until 2010 and Vitek-2 compact system (bioMérieux, France), thereafter. The susceptibility of bacterial isolates to variety of antibiotics was determined by the Kirby-Bauer disk diffusion method.

Results: Of 791 cases reviewed, culture positivity was reported in 388 cases (48.92%). Commonest clinical setting of endophthalmitis necessitating evisceration was post-microbial keratitis (58%), followed by post-trauma and post cataract surgery (14-15%). Commonest isolate was *Streptococcouspneumoniae*, seen in 68 samples overall (17.52%). Hundred and eighty three isolates (47.16%) were gram-positive, 86 (22.16%) were gram-negative and fungi constituted 137 (35.3%) isolates. *Streptococcus pneumoniae* was the commonest gram positive bacterial isolate seen in 68/183 samples (37.15%). Among gram-negative organisms, the commonest was *Pseudomonas aeruginosa* seen in 47/86 (54.65%). *Aspergillus* spp. formed the commonest fungal isolate , 58/137 (42.33%). The susceptibility of the gram-positive bacteria was highest with vancomycin, 136/147 (92.51%) and for gram-negative bacteria was seen best with imipenem 24/29 (82.75%) Susceptibility to ceftazidime was 31/61 (50.81%) in 31/61.

Conclusion: Endophthalmitis due to *Pneumococci*, *Aspergillus* and *Pseudomonas* can be very fulminant and progress to require evisceration in spite of prompt and appropriate treatment.

ELECTRORETINOGRAM CHANGES IN CHILDREN WITH RETINOPATHY OF PREMATURITY MANAGED WITH VARIOUS TREATMENT MODALITIES

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Purpose: To evaluate the global retinal function using full field Electroretinogram (ERG) in subjects of Retinopathy of Prematurity (ROP) managed with various modalities of treatment.

Methods: In a cross sectional observational study we prospectively analyzed the global retinal function of children previously treated for ROP with the help of full field ERG over a period of 1 year.

Results: The study population included 32 eyes of 16 children with age ranging from 1 to 11 years at the time of examination. Twenty eyes belonged to laser alone group, 4 eyes had laser and intravitreal bevacizumab (IVB) combination therapy, 2 eyes had laser, IVB and surgery all in the same eye.ERG changes were observed irrespective of treatment. While spontaneously regressed ROP without sequelae or with minimal involutional changes had normal ERG, the group that received IVB in addition to laser or surgery showed severe photoreceptor and inner retinal dysfunction. The ERG changes in laser alone group ranged from no dysfunction to rod dysfunction alone to severe rod-cone dysfunction. The later was observed in those who had aggressive posterior ROP with very posterior disease location. In laser group whenever both photoreceptors were affected, the rod system showed the maximum damage. None had exclusive cone dysfunction.

Conclusions: Subjects treated for ROP showed photoreceptor dysfunction of different severity depending on the extent of prematurity, severity of the disease at presentation, type and extent of treatment and final involutional changes at regression.

COMPARISION OF PERIPHERAL REFRACTIVE PROFILES IN DIFFERENT REFRACTIVE ERRORS IN INDIAN EYES

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Purpose: To report peripheral refractive profiles in Indian eyes.

Methods: A total of 204 subjects with spherical equivalent (SE) ranging from +2.00D to -10.00D with astigmatism ≤-1.50D participated in the study. Cycloplegic peripheral refractive errors measured with open field auto refractor (NVision K-5001; Shin-Nippon, Tokyo, Japan) from center to ±30˚ eccentricity in horizontal meridian and ±15˚ vertical meridian in 5˚ steps. Relative Peripheral Refractive Errors (RPRE) calculated for example as: At 10^0RPRE = refractive error measured at 10^0-refractive error at 0^0

Results: Subjects mean ± SD of age is 22.5 ± 4.20 yrs. The mean ± SD of SE of Hyperopes, Emmetropes and Myopes is 1.07 ± 0.48Ds,0.015 ± 0.45Ds, and -3.49 ± 2.32Ds respectively. RPRE values were significantly different along horizontal eccentricity in Hyperopes (P<0.05), Emmetropes (P<0.05) and Myopes (P<0.05). RPRE values showed significant difference only at temporal eccentricities at 10˚(p=0.031), and 15˚ - 30˚(P<0.05)between refractive error groups. RPRE values were significantly different in horizontal eccentricities in low myopes, moderate myopes and high myopes (p<0.05). RPRE values were significantly different in nasal eccentricities at 5˚ (p=0.031), 10˚ (p=0.005), 15˚ (p=0.001), 20˚ (p=0.002), 25˚ (p=0.027), and 30˚ (p=0.041) and in temporaleccentricity at 10˚ (p=0.002) and 15˚ (p=0.048).

Conclusion: Indian eyes showed asymmetric RPRE values in horizontal eccentricity. All refractive error groups showed peripheral myopic shift in nasal eccentricity.

OUTCOME OF UNIVERSAL NEW-BORN EYE SCREENING PROGRAMME IN TERTIARY HEALTH CARE CENTRE IN SOUTH INDIA

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Purpose: To evaluate the outcome of universal new-born eye screening programme in tertiary health care centre, Andhra Pradesh, India.

Methods: New-born eye screening was conducted at 2-3 days of their birth in government and private tertiary health care centre by trained ophthalmologist using indirect ophthalomoscope and +20D lens. Data included parent's age, obstetric status, gender, birth weight, oxygen saturation, Apgar score, systemic conditions, anterior and posterior segment findings in a validated questionnaire. Babies were referred to L V Prasad Eye Institute, Tadigadappa, Vijayawada for any ocular pathology found.

Results: We screened 1721 new-born babies. Mean age of father and mother was 28.33 and 23.63 years. There was in-equal distribution of babies in two hospital (75.6% in Government hospital). There was slight male predominance (68%). Ocular pathology was found in 448(26.03%) babies. Preretinal haemorrhage and intraretinal haemorrhage was the most common pathology (327, 19%). Other ocular pathology are retinopathy of prematurity (112, 6.5%), FEVR(1), congenital cataract(2), congenital glaucoma(2), corneal opacity(1), retinohoroidalcoloboma(1), optic atrophy(2). Six babies (6/1721) required immediate surgical intervention.

Conclusion: Though we found significant number of ocular pathology during our screening programme but only 6 babies had to have emergency management at tertiary care. This reflects very negligible benefit of universal screening, but considering the blind year prevention in each baby who received immediate intervention, universal eye screening has some importance.

CHANGING PATTERN OF CHILDHOOD BLINDNESS IN SCHOOLS FOR THE BLIND IN ANDHRA PRADESH, SOUTH INDIA

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Purpose: To determine the changing pattern of causes of severe visual impairment (SVI) and blindness in children in 7 schools for the blind in Andhra Pradesh, India.

Methods: Children aged less than 16 years, attending the residential schools for the blind were examined in 2016-2017. History taking, visual acuity estimation, external ocular examination, retinoscopy, refraction and fundoscopy were done on all children. The anatomical and etiological causes of severe visual impairment (<6/60–3/60) and blindness (<3/60 in the better eye) were classified using the World Health Organization's prevention of blindness programs' record system. Results were also compared with the historical data published 20 years ago.

Results: A total of 249 students were examined from 7 school for the blind and 227 (91.2%) were having SVI or blindness based on best corrected visual acuity. The major anatomical sites of visual loss were whole globe (75, 33.05%), corneal conditions (27, 12.15%), lens related condition (29, 13.05%), and retinal disorders (64, 28.01%). The main aetiological causes were hereditary (37.8%), intrauterine (13.2%) and unknown (37.8%). As compared to historical data, there was decline in avoidable causes of childhood blindness.

Conclusions: Among anatomical causes whole globe, cataract, and retinal conditions account for most of the blindness and among aetiological causes hereditary conditions continue to be most common causes in children. As compared to past, there is change in pattern of etiology and causes of childhood blindness with a avoidable causes being on decline.