27th

ARVO International Chapter Affiliate

ARVO-INDIA

Indian Eye Research Group

Program Book 2021

Special Orations and Keynotes by Eminent Faculty

Orientations on Advances in Eye Research

Vision for the Future

Panel Discussions, Free Papers and E-Posters

Presentations, Awards and Recognitions for Young Researchers
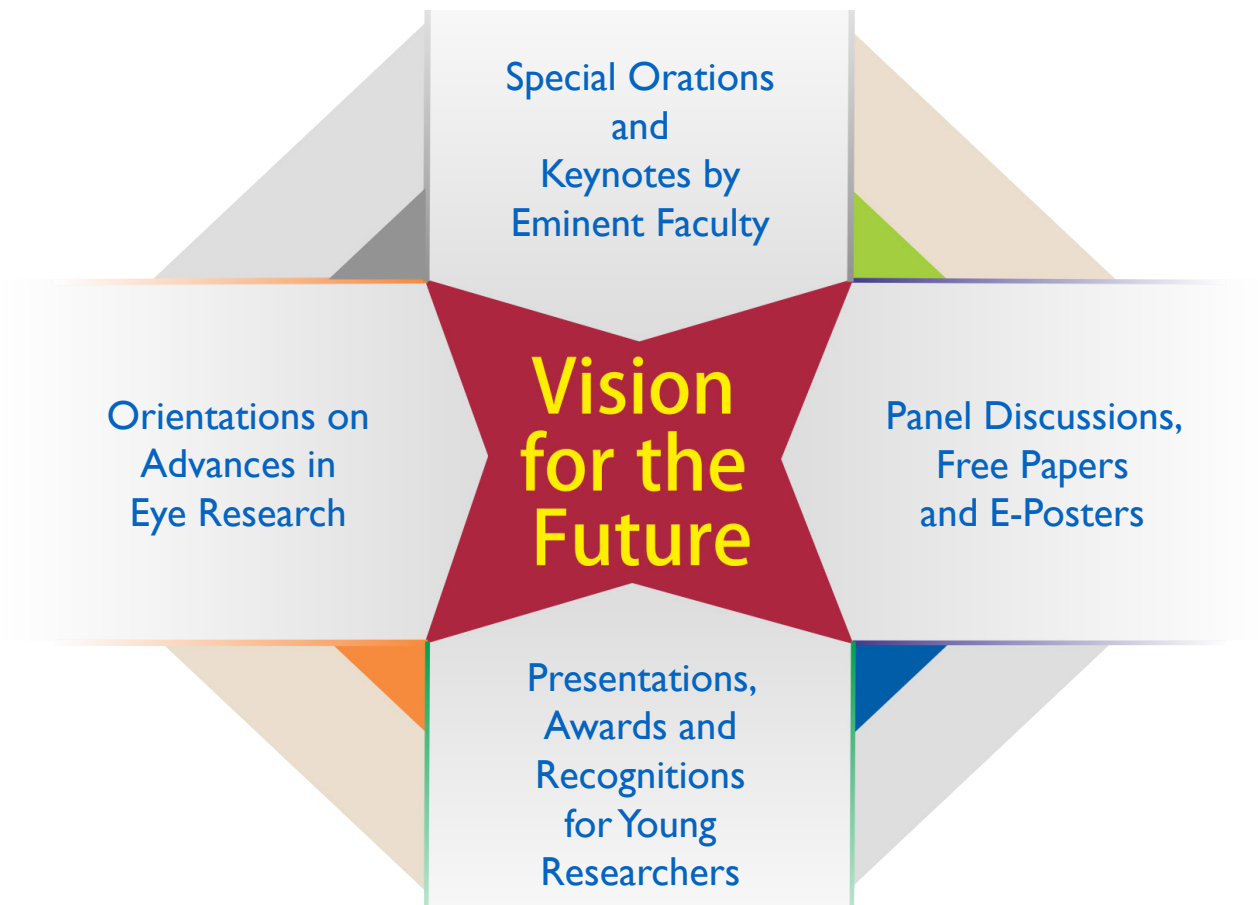
7th-10th October, 2021

Contact Details

Organizers	
Dr Sayan Basu	+91 9989479969
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Organizers

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Pre-Conference Workshops

October 7-8, 2021

Theme: "Advances in Ocular Gene Therapy"

Coordinator: Dr. Arkasubhra Ghosh

Thursday, October 7th, 2021; 10:00 AM - 12:00 PM

Gene therapy is a technique for correcting pathogenic effects of defective genes in cells and organs that drive a variety of inherited and acquired illnesses. With a very large population, high birth rate and consanguineous marriages favoured in many communities, there is a high prevalence of genetic disorders in India. For many diseases such as Leber's Congenital Amaurosis, Retinitis Pigmentosa, Stargardt disease, etc, the only recourse is gene therapy. The principle of gene therapy is to transfer a therapeutic gene using viral or non-viral vectors to the target tissue. Due to their natural ability to transfer DNA or RNA cargo to cells efficiently, viral vectors have emerged as the most efficient vehicles for Gene Therapy.

Inherited retinal dystrophies (IRDs) that are caused by mutations resulting in progressive functional loss of retinal cellular layers including photoreceptors, has been a particularly attractive target for gene therapy development. Treating LCA2 with an AAV vector expressing the RPE65 gene was the first FDA approved gene therapy. Today, there are several hundred gene therapy trials across a variety of diseases using many different vector types in progress around the world. Critical hurdles against success are design of transgenes, capsid or vector selection, production of the clinical grade GTP (gene therapy product), efficacy/toxicity of the vector dosages, patient selection criteria and regulatory processes. During this workshop, we will discuss the following aspects:

1. **Introduction to Gene Therapy:** Dr. Arkasubhra Ghosh (5 min)
2. Introduction to the panel: (5 min)

   - Dr. Geeta Jotwani, ICMR
   - Dr. G. Kumaramanickavel, NNF
   - Dr. P. Sundaresan, AMRF
   - Dr. Debojyoti Chakraborty, IGIB
   - Dr. Indumathi Mariappan, LVPEI

Session I: Choices of therapeutic strategies for ocular gene therapy: current status

3. Status of trials for ocular gene therapy: Dr. Chitra Gopinath (10)
4. Vectors and transgene designs for ocular gene therapy: Dr. Ruchita Selot (10)

5. Gene editing for ocular diseases: Dr. Mayank Bansal (10)

Session II: Which patients are suitable to receive gene therapy?

6. Important considerations regarding patient selection for gene therapy: Dr. G.Kumaramanickavel
7. Patient's perspectives: IRD clinical diagnosis and functional evaluation: Dr.Poornachandra B (10)

8. Patient's perspectives: Genetic counselling for IRD: Dr. Anuprita Ghosh (10)

Session III: Vector production for IRD human trials and regulatory considerations

9. AAV gene therapy vector production and scale up: Dr. Sharath Babu (12)

10. Human trial and regulatory considerations for Gene Therapy: Dr. Geeta Jotwani (15)

    Panel discussion: Way forward for ocular gene therapy in India.
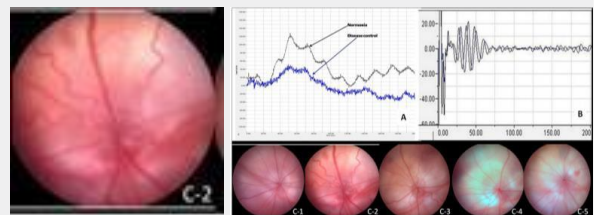
ARVO-INDIA
Indian Eye Research Group

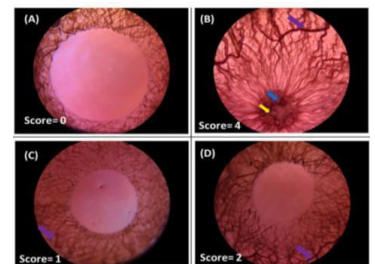## Theme: Advances in Ocular Pharmacology- LIVE WORKSHOP

Coordinators: Dr. T. Velpandian & Dr. N. Halder

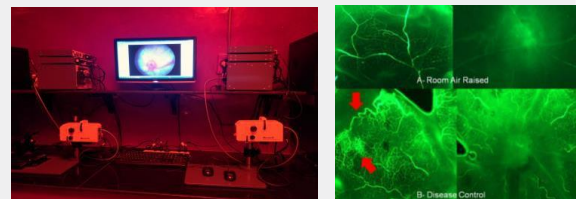### Thursday, October 7th, 2021; 10:00 AM - 12:00 PM

- **Electrophysiology: Rodent retina**

- **Imaging: Endotoxin induced experimental model of uveitis in rats**

- **Imaging: Rat retinal angiogenesis**

- **Interactive session**

Organised by :
Ocular Pharmacology and Pharmacy Div., Dr. Rajendra Prasad Centre for Ophthalmic Sciences, AIIMS, New Delhi

ARVO-INDIA
Indian Eye Research Group

Theme: Animal Models in Eye Research

Coordinators: Dr. Abhinav Reddy Kethiri, Dr. Kiran Kumar Bokara, Dr. Vivek Singh

Friday, October 8th, 2021; 10:00 AM - 12:00 PM

Ocular diseases and therapeutics have been extensively studied in humans. Tissues obtained from human is often limited. Thus, less information is available regarding the pathological mechanisms causing the disease thereby making *in vitro* analysis 'less translatable' to human. Animal models, compared to other experimental methods, e.g., cell and organ cultures allow the study of different pathological factors and therapeutic treatments under *in vivo* conditions, i.e., with the visual and other systems of the body intact. Therefore, holding an educational session in this area will benefit researchers to understand the need of animal models in their research. The course is expected to bring together interested researchers and encourage them to design better animal models of disease and perform therapeutic research.

Scope of the session: This session covers the animal models used in ocular research and would discuss the *in vivo* cell lineage tracking, CRISPR in retinal dystrophies, surgical aspects in animal models and, an overall view of designing the animal experiments. It will give the glimpse of various tools and animal models to answer unsolved questions and to understand the importance of pre-clinical study using animal models.

Time (hrs; IST)	Session Details	Resource Person(s) / Speakers
09:55 – 10:00	Welcome & Introduction	Dr. Abhinav Reddy Kethiri, LVPEI
10:00 – 10:30 (15:30 – 16:00 AEDT)	A multicolor transgenic mouse model to study stem cell fate during corneal development, wound healing and disease	Prof. Nick Di Girolamo University of New South Wales Australia
10:30 – 11:00 (13:00 – 13:30 SGT)	Eye: A Window to the Body- Discovery Tool for Many Diseases	Dr. Veluchamy A. Barathi Singapore Eye Research Institute Singapore
11:00 – 11:30	CRISPR/Cas ribonucleoproteins (RNPs) delivery using non-viral vectors for retinal dystrophies	Dr. Deepak Chitkara BITS, Pilani India
11:30 – 12:00 (08:00 – 08:30 CEST)	Surgical aspects in animal models for RPE cell therapy in AMD	Dr. Boris V. Stanzel Sulzbach / Saar Eye Clinic Germany
12:00	Concluding remarks & discussion	Dr. Vivek Singh, LVPEI Dr. Kiran Kumar Bokara, CCMB Dr. Abhinav Reddy Kethiri, LVPEI

Theme: Clinical Proteomics of the Eye

Coordinator: Prof. K. Dharmalingam

Friday, October 8th, 2021; 10:00 AM - 12:00 PM

Omics approaches in general and Clinical proteomics in particular are becoming indispensable tools in the analysis of the healthy and diseased eye. The workshop on Clinical Proteomics of the Eye will focus on the utility of proteomics analysis in understanding the eye disorders. Main emphasis will be using proteomics in understanding specific diseases, such as ROP, DR, Infectious keratitis etc. and the importance of sample handling issues will be explained in detail. We will also look at briefly how proteomics is being used in other related fields. Exposure to this workshop will be useful to all those interested in using modern technologies, including clinical fellows, to understand and apply proteomics to study eye and its disorders.

Date: October 8th, 2021

Time: 10 AM -12 Noon

Program:

- **Introduction to the workshop**
  Dr. K.Dharmalingam

- **Talks on Clinical proteomics**
  1. Dr. Jeya Maheshwari, AMRF:
     Introduction to Proteomics – a powerful tool to unravel the molecular mystery ofocular diseases.
  2. Dr. Chitra Thangavel. Ganga Orthopaedic Research and Education Foundation:
     Challenges and Impact of extraction buffers on avascular tissue proteomeanalysis
  3. Dr. Inderjeet Kaur, LVPEI:
     Proteomics and Ocular diseases.
  4. Dr. Balamurugan. Alagappa University:
     Model system, Caenorhabditis elegans based proteomics analysis to understandbacterial infections.

- **Panel discussion**
  Moderators
  - Prof. S.Karutha Pandian
  - Prof. K.Dharmalingam
- **Concluding remarks**
  - Prof. K.Dharmalingam

Program Schedule

ARVO-INDIA
Indian Eye Research Group
October 7-10, 2021 (Virtual)

THURSDAY, OCTOBER 7, 2021 | DAY 1

Sessions	Time	Hall 1	Hall 2	Hall 3	Hall 4	E-Poster Hall
Workshops	10.00 - 12.00	**Workshop 1** Arkasubhra Ghosh *Ocular gene therapy*	**Workshop 2** T Velpandian *Ocular pharmacology*			
Inauguration	17.30 - 17.35	Welcome address: Prashant Garg				
	17.35 - 17.45	ARVO India - IERG Reminiscence: Gullapalli N Rao				
	17.45 - 18.00	ARVO during the pandemic and beyond: Iris M Rush				
Free Papers	Session Chairs	Periasamy Sundaresan Sarangapani Sripriya	Srinivasan Senthil Kumari K Aruna Sri	Javed Ali Mudit Tyagi	PremNandhini Satgunam Pavan K Verkicharla	
	18.00 – 18.08	**BSO1** Goutham Pyatla *Involvement of GLIS1 in the pathogenesis of primary congenital glaucoma*	**BSO7** Gowtham Lakshminarayanan *Untargeted metabolomic investigation of glaucomatous patients using high resolution mass spectrometry*	**CLO1** Tanvi Mudgil *Longitudinal changes in corneal epithelial thickness and reflectivity following simple limbal epithelial transplantation: an optical coherence tomography-based study*	**OPO1** Satish Kumar Gupta *Does relative peripheral defocus alter the corresponding peripheral retinal electrical signals?*	All Posters
	18.08-18.16	**BSO2** Harshavardhini G *A spectrum of candidate and mitochondrial gene mutations in Usher syndrome patients from India*	**BS08** Nikhala Shree S *Anti-glaucoma medications lowered decorin and altered profibrotic proteins in human tenon's fibroblast*	**CLO2** Tapas R. Padhi *Retinopathy of prematurity of half zone: Clinical profile and outcome*	**OPO2** Rahul Negi *The grade of relative afferent pupillary defect is dependent on intensity of light stimulation of the pupils*	
	18.16-18.24	**BSO3** Pooja Khamar *Status of SARS-CoV-2 specific pro & anti-viral genes on the ocular surface*	**BS09** Pon Yazhine T S *Exoproteome of clinical isolates of Fusarium solani*	**CLO3** Sharon D'Souza *Classification of ocular surface pain based on discordant signs and symptoms: association of clinical, confocal imaging and tear molecular profiles*	**OPO3** Preetam Kumar *Perception of suprathreshold contrast in keratoconus*	
	18.24-18.32	**BSO4** Prerna Kulshrestha *Global microRNA profiling for understanding the pathogenesis of non-infectious uveitis*	**BSO10** Ramaraj Kannan *Untargeted metabolomics analysis of aqueous humour in fuchs endothelial corneal dystrophy identifies unique signaling modules*	**CLO4** Saumya Srivastav *Non-invasive tear film and meibomian gland assessment in healthy Indian population: effect of age, gender and interparametric relationship*	**OPO4** Prayag Bellur Shashikumar *Visualisation of corneal opacity score through a multi axis wheel diagram*	
	18.32-18.40	**BSO5** Srikrupa Natarajan *Whole exome sequencing identifies SLC6A6 as novel candidate gene in leber congenital amaurosis*	**BSO11** Rebecca Manohar *Elastin interacting partners are differentially expressed in pseudoexfoliation syndrome*	**CLO5** Divya Sree Ramya Achanta *Corneal endothelial cell alterations in variants of Axenfeld-rieger syndrome assessed by specular microscopy*	**OPO5** Chandrika Ravisankar *Fluctuations of steady-state accommodation is a marker for screening spasm of near reflex*	
	18.40-18.48	**BSO6** Swathi Chadalawada *Dysregulated expression of micro RNAs in vitreous humour from intra-ocular tuberculosis patients*	**BSO12** Saumya Srivastava *Identification of antigenic protein of Acanthamoeba castellani for the diagnosis of acanthamoeba keratitis*	**CLO6** Deepanjan Datta *Microneedle contact lens for ocular delivery of Cyclosporine A*	**OPO6** Aiswaryah Radhakrishnan *Objective and subjective changes in color discrimination with and without underwater blur*	
Discussion	18.48-19.00	**Q & A**	**Q & A**	**Q & A**	**Q & A**	
	19.00 - 19.10	**Break**				
Keynotes* Chairs: Rohit C Khanna Soumyava Basu	19.10-19.30	**Keynote 1** Nathan Congdon *ENGINE: Research documenting how vision care can accelerate progress towards the SDGs across the life course*				
	19.30-19.50	**Keynote 2** Anat Galor *What's new in the world of dry eyes?*				
Oration* Chair: Ronnie George	19.50 - 20.50	**SS Badrinath Oration** Usha Chakravarthy *The early detection of neavascular AMD and what has the EDNA study taught us*				

* All the Keynotes & Orations will be in Hall-1

Sessions	Time	Hall 1	Hall 2	Hall 3	Hall 4	E-Poster Hall
Workshops	10.00 - 12.00	**Workshop 3** Vivek Singh, Abhinav Kethiri *Animal models in eye research*	**Workshop 4** K Dharmalingam *Ocular proteomics*			
	Session Chairs	Indumathi Mariappan Sinnakaruppan Mathavan	Mamatha Reddy Ayyasamy Vanniarajan	Charanya Ramachandran Sunita Chaurasia	Shrikant Bharadwaj Srinivas Marmamula	
Free Papers	17.30 – 17.38	**BSO13** Akhil Varshney *Toxicity of endogenous cytoplasmic Alu complementary DNA in age-related macular degeneration*	**BSO19** Janavi Subramani *Retinal pigment epithelium (RPE) cells generated from oculocutaneous albinism type IA (OCA1A) patient-derived induced pluripotent stem cell (iPSC) line mimics disease phenotype of pigmentation defects*	**CLO7** Ashik Mohamed *Effect of topical rho-associated kinase inhibitor ripasudil 0.4% on corneal endothelium*	**OPO7** Rebecca Sumalini *How does vision correlate with overall development in children with cerebral visual impairment?*	
	17.38-17.46	**BSO14** Ankit Jain *Heterogeneity using novel invariant gene expression analysis and lipidomics in retinoblastoma*	**BSO20** Radhika Manukonda *Isolation and characterization of serum derived extracellular vesicles from retinoblastoma patients*	**CLO8** Himanshu Gururani *A study on corneal birefringence behavior using digital photoelasticity*	**OPO8** Rajashekar Varada *Who is paying for cataract surgery of elderly in rural Telangana?*	
	17.46-17.54	**BSO15** Barathi Lenin *In silico identification of putative alternatives to ocular chemotherapeutic agents: high-throughput virtual screening and molecular dynamics studies of natural product inhibitors targeting human DNA topoisomerase II beta*	**BSO21** Ramya Ravi *Paraoxonase 2 protects against the cml mediated mitochondrial dysfunction through modulating JNK signalling pathway in human retinal microvascular endothelial cells*	**CLO9** Kavitha A V *A pilot study on comparative analysis of minimum inhibitory concentration (MIC) and mutant prevention concentration (MPC) of conjunctival bacterial isolate to fluoroquinolones*	**OPO9** Vijitha S Vempuluru *Photo-screening for early detection of retinoblastoma: a proof of concept study*	
	17.54-18.02	**BSO16** Deepak Kumar Sahel *CRISPR/Cas9 nanomedicine therapy for the management of wet-age related macular degeneration*	**BSO22** Revu VL Narayana *Generation and characterization of drug resistant clones of RB Y79 cells*	**CLO10** Lalit Kishore Ahirwar *Elucidating the clinical, microbiological and molecular diagnostic aspects of Macrophomina phaseolina keratitis*	**OPO10** Vijaya Kumari Gothwal *Life in lockdown: impact of COVID-19 lockdown measures on the lives of school-age children and their families in India*	
	18.02-18.10	**BSO17** Ravinarayanan Haribalaganesh *Genome-wide transcriptome profiling of glucocorticoid responder and non responder primary human trabecular meshwork cells using RNA-sequencing after dexamethasone treatment*	**BSO23** Sumaiya Sirajudeen *Differential response of corneal epithelial and stromal cells to the novel chemical cross-linker treatment*	**CLO11** Subhpreet Kaur *Comparison of two corneal epithelial cell lines in respect of their expression of differentiation and stemness markers*	**OPO11** Samir Sutar *The SPA-VVRT (smart phone anaglyph video virtual reality therapy) study- a pilot non-randomized trial of smart phone anaglyph video virtual reality based therapy for treatment of amblyopia in adults*	
	18.10-18.18	**BSO18** Tapas Kumar Roy *Enhanced stability of sodium ascorbate for ocular emergency by using TRANSRECON™*	**BSO24** Susan Immanuel *Isolation and characterisation of exosome mimetics from human corneal epithelial cells*	**CLO12** Swati Singh *Prostaglandin E receptor subtype 3 expression in lacrimal glands of healthy subjects, Stevens-Johnson syndrome and non-specific dacryoadenitis*	**OPO12** Sai Naga Sri Harsha Ch *Tearing mode fracture toughness of cornea*	
Discussion	18.18-18.30	**Q & A**	**Q & A**	**Q & A**	**Q & A**	
Keynotes* Chairs: Inderjeet Kaur N Angyarkanni	18.30–18.50	**Keynote 3** Bikash R Pattnaik *A vision for the future is the treatment for ion channelopathies*				
	18.50-19.10	**Keynote 4** Renu Kowluru *Diabetic Retinopathy: Mitochondria in a Web of Epigenetics*				
	19.10 - 19.20	**Break**				
	19.20 - 19.40	**Keynote 5** Manni Luthra-Guptasarma *Development of tools to explore amelioration of ocular diseases through the manipulation of components of the extracellular matrix*				
Oration* Chair: Raja Narayanan	19.40 - 20.40	**D.Balasubramanian Oration** Miguel Seabra *From gene identification to gene therapy in 20 years: the Choroideremia example*				

All Posters

* All the keynotes & Orations will be in Hall-1

Sessions	Time	Hall 1	Hall 2	Hall 3	Hall 4	E-Poster Hall
Keynotes* Chairs: Sanhita Roy G Chidambaranathan	9.30 - 9.50	**Keynote 6** Eric Pearlman *Fungal and bacterial keratitis – new insights into pathogenesis*				
	9.50 - 10.10	**Keynote 7** Aparna Lakkaraju *Live imaging reveals novel insights into macular degenerations*				
	10.10 - 10.30	**Keynote 8** Taraprasad Das *Application of bed-side and bench research in the advancement of endophthalmitis care*				
Oration* Chair: Savitri Sharma	10.30 - 11.30	**Bireswar Chakrabarti Oration** Fiona Stapleton *Global Epidemiology of Infectious Keratitis*				
	11.30 - 11.40	**Break**				
	Session Chairs	Chitra Kannabiran Bhavani S Kowtharapu	J Jeya Maheshwar T Velpandian	Swathi Kaliki Bhavik Panchal	Vivek Dave Aparna Rao	
Free Papers	11.40 – 11.48	**BSO25** Keya Katare *BCAT1 regulates metabolic flux in retinoblastoma abetting tumor growth*	**BSO31** Animith Venuganti *Evaluating the potential of a biomimetic decellularized matrix (DCM) hydrogel in preventing corneal scarring in an animal model*	**CLO13** Komal Agarwal *Clinical profile and treatment outcomes of infants with exudative retinal detachment as the presenting feature in retinopathy of prematurity*	**CLO19** Deepika C Parameswarappa *Clinical characteristics of comorbid retinal dystrophies and primary angle closure disease*	**All Posters**
	11.48-11.56	**BSO26** Archana Padmanabhan Nair *Intraocular immune profile of dry AMD: a vision for now and future*	**BSO32** Ashu Shukla *Exploring the role of domain variants of fibronectin type III repeats of TENASCIN-C (Tn fnIII) in corneal wound healing–an in-vitro study*	**CLO14** Manisha Malani *Artificial intelligence in predicting the drug-transporter interaction: understanding the entry of systemic drugs into eye*	**CLO20** Dhivya Ashok Kumar *Semilunar sign of scleritis in cornea*	
	11.56-12.04	**BSO27** Apurwa P Samarath *Characterization of type 2 secretion system (T2SS) in ocular clinical isolates of Pseudomonas aeruginosa keratitis*	**BSO33** Athira Ramesh *PIWI/piRNA as a possible interactant in neurons for light perception and sleep cycle*	**CLO15** Sameera Nayak *Sight threatening intraocular infection after hospital discharge for Covid-19 treatment in south India*	**CLO21** Tarjani V Dave *Choroidal vascularity index in thyroid eye disease: comparison with controls and application in diagnosing non-inflammatory active disease*	
	12.04-12.12	**BSO28** Angayarkanni Narayanasamy *Metabolites relevant to pathogenesis of AMD impair phagocytosis machinery in the ARPE-19 cells: mitigating effect of active principles*	**BSO34** Divya Gopal *Synthesizing and characterizing novel peptide as a cargo for targeting human tenon fibroblast cells to modulate the fibrosis*	**CLO16** Swathi Kaliki *Artificial intelligence and machine learning in ocular oncology: Retinoblastoma*	**CLO22** Gazal Patnaik *Clinical profile and histopathological correlation in patients with sympathetic ophthalmia – a 28 years study from a tertiary eye care center in South India*	
	12.12-12.20	**BSO29** Sujithra Shankar *Novel corneal targeting cell penetrating peptide for management of corneal diseases and disorders*	**BSO35** Iswarya Radhakrishnan *Transplanted trabecular meshwork (TM) stem cells home to TM*	**CLO17** Vishal Raval *Macular Retinoblastoma: clinical presentation and treatment outcomes*	**CLO23** Gunjan Sharma *Intraocular immune response in different ocular TB presentations*	
	12.20-12.28	**BSO30** Sruthi Priya M *Effect of neddylation inhibition by MLN4924 on hypoxia induced retinal angiogenesis*	**BSO36** Ujjalkumar Subhash Das *Bio-intelligence in predicting tear penetration using QSPR approach*	**CLO18** Vivek Pravin Dave *Application and validation of a novel inflammatory score in the clinical grading of infectious endophthalmitis: the endophthalmitis management study – Report 2*	**CLO24** Raja Narayanan *Indian health outcomes, public health and economics research centre*	
Discussion	12.28-12.40	Q & A	Q & A	Q & A	Q & A	
iQUEST*	14.00 -15.00	iQUEST (IERG Quiz competition) Co-ordinators: Anthony Vipin Das & Joveeta Joseph				
Sponsor's Talk*	15.00 -15.15	Ivonne Petermann (10x Genomics) Biology at true resolution: Resolving complex biology of the eye at the single cell level				
	15.15 -15.30	Vineet Ratra (Johnson & Johnson) Changing standard of carein monofocal cataract surgery				
	15.30 -15.45	C M Wavikar (Alcon) VIVITY optical principle				

* All the keynotes, Orations, iQUEST & Sponsor's Talk will be in Hall-1

Sessions	Time	Hall 1	Hall 2	Hall 3	Hall 4	E-Poster Hall
Best of ARVO-India*	17.30 -18.00	Best of Vision Research Foundation, Sankara Nethralaya Moderator: Ronnie George				
	18.00 -18.30	Best of Aravind Medical Research Foundation, Dr G Venkataswamy Eye Research Institute Moderator: Kuppamuthu Dharmalingam				
	18.30 -19.00	Best of Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences Moderator: Thirumurthy Velpandian				
	19.00 - 19.10	Break				
Best of ARVO-India*	19.10 - 19.40	Best of Narayana Nethralaya Foundation, Narayana Nethralaya Moderator: Arkasubhra Ghosh				
	19.40 - 20.10	Best of Prof. Brien Holden Eye Research Centre, Hyderabad Eye Research Foundation, L V Prasad Eye Institute Moderator: Sayan Basu				
Valedictory*	20.10 - 20.20	27 years of Vision Research Dorairajan Balasubramanian				All Posters
	20.20 - 20.35	Final words Prashant Garg Ronnie George Kuppamuthu Dharmalingam				
	20.35 - 20.40	Vote of thanks Subhabrata Chakrabarti				

* All the sessions will be in Hall-1

List of E-Posters

ARVO-INDIA 2021
E-Posters: Basic Sciences

Abstract code	Presenter	Abstract code	Presenter
BSP1	A. S. Sriee Viswarubhiny	BSP35	Pinal D Trivedi
BSP2	Aastha Singh	BSP36	Pooja B. Malaviya
BSP3	Abhishek Sahoo	BSP37	Poonam Naik
BSP4	Akepogu Jacquelyn	BSP38	Prabhjot Kaur
BSP5	Akshi Valodara	BSP39	Prabhudatta Das
BSP6	Alice Arati Anthony	BSP40	Priyadarshini Sathe
BSP7	Aloysius Dhivya M	BSP41	Priysha Mishra
BSP8	Ashish Mishra	BSP42	Prakash Chermakani
BSP9	Attem Jyothi	BSP43	Ranjith Kondhuri
BSP10	Bharathi Bhogarurapu	BSP44	Rizza Abdul Nayeem
BSP11	Bharathidevi SR	BSP45	Samayitree Das
BSP12	Damala Mukesh	BSP46	Samir Bera
BSP13	Deeksha Prasad	BSP47	Saranya Pandi
BSP14	Derin Mary Thomas	BSP48	Sarmeela Sharma
BSP15	Dhanwini Rudraprased	BSP49	Saurabh Kumar
BSP16	Divya Ashok Pidishetty	BSP50	Shailja Tibrewal
BSP17	Divya Ranga	BSP51	Shalem Raj Padakandla
BSP18	Vinay Kumar Pulimamaidi	BSP52	Shanthini T
BSP19	Gorati Vani	BSP53	Sharada Ramasubramanyan
BSP20	InduVahi Veernala	BSP54	Sharmila Rajendran
BSP21	Jadi Praveen Kumar	BSP55	Siddharth Narendran
BSP22	Jaishree Gandhi	BSP56	Sima Das
BSP23	Jayasudha Rajagopalaboopathi	BSP57	Simarpreet Kaur
BSP24	Jilu Jaffet	BSP58	Souradip Chatterjee
BSP25	Kamini Khatak	BSP59	Spandita Pal
BSP26	Krishnakumar Subramanian	BSP60	Srilekha Sundaramurthy
BSP27	Lakshmi Priyankka Alagappan	BSP61	Suchita Pandey
BSP28	Lavanya Mayura Priya Easwaran	BSP62	Sudipta Mahota
BSP29	Madhuri Amulya Koduri	BSP63	Suganya Kandeeban
BSP30	Awasthy P Nair	BSP64	Susmita Chowdhury
BSP31	Maynak Chakraborty	BSP65	Swatilekha Hazra
BSP32	Mohammad Salman	BSP66	Trupti Agarwal
BSP33	Moksha L	BSP67	Vijay Kumar Singh
BSP34	Naheed Arfin Borah	BSP68	Waseema Arif

ARVO-INDIA 2021
E-Posters: Clinical Sciences

Abstract code	Presenter
CLP1	Agimanailiu K
CLP2	Akash Belenje
CLP3	Akshay Gopinathan Nair
CLP4	Anamika Patel
CLP5	Ananya Sudhir Nibandhe
CLP6	Anthony Vipin Das
CLP7	Bhupesh Bagga
CLP8	Brijesh Takkar
CLP9	Dhanukurekha Lakshmipathy
CLP10	Dilip Kumar Mishra
CLP11	Esther Sheba
CLP12	Harinee Rajagopalan
CLP13	Himansu Sekhar Behera
CLP14	Illakiya M
CLP15	Jyoti Rajput
CLP16	Jyotirmay Biswas
CLP17	Mani Vimalin Jeyalatha
CLP18	Manju Bhate
CLP19	Manju Varshini B
CLP20	Niveditha Narayanan
CLP21	Pragnya Rao Donthineni

Abstract code	Presenter
CLP22	Pratima Singh Thakur
CLP23	Priyanka Walvekar
CLP24	Rajesh Nagarajan
CLP25	Richa Dharap Wagh
CLP26	Sagarika Dash
CLP27	Saraswathi Kannan
CLP28	Shalini Singh
CLP29	Shanu Kumar
CLP30	Simmy Chaudhary
CLP31	Srishti Ramamurthy
CLP32	Subhadra Jalali
CLP33	Suneetha Gavara
CLP34	Supriya Sharma
CLP35	Sushmasri K
CLP36	Swapna S Shanbhag
CLP37	Ankit Kumar Bhopalka
CLP38	Umesh Chandra Behera
CLP39	Vandhana Sundaram
CLP40	Vineet Joshi
CLP41	Zarin Modiwala
CLP42	Ishwarya Suresh

ARVO-INDIA 2021
E-Posters: Optometry & Public Health

Abstract code	Presenter	Abstract code	Presenter
OPP1	Amithavikram Rugvedi H	OPP20	Raghav Narasimhan
OPP2	Asra Fatima	OPP21	Rahul Prashant Patil
OPP3	Bhagya Lakshmi Marella	OPP22	Ramya Natarajan
OPP4	Brughanya Subramanian	OPP23	Ranindita Saha
OPP5	Dhanalakshmi S	OPP24	Rohit Dhakal
OPP6	Dharani Boopalan	OPP25	Sabyasachi Goswami
OPP7	Janaki Raman P	OPP26	Sangeetha Nagarajan
OPP8	Janani S	OPP27	Shinjini Basak
OPP9	Khaarthiyaa Lokanathan	OPP28	Shravya Sri Durgam
OPP10	Krishnaveni Nagarajan	OPP29	Srinivas Marmamula
OPP11	Maanasi Mahalingam	OPP30	Suchana Shirodker
OPP12	Manoj K. Manoharan	OPP31	Suchismita Rout
OPP13	Mathew Francis	OPP32	Sujoy Mukherjee
OPP14	Monika Thakur	OPP33	Velmurugan K
OPP15	Prabhakar.G.V	OPP34	Venkateshwaran K,
OPP16	Preetirupa Devi	OPP35	Vijay Kumar Yelagondula
OPP17	Pritam Dutta	OPP36	Vijay Reena Durai
OPP18	Priyadarshana Bardoloi	OPP37	Vivek Suganthan R
OPP19	Priyajana		

Orations
&
Keynotes

Oration Awardee

S. S. BADRINATH ORATION

Dr. Usha Chakravarthy

Professor, Ophthalmology and Vision Sciences, Centre for Public Health, Royal Victoria Hospital and Queen's University Belfast, Northern Ireland

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Web: https://www.qub.ac.uk/research-centres/CentreforPublicHealth/Research/HealthServicesGlobalHealth/EyeandVision/Meetourteam/

Areas of interest: Epidemiology and risk factors for retinal diseases

The early detection of neovascular AMD what has the EDNA study taught us

EDNA was a diagnostic accuracy study. Fluorescein angiography was the cornerstone for diagnosing the onset of nAMD for decades. The world has changed since analog image capture with digital technological revolutions in fundus imaging. In EDNA the diagnostic accuracy of 5 commonly used pragmatic tests of retinal function and morphology were compared to FA for detection of new onset nAMD and their sensitivity and specificity assessed over the 3 year period of the study on over 550 participants with nAMD in one eye with the unaffected second eye nominated as the study eye. Comparisons were made on the functional and morphological features at conventional detection (first eye) versus early detection (EDNA study eye). The cost utility benefit was modelled. Lessons from the EDNA study will be presented.

Oration Awardee

D. BALASUBRAMANIAN ORATION

Dr. Miguel Seabra

Professor, Universidade Nova de Lisboa-Nova Medical School and Champalimaud Foundation, Lisbon, Portugal

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Areas of interest: Cellular and Molecular medicine

From gene identification to gene therapy in 20 years: the Choroideremia example

Choroideremia (CHM) is an inherited retinopathy clinically similar to Retinitis Pigmentosa where patients start with night blindness slowly progressing to full blindness by middle age. The CHM locus on the X-chromosome was among the first ten genes identified by positional cloning in the 1980´s by the Ropers lab. Working in a completely different subject, my lab discovered the function of the CHM gene in 1992. Most if not all mutations found in patients are loss-of-function mutations, therefore CHM patients could benefit from replacement gene therapy. Here, I will review our efforts to unravel the molecular pathogenesis of CHM and subsequently the preclinical work that led to the first clinical trial in the UK in 2011.

Oration Awardee

BIRESWAR CHAKRABARTI ORATION

Dr. Fiona Stapleton

Scientia Professor, School of Optometry and Vision Science,
University of New South Wales (UNSW), Sydney, Australia
Email: f.stapleton@unsw.edu.au

Web: https://www.optometry.unsw.edu.au/fiona-stapleton

Areas of interest: Pathogenesis of contact lens related infections

Global Epidemiology of Infectious Keratitis

Infectious keratitis is a rare but potentially severe sight-threatening disease, associated with considerable societal burden, cost and morbidity. This presentation will review recent evidence for the incidence, risk factors and impact of disease, all of which vary widely according to region, access to health care, socioeconomic and environmental factors, predisposing conditions and causative organisms. The frequency and societal impact of infectious keratitis are significantly higher in low-income countries. In non-viral infectious keratitis, bacterial causes predominate in most regions and causative organisms vary with predisposing condition. Fungi, particularly linked with agricultural trauma, are more frequently associated with infectious keratitis in low-income regions, particularly in India and certain African countries. The disease impact is compounded by poverty and limited access to services and treatment. Early diagnosis, access to appropriate treatment, prophylaxis in ocular trauma, availability of eye protection, awareness of risk factors may be associated with reduced disease severity and vision loss. Evidence for the incidence and burden of disease is lacking in certain regions and consequently well-designed epidemiological studies to identify independent risk factors for the disease and those associated with more severe outcomes may better identify causation and guide resource allocation and preventative strategies.

Keynote Lecture 1

Dr. Nathan Congdon

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Areas of interest: Global strategic approaches to eye diseases and designing models of eye care delivery

ENGINE: Research documenting how vision care can accelerate progress towards the SDGs across the life course

- ENGINE (Eyecare Nurtures Good-health, Innovation, driviNg-safety and Education) is designed to leverage high-quality research results from four component studies to drive lasting policy change and achieve improved quality of life for people in low and middle-income countries. The four research projects are:

- STABLE (Slashing Two-wheeled Accidents By Leveraging Eyecare) will assist local partners, including the Vietnamese Ministry of Transport, to combat Vietnam's twin epidemics of uncorrected short-sightedness and motorcycle crashes in the young.

- ZEAL (Zimbabwe Eyecare and Learning) will work with local partners who currently implement the Zimbabwe government's national school vision project to explore how targeting long-sighted children with novel, low-cost screening can add to the academic impact of the programme.

- CLEVER (Cognitive Level Enhancement through Vision Exams and Refraction) supports the Indian government's strategy of finding scalable, low-cost means of preventing dementia, the management of which currently consumes 1.5% of national GDP.

- THRIFT (Transforming Households with Refraction and Innovative Financial Technology) will capitalise on the Bangladesh government's novel and forward-looking plan to digitise all social safety net payments to the elderly by providing free glasses and training to help them better cope with unfamiliar smartphones, thus improving financial independence.

- CLEVER and STABLE are the first trials of their kind, examining how low-cost vision care can slow the pace of cognitive decline with aging and deliver safe roads in low-resource settings. Potential benefits of ENGINE extend far beyond the borders of participating countries:

- Traffic injuries are the leading cause of death globally between ages 5 and 29. While only 60% of vehicles are found in low and middle-income countries, 90% of traffic deaths occur there.

- The World Bank and others suggest Bangladesh's novel strategy of delivering safety net payments through e-banking to the elderly provides a model for many other countries, especially during COVID, if visual challenges for users are successfully met.

Keynote Lecture 2

Dr. Anat Galor

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Keynote Lecture 3

Dr. Bikash R. Pattnaik

RRF M.D. Matthews Professor, McPherson Eye Research Institute, Departments of Pediatrics, Ophthalmology & Visual Science, University of Wisconsin-Madison, Madison, USA

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Areas of interest: Ion channellopathy and intracellular signaling in ocular diseases

A vision for the future is the treatment for ion channelopathies

Inherited or spontaneous single-nucleotide mutations that produce premature termination codons (PTC) in retinal ion channel genes are a known cause of severe blindness. Such 'nonsense' mutations terminate RNA translation, leading to non-functional ion channel proteins that are otherwise essential to robust phototransduction. Depending on the location of a nonsense mutation, either no protein product will be produced, or a truncated protein product will be made that isn't assembled and/or trafficked normally. Current small molecule strategies to repair PTC result in the insertion of near cognate amino acid, resulting in missense mutations. These corrected missense mutations are poorly tolerated because of the complexities associated with precise post-translational modifications, carefully regulated expression, and assembly of ion channels. Further, small molecule read-through drugs, such as Ataluren, have short-lived pharmacological utility and have displayed poor *in vivo* efficacy. Although gene augmentation or editing have demonstrated potential, several problems remain to be addressed for these and other gene-based therapeutics (e.g., accommodating larger or more complex genes, off-target effects, and/or limited efficiency of targeted delivery). Within the retina, PTC mutations that disrupt the expression of ion channel genes cause severe forms of blindness. There are limited therapeutic interventions for PTC-related ocular diseases. The presentation will discuss recent developments that explore treatments for nonsense and rare mutations.

Keynote Lecture 4

Dr. Renu Kowluru

Professor and Director of Translational Research, Ophthalmology,
Visual & Anatomical Sciences, Wayne, State University, Detroit, MI, USA

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Areas of interest: Molecular mechanisms and metabolic memory in DR

Diabetic Retinopathy: Mitochondria in a Web of Epigenetics

Diabetic retinopathy remains the leading cause of acquired blindness in working-age adults. Despite cutting edge research by many leading laboratories, the exact molecular mechanism of this devastating disease remains unclear, limiting therapeutic options. Our laboratory has shown that retinal mitochondrial homeostasis is imbalanced in diabetic retinopathy, and mitochondrial dysfunction plays a central role in the pathogenesis of this sight-threatening disease. Emerging evidence suggests that the expression of a gene can also be regulated by epigenetic modifications, and diabetes facilitates many epigenetic modifications. This presentation will discuss the role of DNA methylation and long noncoding RNAs in mitochondrial dysfunction in diabetic retinopathy.

Dr. Manni L Guptasarma

Professor, Department of Immunopathology,

Postgraduate Institute of Medical Education and Research (PGIMER) Chandigarh, India

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Areas of interest: Protein misfolding-related diseases; ocular pathology; antibody engineering; DNA-based (molecular) diagnostics

Development of tools to explore amelioration of ocular diseases through the manipulation of components of the extracellular matrix

The components of the extracellular matrix (ECM) determine cellular behavior, in that modifications of ECM molecules end up signalling cells in ways that can effect a pathological condition. Over the years, we have contributed to the rational design and use of protein-engineered reagents (including single chain variable fragment or scFv antibodies) to titrate modified components with a view to ameliorating certain pathological conditions. We have demonstrated the success of this approach through the manipulation of the components of the ECM in the cornea, lens, vitreous and retina, with a focus on the bidirectional effector relationship that exists between the cells and ECM in these tissues, in the following three contexts: proliferative vitreoretinopathy (PVR), posterior capsular opacification (PCO) and posterior vitreous detachment (PVD). We use a combination of Protein Biochemistry, Biophysics and Protein Engineering on the one hand and tools and techniques of Molecular and Cellular Biology, including proteomics, transcriptomics and imaging on the other hand, in addition to histopathological and other techniques.

Keynote Lecture 6

Dr. Eric Pearlman

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Areas of interest: Innate immune response to parasitic, fungal, and bacterial infections

Fungal and bacterial keratitis – new insights into pathogenesis

Pseudomonas aeruginosa and the pathogenic fungal species *Fusarium* and *Aspergillus* are the major causes of corneal ulcers in India and worldwide. However, corneal disease is also a consequence of the immune response, especially the profound neutrophil infiltration at early stages of infection. Recent findings on microbial virulence factors and neutrophil activation provide increased insights into the pathogenesis of disease and identify potential targets for therapeutic intervention.

Keynote Lecture 7

Dr. Aparna Lakkaraju

Professor of Ophthalmology, School of Medicine, University of California, San Francisco, USA

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Areas of interest: Cellular mechanisms andidentifying therapeutic targets in AMD

Live imageing reveals novel insights into macular degenerations

Injury to the retinal pigment epithelium (RPE) eventually culminates in vision loss in age-related macular degeneration (AMD). Therefore, drugs that preserve RPE health and function could halt AMD at its earliest stages. We have recently identified ceramide as a novel drug target in AMD because it regulates complement activity, metabolic dysfunction, and inflammation, three pathways implicated in AMD pathogenesis. This presentation will discuss our recent studies using high-resolution live imaging, mouse models, and human donor tissues which demonstrate that clinically approved drugs that decrease RPE ceramide correct multiple dysfunctional pathways implicated in early AMD and safeguard RPE integrity.

Keynote Lecture 8

Dr. Taraprasad Das

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Areas of interest: Vitreo Retinal Diseases

Application of bed-side and bench research in the advancement of endophthalmitis care

Over the years, the incidence of endophthalmitis, particularly after intraocular surgery, has declined from 2% in the early 1900s to the current less than 0.1%. It owes to superior prophylaxis and targeted therapy. The current strategies are based on our ability to raise and answer relevant bedside questions through appropriate clinical and laboratory studies. These include understanding the worldwide variation in microbiology, choice of intravitreal therapy, the timing of vitrectomy, pre-operative antiseptic preparation of the eye, and intracameral antibiotic prophylaxis.

Our research has shown that (1) the spectrum of infective endophthalmitis in India is different from northern America and Europe with a higher gram-negative and fungal infection; (2) up to 5% of instances it could be multi-drug resistant; (3) cluster endophthalmitis, not uncommon in India, could often be traced using advanced molecular microbiology techniques; (4) intravitreal dexamethasone is beneficial in acute bacterial endophthalmitis; (5) intracameral moxifloxacin is as good as intracameral cefuroxime; (6) topical antibiotics may not be necessary after intracameral antibiotic; (7) endophthalmitis occurring after intracameral moxifloxacin responds better to the standard of care; (8) fungi are also ocular surface commensal; (9) many fungi are harvested in culture-negative endophthalmitis by the next-generation sequencing; (9) fungal infection needs a different treatment; and (10) specific inflammatory markers could help in rapid diagnosis. Further research is needed to identify and reduce nosocomial infection; fashioning intraocular drug depository formulation for short-term antibiotic delivery into the eye; discovery of new antibiotics that do not develop resistance quickly; and further insight into culture-negative endophthalmitis.

ABSTRACTS
(FREE PAPERS)

BASIC SCIENCES

INVOLVEMENT OF *GLIS1* IN THE PATHOGENESIS OF PRIMARY CONGENITAL GLAUCOMA

Goutham Pyatla[1], Samir Bera[1], Laura M. Leon[4], Alice Anthony[1], Anil K Mandal[2,3], Ashish Mishra[1], Hameed Syed[1], Sneha Kumari[1], Sirisha Senthil[2,3], Inderjeet Kaur[1], Hemant Khanna[4], Subhabrata Chakrabarti[1]

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Purpose: Primary congenital glaucoma (PCG) is an autosomal recessive disease that occurs due to developmental defects in the trabecular meshwork (TM) and anterior chamber angle, resulting in raised intraocular pressure (IOP) along with damages to the optic nerve. The known candidate genes *(CYP1B1, LTBP2, TEK, MYOC* and *FOXC1)* contribute to <50% of these cases. Recently, it was shown that *GLIS1,* a kruppel like zinc finger transcription factor, plays a critical regulatory role in maintaining the structure of the TM and regulating IOP. The present study aimed to identify the possible involvement of *GLIS1* in PCG pathogenesis.

Methods: All the exons of *GLIS1* underwent bi-directional sequencing in PCG cases (n=354) and controls (n=354) using the BigDye chemistry. The data was analyzed in conjunction with other PCG-associated candidate genes. Ingenuity pathway analysis (IPA) was undertaken to understand the functional interactions of *GLIS1*.

Results: We identified 13 non-synonymous rare variants in 27 PCG cases (3.67%) of which 8 (p.S252F, p.R294Q, p.P340L, p.R414H, p.Y415C, p.R515H, p.P605L and p.Y706H) were predicted to be pathogenic and were either absent or rarely observed in controls and global databases. Overall, 16/27 PCG cases exhibited co-occurring mutations in the candidates along with *MMP9*. IPA suggested that *GLIS1* interacted through *wnt*, PI3K/AKT and Nf-κB pathways and regulated *MMP9*.

Conclusions: The *wnt* and PI3K/AKT pathways are known to maintain TM integrity and aqueous humor outflow along with protection of glaucomatous axonal damage. Thus, mutations in *GLIS1* and candidate genes may contribute to developmental defects and RGC death in PCG.

A SPECTRUM OF CANDIDATE AND MITOCHONDRIAL GENE MUTATIONS IN USHER SYNDROME PATIENTS FROM INDIA

Harshavardhini G[1], Porkodi Periyasamy[1], Rajiv Raman[2], Akila Ramkumar[3], Vikas Khetan[2], Parveen Sen[2], Sripriya S[1]

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Purpose: Usher syndrome (USH) is a rare syndromic form of retinitis pigmentosa (RP) associated with deaf-blindness inherited in an autosomal recessive manner. The abstract describes the spectrum of mutations observed in USH patients from India.

Methods: The patients with confirmed diagnosis of USH were recruited based on the detailed clinical evaluation. Targeted re-sequencing of both IRD and non-syndromic deafness genes was performed in Illumina HiSeq platform. The data obtained was annotated and filtered for pathogenic variations in candidate genes. In addition, the mitochondrial variation spectrum was also assessed in the study cohort.

Results: Novel disease causing mutations were observed in 53 out of 73 patients in USH2A (28%), *MYO7A* (18%), *CDH23* (9%), *GPR98* (8%), *PCDH15* (4%), *USH1C* and *CLRN1* (3% each) and *USH1G* (1%) genes. Five percent of the patients had either a single heterozygous (N=4) or digenic mutations (N=1) in USH genes. A higher frequency of mitochondrial variants with MAF$\leq$0.0005 were observed in patients with USH2A, MYO7A and CDH23 gene mutations and early disease onset compared to the others.

Conclusions: Disease segregating recessive mutations in USH genes were observed in 73% of the study cohort and USH2A gene was frequently mutated (28%). We were able to classify the cohort into Type I (32%), Type II (34%) and Type III (2%) based on the molecular spectrum. This is the first genetic study in a large cohort of Usher syndrome patients from India which gains importance in the context of emerging clinical trials for gene therapy in these patients.

STATUS OF SARS-COV-2 SPECIFIC PRO- & ANTI-VIRAL GENES ON THE OCULAR SURFACE

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Purpose: To investigate the expression of SARS-CoV-2-specific host attachment, entry and anti-viral response genes in the various layer of donor eyes, including the ocular surface.

Methods: Cadaver eyes from health controls (n=5), COVID-19 infected (n=5) and COVID-19 recovered (n=2) donors were used for the study. Tissue sections were prepared from the donor eyes for immunofluorescence imaging. Dissection of ocular tissues based on different anatomical regions of the eye also performed was used for RNA extraction. Impression cytology from controls (n=8) and dry eye disease (DED) patients (n=7) obtained during pre-pandemic period was used to for RNA extraction. Expression of ACE2, TMPRSS2, CTSL, type 1, 2 and 3 IFNs (interferons) were assessed by immunofluorescence microscopy and/or quantitative PCR.

Results: Increased expression of ACE2, TMPRSS2, CTSL were higher in ocular surface layers compared to other regions/layers of the eye. Further, increased ocular surface expression of ACE2 and TMPRSS2; along with decreased expression of type 1 IFNs (IFN alpha) was observed in COVID-19 infected eyes compared to healthy controls and COVID-19 recovered eyes. A similar expression pattern was observed on the ocular surface of DED patients compared to controls.

Conclusions: The expression of host factors aiding the attachment and entry of SARS-CoV-2 and interferon signatures were observed to be higher in the ocular surface compared to other regions of the eye. Ocular surface of DED subjects exhibits higher expression of ACE2, CTSL and TMPRSS2. Hence, topical agents that alters such profile would be beneficial in the prophylaxis against ocular manifestation associated with COVID-19.

GLOBAL MICRORNA PROFILING FOR UNDERSTANDING THE PATHOGENESIS OF NON-INFECTIOUS UVEITIS

Prerna Kulshrestha[1,2], Pallavi Goel[1], Somasheila Murthy[3], Soumyava Basu[4], Mudit Tyagi[4], Pratik Gogri[5], Inderjeet Kaur[1]

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Purpose: Uveitis is a major sight-threatening disorder. It can be autoinflammatory, autoimmune, infectious disease or associated with any systemic conditions, resulting from immune dysregulation. Recently, microRNAs (miRNAs) have been identified as important regulators in various inflammatory disorders and autoimmune responses. We aimed to identify the miRNAs involved in uveitis pathogenesis and as future predictive markers for its progression.

Methods: Total RNA was extracted from blood samples collected from non-infectious uveitis and controls (n=6 in each category). RNA quality and quantity was measured by Agilent Bioanalyzer and Qubit 2.0 followed by short RNA sequencing on Illumina HiSeq platform. The raw data was checked for quality of sequencing and then analysed (including alignment, differential expression analysis and pathway analysis) using PARTEK software.

Results: Initial data analyzed for a subset of 8 samples (4 in each category) revealed 457 differentially expressed miRNAs, of which, 23 are found to be significantly deregulated with a p-value $\leq$0.05 and fold change of 2 or more. 11 miRNAs are downregulated whereas 12 are found to be upregulated. Several novel miRNAs are also identified that are being further characterized. The major pathways associated with the deregulated miRNAs included nuclear factor-κB (NF-κB), PTEN-AKT-mTOR, MAPK/EGR, JAK–STAT and Complement pathway. Further characterization by multiplex ELISA confirmed the activation of complement pathway proteins in the eye.

Conclusions: MiRNA expression profiles of uveitis patients are significantly different from the controls. Complement activation leading to inflammation further mediated by the nuclear factor-κB (NF-κB) pathway contributes to chronic inflammation seen in these patients.

WHOLE EXOME SEQUENCING IDENTIFIES *SLC6A6* AS NOVEL CANDIDATE GENEIN LEBER CONGENITAL AMAUROSIS

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Purpose: The overlapping clinical feature among retinal degenerative diseases (RDD) makes it difficult for differential diagnosis. In this study, whole exome sequencing (WES) was done to identify the causative gene(s) among the initially diagnosed Leber Congenital Amaurosis (LCA) cohort that did not show known variants associated with LCA by targeted resequencing.

Methods: WES was performed in Illumina HiSeq 2500 platform for 22 families (N=44) that were excluded for mutations in LCA genes. The pathogenicity of the identified variants were confirmed by co-segregation analyses, control screening and *insilico* prediction. Homology modelling using Modeller-9.23 and molecular simulation in membrane system was performed for the novel mutation in *SLC6A6 gene.*

Results: We obtained approximately 8 Gb data/ sample, with ~100X coverage and Q30 quality score. Disease causing pathogenic variants were identified in 17 families (77%)- 65% in LCA candidate genes *(AIPL1, ALMS1, CRB1, LCA5, RPGRIP1, SPATA7)*, 29% in other retinal degenerative disease genes *(AHI1, CNGA3, CNNM4, MYO7A, RPGRIP1L)*. Also, an extensive analysis of exome sequencing data revealed anovel p.(Pro82Leu) variant in *SLC6A6* gene. By homology modelling and molecular simulation, the mutation resulted in higher number of α-helical structure around the TM2helix when compared to the wild type. This transition could potentially hinder the regular function of the taurine transport cycle, probably causing disruption in transport oftaurine across the membrane leading to disease phenotype.

Conclusions: We report the first case of *SLC6A6* gene mutation in LCA from India. Among the other cases, molecular testing helped in re-diagnosis of 29% cases as eithersyndromic or non-syndromic IRDs.

DYSREGULATED EXPRESSION OF MICRORNAS IN VITREOUS HUMOUR FROM INTRA-OCULAR TUBERCULOSIS PATIENTS

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Purpose: Dysregulated expression of microRNAs (miRNAs), by *Mycobacterium tuberculosis* (MTB) infection, was reported to modulate the host defence mechanism and also helpsto distinguish between systemic TB or latent TB patients with healthy individuals. In this study, we aim to identify the altered expression of miRNA expression in vitreous humour (VH) of patients with intra-ocular TB (IOTB).

Methods: In total of 500 µl of VH was collected from each sample from IOTB (n = 9) and macular hole (n = 9) patients. Three IOTB and macular hole samples were selected for small-RNA deep sequencing. Total RNAs were extracted, libraries were prepared with GenXPro low-input small RNA library preparation kit and sequenced on a Hiseq 2000Illumina system. Differential miRNA expression analysis was carried out in-house bioinformatics pipeline. Further, the identified miRNAs were validated using quantitative PCR (RT-qPCR). miRNA targets were predicted using miRWalk and theirfunctional enrichment analysis was carried out using DAVID and g:profiler.

Results: Totally, forty-three miRNAs were upregulated and thirty-eight were downregulated. Among them fourteen miRNAs were selected for RT-qPCR analysis. Three miRNAs hsa-miR-21-5p, hsa-miR-26b-5p, and hsa-miR-150-5p were showed significant upregulation with fold change of above two and P-value <0.05. Pathway enrichment analysis showed a significant association of all three miRNAs with TB related pathways.

Conclusions: Our study is first to report the dysregulated miRNAs in intra ocular tuberculosis (IOTB) that may play a major role in pathogenesis of IOTB. The confirmation of selected miRNAs with large cohorts, could provide a better diagnostic marker for IOTB.

UNTARGETED METABOLOMIC INVESTIGATION OF GLAUCOMATOUS PATIENTS USING HIGH RESOLUTION MASS SPECTROMETRY

Lakshminarayanan Gowtham[1], Nabanita Halder[1], Dewang Angmo[2], Sundararajan Baskar Singh[3], Rama Jayasundar[4], Tanuj Dada[2], Thirumurthy Velpandian[1]

[1]Ocular Pharmacology and Pharmacy Division, [2]Department of Ophthalmology, Dr. Rajendra Prasad Centre for Ophthalmic Sciences; [3]Department of Biophysics, [4]Department of NMR, All India Institute of Medical Sciences, New Delhi, India
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Purpose: To understand the metabolic dysfunction underlying complex pathological mechanisms of glaucoma may help discover disease pathways, novel biomarkers and rationalize newer therapeutics. This study aims to compare the metabolomic alterations in the aqueous humor and plasma of primary glaucomatous patients.

Methods: This study cohort comprised of primary open-angle (POAG), primary angle-closure (PACG) glaucomatous groups, and age as well as sex-matched cataract control. Aqueous humor (70 to 100µL) and plasma (2mL) samples were collected during trabeculectomy and cataract surgeries, snap-frozen at -80°C, and subjected for high-resolution mass spectrometry (HRMS) analysis. Spectra were processed and the data were acquired using Xcalibur and Compound Discoverer (V4.1) respectively. Univariate and multivariate statistical analyses were carried out using Metaboanalyst ver 5.0.

Results: Overall 12 and 9 metabolites were found to be significantly altered ($p<0.05$, variable importance of projection >1 and log2 fold change $\geq 0.58/\leq -0.58$) in the aqueous humor of POAG and PACG patients, respectively. Plasma metabolomics helped to rule out the metabolites altered in the aqueous humor under the systemic influence. Interestingly, 46.6% and 56% of metabolites found its unique association in the plasma of POAG and PACG, respectively. Pathway analysis has revealed altered galactose, amino sugar, and nucleotide sugar metabolism in the aqueous humor of PACG patients. Altered TCA cycle, sphingolipids, glutamate, and glutamine metabolism are the pathways with significant impact in POAG.

Conclusion: This study provides newer insights into the distinctly localized metabolites of aqueous humor differentially altered in the POAG and PACG from their respective alterations in the systemic circulations.

ANTI-GLAUCOMA MEDICATIONS LOWERED DECORIN AND ALTERED PROFIBROTIC PROTEINS IN HUMAN TENON'S FIBROBLAST

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Purpose: Long term exposure to anti-glaucoma medications (AGMs) lead to the increase in extracellular matrix (ECM) accumulation in primary glaucoma patients. This study aims to evaluate the effect of topical AGMs in primary human tenon's fibroblasts (HTFs) and analyze the expression on profibrotic and anti-fibrotic proteins.

Methods: Primary HTFs were cultured from patients undergoing cataract (control) and trabeculectomy. The different types of AGMs in single/multiple combinations (BB, PG, AA, CAI, CH, combinations of 3- PG+AA+CAI, 4A- BB+PG+AA+CAI, 4B-BB+PG+CAI+CH, 5- BB+PG+AA+CAI+CH) on chronic exposure were tested for cell viability using MTT assay and morphological alterations. Profibrotic proteins mainly SPARC, LOXL2, COL1A1 and anti-fibrotic DCN were analyzed in treated HTFs using q-PCR and ELISA. Sirius red staining and collagen gel contraction (CGC) assay were performed to assess collagen synthesis and the contractility of HTFs, respectively.

Results: Except AA and CH, the other AGMs at a higher concentration were found to decrease the cell viability of HTFs. The morphology of HTFs were altered on exposure to BB, CH and AA; Profibrotic proteins i.e., SPARC, LOXL2 and COL1A1 were significantly increased ($p < 0.05$) on exposure to combination of AGMs with TGF-β1, whereas the anti-fibrotic DCN expression was significantly lowered ($p < 0.05$) in single/multiple AGM. Sirius red staining showed increased collagen synthesis with CAI and combinations of AGMs with TGF-β1. Meanwhile HTFs showed increased collagen gel contraction with TGF-β1, CAI and CH.

Conclusion: This study reveals that altered profibrotic proteins, with significantly lowered DCN on chronic exposure of AGMs in HTFs.

EXOPROTEOME OF CLINICAL ISOLATES OF *FUSARIUM SOLANI*

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Purpose: Exoproteome of clinical isolates of *F. solani* isolated from keratitis patients who respond and those fail to respond to antifungal treatment.

Methods: Genomic DNA was extracted from the fungal cultures (n=36) isolated from fungal keratitis patients and sequenced using ITS1/4 primers. The fungal species were identified from the sequences using NCBI BLAST. The fungal exoproteome was extracted by SSF methodfrom different corneal isolates of two groups (group 1: responders to antifungal treatment; group 2: non-responders to antifungal treatments) and the 1D & 2D protein profiling was done. The total proteins were identified by shotgun LC-MS/MS and the differences in the exoproteome werecompared between the two groups.

Results: Sequencing data showedthat66.7% of the species identified were *Fusarium solani,* thereby being thepredominant pathogen causing fungal keratitis. The 1D and 2D protein profiles of the responders and non-responders were compared for notable changes in the band patterns. The 1D profile didn't show any significant difference whereas the 2D profile showed variation in the protein spots among different clinical isolates. Mass spectrometric data showed that more than 1000 proteins were identified in each group, and among the identified proteins ~50% are common proteins. The functional annotation revealed that oxidoreductases and hydrolases form the predominant classes of proteins in the fungal exoproteome.

Conclusions: Our study revealed that *F. Solani* secretes a complex protein profile, which is distinct in the isolates from responders and non-responders. Secretome represents proteins involved in signaling, pathogenesis etc.

UNTARGETED METABOLOMIC ANALYSIS OF AQUEOUS HUMOUR IN FUCHS ENDOTHELIAL CORNEAL DYSTROPHY IDENTIFIES UNIQUE SIGNALLING MODULES

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Purpose: Fuchs endothelial Corneal Dystrophy (FECD) is a degenerative corneal disease resulting in loss of vision. The loss of endothelial cells and formation of intracellular guttae on the Descemet's membrane are the primary hallmarks of the disease. In order to elucidate whether the cellular dysfunction is associated with altered secretion of metabolites, we performed a global metabolomics assay in aqueous humour from patients. Pathways affecting altered metabolic profiles may reveal possible new druggable targets.

Methods: In the current study we used aqueous humour collected from 6 FECD patients and 6 cataract age/sex matched controls to study the metabolite profile. Metabolites were precipitated using Methanol-Ethanol (1:1) followed by Triple TOF Mass spectrometer analysis in both positive and negative mode. Markerview v1.3.1 software was used for statistical analysis and KEGG database was used for pathway analysis.

Results: Within the identified 9890 spectra, 156 and 106 metabolites were found to be significantly (p-value < 0.05) downregulated and upregulated respectively (Log Fold change <-1 and >1), between FECD samples as compared to control. 6 metabolites were uniquely expressed in FECD patient samples. The differentially expressed metabolites were found to be majorly involved in the PPAR signalling pathway, necroptosis, arachidonic acid metabolism, linoleic acid metabolism and sphingolipid signalling pathway.

Conclusions: The differential metabolite expression indicates the role of aberrant cellular signalling such as apoptosis, mitochondrial dysfunction, oxidative stress and inflammation as key players in FECD pathogenesis. In addition, the identified differential metabolites can serve as potential biomarkers for FECD.

ELASTIN INTERACTING PARTNERS ARE DIFFERENTIALLY EXPRESSED IN PSEUDOEXFOLIATION SYNDROME

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Purpose: Pseudoexfoliation syndrome (PXF) is a complex late-onset elastosis disorder of unknown etiology, primarily affecting the eye. Accumulation of Pseudoexfoliative material (PXM) predisposes to PXF-glaucoma (PXF-G). Elastin dysregulation associated with the disease is incompletely understood. This study assessed the differential elastin binding proteins in PXF to further the understanding of the disease mechanism.

Methods: Aqueous humor (aqH) samples (age/sex-matched) were collected from Cataract, PXF and PXF-G cases undergoing extracapsular cataract extraction for Cataract. The aqH samples were subjected to immunoprecipitation (IP) of Elastin, followed by SDS-PAGE of the pellet protein, in-gel digestion and peptide profiling by MALDI-TOF analysis to identify interacting partners. Interacting partners of Elastin were also obtained in silico based on string analysis and based on structural homology with Elastin derived peptides. Further, the KEGG pathway analysis of the identified interacting proteins was done using the DAVID annotation tool.

Results: SDS-PAGE analysis of the IP protein resulted in 3 major bands ~ 70 kDa, 50 kDa and 25 kDa. The proteomic analysis of the bands revealed, 88 protein characteristic of PXF. The biological functions of these proteins were chiefly related to cellular processes (29.9%) metabolic processes (25.3%) and biological regulation (12.6%). The major pathways involved included inflammation (22.2%) followed by apoptosis signaling, TGFβ signaling, p53, and dopamine receptor-mediated pathway. Further, the involvement of nuclear binding proteins and integrin signaling is highlighted both by wet and dry lab studies.

Conclusion: Thus the study revealed inflammation and other novel pathways involved in the PXF disease mechanism.

IDENTIFICATION OF ANTIGENIC PROTEIN OF *ACANTHAMOEBA CASTELLANI* FOR THE DIAGNOSIS OF ACANTHAMOEBA KERATITIS

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Purpose: Acanthamoeba keratitis (AK), is a rare, vision threatening, parasitic infection caused by *Acanthamoeba castellani*. The major challenge in developing an accurate, rapid diagnostic assay is the selection of a specific diagnostic marker. Due to localized parasitic infection, detection of serum antibodies to AK antigens is impossible. Therefore, there is a need to identify an antigen and design a rapid diagnostic platform. Mannose-binding protein (MBP) of *Acanthamoeba* plays a key role in pathogenesis by mediating adhesion of parasites to host cells. Here we have identified the highly antigenic determinant of MBP as a diagnostic marker.

Methods: *A. castellani* was maintained on 2% non-nutrient agar plate seeded with active 48-h *E. coli* at 30°C. DNA was isolated and subjected to PCR for species confirmation using 18S rRNA gene. Linear B-cell epitope prediction of MBP was performed by using sequence retrieved from NCBI.

Results: Trophozoites were identified using inverted microscope. Species confirmation (500 bp) was done by PCR followed by gel electrophoresis. The sequence was found to be highly accessible in nature by Emini Surface Accessibility Prediction and showed that the maximum residues were having more than 1 score. Antigenicity analysis by Kolaskar & Tongaonkar showed the maximum residues having score more than 1, implying the sequence is highly antigenic in nature. Further studies are in progress.

Conclusions: MBP is highly antigenic and accessible in nature, and could act as a diagnostic marker for AK. We thank to ICMR for providing fellowship to Dr Saumya for this work.

TOXICITY OF ENDOGENOUS CYTOPLASMIC ALU COMPLEMENTARY DNA IN AGE-RELATED MACULAR DEGENERATION

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Purpose: Geographic atrophy (GA) is an untreatable late stage of age-related macular degeneration (AMD). Accumulation of toxic Alu RNA transcripts in the retinal pigment epithelium (RPE) is involved in the pathogenesis of GA. Recently, it was reported that Alu RNA toxicity mediated via the synthesis of long interspersed nuclear element-1 (L1) reverse transcriptase (RT)-mediated synthesis of Alu cDNA in the cytoplasm. The purpose of this study was to determine the mechanism of Alu cDNA-mediated RPE toxicity in GA, which has been unknown.

Methods: L1 RT activity was tested in nuclear and cytoplasmic fractions to identify the site of Alu cDNA synthesis. L1 was targeted by siRNA. Alu RNA was subretinally transfected into wild-type C57BL/6J mice or L1-extinct rice rats (Oryzomys palustris). RPE degeneration was assessed by fundus photography and ZO-1 immunostaining.

Results: An siRNA targeting L1 prevented the formation of Alu cDNA in primary human RPE cells and also blocked Alu RNA-induced RPE degeneration in mice. Alu cDNA is engaged by the DNA sensor cGAS to induce cytosolic mitochondrial DNA escape, which amplifies cGAS activation, triggering RPE degeneration via the inflammasome. The L1-extinct rice rat (Oryzomys palustris) was resistant to Alu RNA-induced Alu cDNA synthesis and RPE degeneration, which were restored upon L1-RT overexpression.

Conclusions: Non-genomic Alu cDNA was detected in the macular RPE of human eyes with GA. These findings may reveal a novel mechanism of the life cycle of Alu RNAs in GA, and also provide a molecular rationale for evaluating the potential therapeutic activity of nucleoside reverse transcriptase inhibitors (NRTIs), which inhibit both L1-RT and inflammasome activity, to block Alu cDNA toxicity in GA.

HETEROGENEITY USING NOVEL INVARIANT GENE EXPRESSION ANALYSIS AND LIPIDOMICS IN RETINOBLASTOMA

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Purpose: To study drug resistance to cell cycle inhibitors and tumor heterogeneity in Retinoblastoma

Methods: We have developed invariant differential expression analysis (iDEA) that improves the state of the art in differential expression analysis (DEA). iDEA uses strong Boolean implication relationships in a large diverse human dataset GSE119087 (n = 25,955) to filter the noisy differentially expressed genes (DEGs). iDEA was applied to RB datasets and a gene signature was computed that led to prediction and mechanism of drug sensitivity. The prediction was confirmed using drugs-sensitive/resistant RB cell-lines and mouse xenograft models using CDC25 inhibitor NSC663284. iDEA improved reproducibility of differential expression across diverse retina/RB cohorts and RB cell-lines with different drug sensitivity (Y79/Weri vs NCC Rb 51). Mass spectrometry based lipidomics was done on MIOM1 (normal), WERI (less aggressive) and NCC Rb 51 (more aggressive drug resistant phenotype) using reversed phase HPLC connected to Q Exactive plus Mass spectrometer.

Results: Pathway analysis revealed WNT/β-catenin involved in distinguishing drug sensitivity to CDC25 inhibitor NSC663284. NSC663284 inhibited tumour cell proliferation in mouse xenograft model containing Y79 cells indicating novel therapeutic option in RB. Differential gene expression involved in lipid pathway were observed by DNA microarrays between Retina, Non-invasive and Invasive RB samples. Lipidomic studies reveal dysregulation of ceramide and diacyl glycerol compounds in cancer samples compared to MIOM1.

Conclusions: Our results indicate heterogeneity in RB tumours/cells for drug resistance, gene expression and lipidomic expression especially ceramides. Our results should pave way for targeted therapy in RB.

IN SILICO IDENTIFICATION OF PUTATIVE ALTERNATIVES TO OCULAR CHEMOTHERAPEUTIC AGENTS: HIGH-THROUGHPUT VIRTUAL SCREENING AND MOLECULAR DYNAMICS STUDIES OF NATURAL PRODUCT INHIBITORS TARGETING HUMAN DNA TOPOISOMERASE II BETA

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Purpose: Topoisomerase II poisons like etoposide and doxorubicin are a routine part of the chemotherapeutic regime used for treating ocular malignances. However, their benefits are outweighed by side effects such as the development of cardiotoxicity, secondary malignancies, and multidrug resistance. Therefore, catalytic inhibitors targeting the nucleotide-binding cavity of the enzyme serve as safer alternatives due to their less deleterious mechanism of action. In this study, we carried out virtual screening of natural compounds followed by comprehensive validation to identify potent catalytic inhibitors of human DNA topoisomerase II beta (hTopIIβ).

Methods: The ATPase domain of hTopIIβ was comparatively modelled using Modeller v 9.20. Structure-based HTVS of the NPASS database was carried out using the Glide docking workflow (Schrodinger software suite). Binding free energy and ADMET properties of the top 100 hits were evaluated through various tools. Finally, two favourable hits were subjected to 50 ns molecular dynamics simulations using the Desmond package and the ligand-binding affinities were analysed.

Results: We identified two natural compounds, Penicacid C and Caffeoylmalic acid as top hits with docking scores of -13.88 kcal/mol and -15.33 kcal/mol, higher than reference inhibitor Salvicine (-6.89 kcal/mol). MM/GBSA and ADMET analyses were promising for these compounds. The compounds were stable during MD simulations and showed a good hydrogen bond interaction pattern within the ligand-binding cavity.

Conclusions: Natural compounds are ideal starting points for the development of new drugs. We have identified two novel hTopIIβ inhibitors which may serve as potent and safe alternatives for chemotherapy drugs.

CRISPR/CAS9 NANOMEDICINE THERAPY FOR THE MANAGEMENT OF WET-AGE RELATED MACULAR DEGENERATION

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Purpose: Since wAMD patients fail to respond to anti-VEGF injections, an alternative therapy is needed. CRISPR/Cas9 Ribonucleoprotein (RNP) mediated knockout of the VEGF-A gene could be a potential approach. Considering the viral vector's drawbacks in delivering Cas9, a non-viral nano-carrier could provide a better strategy for treating wAMD.

Methods: An amphiphilic block copolymer was synthesized by ring-opening polymerization and then grafted with functional ligands such as a cationic chain, cholesterol, and morpholine. The copolymer was utilized to make blank cationic nanoplexes, followed by incubation with RNPs to produce RNPs nanoplexes. Particle size, zeta potential, TEM, complexation efficiency, transfection efficiency, nuclear localization, and *in vivo* retinal fate were studied to evaluate the efficiency of RNPs nanoplexes.

Results: The obtained RNPs lipopolymeric nanoplexes were found to be spherical with a size and zeta potential of 117.3 nm and +6.17 mV, respectively. The lipopolymer formed complexes with RNPs at a ratio of 1:10 (w/w). The nanoplexes were able to efficiently transfect ARPE-19 and NIH3T3 cells after 6 h of incubation with a retained DNA binding/endonuclease property, as per CasFISH assay findings. The RNPs nanoplexes also successfully enter the retinal cells after 48 h of intravitreal injection in rats.

Conclusion: Cas9 RNPs loaded lipopolymeric nanoplexes fulfilled the necessary measures for its use in retinal delivery.

GENOME-WIDE TRANSCRIPTOME PROFILING OF GLUCOCORTICOID RESPONDER AND NON-RESPONDER PRIMARY HUMAN TRABECULAR MESHWORK CELLS USING RNA-SEQUENCING AFTER DEXAMETHASONE TREATMENT

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Purpose: To investigate genes and pathways involved in the differential glucocorticoid (GC) responsiveness in human trabecular meshwork (HTM) cells using RNA sequencing (RNA-seq).

Methods: In a paired human donor eyes, human organ-cultured anterior segment (HOCAS) was established in one eye to characterize their GC responsiveness based on the IOP change (>5mmHg) and primary HTM cell culture was established in the other eye. Total RNA was extracted from cultured HTM cells with known GC responsiveness (GC-responder (GC-R; n=4) and GC-non-responder (GC-NR; n=4) for RNA-seq after treatment with either 100nM dexamethasone (DEX) or ethanol (ETH) for 7 days. Differentially expressed genes (DEGs) were compared among 5 groups (Group#1: ETH vs DEX-treated GC-R; #2: ETH vs DEX-treated GC-NR; #3: overlapping DEGs between Group #1 and #2; #4: Unique DEGs of GC-R and #5: Unique DEGs of GC-NR) and validated by qPCR array.

Results: The DEX-treated eyes showed significant elevated IOP in 7/16 eyes (43.7% responders). In total, 616 and 216 genes were significantly dys-regulated in Group #1 and #2 respectively. Around 80 genes were commonly dys-regulated in Group #3 whereas 536 and 136 genes were uniquely expressed in GC-R (#4) and GC-NR HTM (#5) cells respectively. Pathway enrichment analysis revealed that WNT signaling, TGF-β signaling, cell adhesion, MAPK, drug metabolism and cytochrome p450 signaling were associated with GC responsiveness.

Conclusions: This is the first study reporting a distinct gene signature and their associated pathways for GC-R and GC-NR HTM cells. This will help in identifying suitable molecular targets for the management of GC-induced glaucoma.

ENHANCED STABILITY OF SODIUM ASCORBATE FOR OCULAR EMERGENCY BY USING TRANSRECON™

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Purpose: Ascorbate eye drops are used in emergency management of corneal burn. Extemporaneous drug dispensation requires qualified pharmacists and sterile techniques. Maintaining physicochemical and biological stability of reconstituted formulations during storage is a constraint. TransReCon™, an invention allows instant onsite, sterile preparation of an aqueous solution/suspension of the active ingredient without additional need of any skilled personnel. Freshly ascorbate aqueous solution is highly unstable and its sterile emergency preparation is a practical difficulty in remote ophthalmologist's clinic. Therefore the objective of present study is the development of a stable formulation of ascorbate for its instant sterile reconstitution and use in clinics.

Methods: Innovative TransReCon™ technique has been adopted for the immediate reconstitution of sterile formulation of 10% ascorbate made in sterile conditions in aseptic pharmaceutical drug dispensing facility. Approved chemical stabilizers were tested for their utility to increase the shelf life of ascorbate after its reconstitution. Ascorbate content in the formulation were analysed on 0 and 7 days using HPLC with UV detection. Osmolarity and pH were also recorded to monitor formulation stability.

Results: The use of 0.01% of sodium metabisulphite significantly increased the stability of 10% ascorbate up to day 7 within pharmaceutically accepted limits. No significant decrease in pH and osmolarity were observed in the same indicating it's utility in clinics.

Conclusions: TransReCon™ is a simple, cost effective and unskilled person-friendly device for ocular emergencies. Moreover, the successfully developed reconstituted formulation was found to increase the shelf life upto 7 days for its use in rural setup.

RETINAL PIGMENT EPITHELIUM (RPE) CELLS GENERATED FROM OCULOCUTANEOUS ALBINISM TYPE IA (OCA1A) PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL (iPSC) LINE MIMICS DISEASE PHENOTYPE OF PIGMENTATION DEFECTS

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Purpose: Oculocutaneous albinism (OCA) is a group of rare inherited autosomal recessive disorders caused by lack of melanin biosynthesis. OCA1A subtype represents the most severe form with mutations on TYR gene resulting in lack of pigmentation in skin, hair and eyes. Further, it is manifested by low visual acuity and intense photophobia due to defective foveal development. The purpose of this study was to model OCA1A in vitro and identify drugs to improve pigmentation.

Methods: Peripheral blood mononuclear cells from an OCA1A patient were reprogrammed using episomal vectors (Yamanaka factors) to create OCA1A-iPSC line. Healthy iPSC (control) and OCA1A-iPSC were differentiated to retinal pigment epithelium (RPE) and detailed characterization was done using stage-specific markers. Melanin pigment and tyrosinase activity were quantified in OCA1A-RPE and compared with control-RPE. Electron microscopy was performed to show presence of apical cilia and distribution of melanosomes in control-RPE and OCA1A-RPE.

Results: Morphologically, OCA1A-RPE were indistinguishable from control-RPE until they reached maturity. Control-RPE produced pigmentation whereas OCA1A-RPE cultures were completely devoid of pigmentation recapitulating the disease phenotype in vitro. Although, OCA1A-RPE expressed committed RPE markers like PAX6, RX, ZO-1, MITF, TYRP1, and RPE65; key proteins related to pigmented RPE such as TYR, PMEL17 were absent. Melanin quantification corroborated the lack of pigment production in OCA1A-RPE. Low Tyrosine activity further indicated that OCA1A-RPE produces non-functional tyrosinase resulting in complete lack of pigmentation. Ultra-structural analysis demonstrated absence of melanosomes in OCA1A-RPE when compared to control-RPE.

Conclusions: We established a 'disease-in-a-dish' model of OCA1A that faithfully recapitulates the clinical phenotype of pigmentation defect in the eye that can advance our understanding of this incurable disease. Ongoing studies using this model are directed towards rescuing the pigmentation defect of OCA1A-RPE cells.

ISOLATION AND CHARACTERIZATION OF SERUM DERIVED EXTRACELLULAR VESICLES FROM RETINOBLASTOMA PATIENTS

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Purpose: To isolate and characterize the serum derived extracellular vesicles (EVs) from retinoblastoma (RB) patients and healthy controls (HCs)

Methods: EVs isolated from serum of 12 RB patients prior to chemotherapy (n=9) and post-chemotherapy (n=3) and 6 HCs using total exosome isolation reagent (Invitrogen™). The size, concentration, and zeta potential were quantified by using transmission electron microscopy (TEM) and nanoparticle tracking analysis (NTA). Total RNA and protein was extracted from EVs using total exosome RNA & protein isolation kit (Invitrogen™). Western blotting (WB) was done for EV specific proteins (CD9, TSG101 & Hsp-70). RNA sequencing was performed for profiling of different RNAs present in EVs and to detect RB specific tumor signatures present in serum EVs.

Results: EV isolated from patients and HCs were in the concentrations of 5-$5.6×10^{11}$/ml blood measured and the size ranged between 100-140 nm. The zeta potential of serum EVs for RB patients post-chemotherapy was higher (14.5±0.98mV) vs (12.2±1.02 mV) prior to chemotherapy and controls (11.1±0.33mV). Western blot showed EV-associated proteins CD9, TSG101 and Hsp-70. RNA sequencing analysis revealed the presence of mRNA's (92-94%), microRNAs (3-4%) and long noncoding RNAs (3-4%). Compared to HCs metabolic, cAMP, cGMP-PKG, P53 and RAP1 were the most dysregulated signaling pathways in RB.

Conclusions: The study demonstrates that the serum derived EVs from RB patients show distinct transcriptome profiles mostly related to cell growth, proliferation and metabolism. Further studies are warranted for evaluating their functional role and as a source of biomarkers to monitor RB tumor progression and response to treatment.

PARAOXONASE 2 PROTECTS AGAINST THE CML MEDIATED MITOCHONDRIAL DYSFUNCTION THROUGH MODULATING JNK SIGNALLING PATHWAY IN HUMAN RETINAL MICROVASCULAR ENDOTHELIAL CELLS

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Purpose: Paraoxonase-2 (PON2) a known anti-apoptotic protein, has not been explored against N$^\varepsilon$-(carboxymethyl)lysine (CML), induced mitochondrial dysfunction and apoptosis in human retinal microvascular endothelial cells (HRECs). Hence, this present study aims to investigate the potential role of PON2 in mitigating CML-induced mitochondrial dysfunction.

Methods: PON2 expression was quantified in cadaveric diabetic retina vs respective controls. For *In vitro* studies, HRECs were exposed to CML for 24 h and expression of PON2 was quantified. ROS production, mitochondrial membrane potential (MMP), mitochondrial permeability transition pore (mPTP) opening, release of Cyc-c, Bax, Casp-3, Fis1, Mfn1, Mfn2, mitochondrial morphology, and the signaling pathway was assessed using DCFDA, JC-1, $CoCl_2$, immunofluorescence or western blotting analysis in both loss-of-function or gain-of-function experiments.

Results: PON2 protein was significantly downregulated in HREC cells upon CML treatment as well as in the diabetic retina (p=0.035). Decrease in PON2 augments Fis1 expression resulting in fragmented mitochondria and enhances the ROS production, decreases MMP, facilitates mPTP opening, and induces release of Cyt-c, which activates pro-apoptotic pathway. Whereas PON2 overexpression similar to SP600125 (a specific JNK inhibitor) was able to decrease Fis1 (p=0.036) and reverse the Bcl-2 and Bax ratio, and inhibit JNK1/2 signaling pathway.

Conclusions: Our results confirm that anti-apoptotic role of PON2 against the CML mediated mitochondrial dysfunction. This is the first study to show PON2 protects against the CML activated mitochondrial dysfunction through modulating JNK pathway in HRECs. **General Significance:** We hypothesis that enhancing PON2 may provide a better therapeutic potential against diabetic vascular disease.

GENERATION AND CHARACTERIZATION OF DRUG RESISTANT CLONES OF RB Y79 CELLS

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Purpose: Tumor heterogeneity, specifically with reference to chemotherapy induced resistant cells are responsible for tumor relapse, is a huge challenge to overcome. We herein developed and evaluated a drug resistance clone of Y79 cells through *in-vitro* and *in-vivo* assays, with specific reference to Cancer stem cell properties and influence of hypoxia.

Methods: Y79/DR cells were generated form parental cells with repeated exposure of Carboplatin. Hypoxia induction carried out by using 100μM CoCl2. In-vitro CSCs properties were evaluated by cell proliferation (MTT assay), Colony forming and migration ability and Gene expression (CD133, CD44, ABCG2, OCT4, Glut1 and VEGF). *In-vivo* tumorogenicity and metastasis was evaluated by CE-CAM assay.

Results: Y79/DR cells showed 10folds resistance to carboplatin as well as etoposide and vincristine compared to parental Y79 cells. The Y79/DR cells showed lower clone forming ability, proliferation (P<0.05), migration (P=0.02) compared to parental cells. Under hypoxia, Y79/DR cells escalates proliferation, colony forming efficiency, migration capacity and higher expression of CD44, ABCG2, OCT4, Glut1 and VEGF, lower CD133 expression were noted compared to Y79 cells (P<0.05). The Chick Embryo model demonstrated the hypoxic primed Y79/DR cells showed higher tumor forming ability and metastatic potential compared to their parental cells.

Conclusions: This study demonstrates the repeated exposure of chemotherapeutic drugs to Y79 cells generates drug resistant clones that show enhanced CSC properties. Both *in-vitro* and *in-vivo* studies show that the hypoxic state enhances the CSCs characteristics. Thus this study provides evidence of drug resistant clones with CSC properties are further nurtured by the hypoxic microenvironment.

DIFFERENTIAL RESPONSE OF CORNEAL EPITHELIAL AND STROMAL CELLS TO THE NOVEL CHEMICAL CROSS-LINKER TREATMENT

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Purpose: We have developed a novel, PBS soluble chemical crosslinker to treat keratoconus. Preliminary studies have established that the cross-linker can stiffen the keratoconus cornea with negligible cytotoxicity and morphological changes to the corneal tissue. The purpose of the current study is to analyse few of the molecular changes occurring in the corneal epithelial and stromal layers following treatment with the cross-linker.

Methods: Human corneal epithelial cell line (HCE) and stromal cells derived from keratoconus cornea were used to analyse transcripts of *IL-6*, *MMP-2*, *MMP-9* and *Col1A1* before and after cross-linker treatment. Inflammatory conditions akin to keratoconus were induced in HCE cells in-vitro by treating the cells with 10ng/ml TNF-α. Secreted MMP-2 and MMP-9 levels from cell supernates of HCE and stromal cells were measured using ELISA, and activity was determined by gelatin zymography.

Results: In HCE cells, *IL-6* and *MMP-9* expression increased marginally with 10ng/ml TNF-α treatment compared to control, while *Col1A1* expression decreased. *MMP-2* was not expressed in HCE cells. The expression of *IL-6* and *MMP-9* increased 1.5-folds while the expression of Co l1A1 decreased further after cross-linker treatment. In stromal cells, the expression of *MMP-2* decreased by 1.45 folds at transcript level. MMP-2 activity was inhibited following crosslinker treatment. *Col1A1* showed decreased expression by 1.25 fold while MMP-9 expression was not seen both at transcript and protein level.

Conclusion: The novel chemical cross-linker regulates signalling pathways involving MMP-9 in the epithelium and MMP-2 in the stroma to induce stiffening of the cornea.

ISOLATION AND CHARACTERISATION OF EXOSOME MIMETICS FROM HUMAN CORNEAL EPITHELIAL CELLS

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Purpose: Exosomes are naturally secreted extracellular entities with unique targeting ability that widely attracted their use in targeted drug delivery. However, the use of exosomes has been limited due to the tedious extraction process and poor yield. Exosome mimetics, also known as cell derived nanovesicles (CDNs) are promising alternatives to exosomes which are considered to be similar in their properties (size, targeting ability, biocompatibility, non-immunogenicity).

Methods: HCE transformed cell line (put proper descriptor) was cultured until it reached 80% confluency. The cells were trypsinized, washed with 1X PBS twice and resuspended in 1X PBS containing protease inhibitor cocktail. The cells were then subjected to a rapid freeze- thaw cycle, followed by a series of extrusion steps (10 μm, 8 μm, 0.45 μm, 0.2 μm) and Size exclusion chromatography (SEC). 1D SDS-PAGE and Mass spectrometry for the analysis of Exosome mimetics proteome.

Results: Shotgun and analytical Mass spectrometric analysis showed ~1700 proteins in exosome mimetics sample . A comparison between the proteome of HCE exosomes (Mckay *et al.* 2020) and exosome mimetics revealed >50 % common proteins.

Conclusions: In this study, we report the isolation of cell derived nanovesicles from HCE for the first time. Preliminary proteome analysis has revealed proteins with pathways similar to extracellular exosomes. Detailed analysis will be presented.

BCAT1 REGULATES METABOLIC FLUX IN RETINOBLASTOMA ABETTING TUMOR GROWTH

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Purpose: Retinoblastoma tumor growth requires alternative fuels, met by fatty acid oxidation and repurposing amino acid TCA intermediates. BCAT1 is a branched chain amino transferase catalyzing de-amination of branched chain amino acids(BCAA) into keto-acids and glutamate. We here investigate BCAT1 contribution to metabolic flux aiding tumor growth in retinoblastoma.

Methods: Expression of proteins, BCAT1, Rb1, HK1, E2F1/2 was evaluated by immunohistochemistry in tumor tissues. The Rb-/- Y-79 and WERI-RB1 cells were transfected to establish gain or loss-of-function models of BCAT1. The effect of BCAT1 modulation was analysed by immunoblotting, cancer hallmark assays and mRNA analysis.

Results: IHC analysis from patient tissues revealed BCAT1 upregulation compared with pediatric retina, despite heterogeneity across different areas. E2F proteins were upregulated, but HK1 was reduced. BCAT1 over-expression in Y79 cells enhanced cell proliferation and spheroid formation. BCAT1 ablation displayed reduction in proliferation similar to wtRB1 over-expressing cells. The BCAT1 ablation reduced E2F transcription factors. BCAT1 reduction also decreased mTOR phosphorylation at Ser-2448, phosphorylated by Akt pathway to growth factor response. Since loss of BCAT1 enhanced HK1 and LDHA proteins, the metabolic flux was affected, possibly reducing the proliferation rates.

Conclusions: In the absence of Rb protein, BCAT1 expression is increased in tumor cells that supports enhanced proliferation. Since BCAT1 phosphorylates mTOR and reduces HK1, it can control the metabolic flux through these proteins likely by controlling BCAA levels. Therefore, targeting BCAA metabolism and bioenergetic pathways may be a novel therapeutic modality for Rb tumors.

INTRAOCULAR IMMUNE PROFILE OF DRY AMD: A VISION FOR NOW AND FUTURE

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Purpose: Dry Age-Related Macular degeneration (dAMD) is a progressive multifactorial retinal degenerative disorder affecting vision with no effective treatment till date. Emerging knowledge suggests the relevance of aberrant inflammation in dAMD pathobiology. However, the status and contribution of intraocular immune status is yet to be explored.

Methods: Aqueous humor (AH) was collected from subjects with (n=13, dAMD) and without (n=15, controls) dAMD at the time of cataract surgery without deviation from standard of care. Samples were collected following informed consent. AH immune cell subsets and 40 soluble factors were measured by immunophenotyping and bead-based multiplex ELISA, respectively using flow cytometry. Immune cells were identified using fluorochrome-conjugated antibodies specific for leukocytes (CD45+), neutrophils (CD66b+), monocytes (CD14+), Natural killer–NK cells (CD56+), pan-T cells (CD3+), NKT cells (CD3+CD56+) and pan-B cells (CD19+).

Results: Significantly (*P<0.05) increased proportions of neutrophils (total, quiescent and activated), NK cells and mature B cells were observed in AH of dAMD subjects compared to controls. IL-1α, IL-12p70, IFNγ, TGF-β1 NGAL, MMP2, MMP9, TIMP1 and Perforin were significantly high in AH of dAMD subjects compared to controls. On the contrary, IL-17F, IL-21 and CCL5 were significantly lower in AH of dAMD subjects compared to controls. A significant positive correlation was observed between NK cells and Perforin (r=0.621*); and activated neutrophils and MPO (r=0.499*), NGAL (r=0.482*) in AH.

Conclusions: AH immune profiling suggests dysregulated intraocular immune status in dAMD. Targeting neutrophils and NK cells may be novel therapeutic strategies in the management of dAMD.

CHARACTERIZATION OF TYPE 2 SECRETION SYSTEM (T2SS) IN OCULAR CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* KERATITIS

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Purpose: *Pseudomonas aeruginosa* keratitis is the most common type of bacterial keratitis and causes serious corneal infections leading to severe inflammation and visual disability. *P. aeruginosa* is notorious in causing infection by a combination of several virulent factors such as biofilm formation and production of toxins such as pyoverdine and pyocyanin. The bacterium also consists of two major virulent systems, of which type II secretion system (T2SS) is known to secrete range of toxin and effectors contributing in pathogenesis. Therefore, the aim of the current study is to characterize the clinical isolates for various virulent factors and their correlation with T2SS.

Methods: PAO1, ΔT2SS strain and 16 clinical isolates were genotyped for T2SS virulent genes and further characterized for virulent factors such as biofilm formation by crystal-violet method, production of pyoverdine, pyocyanin and elastase by biochemical assay.

Results: Amongst the various T2SS genes, we found *xcpQ* (secreton) and *plcH* (phospholipase) absent in 31.25% and 25% of the isolates respectively. The clinical isolates of *Pseudomonas* showed variation in their capabilities of forming biofilm, production of pyoverdine, pyocyanin and elastase.

Conclusions: The data suggest that the isolates have shown variations in T2SS gene expression as well as in their capability of forming biofilm, production of pyoverdine, pyocyanin and swarming motility. However, more of clinical isolates further need to be characterized to have a complete understanding.

METABOLITES RELEVANT TO PATHOGENESIS OF AMD IMPAIR PHAGOCYTOSIS MACHINARY IN THE ARPE-19 CELLS: MITIGATING EFFECT OF ACTIVE PRINCIPLES

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Purpose: AMD pathogenesis involves impaired phagocytosis causing drusen accumulation. Therapeutic agents that improve phagocytosis by RPE is potentially beneficial. This study evaluated the metabolites associated with AMD in an in-vitro model of ARPE-19 cells as well as screened active principles to check phagocytosis promoting activity.

Methods: ARPE-19 cells (ATCC-CRL-2302) in DMEM/F12 /1% FBS) after overnight serum starvation were subjected to pathological insults (Homocysteine (Hcys): 100µM; Advanced Glycation End products (AGE): 100µg; oXLDL: 50µg, for 3h) with/without supplements pre-treatmed (Chebulagic acid (CI); Chebulinic acid (CA); alcoholic extract of Triphala (AE); curcumin (CUR) and lutein (LT)); vehicle controls, followed by spectrofluorimetric analysis of FITC-labelled total photoreceptor outer segment (POS) uptake by ARPE-19 cells in a transwell assay. For the immunofluorescence assay (IF) uptake was evaluated after 6h of treatment with FITC-labelled POS in dark the after fixation of the cells in 24-well plates. Cell viability was assessed by MTT assay and apoptosis by caspase 3 expression.

Results: POS uptake was significantly decreased in Hcys ($P<0.001$); AGE ($P<0.01$) and oxLDL ($P<0.01$) treatment. Supplementation with CUR ($p<0.001$) and LT (LT: $p<0.001$) increased the POS uptake significantly in Hcys treated cells; CA, LT and CUR ($P<0.001$) and CI and AE ($P<0.05$) improved it in oxLDL and AGE treated cells. These observations were supported by the IF study as well. Treatment with supplements also decreased the Casp3 gene expression significantly by >2fold.

Conclusions: The pro-oxidants Hcy, AGE and oxLDL impaired the phagocytosis by ARPE-19 cells. The active principles from natural products studied significantly improved the phagocytosis by ARPE-19 cells.

NOVEL CORNEAL TARGETING CELL PENETRATING PEPTIDE FOR MANAGEMENT OF CORNEAL DISEASES AND DISORDERS

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Purpose: Cell penetrating peptides are biodegradable and generally non-toxic and have emerged as a potent drug nanocarrier. Purpose of the current study is to describe a novel cornea targeting peptide for efficient drug delivery to anterior ocular tissues.

Methods: A novel 11 mer peptide sequence, Corneal Targeting Sequence 1 (CorTS 1), has been developed by modifying leucine rich repeat (LRR) motif ensuring that it interacts with small leucine rich proteoglycans and collagen present in the corneal stroma. The activity of peptide has been characterized by carrying out *in vitro* uptake experiments, cell viability assays, cargo delivery assessment, mechanistic study of the peptide uptake, *ex vivo* tissue uptake in bovine corneas and assessment of antimicrobial activity in vitro.

Results: CorTS 1 exhibited dose dependent cellular translocation from 5 μM in Human Corneal Epithelial cell line (HCE) with no cytotoxicity. CorTS 1 also successfully delivered protein cargo inside HCE cells. *Ex vivo* tissue penetration study of CorTS 1 demonstrated in bovine eyes revealed an augmented accumulation of peptide in the stromal region of cornea than in aqueous humor. Interestingly, CorTS 1 showed an antimicrobial activity against MRSA and *Fusarium dimerum*.

Conclusions: CorTS 1 has been designed with an aim to develop better and efficient strategies for the treatment of diseases of the anterior segment of the eye. The present study distinctly shows that the dual nature of the peptide (CPP as well as AMP) can have various applications in the field of ocular therapeutics.

EFFECT OF NEDDYLATION INHIBITION BY MLN4924 ON HYPOXIA INDUCED RETINAL ANGIOGENESIS

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Purpose: The oxygen and essential nutrients to the eye are furnished by an ingenious network of intricate blood vessels forming the ocular vasculature system. Pathological angiogenic switch triggered mainly under conditions of hypoxia sets off an abnormal proliferation of these blood vessels leading to vision-threatening intraocular vascular diseases. Recent evidence shows that neddylation, a post translational modification plays a vital role in tumor angiogenesis. The current work aims to identify a novel molecular signalling cascade of suppressing ocular angiogenesis by inhibiting the neddylation-HuR signalling pathway using a neddylation inhibitor, MLN4924.

Methods: Primary Human Retinal Microvascular Endothelial Cells were treated either with Vehicle control/ Cobalt Chloride ($CoCl_2$) / $CoCl_2$ along with MLN4924 and the effect of neddylation inhibition on hypoxia induced angiogenesis was verified by the real time PCR, western blotting, immunofluorescence and functional assays.

Results: Angiogenic assays showed that treatment with MLN4924 could suppress hypoxia induced angiogenesis by down regulation of both mRNA and protein levels of VEGFR2, without altering the expression of VE-Cadherin. Neddylation inhibition inhibited the translocation of HuR to cytoplasm, decreased its binding to mRNAs of pro-angiogenic markers and destabilized them as verified by RNA immunoprecipitation and Actinomycin D experiments respectively.

Conclusions: Treatment with MLN4924 in HRMVECs destabilized HuR protein and inhibited its translocation into the cytoplasm upon hypoxia treatment, which is required for its binding to the mRNAs of pro-angiogenic factors. Thus, neddylation inhibition could suppress hypoxia induced angiogenesis via down-regulation of VEGFR2 without affecting the barrier integrity of the cells.

EVALUATING THE POTENTIAL OF A BIOMIMETIC DECELLULARIZED MATRIX (DCM) HYDROGEL IN PREVENTING CORNEAL SCARRING IN AN ANIMAL MODEL

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Purpose: Corneal scarring is one of the leading causes of blindness that affect millions every year. Though corneal transplantation is a go-to option, shortage of donortissue creates a void in treating scars. This study is to evaluate the potential effects of a biomimetic and biocompatible DCM hydrogel of human and bovine origin in preventing scar formation.

Methods: The experiment included 12 rabbits; in which three sets are treated with human DCM, bovine DCM, collagen, and one as control group (n=3 in each). A central corneal wound was created in the left eye of rabbits by surgically removing 150-200 ￿m of the stroma, followed by treatment. All groups underwent clinical follow-ups where the corneal scar progression was assessed using anterior segment optical coherence tomography (AS-OCT). The animals were sacrificed after 12 week follow-up and the corneas were assessed by histopathological examination.

Results: The DCM hydrogel treated sets restored the corneal thickness (335±3.74; 334±14.79) to baseline thickness (337±22.73), while the collagen and control groups had shown significant reduction in corneal thickness (288±16.17; 278±16.26). Similarly, the epithelial to stromal reflectivity ratio (8 week) of DCM hydrogel treated groups (0.82±0.01; 1.07±0.01) was close to baseline ratio (1.13±0.15), while collagen and control groups had shown reduced ratios (0.72±0.13; 0.78±0.08). Histopathological examination revealed that the stromal recovery is more evident in DCM hydrogel treated eyes.

Conclusion: The study established that the DCM hydrogel had prevented the scar formation and corneal opacification. This can be further evaluated for its use in treating corneal scars in humans.

EXPLORING THE ROLE OF DOMAIN VARIANTS OF FIBRONECTIN TYPE III REPEATS OF TENASCIN-C (TN FNIII) IN CORNEAL WOUND HEALING - AN *IN-VITRO* STUDY

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Purpose: Restoration of corneal integrity is important for normal vision following any injury. Fibronectin as well as Tenascin-C (TnC) get deposited during corneal re-epithelialization, serving as scaffold for cellular migration. Different domains of fibronectin type III repeats of TnC (Tn fnIII) have been detected in various pathological conditions, which interact with fibronectin, resulting in cell signaling events. The present study aims to explore the roles of domain variants of TnC fnIII in corneal wound healing.

Methods: TnC fnIII domains (Tn fnIII 1-5, Tn fnIII 3-5, Tn fnIII 4-5, Tn fnIII 3, Tn fnIII 4 and Tn fnIII 5) were expressed and purified. Cell proliferation, adhesion and wound closure was examined *in-vitro* using human corneal epithelial cells (HCEC), and primary human corneal fibroblasts (HCF). Th effect of these domains on expression of fibrotic markers and extracellular matrix (ECM) proteins was analyzed in corneal fibrosis model using real-time PCR.

Results: Tn fnIII 1-5, Tn fnIII 4-5 and Tn fnIII 5 resulted in increased migration, adhesion and proliferation of HCECs. Tn fnIII 3 promoted migration and proliferation of HCFs. The pro-fibrotic nature of Tn fnIII 1-5, Tn fnIII 4-5 and Tn fnIII 3 domains was established using the *in-vitro* corneal fibrosis model, with increased expression of fibrotic markers as well as ECM proteins. However, Tn fnIII 5 domain resulted in significantly reduced expression of these markers.

Conclusion: The fifth fibronectin type III domain of TnC (Tn fnIII 5) holds promise as a potential anti-fibrotic candidate in the context of corneal wound healing.

PIWI/PIRNA AS A POSSIBLE INTERACTANT IN NEURONS FOR LIGHT PERCEPTION AND SLEEP CYCLE

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Purpose: PiRNA, a small ncRNA and its binding protein, Piwi are predominantly seen in germline cells, facilitating in stem cell maintenance, differentiation, genomic stability and transposon silencing. Recently, the presence of Piwi like protein and its isoforms are identified in *Aplysia* neurons, mouse brain, and *Drosophila* brain. Even though, they have been identified in neurons, their functions are not well understood. However, the significance of microRNAs (miRNAs) a similar small ncRNA acts in the brain as the regulator of light-induced clock resetting (Cheng *et al*.,2007) and in guiding/remodeling of neuronal arborizations from the brain to optic lobes and to photoreceptors, that, in turn, helps in light perception(Cusumano P *et al*.,2018) are already known.

Methodology & Results: Our behavioral studies were executed in *Drosophila melanogaster*. Interestingly, in our behavioural and locomotory analysis with *Drosophila*, when Piwi protein is downregulated in specific circadian neurons, high night activity was observed in Light/Dark cycle at different light intensities, 250lux and 30lux. We would like to put forward the possibility that piwi/piRNA might also exhibit similar role as miRNAs through our studies in flies and with bioinformatic support. And, also like to propose a visualization technique to check the orientation of neuronal arborizations with the help of Mueller polarimetry (LSMP).

Conclusions: Taken together, we would like to indicate the possibility of the involvement of Piwi/piRNA in the regulation of sleep cycle and related disorders and light perception moreover, as a link between circadian system and eye.

SYNTHESIZING AND CHARACTERIZING NOVEL PEPTIDE AS A CARGO FOR TARGETING HUMAN TENON FIBROBLAST CELLS TO MODULATE THE FIBROSIS

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Purpose: Trabeculectomy is done at severe stage of glaucoma eye disorder to drain the excess intraocular fluid. Fibrosis a condition that occurs post-surgery where antimetabolite like Mitomycin C and 5 fluorouracil are employed to reduce fibrosis and scar formation. Hence, sustained drug release on the surgery to reduce the side effects of the drug is much needed.

Methods: Using subtractive proteomic approach cell penetrating peptide VRF2019 were designed. The peptide was characterized using circular dichroism spectroscopy. The peptide and the drug was conjugated, through EDC/NHS chemistry and analysed by HPLC and Mass spectrometry. Further, the conjugate and Polycaprolactone (PCL) were electrospun to form a composite nanofiber which is further characterised by Scanning electron microscopy (SEM). The conjugatewas also tested for the cytotoxicity and drug release on human tenon fibroblast cells.

Results: VRF2019 showed α-helical structure. The peptide's cellular uptake was found to be translocation in human tenon fibroblast cells. HPLC and Mass spectrometry analysis confirmed conjugate. Further, SEM revealed the morphology of PCL nanofiber composite. Sustainedconjugate release was observed. The released conjugate showed 20 fold efficacy than native drug.

Conclusion: Peptide drug nanofiber matrix showed sustained release of conjugate and 20 fold efficacy.

TRANSPLANTED TRABECULAR MESHWORK (TM) STEM CELLS HOME TO TM - A STUDY IN CELL LOSS HUMAN ORGAN CULTURE ANTERIOR SEGMENT (HOCAS) MODEL OF GLAUCOMA

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Purpose: To evaluate the efficacy of *in vitro* expanded trabecular meshwork stem cells (TMSCs) in the regeneration of trabecular meshwork (TM) in a cell loss human organ culture anterior segment (HOCAS) model for glaucoma.

Methods: TM cells cultured in TM media and stem cell growth media (SCGM) were analyzed for the stem cell content based on the marker expression (high ABCG2 and p75) and sphere forming ability. A cell loss HOCAS model of glaucoma was created using 0.002% saponin. Qtracker labelled cultured TM cells (3,00,000 cells in 100µl) were transplanted in cell loss HOCAS model of glaucoma and the intraocular pressure (IOP) changes were monitored periodically. After a week the tissues were subjected to immunohistochemistry to identify the localization of transplanted cells.

Results: SCGM was found to maintain TMSCs in culture based on the presence of 65.6±6.68 % of cells expressing high level of ABCG2 as well as p75 and higher ability to form neurospheres-0.24±0.1% compared to those cultured in TM media (0% stem cell marker expression and 0.02±0.01% sphere forming ability). In HOCAS, the saponin treatment resulted in 26.97±0.35% TM cell death and increase in IOP, thus a cell loss glaucomatous model was established. Transplantation of cultured TMSCs in this cell loss model reduced the IOP increase due to saponin treatment. Confocal analysis confirmed the homing of transplanted cell population in both filtering and non-filtering regions of the TM.

Conclusions: In cell loss HOCAS model of glaucoma, homing and integration of transplanted cells to the TM resulted in the reduction of IOP which was increased upon saponin treatment.

BIO-INTELLIGENCE IN PREDICTING TEAR PENETRATION USING QSPR APPROACH

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Purpose: Understanding molecular dynamic and physicochemical properties of xenobiotics would be useful to build a simple QSPR model for prediction of tear levels after systemic administration. Also, it is an essential goal of ophthalmic drug discovery to screen newer agents for tear penetration. Therefore, the proposed study aims to develop QSPR models for penetration of xenobiotics in tear compartment in rabbits.

Methods: New Zealand albino rabbits of either sex weighing (1.5-2 Kg) were divided into five groups (n=12 each) and received intravenous cassette dosage of antifungals (n=8), calcium channel blockers (n=8), beta adrenergic receptor blockers (n=8), statins (n=7) and fluoroquinolones (n=10) at an equimolar dose of 0.1 µM/Kg. Tear and blood samples were withdrawn at 30, 60 and 120 min. All samples were quantified using validated LC-MS/MS method. Log_{10} tear-to-plasma (t/p) ratio were calculated and correlated with molecular descriptors at different time points. Multiple linear regression (MLR) model was derived with best correlated molecular descriptors using Discovery Studio (ver 17.2, Dassault Systemes, BIOVIA).

Results: Differential penetration in tear compartment was observed at sub-therapeutic dose in rabbits. MLR model (120 min) generated using parameters viz., band gap energy, molecular weight, number of double bonds, number of hydrogen donors and plasma concentration showed best prediction (R^2=0.672, Q^2=0.561, RMSE=0.442, n=40).

Conclusions: For the first time, a QSPR model was constructed for prediction of penetration of experimental compounds *in-vivo* in rabbit's tear compartment after systemic administration. Further the model is under validation for the prediction of tear levels of external set of compounds.

CLINICAL SCIENCES

LONGITUDINAL CHANGES IN CORNEAL EPITHELIAL THICKNESS AND REFLECTIVITY FOLLOWING SIMPLE LIMBAL EPITHELIAL TRANSPLANTATION: AN OPTICAL COHERENCE TOMOGRAPHY-BASED STUDY

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Purpose: To describe changes in corneal epithelial thickness and reflectivity following simple limbal epithelial transplantation (SLET) using anterior segment optical coherence tomography (ASOCT).

Methods: This was a prospective study of 31 eyes who had undergone SLET for limbal stem cell deficiency and had stable, avascular surfaces postoperatively. ASOCT scans of all four quadrants were taken pre-operatively and at the 1-week, 1-month, 3-months, 6-months and 1-year post-operative time points in the eyes undergoing SLET. Baseline scans were also taken from the normal. The following parameters were obtained from the scans: (i) epithelial thickness (ET), (ii) stromal thickness, (iii) total corneal thickness, (iv) epithelial reflectivity(ER), (v) stromal reflectivity, (vi) epithelial/stromal (ES) reflectivity ratio.

Results: Chemical injury (24 eyes, 77.4%) was the most common indication for surgery. There was a significant improvement in the ET (184.8±117.1 vs 60.3±10 μm, p<0.0001) and ER (144.5±26.4 vs 120.9±28.9, p<0.0001) within the initial postoperative period following SLET which remained stable at the end of one year follow up. There was no difference in the ET of the post SLET and normal eyes after the 3-months timepoint. A significant normalization was noted in ES reflectivity ratio at the end of 1 year (2.1 ±0.8 vs 1±0.2, p<0.001). A significant correlation was found between final visual acuity and total corneal thickness (r=0.942, p=0.005).

Conclusions: There is significant improvement in the epithelial thickness and reflectivity of the cornea in eyes undergoing SLET. ASOCT provides a reliable objective measure of these changes and can be used to monitor outcomes in these eyes postoperatively.

RETINOPATHY OF PREMATURITY IN HALF ZONE: CLINICAL PROFILE AND OUTCOME

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Purpose: To analyze the clinical profile and outcome of Retinopathy of Prematurity in Half zone (Zone I posterior)

Methods: In a partly retrospective and partly prospective study the babies with ROP in Zone 1 posterior were analyzed over 10 years at a tertiary care Institute serving babies from Eastern India. The demographic profile, significant perinatal risk factors,anatomical outcome of those treated were analyzed. By Zone 1 posterior, we meant the posterior most extent of the disease lying behind the fovea or presumed fovea.

Results: A total of 65 babies (130 eyes) were included. The average gestational age (GA) and birth weight (BW) were 27.93 weeks and 1174.46 gm respectively. The mean Post menstrual age (PMA) at first presentation was 37.7 (31.5 to 48) wks. Majority hailed from civil neonatal intensive care unit (NICU)s and received unblended oxygen for more than a week. The pattern of ROPs included Aggressive ROP followed by threshold and Hybrid ROP.The disease was circumferential in most instances with retinal vascular tortuosity more than the dilatation in all quadrants.Majority(80.3%) were treated with intravitreal antiVEGF followed by combination therapy followed by laser alone. The eyes with atypical morphologies like bleb like exudative detachments, indistinct central retinal vascular trunks were associated with poor outcome.

Conclusions: The half zone ROPs had many interesting observations that are different from the staged ROP and urges for relooking at the pathogenesis.

CLASSIFICATION OF OCULAR SURFACE PAIN BASED ON DISCORDANT SIGNS AND SYMPTOMS: ASSOCIATION OF CLINICAL, CONFOCAL IMAGING AND TEAR MOLECULAR PROFILES

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Purpose: Ocular surface conditions including dry eye disease often present with pain including hyperalgesia or allodynia. Sometimes it is clinically difficult to distinguish between etiopathologies due to discordance between symptoms and signs. Thus, a comprehensive approach based on multiple parameters can assist diagnosis and guide appropriate therapy. In this study we stratified subjects with ocular surface pain using a new classification – Discomfort concordance to Signs (DCS), based on clinical features.

Methods: All subjects were clinically evaluated by slit lamp examination, ocular surface disease index (OSDI) scoring, Schirmer's test I, tear break up time and ocular surface staining. Subjects were grouped as (i) without symptoms and no clinical signs; (ii) without symptoms but with signs; (iii) with similar grades of symptoms and signs; (iv) with symptom grade higher than the grade of signs. We evaluated corneal IVCM (in vivo confocal microscopy) features such as corneal dendritic cells (cDC), sub-basal nerve plexus features (SBNP) and microneuroma along with tear molecular factors (by multiplex ELISA).

Results: The cDC density was significantly increased in groups with severe signs and symptoms, and higher in subjects with discordant symptoms to signs. Significantly higher proportion of subjects with microneuroma were in the group with discordant symptoms. Among the tear fluid soluble factors, higher levels of IL-17A were found in the group with more symptoms.

Conclusions: Composite clinical classification of ocular surface pain integrating clinical features, IVCM and tear molecular factors can help clinicians improve accuracy of diagnosis or treatment planning targeted towards the underlying pathology.

NON-INVASIVE TEAR FILM AND MEIBOMIAN GLAND ASSESSMENT IN HEALTHY INDIAN POPULATION: EFFECT OF AGE, GENDER AND INTERPARAMETRIC RELATIONSHIP

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Purpose: To investigate age- and gender- related differences in tear film parameters and study interparametric relationship in a normal Indian population.

Methods: Healthy subjects with no ocular disease (median ocular surface disease index=0), were subjected to an automated evaluation of tear meniscus height (TMH), non-invasive tear breakup time (NIBUT), and infrared meibography using Keratograph 5M (OCULUS GmbH, Wetzar, Germany). Tear osmolarity was measured using the TearLab Osmolarity System (TearLab Corporation, California, USA). A mixed effects model with random intercepts at the level of patient was used for evaluating the relationships between explanatory (age, gender and tear osmolarity) and outcome variables (TMH and NIBUT).

Results: A total of 154 subjects were enrolled with mean age of 38±14 years; 74% were males. The mean values of TMH, NIBUT, and tear osmolarity were 0.35 ± 0.09 mm, 10.78 ± 2.01s and 288.8 ± 6.8 mOsm/L, respectively. Age had a significant positive relationship with TMH ($p<0.0001$; 0.003 mm/year) and NIBUT ($p = 0.04$; 0.03 s/year), but there was no effect on tear osmolarity ($p = 0.09$). There was no effect of gender on tear film parameters. TMH had a significant positive relationship with NIBUT ($p = 0.03$; 3.40 s/mm). Tear osmolarity showed no relationship with TMH ($p = 0.35$) or NIBUT ($p = 0.48$). Meibography grade was zero in all subjects.

Conclusions: In a normal Indian population, tear film stability is affected by age and tear meniscus height and is independent of tear osmolarity. Tear osmolarity shows no age-related changes.

CORNEAL ENDOTHELIAL CELL ALTERATIONS IN VARIANTS OF AXENFELD-RIEGER SYNDROME ASSESSED BY SPECULAR MICROSCOPY

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Purpose: To evaluate the corneal endothelial cellular alterations in Axenfeld Rieger Syndrome (ARS) variants and age-matched healthy controls**.**

Methods: This was a retrospective cross-sectional study involving 52 eyes of 30 subjects with variants of ARS. The endothelial cell parameters were evaluated by specular microscopy. The endothelial data in ARS were compared with those of 36 eyes of 21 age-matched healthy controls.

Results: ARS comprised the following variants: 7 eyes (13.4%) of Axenfeld anomaly (AXA), 18 eyes (34.6%) of Reiger anomaly (RGA), and 27 eyes (52%) of Axenfeld Reiger anomaly (AXR). Median age at investigation was 21.5 years (IQR ,13.8- 33.3 years). Sixteen (53.3%) participants were females. Systemic associations were reported in 8 subjects (26.7%). Mean endothelial cell density (ECD) for controls, AXA, RGA and AXR were 2909.7 ± 86.7 cells/mm^2, 2440.4 ± 198.3 cells/mm^2, 2283 ± 120.3 cells/mm^2 and 1903.2 ± 102.1 cells/mm^2 respectively. ECD was significantly ($p<0.001$) lower in RGA and AXR variants than controls. In average cell size ($p<0.001$), coefficient of variation ($p= 0.008$), maximum cell area ($p<0.001$) and minimum cell area ($p=0.002$) only AXR was significantly higher than controls. Corneal involvement and glaucoma were present in 19.2% and 42.3% of the eyes, respectively.

Conclusions: Corneal endothelial cellular alterations were more pronounced in eyes with AXR than the other variants. These findings highlight that patient with ARS spectrum require close follow-up and monitoring throughout infancy and into adulthood.

MICRONEEDLE CONTACT LENS FOR OCULAR DELIVERY OF CYCLOSPORINE A

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Purpose: The purpose of the present study was to fabricate and evaluate polymeric microneedle contact lens (MCL) containing cyclosporine A (CsA) for effective ocular administration.

Methods: Microneedles (MNs) were prepared using polyvinyl pyrrolidone (PVP K90, 21% w/v) by micromolding technique with contact lens design features. CsA was formulated in a mixture of castor oil, polyethylene glycol 400, ethanol and acetone to enhance its solubility from 0.03 mg/ml (in water) to 25 mg/ml. CsA was entrapped in 25 MNs by micromolding technique. MCL was characterized for physical and mechanical properties, CsA loading, dissolution, corneal insertion and CsA distribution in excised porcine eye globe. The commercially available Cyclomune® eye drops were used as the control formulation.

Results: Stereomicroscope and SEM images showed formation of 25-cone shaped MNs. The average length, width at base and tip of MNs were found to be 560±43, 286±28 and 19.7±5µm, respectively. Compression strength of 25-MNs of CsA-MCL was 57.3±13.7N. The average force required for insertion of 25 CsA loaded MNs in excised porcine cornea was (10.9±0.91N). The average amount of CsA loaded within each MCL was found to be (50.3±1.10µg). The amount of CsA retained within the cornea after 2h MCL application (81.1±6..2 µg) was found to be significantly ($p < 0.05$) greater compared with eye drops application (53.5±3.0µg). The decreasing order of CsA disposition within different tissues of porcine eye globe after 2h MCL application was cornea>aqueous humor>lens>vitreous humor>sclera>choroid-retinal complex.

Conclusion: Microneedle contact lens can be developed to deliver CsA through cornea.

EFFECT OF TOPICAL RHO-ASSOCIATED KINASE INHIBITOR RIPASUDIL 0.4% ON CORNEAL ENDOTHELIUM

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Purpose: Rho-associated protein kinase inhibitor Ripasudil 0.4% (Ripatec, Ajanta Pharma Limited, Mumbai, India) has been approved for clinical use in glaucoma to reduce intraocular pressure by promoting aqueous humor outflow. The purpose of this study is to evaluate the effect of ripasudil on normal corneal endothelium.

Methods: This prospective observational study included 14 healthy volunteers. After a baseline specular microscopy, a single drop of ripasudil was instilled in randomly chosen one eye of every subject. Serial specular microscopy was performed after 1, 3, 6 and 24 hours. The endothelial cell morphology and quantitative parameters were analyzed.

Results: Mean age was 31.0 ± 5.8 years. As a function of time, there were no statistically significant changes in the mean number of endothelial cells, endothelial cell density, average cell size, coefficient of variation and hexagonality. A dark light reversal pattern was apparent in the eye after ripasudil instillation. The dark light reversal pattern which was 0% at baseline, significantly ($p<0.001$) increased to 90% in 1 hour, then significantly ($p<0.001$) decreased to 10% in 3 hours and reached 0% by 6 hours.

Conclusions: Specular microscopy revealed no changes in the quantitative parameters after ripasudil administration. However, transient morphological changes were observed in one hour following administration of ripasudil that reverted back to normal in 6 hours. Clinicians must be wary of these changes to the dark light reversal pattern in endothelial cell morphology when advising and interpreting specular microscopy findings.

A STUDY ON CORNEAL BIREFRINGENCE BEHAVIOR USING DIGITAL PHOTOELASTICITY

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Purpose: To characterize the birefringence behavior of cornea using digital photoelasticity technique.

Methods: A set of five rejected human corneoscleral segments was subjected to intraocular pressure using an in-house designed anterior chamber. Then, they were imaged usinga polariscope in the transmission mode under white light. The images were captured at 0 and 20 mm of Hg pressure. The ten-step phase-shifting technique was employedand and the obtained phase maps were then unwrapped to obtain the full-field birefringence data.

Results: Two types of isochromatic patterns were observed, one with a diamond-like and the other with circular-like morphology. These isochromatic form their skeleton around thepoints of zero retardation. In photoelastic literature, these points are known as isotropicpoints. The corneal isoclinics were found to be distributed around the isotropic pointsas they pass through it. The effect of pressure increment was seen to be significant inthe peripheral regions. However, the spatial location of the isotropic points remain unaltered under pressure loading.

Conclusions: The techniques such as ten-step phase shifting, unwrapping algorithms, provide accurate full-field birefringence data. The stability of isotropic points is an implication of stable microstructure and curvature even under pressure loading. Therefore, it is proposed that tracking the movements of these points during various interventions would be beneficial to the clinical practice. Further, the analysis based on digital photoelasticity could be translated into the clinical usage due to its simplicity and the accuracy of data that it provides.

A PILOT STUDY ON COMPARATIVE ANALYSIS OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MUTANT PREVENTION CONCENTRATION (MPC) OF CONJUNCTIVAL BACTERIAL ISOLATES TO FLUOROQUINOLONES

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Purpose: The purpose of the study was to compare the minimum inhibitory concentration and mutant prevention concentration of fluoroquinolones such as ciprofloxacin, moxifloxacin and gatifloxacin against Methicillin Resistant and Methicillin Sensitive *Staphylococcus aureus* and *Staphylococcus epidermidis (MRSA, MSSA, MRSE, and MSSE)* isolated from conjunctival swabs.

Methods: Twenty five isolates of *Staphylococcus spp* isolated from specimens received in the Microbiology department were included in this study. Identification and confirmation of MRSA, MSSA, MRSE and MSSE were done by standard microbiological techniques as per CLSI guidelines. Determination of MIC and MPC was done by agar dilution method according to previous studies and analyzed. MIC_{50}, MIC90 values, MPC_{50} and MPC_{90} values of three fluoroquinolones were calculated.

Results: Out of 25 isolates, 20 were *S. epidermidis* and 5 were *S. aureus*. In our study, MIC and MPC values were found to be lowest for gatifloxacin in all the isolates when compared to ciprofloxacin and moxifloxacin. MPC_{50} and MPC_{90} of gatifloxacin were found to be lower when compared to ciprofloxacin and moxifloxacin. Our study reveals that MIC and MPC values for gatifloxacin was lower than ciprofloxacin and moxifloxacin comparatively. Moreover, MPC of ciprofloxacin, moxifloxacin and gatifloxacin exhibited broader range of distribution than the MIC.

Conclusions: Gatifloxacin exhibited effective inhibition of resistant mutant strains at a lower concentration when compared to Ciprofloxacin and Moxifloxacin. Further, future study with large no of isolates including pharmacokinetic/pharmacodynamic parameters will provide necessary information on therapeutic outcome and resistance prevention.

ELUCIDATING THE CLINICAL, MICROBIOLOGICAL AND MOLECULAR DIAGNOSTIC ASPECTS OF MACROPHOMINA PHASEOLINA KERATITIS

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Purpose: To report the clinico-microbiological profile of six cases of keratitis caused by *Macrophomina phaseolina*-an uncommon dematiaceous fungus.

Methods: Between June 2020 and February 2021, six microbiologically diagnosed unidentified dematiaceous fungal isolates obtained from corneal tissue of patients with fungal keratitis were subjected to Sanger DNA sequencing of ITS1-5.8S-ITS2 (ITS) region and phylogenetic analysis. The isolates were further reconfirmed by specific PCR for *M. phaseolina*. Minimum inhibitory concentrations (MICs) of six antifungal drugs against the isolates were determined by mircrobroth dilution method. Patients were treated with topical and systemic antifungals. A failed medical therapy necessitated therapeutic penetrating keratoplasty(TPK). Corneal buttons were processed for histopathology. Clinical data was retrieved from electronic medical records and analysed.

Results: The BLAST analysis for ITS sequences of all fungal isolates revealed their identity as *M. phaseolina,* but when limited to sequences from type material it showed *M. pseudophaseolina.* Phylogenetic analysis could not differentiate between these two species while PCR assay of *MpCal* (calmodulin) gene reconfirmed *M. phaseolina*. The MICs of voriconazole and posaconazole were lowest (0.03 to 2 and 0.1 to 2µg/mL respectively) followed by natamycin and others. All cases except one worsened on medical therapy requiring TPK. Histopathology of 3 out of 4 infected tissues showed fungal filaments.

Conclusions: The ITS sequencing and PCR assay of *MpCal* allow specific diagnosis of *M. phaseolina* keratitis. Despite low MIC, clinical response to commonly used antifungals is not promising and patients may require TPK for resolution of infection in *M. phaseolina* keratitis.

COMPARISON OF TWO CORNEAL EPITHELIAL CELL LINES IN RESPECT OF THEIR EXPRESSION OF DIFFERENTIATION AND STEMNESS MARKERS

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Purpose: Human corneal epithelial cell lines have been widely used as alternatives to primary cultures for differentiation-related studies in the context of the cornea. However, maintenance of similar profile of differentiation markers in these cell lines is necessary to mimic the *in vivo* scenario. This study was conducted to compare the SV40-immortalized corneal epithelial cell line (HCE-T) and telomerase-immortalized corneal limbal epithelial cells (HCLE) cultured under different conditions to assess the suitability of cell type and culture conditions to be used for corneal reprogramming studies.

Methods: HCE-T cell line was cultured under two culture conditions: (a) proliferation medium and (b) differentiation medium. HCLE cells were cultured under three culture conditions: (a) proliferation medium (b) growth medium and (c) stratification medium. Expression levels of markers for differentiation (KRT 3, KRT 12 and PAX6), stemness (ABCG2 and p63) and pluripotency (OCT 4, SOX 2 and NANOG) were analyzed using real time PCR.

Results: HCLE cells cultured under any condition showed increased expression of KRT 3 and PAX 6 as compared with HCE-T. KRT 12 expression was comparable in both cell lines. Limbal stem cell marker, p63 was found to be upregulated in HCLE under all culture conditions. However, ABCG2 expression was found to be higher in HCE-T than HCLE. Moreover, HCE-T showed increased expression of OCT 4, SOX 2 and NANOG as compared to HCLE.

Conclusions: HCLE cells are more differentiated as compared to HCE-T cells. Thus, HCLE cells are more suited for use in corneal reprogramming studies.

PROSTAGLANDIN E RECEPTOR SUBTYPE 3 EXPRESSION IN LACRIMAL GLANDS OF HEALTHY SUBJECTS, STEVENS-JOHNSON SYNDROME AND NON-SPECIFIC DACRYOADENITIS

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Purpose: Prostaglandin E2 receptor subtype 3 (EP3) expression has not been described in the lacrimal glands before. To compare EP3 distribution in the main and accessory lacrimal glands of normals, non-specific dacryoadenitis, and chronic Stevens-Johnson syndrome (SJS) patients.

Methods: Biopsies from lacrimal glands of four chronic SJS patients (mean age, 21 years) with severe dry eye disease (Schirmer=0 mm), four dacryoadenitis patients (mean age= 26 years; mean Schirmer= 15mm), and five fresh body donors (mean age= 41 years) were assessed for EP3 expression using the immunohistochemical technique. The evaluated parameters were the percentage of acini expressing EP3, the intensity of immunostaining, and the degree of mononuclear cell infiltration.

Results: There was a strong nuclear and cytoplasmic expression of EP3 in the majority (>75%) of acini of main and accessory lacrimal glands with no ductular expression in normals. Lacrimal glands of dacryoadenitis and SJS patients showed variable inflammatory cell infiltration (10-20/HPF in dacryoadenitis, 5-20/HPF in SJS). In dacryoadenitis, EP3 expression was similar to normal glands (>75% of acini) with no immunostaining of mononuclear cells except in one dacryoadenitis patient with no visible acini. However, lacrimal glands from SJS patients showed a weak and reduced (<10% acini) EP3 expression within acinar cells. The reduction in intensity was more in glands with higher mononuclear cell infiltration (>10/HPF).

Conclusions: Normal human main and accessory lacrimal glands expresses EP3 within acinar cells. There is downregulation of EP3 expression in the lacrimal glands of SJS patients, whereas EP3 expression is preserved in non-specific lacrimal gland inflammations.

CLINICAL PROFILE AND TREATMENT OUTCOMES OF INFANTS WITH EXUDATIVE RETINAL DETACHMENT AS THE PRESENTING FEATURE IN RETINOPATHY OF PREMATURITY

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Purpose: To describe the clinical profile and treatment outcomes of infants with retinopathy of prematurity (ROP) presenting with exudative retinal detachment.

Methods: Retrospective interventional case series. Preterm infants diagnosed with ROP having exudative retinal detachment (ERD) at presentation were included. All demographic details, clinical findings and treatment given were noted. Anatomical outcome was categorized as good, fair and poor. Refractive outcome was categorized into mild, moderate and severe according to spherical equivalent at last visit.

Results: 15 eyes (8 patients) were included. Mean GA was 31.3 weeks and birth weight was 1462.6gms. All infants had history of respiratory distress syndrome. All eyes presented with APROP. Yellowish white patches of retinal edema in avascular retina were seen in all eyes. 86.6% eyes had vascular sclerosis. 99.3% eyes had subretinal exudates. 13 eyes were treated with intravitreal bevacizumab, 2 eyes required only laser photocoagulation. Anatomical outcome was good in all eyes. 40% eyes had mild refractive error.

Conclusions: ERD in ROP is rare. Use of oxygen can be contributory factor. Vascular sclerosis is consistent with hyperoxia induced retinopathy models. Retinal edema and subretinal exudates point towards disrupted inner and outer blood retinal barrier. Treatment outcomes are good when diagnosed and treated in time.

ARTIFICIAL INTELLIGENCE IN PREDICTING THE DRUG-TRANSPORTER INTERACTION: UNDERSTANDING THE ENTRY OF SYSTEMIC DRUGS INTO EYE

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Purpose: To understand the role of membrane transporter (Organic Cation Transporter-1(OCT-1)) in the transport of systemic drugs into eye which causes ocular toxicity, using artificial intelligence (AI)-based models. AI models will predict the interaction between drug and transporters based on their structural and physicochemical properties.

Methods: AI models were developed based on supervised-learning algorithms and artificial neural networks (ANN). Briefly, a dataset comprising of 193 drugs (substrate/non-substrates for OCT-1) were used to train the models. Various physicochemical properties such as molecular weight, volume, polar surface area, CLogP value, etc., were used as input features and threshold values were decided based on their range of occupancy. Dataset was divided into training-testing dataset (80:20). To assess the performance of developed models, accuracy and precision were measured. To improve the accuracy and stability, consensus model was developed with k-fold cross validation (k=5) and logistic regression. ANN model was developed with one input (seven-neurons), two hidden (six-neurons) and one output layer (one-neuron). Developed models were used to predict the interaction of OCT-1 with systemic drugs (n=142) causing ocular toxicity.

Results: Logistic regression model and ANN model showed accuracy of 81% and 83% respectively. Among the 142 systemic drugs causing ocular toxicity screened through developed model, 77 molecules were predicted as substrate (54%).

Conclusions: Many drugs were predicted as substrate for OCT-1 which were not known earlier, but causes visual impairment. Further studies will be carried out using invivo models to confirm these predictions and understand the functional role of transporters.

SIGHT THREATENING INTRAOCULAR INFECTION AFTER HOSPITAL DISCHARGE FOR COVID-19 TREATMENT IN SOUTH INDIA

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Purpose: The study purpose was to analyse the clinical and microbiological data of laboratory-confirmed COVID-19 patients from April 2020 to January 2021, presenting with features of endogenous endophthalmitis within 12 weeks of discharge from the hospital in two neighbouring states in South India.

Methods: Retrospectively retrieved data from electronic medical record system of COVID-19 patients included demography, systemic comorbidities, COVID treatment details, time to endophthalmitis symptoms, the microbiology of systemic and ocular samples, ophthalmic management, and outcomes.

Results: The mean age of 24 patients (33 eyes) was 53.6 $\pm$ 13.5 (range, 5 to 72) years; 17 (70.83%) patients were male; 22 (91.6%) patients had systemic comorbidities; and the median period of hospitalization for COVID-19 treatment was 14.5 $\pm$ 0.7 (range 7-63) days. The COVID- 19 treatment included broad-spectrum systemic antibiotics (all), antiviral drugs (22, 91.66% patients), systemic corticosteroid (21, 87.5% patients), supplemental oxygen (18, 75% patients), low molecular weight heparin (17, 70.8% patients), admission in intensive care units (16, 66.6% patients), and interleukin-6 inhibitor (tocilizumab) (14, 58.3% patients). Five (20.8%) patients died of COVID-19 related complications during treatment for endophthalmitis; one eye progressed to pan ophthalmitis and orbital cellulitis; 8 eyes regained vision > 20/400. Fourteen of 19 (73.7%) vitreous biopsies were microbiologically positive (culture, PCR, and microscopy), and the majority (11 patients, 78.5%) were fungi.

Conclusions: Intraocular infection in hospitalized COVID patients is predominantly caused by fungi. We suggest a routine eye examination be included as a standard of care of COVID 19.

ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN OCULAR ONCOLOGY: RETINOBLASTOMA

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Purpose: To explore the utility of artificial intelligence (AI) and machine learning in the diagnosis and grouping of intraocular retinoblastoma (iRB)

Methods: AI and Machine learning, Computer Vision (Open CV)

Results: Of 771 fundus images of 109 eyes, 181 images had no tumor and 590 images displayed iRB based on review by 2 independent ocular oncologists (with an inter-observer variability of <1%). The sensitivity, specificity, positive predictive value, and negative predictive value of the trained AI model was 85%, 99%, 99.6%, and 67% respectively. Of 109 eyes, the sensitivity, specificity, positive predictive value, and negative predictive value for detection of RB by AI model was 96%, 94%, 97%, 91% respectively. Of these, the eyes were normal (n=31) or belonged to group A (n=1), B (n=22), C (n=8), D (n=23), and E (n=24) RB based on review by 2 independent ocular oncologists (with an inter-observer variability of 0%). The sensitivity, specificity, positive predictive value, and negative predictive value of the trained AI model was 100%, 100%, 100%, and 100% for group A; 82%, 98%, 90%, and 96% for group B; 63%, 99%, 83%, and 97% for group C; 78%, 98%, 90%, and 94% for group D, and 92%, 91%, 73%, and 98% for group E respectively.

Conclusion: The AI model for iRB is highly sensitive in detection of RB with high specificity for classification of iRB.

MACULAR RETINOBLASTOMA: CLINICAL PRESENTATION AND TREATMENT OUTCOMES

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Purpose: To describe the clinical features and treatment outcomes in eyes with retinoblastoma (RB) involving macula

Methods: Retrospective study of 47 eyes in 41 patients with macular RB

Results: Of 41 patients, 20 were male and 21 were females. The mean age at diagnosis was 16 months (median, 11 months; range, 1 to 60 months). Bilateral presentation was seen in 6 patients. At presentation, the macula was completely covered with tumor in 22 (47%) eyes and was partially covered with fovea being spared in 13 (28%) eyes and fovea involved 12 (25%) eyes. Based on Intraocular Classification of Retinoblastoma (ICRB), 25 (53%) eyes belonged to Group B and 22 (47%) belonged to Group C. The tumor exhibited exophytic features in 36 (77%) eyes. The mean tumor basal diameter and thickness was 10 mm and 5.6 mm respectively. Associated features included subretinal seeds (n=10; 21%) and surrounding subretinal fluid (n=16; 34%). Of 47 eyes, 43 (92%) eyes were treated with intravenous chemotherapy (IVC), 2 (4%) with intra-arterial chemotherapy and 2 (4%) with transpupillary thermotherapy. Local tumor control was achieved in 44 eyes (94%) with 33 eyes (70%) showing type 3 regression pattern. Over a mean follow-up period of 23 months (median, 23; range, 1 to 48 months), tumor recurrence occurred in 19 eyes (40%), globe salvage was achieved in 100% eyes with associated foveal atrophy (n=36; 77%) and three (7%) patients died.

Conclusion: Macular RB has good prognosis for globe salvage while vision salvage is compromised due to associated foveal atrophy.

APPLICATION AND VALIDATION OF A NOVEL INFLAMMATORY SCORE IN THE CLINICAL GRADING OF INFECTIOUS ENDOPHTHALMITIS: THE ENDOPHTHALMITIS MANAGEMENT STUDY – REPORT 2

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Purpose: To describe and validate a novel inflammatory score (IS) system in the management of infectious endophthalmitis.

Methods: A prospective comparative non-interventional observational study. The study included the patients with clinical signs and symptoms of acute post-cataract surgery endophthalmitis (surgery within 6 weeks) with visual acuity from 6/18 to light perception. IS was scored by the clinical picture at two-levels of four ocular tissues on a scale of 0 (normal) to 4 (severe). Four masked graders of different levels of experience evaluated slit lamp photographs. The concordance correlation coefficient was assessed between the slit lamp clinical grading and photographic grading. We measured the concordance correlation coefficient, Pearson's correlation (indicating precision), and the bias correction factor (indicating the accuracy).

Results: The study included 43 eyes of 43 patients. The concordance correlation coefficient was 0.99 (95% C.I. 0.995 to 0.998). Both Pearson's correlation coefficient and the bias correction were 0.99. The interclass correlation coefficient (ICC) was measured. The intra-rater ICC was 0.833 with good agreement (95% CI, 0.711 to 0.906), $p < 0.001$. Inter-rater ICC for consistency was 0.92 (95% C.I. 0.87 to 0.95). Inter-rater ICC for absolute agreement was 0.86 (95% C.I. 0.66 to 0.93).

Conclusion: Inflammatory score is a reliable, reproducible and easy-to-apply scale to measure inflammation severity in endophthalmitis.

CLINICAL CHARACTERISTICS OF COMORBID RETINAL DYSTROPHIES AND PRIMARY ANGLE CLOSURE DISEASE

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Purpose: To assess the clinical characteristics of comorbid retinal dystrophies and primary angle closure disease.

Methods: This retrospective study from January 1992 to June 2020 study included 92 eyes of 46 patients with comorbid retinal dystrophies and primary angle closure disease (PACD) that included eyes with primary angle closure suspect, primary angle closure and primary angle closure glaucoma. Demographic profile, clinical characteristics of PACD and its association with retinal dystrophies are described.

Results: The study included 46 patients(92 eyes). Males were majority, 63%. Mean (± standard deviation) age when retinal dystrophy was diagnosed was 29.6 ± 9.4 years and PACD was diagnosed at 32.23±7.92 years. Mean BCVA at presentation was 1.07±0.87 log MAR (95% confidence interval (CI):0.87,1.26). Mean Intraocular pressure at diagnosis of glaucoma was 27±16 mmHg (95% CI:23.5,31.5 mmHg). The most common retinal dystrophy associated with PACD was retinitis pigmentosa (RP) followed by RP with retinoschisis. The hospital-based prevalence of PACD among all patients with RP and retinoschisis was 0.19% and 0.15% respectively. Laser peripheral iridotomy (LPI) was performed in 74 eyes (80.5%). Glaucoma was managed medically in majority of the eyes (58 eyes, 63.04%) and minority required surgical management with trabeculectomy(11, 11.9%).

Conclusions: Retinitis pigmentosa is the most common retinal dystrophy associated with PACD. Comorbid PACD in eyes with retinal dystrophies was observed in 2nd to 3rd decade of life. This calls for screening for angle closure in eyes with retinal dystrophies from second decade onwards to identify the comorbid PACD and treat or refer them appropriately.

SEMILUNAR SIGN OF SCLERITIS IN CORNEA

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Purpose: To report and analyze the 'semilunar sign' in non-infectious anterior scleritis using multimodal imaging.
x
Methods: Posterior cornea was visualized using the digital slit lamp photography, spectral domain Optical coherence tomography and specular analyzer. "semilunar sign" was defined by the (1) Presence of posterior corneal opacity (2) Concave semilunar pattern (3) Absent blood vessels

Results: A total of 76 eyes of 72 patients of anterior scleritis were recruited. Overall 15 eyes of 11 patients (15.3%) presented with semilunar sign. The scleritis was non-necrotizing healed (n=6), nonnecrotizing active (n=2), necrotizing healed (n=6) and necrotizing active (n=1). Only 3 out of 15 eyes were symptomatic. The laboratory test showed positive tests for Mantoux skin test (54.5%, n=6), Rheumatoid factor (n=1, 9%) and Antinuclear antibody (n=1, 9%). The semilunar sign extent was directly related to the extent of the scleral disease (p=0.002).The BCVA was 0.63±0.4decimal equivalent. The mean posterior corneal opacity thickness measured 212.5±129.3 microns on OCT. The mean central endothelial cell density (ECD) was 2540.8±351.7 cells/sq. mm which was significantly higher than the involved peripheral cornea (p=0.05). The mean surface area of the endothelial opacity was 7.7±5.2 sq mm. There was no statistically significant correlation between the opacity thickness and the BCVA (P=0.895, r= -0.39), the ECD (p=0.52, r= -0.188) and the Mantoux (p=0.696, r=-0.142).

Conclusions: Semilunar sign of non infectious anterior scleritis involved the endothelium and the posterior stroma and remained asymptomatic. Multimodal analysis can aid in clinical assessment and follow up.

CHOROIDAL VASCULARITY INDEX IN THYROID EYE DISEASE: COMPARISON WITH CONTROLS AND APPLICATION IN DIAGNOSING NON-INFLAMMATORY ACTIVE DISEASE

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Purpose: To report the differences in choroidal vascularity index (CVI) in thyroid eye disease (TED) and normal eyes and its discriminatory value for differentiating various stages of TED.

Methods: Prospective, cross sectional, non-interventional imaging study. Ninety-four eyes of 54 patients were included and divided into 5 groups – normal controls (C), inactive TED (I), active TED (A), non-inflammatory active TED (NIA) and systemic hyperthyroid disorder but no TED (SYS). Choroidal images were acquired using the swept-source optical coherence tomography and the choroid was binarized to calculate the CVI.

Results: Ninety-four eyes were included. Mean age was 44.52±10.02 years (median 46 years, range 19-65 years). Mean IOP was 16.1±3.37 mm Hg (median 16 mm Hg, range 16 – 24 mm Hg). Mean SE was -0.08±1.86 diopters (median 0, range -2.5 to +2.25). Intra-rater agreement was 0.84 ($p<0.001$). Inter-rater agreement was noted to be 0.85 ($p<0.001$) for consistency and 0.77 ($p<0.001$) for absolute agreement. CVI in the A group was 70.11±3.38% and in the NIA group was 69.32±3.5%. Both were comparable to each other and significantly higher than the C, I and SYS groups ($p<0.001$). Multiple regression showed that the CAS had a positive effect and spheroequivalent had a negative effect on the CVI. At CVI of 66.83%, active TED can be diagnosed with sensitivity of 91.67% and specificity of 82.14%.

Conclusions: CVI is significantly higher in active TED and NIA TED compared to other groups. It has a good value in differentiating the non-inflammatory active TED eyes from the inactive eyes.

CLINICAL PROFILE AND HISTOPATHOLOGICAL CORRELATION IN PATIENTS WITH SYMPATHETIC OPHTHALMIA – A 28 YEARS STUDY FROM A TERTIARY EYE CARE CENTRE IN SOUTH INDIA

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Purpose: Clinical profile and histopathological correlation of patients with sympathetic ophthalmia (S.O.) in a tertiary eye care centre.

Methods: A single centre retrospective case series of 40 patients with SO during the 28 years period. The demographics, inciting stimulus, various imaging characteristics and the treatment response were analysed. Histopathological analysis was possible in 10 patients.

Results: Male to female ratio is 2: 1. A mean duration of 75 days was noticed between the injury and the onset of SO. Mean duration of follow up is 3.59 years. Exudative retinal detachment was the most common presentation. Most common inciting event was found to be penetrating trauma in 23 patients (57.5%) followed by vitreoretinal surgeries in 7 patients (17.5%). Ultrasonography, optical coherence tomography (OCT), fundus fluorescein angiography, fundus autofluorescence and indocyanine green angiography revealed various features during the active and the chronic phases of the disease. Choroidal thickening on OCT is the most consistent finding. Histopathological examination demonstrated non – granulomatous inflammation in 50% cases. All the patients were managed with high dose of systemic corticosteroids and immunomodulatory agents. Twelve patients received intravenous methyl prednisolone pulse therapy.

Conclusions: Pars plana vitrectomy has been found to be an important factor for trigerring SO in recent era. Different imaging modalities help confirm the diagnosis as well as prognosticate the disease and its severity. Intravenous methyl prednisolone pulse therapy in acute cases and early institution of immunosuppressive agents has been associated with a better visual prognosis. Histopathological study revealed non - granulomatous reaction in majority of the ocular specimens.

INTRAOCULAR IMMUNE RESPONSE IN DIFFERENT OCULAR TB PRESENTATIONS

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Purpose: To demonstrate differences in intraocular immune phenotypes between ocular tuberculosis (OTB) and TB-immunoreactive uveitis of unknown origin (UNK).

Methods: TB-immunoreactive patients with different uveitis presentations were included in the study. TB-immunoreactivity was measured by the tuberculin skin test $\pm$ QuantiFERON TB-Gold test. We achieved tight clinical discrimination between the OTB and UNK phenotypes by including patients with characteristic ocular signs, and positive TB-polymerase chain reaction $\pm$ active systemic TB, in the OTB group. Other TB-immunoreactive patients were included in the UNK group. Vitreous samples were collected by pars plana vitrectomy, intraocular T cells were isolated from the samples, activated with *Mycobacterium tuberculosis*- specific antigen ESAT-6 or retinal self-antigen interphotoreceptor retinal binding protein (IRBP) and cytokine response analyzed.

Results: In ESAT-6 treated cells, cytokine levels of TNF-α, IL-17, and IFN-□ were highest in the UNK group. IRBP treatment significantly increased IL-17 in OTB as compared to UNK ($p = 0.046$). Interestingly, the non-TB group showed the highest IL-17 levels and the difference was significant between the Non-TB and UNK groups ($p = 0.021$). Levels of dual positive (TNF-α and IFN-□) cells after ESAT-6, showed strong upregulation in the UNK group, much higher than other groups ($p = 0.001$, UNK vs OTB, and $p = 0.006$, UNK vs non-TB). However, levels of dual positive cells were similar in all the groups after IRBP treatment.

Conclusion: This study points that UNK group generates more pro-inflammatory cytokines than OTB group after ESAT-6 treatment, while no difference after IRBP treatment was observed.

INDIAN HEALTH OUTCOMES, PUBLIC HEALTH AND ECONOMICS RESEARCH CENTRE

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Purpose: To report the initial progress of a multi-disciplinary, multi-institutional Indian Health Outcomes, Public Health and Economics (IHOPE) Research Centre.
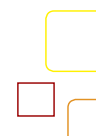
Methods: IHOPE has been set up with the help of a clinical research center grant of INR 10,00,00,000 (USD 1.5 million) over 5 years, funded by the DBT/Wellcome Trust India Alliance. This first of its kind center in the country brings together L.V. Prasad Eye Institute, Indian Institute of Public Health, Hyderabad and the Indian Institute of Management, Ahmedabad with the aim to develop a self-sustainable Centre of Knowledge Creation and dissemination for Clinical and Public Health Research through Big Data.

Results: One year since its inception, IHOPE has taken the first steps towards achieving its objectives in a systematic and focussed manner. An advisory board (Steering Committee) with eminent national and international scientists has been constituted. The Centre has developed skilled human resource with appropriate technology for end-to-end support for scientists. Seven studies have been published till date in peer reviewed journals by the investigators. Three fellows have been inducted into the Clinical Research Training Fellowship Programme for mentored training, with supplementary funding by the India Alliance. Moving into the second year, IHOPE has been included as a key collaborator to execute an independent public health trial funded by the Wellcome Trust.

Conclusions: Strategic funding by granting agencies can help develop multi-disciplinary clinical research centres, which can generate evidence in all aspects of preventive and curative medicine. Sustainability of such centres beyond the grant period should be addressed.

OPTOMETRY
&
PUBLIC HEALTH

DOES RELATIVE PERIPHERAL DEFOCUS ALTER THE CORRESPONDING PERIPHERAL RETINAL ELECTRICAL SIGNALS?

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Purpose: As the mechanisms regulating ocular growth are known to operate locally in a selective manner across the retina, we investigated the effect of naturally occurring retinal defocus on the multifocal electroretinogram (mfERG) responses from central to peripheral retina in different refractive states.

Methods: Central and peripheral refraction using an open-field autorefractor and mfERG responses using an electrophysiology stimulator were recorded for right eyes of 20 non-myopes (>-0.50 to ≤+1.00D) and 24 myopes (≤-0.50 to >-6.00D) aged 18-36 years. The N1, P1, and N2 components of mfERG waveform were compared with the best-matched natural defocus at eleven corresponding locations: fovea (0°), horizontal (±5°, ±10°, and ±25°) and vertical meridians (±10° and ±15°).

Results: Myopes reported significantly smaller mfERG amplitudes than non-myopes at peripheral eccentricities. Mean differences in N1, P1 and N2 amplitudes (nV) between non-myopes and myopes were -100±16, -161±26, and -150±31, respectively at temporal +25° (p≤0.04) and -128±11, -145±11, and -130±10, respectively at nasal -25° (p≤0.04). The mfERG responses did not show any trend with relative peripheral emmetropia, myopia or hyperopia at peripheral eccentricities (p≥0.20). Sub-analysis of mfERG responses at fovea and extreme peripheral eccentricities when both these locations experienced an equal magnitude of absolute retinal defocus, revealed significantly smaller absolute mfERG amplitudes at periphery than foveal amplitudes in both meridians (p≤0.001).

Conclusions: Myopes exhibited smaller absolute mfERG amplitudes at peripheral retina. Non-significant differences in retinal responses between relative peripheral myopic or hyperopic defocus may indicate that the peripheral retina is less sensitive in altering the retinal electrical signals.

THE GRADE OF RELATIVE AFFERENT PUPILLARY DEFECT IS DEPENDENT ON INTENSITY OF LIGHT STIMULATION TO THE PUPILS

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Purpose: The light intensity required to grade the severity of relative afferent pupillary defect (RAPD) during eye examination is not standardized. This study determined the impact of varying light intensities on the grade of RAPD in neuro-ophthalmic pathology, vis-à-vis, age-matched controls.

Methods: Pupillary light reflex of patients with clinically diagnosed right (n=24) and left eye RAPD (n=28; 12 – 72yrs) and 29 controls (14 – 50yrs) were measured at 8 different light intensities (6.4 to 1200lux) in random order using two on-axis infrared sensitive cameras at 15Hz. Pupil constriction in each eye was elicited twice with 1sec-long light pulses from broadband LEDs placed 80 mm before the eye, independent of fellow eye, followed by 3sec-long epochs of darkness. RAPD was calculated as the ratio of pupillary light reflex to direct stimulation of the left and right eyes.

Results: Median (25th – 75th IQR) RAPD scores for controls were 0.99 (0.95 – 1.1), independent of light intensity (p>0.05). Median scores in left eye RAPD was 0.46 (0.43 – 0.61) for the lowest light intensity and progressively increased to reach control levels with increasing intensity [0.89 (0.63 – 0.94)] (p<0.01). Median scores in right eye RAPD showed the opposite trend [score at lowest light intensity: 1.76 (1.23 – 1.98); score at highest light intensity: 0.99 (0.81 – 1.14)] (p<0.01). Consensual reflexes across cohorts were expectedly unremarkable.

Conclusions: Unlike controls, RAPD is amplified at low light intensities and attenuated at high light intensities in neuro-ophthalmic pathology. Standardization of light intensity is therefore necessary for ambiguity-free estimation of RAPD severity during eye examination

PERCEPTION OF SUPRATHRESHOLD CONTRAST IN KERATOCONUS

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Purpose: Threshold level spatial vision is known to be reduced in keratoconus. However, suprathreshold performance has rarely been investigated. Using the well-known contrast constancy paradigm, this study hypothesized that suprathreshold contrast matches for keratoconics will show deficiencies that may be predicted from the pattern of loss in their contrast sensitivity function (CSF).

Methods: Apparent contrast matches were determined at 10% and 50% contrast in five unilateral keratoconic cases (24 – 29yrs) and 10 age-matched controls using an adaptive staircase procedure with 8 reversals. The match was determined between a "standard" Gabor grating, with spatial frequency corresponding to the peak of the subject's CSF, and "test" gratings with frequencies at one-third, one-half, twice or three times that of the standard grating.

Results: For both suprathreshold contrast levels, the matching contrasts of test gratings were within ±20% of the contrast of the standard grating for spatial frequencies greater than the standard grating (p=0.12 for both). In comparison, the contrast matches were significantly higher (~40 – 60%) for spatial frequencies lower than the standard grating (p<0.05 for both frequencies). Similar pattern was observed in controls. At threshold, cases showed significantly deteriorated CSF (mean±1SD area under CSF: 1.25±0.37log units), relative to controls (2.23±0.24log units) (p<0.001).

Conclusion: Suprathreshold contrast perception appears to remain unaltered in keratoconus for spatial frequencies higher than the peak of the CSF, even though contrast thresholds at these frequencies were significantly deteriorated. In comparison, suprathreshold contrast losses may be seen in both keratoconics and controls for lower than peak spatial frequencies.

VISUALIZATION OF CORNEAL OPACITY SCORE THROUGH A MULTI AXIS WHEEL DIAGRAM

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Purpose: Corneal opacity measured by a topographer usually incorporates several important diagnostic modalities such as pachymetry, corneal topography and 3D analysis of the anterior chamber. However, these multi readout instruments have limited visualization capabilities. Herewith we design a methodology to visualize a cumulative multi-axis opacity map by incorporating a uni-axis corneal opacity score.

Methods: Rabbits with deep stromal alkali injury (Penetrating lamellar keratoplasty 5mm wide and 150 µm depth using guarded trephine followed by 0.75N NaOH alkali burn) were imaged using the Galilei (G4 colorZ, Ziemer Ophthalmic Systems AG, Switzerland) to obtain the corneal densitometry score which ranges from 1-100 with 100 being fully opaque. The opacity values were obtained in eight individual axes at 45^0 apart. Specific values of opacities at radii of 2mm, 5mm, 7mm, 9mm and center of the cornea were recorded and transferred to a template. A custom Matlab script (MATLAB. R2020b, The MathWorks Inc., Natick, Massachusetts, United States) was generated to plot a scatter chart in polar coordinates.
Results: The multi-axis polar scatter chart having the opacity scores was plotted for rabbits with scars of diameter 5mm. The scars were of various grades such as nebular, macular and leucomatous. The progress of re-epithelization of the wound and therefore the degree of opacity was visualized over the study period. The average opacity score was calculated for each chart within the wound region. Hence as the scar regenerated, the average opacity score inside the wounded region gradually reduced to ≤20, indicative that the cornea was more transparent.

Conclusions: These results demonstrate temporal changes in the process of scar healing through opacity scores. The multi axis representation of the densitometry map provides a conclusive visualization of the degree of opacity over the entire corneal region.

FLUCTUATIONS OF STEADY-STATE ACCOMMODATION IS A MARKER FOR SCREENING SPASM OF NEAR REFLEX

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Purpose: To determine the utility of root mean squared (RMS) deviations of steady-state accommodation as a non-cycloplegic marker for spasm of near reflex (SNR), vis-à-vis, regular refractive errors.

Methods: Binocular steady-state responses of accommodation, pupil and vergence of 20 patients with accommodative spasm subtype of SNR (SNR-A; 9-23yrs) and 91 with regular refractive errors (29 emmetropes, 41 myopes, 21 hyperopes; 19–38yrs) was recorded *in the* uncorrected refractive error state for 120sec using a dynamic (50fps), infrared photorefractor. Mean and RMS deviation of raw data was calculated for three 20sec–long epochs and their diagnostic utility was determined using standard ROC curves.

Results: RMS deviations of accommodation increased with mean refractive error in SNR-A ($y = -0.23x + 0.38$; $r^2 = 0.69$; $P < 0.001$) and regular refractive error ($y = -0.02x + 0.10$; $r^2 = 0.14$; $P = 0.002$) cohorts, albeit with steeper slope and higher y-intercept in the former rather than the latter cohort. RMS deviation of 0.19D reliably distinguished SNR-A from regular refractive errors with a sensitivity and specificity of 95.2% and 92.2%, respectively [mean (±1 SEM) area under ROC curve: 0.98 ± 0.01]. The sensitivity, specificity, and area under ROC curve for RMS deviations of pupil (66.7%, 80%, and 0.70 ± 0.09) and vergence (52.4%, 84.6%, and 0.68 ± 0.08) were smaller than accommodation.

Conclusions: RMS deviations of steady-state accommodation is a robust noncycloplegic marker for differentiating SNR-A from regular refractive errors. Pupil and vergence fluctuations have limited utility in this regard.

OBJECTIVE AND SUBJECTIVE CHANGES IN COLOR DISCRIMINATION WITH AND WITHOUT UNDERWATER BLUR

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Purpose: Scattering of light and color change are two major sources of distortion which affects human's visual performance underwater. The aim of this study is to evaluate the changes in objective and subjective color discrimination with and without underwater blur.

Methods: For objective color discrimination, the changes in RGB coordinates for Macbeth ColorChecker Chart (24 color patches) with and without underwater blur (UWB) was measured using a Colormeter v1.0.2 app. The color patches were displayed on a Display++ monitor using a MATLAB program. Underwater blur was simulated by placing a water filled glass tank between the monitor and the Colormeter. In each condition total of 300 readings were obtained for each color and the average was used for the analysis. Ten young normal subjects performed a computer based FM100 color discrimination test with and without underwater blur, for obtaining the subjective measurements. For both objective and subjective measurements, the effect of glass tank was measured in a separate session and the color scores were adjusted accordingly.

Results: For the objective measurements, each RGB coordinates with underwater blur were significantly less than without underwater blur condition ($p < 0.01$). There was an increase in slope between reference coordinates and measured coordinates with underwater blur condition (Mean increase in slope: 0.56 ± 0.01). The red coordinates were more affected than other coordinates ($p < 0.05$). The subjective measurements (mean error scores for FM100) were not significantly different with and without underwater blur (1.6 ± 7.3, $p = 0.45$).

Conclusions: Our results suggest that the objective color coordinates decrease with underwater blur. The subjective discrimination of color is not affected by these changes induced by underwater blur.

HOW DOES VISION CORRELATE WITH OVERALL DEVELOPMENT IN CHILDREN WITH CEREBRAL VISUAL IMPAIRMENT?

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Purpose: Vision contributes significantly to the overall development of children. Children with neurological conditions such as cerebral visual impairment (CVI) are likely to have overall developmental delays. Through this study, we determine the correlation between vision loss and overall development of children with CVI by comparing vision-related parameters with developmental quotients (DQ).

Methods: A prospective cross-sectional study was conducted on children with CVI visiting a paediatric neurology clinic in India. Grating acuity (Teller Acuity Cards), contrast sensitivity (Ohio Contrast Cards), functional vision assessment (Roman-Lantzy's CVI range; phase 1=building visual behaviour, phase 2=integrating vision with functions, phase 3=resolution of CVI characteristics) and DQ (Denver Developmental Screening Test-II) were assessed.

Results: Thirty-four children (males=25) with CVI were included with a mean chronological age of 2.9±1.8 years. The mean binocular grating acuity was 1.37±0.62 logMAR (range=2.27 to 0.37 logMAR) and mean binocular contrast sensitivity was 0.43±0.53 logCS (range=0.0 to 1.66 logCS). Grating acuity (r=-0.36, p=0.04) and functional vision (r=0.51, p=0.02 Spearman's rho) were significantly and moderately correlated with DQ. Weak correlation was noted between the contrast sensitivity and DQ (r=0.25, p=0.14). Developmental quotients were found to be significantly different across the three phases of CVI (p=0.04, Kruskall Wallis).

Conclusions: Vision loss correlates with the developmental quotient in children with CVI. Although there is no separate vision component in most developmental screening tools, psychologists could consider referring particularly those with lower developmental scores for vision assessment and management. Eye-care professionals should consider providing appropriate referrals for the management of child's overall development.

WHO IS PAYING FOR CATARACT SURGERY OF ELDERLY IN RURAL TELANGANA?

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Purpose: To understand the paying patterns for cataract surgery among the elderly in rural eye care facilities of LVPEI.

Methods: All the patients aged 60 years and older who underwent paid cataract surgery in September 2020 of six LVPEI rural eye care facilities were interviewed by trained investigator. The interview included collection on information on their family structure, occupation, economic dependency and source of funds for payment for their cataract surgery.

Results: The response rate was 72.7% (n=392); 75% (n=295) were independent for their daily living of which 48% (n=141) are females. But 65% (n=198) of those who were independent reported dependence on their immediate family members for payment for cataract surgery. Overall, 25.5% (n=100) of them are paid by themselves and 74.5% (n=292) of them are paid by their family members.

Conclusions: Though most elderly were independent of their daily living, majority of them needed support from external sources for cataract surgery.There is an immediate attention needed from the policy makers / care givers in providing health care at no cost for all those who are not covered in the insurance or any government schemes.

PHOTO-SCREENING FOR EARLY DETECTION OF RETINOBLASTOMA: A PROOF OF CONCEPT STUDY

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Purpose: To determine if infrared (IR) photo reflex can be an effective screening tool for retinoblastoma (RB).

Methods: Subjects visiting Orbit & Ocular Oncology services underwent IR video recording from September 2020 to December 2020 with diagnosis of RB. All subjects were divided into two groups. Group-1 had diagnosis of RB, and Group-2 other eye of subject with no tumor.

Results: Of 34 eyes from 23 subjects, 25 eyes had RB, and nine had no tumors. We extracted image frames from video recordings of 34 eyes; 75 central gaze images were eligible for measurement of pixel intensity (0 to 255). Of these, 57 images (35 undilated pupil images, 22 with dilated pupil) had RB, and 18 images had no tumor. The mean pixel intensity within the pupil in eyes with RB was 109 (median, 90; range, 22 to 254); and in eyes with no tumor was 70 (median, 67; range, 26 to 125). We found the difference to be statistically significant (p, 0.015; Mann-Whitney U test). The mean pixel intensity in images from undilated eyes with RB was 97 (median, 77; range, 23 to 254), and dilated eyes were 130 units (median, 114; range, 46 to 252). Although the pixel intensity was higher in images from dilated pupils, the difference was not statistically significant (p, 0.9; Mann-Whitney U test).

Conclusion: Technology combining IR imaging and pixel intensity measurement within the pupil can translate into a potential screening tool for early detection of RB.

LIFE IN LOCKDOWN: IMPACT OF COVID-19 LOCKDOWN MEASURES ON THE LIVES OF SCHOOL-AGE CHILDREN AND THEIR FAMILIES IN INDIA

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Purpose: The COVID-19 outbreak has adversely impacted all societal domains, including education. Home confinement, school closures, and distance learning impacted children, teachers, and parents' lives worldwide. The aim of our study was to examine the impact of COVID-19 lockdown on the lives, including education, of school-age children with vision impairment (VI) and their parents in India.

Methods: School-age children with VI (7-19 years) and their parents were recruited from Institute for Vision Rehabilitation, L V Prasad Eye Institute, Hyderabad, India. A questionnaire with open-ended questions was employed to explore experiences of school closure and its impact on education, and attending online classes during the COVID-19 lockdown. Content analysis was used to identify themes pertinent to the cohort studied.

Results: 48 child-parent dyads were included. Inherited retinal disorders were the major cause of VI (40%). Visual acuity (better eye) ranged from 6/12 to 6/750. Six major themes were identified: (1) accessibility of technology (online learning and technology); (2) parental beliefs/concerns (harmful effects of technology, prioritization of normally sighted children, online classes considered a distraction); (3) support (peers, parents, teachers, private tuition); (4) socialization and physical activity, (5) socioeconomic status, and (6) near vision.

Conclusions: Our study provides an understanding of adverse impact of lockdown on lives of children with VI and their parents, especially related to education in India. The study identified critical factors that affect online learning and participation of children with VI in these sessions. Policy makers and educators should implement effective measures for supporting online classes.

THE SPA-VVRT (SMART PHONE ANAGLYPH VIDEO VIRTUAL REALITY THERAPY) STUDY: A PILOT NON-RANDOMIZED TRIAL OF SMART PHONE ANAGLYPH VIDEO VIRTUAL REALITY BASED THERAPY FOR TREATMENT OF AMBLYOPIA IN ADULTS

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Purpose: To study the utility of a smart phone based virtual reality system using anaglyph videos in adults with anisometropic amblyopia.

Methods: Patients >/= 18 years of age diagnosed with anisometropic amblyopia, complaint with use of glasses or contact lenses and with no prior history of amblyopia therapy (patching, penalization etc.) were included in this non-randomized prospective interventional study. After a comprehensive ocular examination, all the patients were subjected to 4 hours of therapy every day. The therapy was home based and involved viewing the anaglyph videos in a smartphone (placed within a VR box) through red-blue filters. Visual acuity, stereopsis, contrast sensitivity and near point of accommodation were recorded at presentation, 3 months, and 6 months after undergoing therapy.

Results: A total of 12 patients were included. Mean age was 20.33 ± 1.96 years (18-23). 8 had hyperopia (mean = +6.12 D ± 1.06, range: +5.00D to + 8.00D). 4 had myopia (mean: -6.62 D ± 2.49, range: -10.00D to -4.5D). The mean BCVA improved from 1.22 LogMAR to 0.9 and 0.8 LogMAR at 3 and 6 months respectively. Stereopsis, contrast sensitivity, near point of accommodation improved from 600 seconds of arc, 0.61 log units, and 20.33 cm to 263.33, 1.12 and 16.33 respectively at 6 months. The changes in all the parameters were statistically significant (P<0.05).

Conclusions: The SPA-VVRT (Smart Phone Anaglyph Video Virtual Reality Therapy) was found to be effective in adults with anisometropic amblyopia and showed significant improvement in visual acuity, stereopsis, contrast sensitivity and accommodation.

TEARING MODE FRACTURE TOUGHNESS OF CORNEA

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Purpose: To investigate the fracture toughness of human cornea under tearing mode to evaluate the defect tolerance of the cornea.

Methods: 30 corneas with an initial crack length of 2.5 mm from limbus were tested under trouser tearing mode at strain rates 3, 30, and 300 mm/min. Tests were carried in a universal tensile testing machine with a 50 N load cell. After the tear tests, one- half of the torn samples were subjected to tensile load to check the relation between the fracture toughness and stiffness.

Results: Fracture toughness of the cornea is found to vary from 5.87±1.13 KJ/m^2 at 3 mm/min strain rate to 7.03±1.19 KJ/m^2 at 300 mm/min strain rate. Tearing energy varies along the tear path, from the center of the cornea towards the periphery, an increase in tearing energy is observed. The relation between fracture toughness and cornea stiffness was linear, which is probably due to the microstructure of the cornea.

Conclusions: Fracture toughness of cornea under tear mode is found. The peripheral part of the cornea is tougher than the central part.

ABSTRACTS (POSTERS)

BASIC SCIENCES

TARGETED PANEL-BASED GENE SCREENING FOR A LARGE COHORT OF PATIENTS WITH LCA IN SOUTHERN INDIA

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Purpose: Leber Congenital Amaurosis (LCA) is a highly heterogeneous inherited retinal disease. The current study aims to reveal the genetic etiology of south Indian LCA patients using panel-based targeted sequencing.

Methods: A targeted panel composed of 30 LCA candidate genes was established and utilized to screen 109 unrelated south Indian LCA patients. The data were analyzed using an *in-house* bioinformatics pipeline and identified mutations as per the ACMG guidelines. Segregation analysis was also performed on the family members through Sanger sequencing.

Results: Among 109 patients, 91 were identified with mutations in 19 LCA candidate genes, of which forty patients possessed a novel mutation. The mutation detection rate was 83%, consisting of 60% pathogenic, 18 % likely pathogenic and, 22% variants of uncertain significance. Among 19 genes, *GUCY2D* has the highest mutation rate of 21%, followed by *LCA5* and *CRB1* (10%), *AIPL1* and *RPGRIP1* (8%), *RPE65* and *ALMS1* (6%), *NMNAT1* and *CRX* (3%), *CEP290* (2.5%), and other genes *SPATA7, IMPDH1*, *RD3*, *KCNJ13, IQCB1*, *CLUAP1*, *IFT140*, *LRAT*, and *CWC27* contributes for less than 1%. Also, this is the first study to report mutations in the LCA genes *IMPDH1*, *CLUAP1, CEP290, IFT140*, *LRAT,* and *CWC27* from the Indian population.

Conclusion: In conclusion, targeted sequencing with an expanded gene panel has significantly increased the resolution of the mutation detection rate to 83% compared to previous studies. Moreover, molecular diagnosis helps to understand the genetic etiology, which would further help to provide an accurate clinical diagnosis, genetic counseling and pave the way for gene therapy.

DEVELOPMENT AND CHARACTERIZATION OF RABBIT CORNEAL ULCERATION MODEL FOR PRE-CLINICAL STUDIES

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Purpose: Here we enumerate hallmarks of corneal fibrosis and ulceration: fibrosis and extracellular matrix deposition (ECM), angiogenesis and inflammation. This study highlights the ophthalmological, clinical and histopathological changes throughout the progression of deep stromal alkali injury in the rabbit cornea.

Methods: Albino New Zealand rabbits were used for the study. Penetrating lamellar keratoplasty was performed on rabbit cornea (n=3) using guarded trephine. 0.75N NaOH was applied followed by irrigating with normal saline. Animals were imaged using the ophthalmological parameters; i) OCT, ii) Slit lamp and iii) Densitometry over the period of 3 weeks. At 3rd week rabbits were sacrificed and eyes were enucleated for histology and histopathology.

Results: The parameters used in clinics for evaluating patients were used to evaluate and grade the scars into nebular, macular and leucomatous. Slit lamp reveled re-epithelization of the wound in first 5-6 days followed by incidences of epithelial defects, inflammation, and opacification. Neovascularization was observed after 14-15 days of alkali burn. OCT: pachymetry wide and raster scans reveled extensive edema and thickening of the central cornea. Densitometry images reveled stable scar formation with the opacity score ≥75, condition of legal blindness. Histology and histopathology supported the ophthalmological evaluation.

Conclusion: These results demonstrate dynamic changes in the process of scar development post alkali burn. Using the advance clinical techniques of OCT and densitometry used we captured the temporal changes during the process of stable scar formation. Based on these results we suggest it is important to follow the clinical parameters while evaluating the pre-clinical animal model for corneal ulceration.

OPTIMIZING THE XENO-FREE TECHNIQUES OF HUMAN LIMBUS-DERIVED MESENCHYMAL/STROMAL STEM CELL EXPANSION USING 3D CULTURE METHODS

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Purpose: Human limbus-derived mesenchymal/stromal stem cells (hLMSCs) are a promising alternative to the conventional corneal transplantation for treating blinding corneal pathologies. However, the methods of cultivating these cells using xeno-free methods is not well established. This study aims to optimize the methods of expanding hLMSCs *in vitro* using xeno-free 3-dimensional (3D) cell culture methods.

Methods: Limbal tissues were excised from the therapeutic grade corneoscleral rims and expanded till tertiary culture using standard serum-based methods followed by culture in different serum-free media. The cells grown in serum free media were analyzed for MSC specific markers using FACS and immunostaining. These hLMSCs were also expanded in different commercial hydrogel compositions for 3D(spheroid) culture and analyzed by immunostaining.

Results: All the three different serum-free media have supported growth of hLMSCs. Cells have retained their morphological and phenotypic characteristics. The FACS analysis have shown that ≥80% hLMSCs were positive for CD90, CD73, ABCG2 and ≤4% were positive for CD45, HLADR. The cells grown in hydrogels attained spheroid morphology and have retained the characteristic phenotype (CD105$^+$, CD90$^+$, CD73$^+$, ABCG2$^+$, VIMENTIN$^+$, CD45$^-$, HLADR$^-$) with respect to the monolayer culture (control) confirmed through immunostaining.

Conclusions: The findings of our study suggest that the hydrogel-based 3D methods enable the hLMSCs to be expanded with increased stemness. That hLMSCs can successfully be cultivated in serum deprived media to prevent xenogeneic contamination, enabling them to be utilized in a clinical trial in their full potential, overcoming the regulatory issues is also one of the potential outcomes of this project.

REPAIR OF ULTRA-VIOLET LIGHT INDUCED DNA DAMAGE IN HUMAN CORNEAL TISSUES

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Purpose: To study the mechanism involved in repair of ultra-violet (UV) induced DNA damage in the corneal tissues of Xeroderma pigmentosa (XP) patients.

Methods: XP patient corneas were procured from the pathology department at the L. V. Prasad Eye Institute (n=5) after obtaining IRB approval. Healthy corneas (n=3) were obtained from Ramayamma International Eye Bank and served as controls. Immunohistochemistry was performed on sections from these samples using specific antibodies against proteins involved in DNA repair including phospho-ataxia telangiectasia mutated (ATM), -ataxia telangiectasia and Rad3-related protein (ATR), -breast cancer 1 (BRCA1), -checkpoint kinase 1&2 (Chk1&2), -Histone H2A.X, -p53, anti-thymine dimer antibody [H3] and anti-rabbit/mouse secondary antibodies. Confocal microscope was used for imaging the sections.

Results: Control corneas showed positive expression of thymine dimer and H2AX only in the epithelial layers unlike XP samples wherein the keratocytes and endothelial cells also showed positive expression. Unlike control corneas, positive expression of specific markers of the DNA repair pathway were noted in all cell layers indicating accumulation of DNA damage that affected the deeper corneal layers. More cells expressed Chk1 as compared to Chk2 protein which suggests that the double stranded and single stranded breaks led to the phosphorylation of BRCA1 through activation of ATM/ATR which ultimately led to apoptosis of these cells as evidenced by the positive staining for p53.

Conclusion: The study shows conclusively the presence of DNA damage in all cell layers of the cornea in XP patient samples and the impaired repair mechanism likely result in their death.

POLYMORPHISM IN THE PROMOTER REGION OF CATALASE GENE IN THE WESTERN INDIA DIABETIC POPULATION WITH RETINOPATHY

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Purpose: Type 2 ddiabetes mellitus (T2DM) is the greatest burden in developing and developed countries because of rapid increase in number of diabetic population. T2DM is associated with several other anomalies including diabetes retinopathy. In present study we have evaluated polymorphism in catalase gene promoter region in West Indian population and its effect on expression of catalase gene.

Methods: 100 subject each of non-diabetics, T2DM and T2DM + DR were included in the study. Catalase activity in blood was determined by spectrophotometric assay. Polymorphism in promoter region was determined by PCR followed by DNA sequencing. Promoter region of catalase gene and associated transcription factor binding sites were determined by Transfec software. Effect of polymorphism on promoter activity was determined by mutagenesis analysis.

Results: Out of 100 subjects with T2DM, we have detected -21 A/T SNP in promoter region of catalase gene in 9 subjects while in T2DM + DR it was detected in 18 subjects. Catalase activity in T2DM subjects with polymorphism was 61% to that of no polymorphism and T2DM + DR was 33% to that of no polymorphism and 75% to the subjects of T2DM. Transfec analysis suggested alteration in binding of transcription factors due to polymorphism. Mutagenesis studies revealed that SNP leads to 25% decrease in activity of promoter.

Conclusions: Catalase activity is reduced in subjects of T2DM and T2DM with complication such as DR. Alteration in catalase activity in T2DM and T2DM with DR subjects may be due to SNP -21A/T.

GENETIC PROFILE OF PRIMARY ANGLE CLOSURE DISEASE IN A LONGITUDINAL COHORT FROM SOUTHERN INDIA

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Purpose: Primary angle closure disease (PACD) comprises of complex phenotypes that are largely characterized by the closure of the iridocorneal angle ($\geq 270°$) resulting in the obstruction of aqueous outflow pathway and increased intraocular pressure (IOP). Various Genome wide association studies (GWAS) have identified genetic variants associated with PACG in different populations. In this study, we aimed to identify the genetic profile of PACD in a longitudinal cohort from Southern India.

Methods: The overall incidence of PACD in the longitudinal Andhra Pradesh Eye Disease (APEDS) cohort was estimated to be 16.47/100 person years. Among these, 167 cases of PACD along with 1759 ethnically matched controls were screened by deep sequencing (Ion ampliseq chemistry) using a targeted gene panel containing previously associated variants in PLEKHA, HGF, ABCC5, GLIS3, DPM2-FAM102A, TNFa, GAS7, TMCO1, and CDKN2B-AS1 genes. Appropriate quality control measures and analysis tools were used to assess the involvement of the gene variants. The variants were analysed in conjunction with other global datasets.

Results: The data revealed that variants in PLEKHA (rs11024102; p=5.89E-05), HGF {(rs17427817; p=1.55E-06 and rs17427817, p=4.43E-02)}, DPM2-FAM102A (rs3739821; p=1.97E-02), TNF {(rs1799724; p=0.049375511), (rs1800629; p=0.014636981)}, and CDKN2B-AS1 (rs1063192; p=8.22375E-14 and rs2157719, p=0.003122856) were found to be significantly associated with all forms of PACD. The GWAS-associated variants across the remaining genes did not exhibit any association to PACD.

Conclusions: Our study indicated that variants in PLEKHA, HGF, DPM2-FAM102A, TNFa, and CDKN2B-AS1 genes could be involved in different forms of PACD in the APEDS cohort, as observed in other cross-sectional studies. Further implications of these variants in disease progression needs to be explored.

HIGH GLUCOSE INDUCED INFLAMMATION IS INHIBITED BY COPPER CHELATION VIA RESCUING MITOCHONDRIAL FUSION PROTEIN 2 IN RETINAL PIGMENT EPITHELIAL CELLS

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Purpose: Altered trace element homeostasis is associated with diabetic complications. Studies show elevation of serum copper levels in diabetes. Copper chelation has been shown to prevent or reverse diabetic organ damage and develop as a new treatment strategy for treating diabetic complications. Diabetic retinopathy (DR) is the major vision-threatening complication of diabetes. Disruption of the outer blood-retinal barrier function by hyperglycemia in DR induces structural alterations in Retinal Pigment Epithelial (RPE). Recent studies have reported copper to be elevated in the serum of patients with DR.

Methods: Here in this study, we attempt to unravel the role of copper chelator penicillamine in RPE cells exposed to high glucose and copper as a model for diabetic retinopathy. The cells were exposed to osmotic control (OC – control), High glucose (HG – 25 mM), HG co-treated with copper (HG+Cu – 50 µM), HG co-treated with penicillamine (HG+P – 800 µM) and HG co-treated with copper and penicillamine (HG+Cu+P). Transcript levels were studied using qPCR, while western blotting and immunofluorescence were used for protein expression.

Results: We have found that high glucose by itself and along with copper alters the mitochondrial morphology, reduces the expression of the mitochondrial fusion protein (MFN2), and induces endoplasmic reticulum stress and inflammation. Copper chelation with penicillamine reduced all these changes in mitochondria, thereby rescuing the cells from mitochondrial damage and inflammation.

Conclusion: Copper chelation could be used as a treatment approach in DR cases with RPE damage.

CONCORDANCE AND RELIABILITY OF DEEP SEQUENCING DATA GENERATED FOR MUTATION SCREENINGS

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Purpose: Next generation sequencing (NGS) of clinical samples have significantly expedited the discovery of genetic variants. However the authenticities of the observed variants are contingent upon multiple quality assessment parameters along with corresponding validations by Sanger sequencing to rule out discordant data. The present study aims to understand the concordance of variants obtained through high throughput NGS to that of bi-directional sequencing data generated from Sanger sequencing.

Methods: Rare variations observed in five candidate genes (*CYP1B1, LTBP2, TEK, MYOC,* and *FOXC1)* involved in primary congenital glaucoma (n=586) generated either through NGS (Ion Ampliseq chemistry) or resequncing (BigDye chemistry) were considered. Both the datasets were validated in the corresponding platforms and subjected to statistical analysis for sensitivity, specificity, positive and negative predictive values in order to assess reliability.

Results: It was observed that the sensitivity of the data obtained from NGS was 83.2% and specificity was 99.68%. The positive and negative predictive scores were 97.64% and 97.41%, respectively. False positives or artifactual variants resulted due to their nature and position in the genome and the inability of the platform to capture such changes.

Conclusions: Overall, our data suggested a slightly low sensitivity for NGS but considering the throughput it stays put as a rapid screening method. The data generated from NGS should be interpreted with caution when novel variants are identified and appropriately validated for a better outcome.

EXOSOMES ISOLATION AND CHARACTERIZATION FROM RETINOBLASTOMA CELL CULTURES

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Purpose: Exosomes are subclass of extracellular vesicles secreted by all types of cells including tumor cells. They contain the cargo that not only facilitate the tumor progression but also facilitates inter and intracellular communication at target sites which may initiate and promote metastasis. We attempted to evaluate and characterize the exosomes derived from conditioned media of RbY79 cells lines (Riken: RCB1645 Y79) and its organoids.

Methods: The culture media 100ml of RbY79 cell lines was used to isolate the exosomes, using three different exosomes isolation methods ultracentrifugation (UC), commercial kit (Total exosomal isolation reagent) and UC followed by kit. The exosomes were evaluated for morphology and size distribution using Field Emission Scanning Electron Microscopy, Transmission Electron Microscopy and Dynamic Light Scattering. Exosomal protein using SDS-PAGE and exosomal surface markers were identified by Immunoblotting.

Results: As compared to kit and combined method, the exosome yield was higher (0.4µg/8µl) from conditioned media of RbY79 cell lines using ultracentrifugation method. The exosomes were spherical (cup shape), measuring 30nm to 100nm. Immunoblotting revealed immuno positivity exosomal surface markers (CD63, CD81). However, to isolate detected quantity of exosomes we need 50 to 100 ml media.

Conclusion: The study demonstrates the feasibility of isolating exosomes and limitation from Rb cell cultures which could be reliable source to pave way for further *invitro* and *in vivo* studies.

DESIGN AND CHARACTERISATION OF SHORT ANTIMICROBIAL PEPTIDE TO TREAT FUNGAL KERATITIS.

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Purpose: Fungal keratitis refers to corneal infection caused by fungi. It requires upgraded treatment as available treatments are challenged by limited availability of therapeutics, toxicity and rise of antimicrobial resistance. Antimicrobial peptides (AMPs) gained importance as they have broad spectrum, rapid killing nature, less chance of gaining resistance by microbes. We have seen efficient inhibition of fungal growth with a host defense peptide that we are currently working on. However, being a host-peptide, it is large in size and is difficult to penetrate the cornea when administered topically. On the basis of the host peptide, we have bioinformatically designed and synthesized a short peptide of 15 amino acids (SA-XV). The purpose of this study is to characterize and determine the anti-microbial activity, immunological response of SA - XV against different fungal species.

Methods: SA - XV has been characterised by circular dichroism. The anti - fungal activity against several species like *Fusarium, Aspergillus* is measured by MIC, CFU. The peptide has also been tested to see its efficiency in curbing the biofilm formation. The membrane disruption study by the peptide has been done with propidium iodide.

Results: SA-XV has found to be non-cytotoxic to human corneal epithelial cells. Following treatment with SA-XV, the growth of fungal hyphae is significantly reduced compared to control. SA-XV has been found to efficiently reduce the biofilm formation by fungal species. The peptide has also been found to efficiently disrupt the fungal membrane.

Conclusion: Our results demonstrate the fungistatic activity of SA-XV against *Fusarium spp*.

ESTIMATION OF SERUM HYDROGEN SULFIDE IN AGE RELATED MACULAR DEGENERATION

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Purpose: Age-related macular degeneration (AMD) is one of the major causes of global blindness and vision impairment. A recent study has proposed hydrogen sulphide (H_2S) as a potent molecular target to be explored in AMD. Chronic low-grade inflammation is associated with AMD. H_2S has a controversial role in inflammation. This pilot study was done to estimate the serum levels of H_2S in AMD subjects and associate with inflammatory marker interleukin-6 (IL-6) in the south Indian population.

Methods: Twenty-six blood samples were collected, 12 control and 14 AMD subjects, the serum obtained from these samples were used for the measurement of H_2S by methylene blue assay and IL-6 using ELISA.

Results: We observed that both the levels of H_2S ($p = 0.04$) and IL-6 ($p = 0.002$) to be elevated in AMD subjects when compared to control and H_2S and IL-6 showed a positive correlation ($r = 0.4569$; $p = 0.0325$).

Conclusion: We speculate H_2S to be an early marker in AMD. Future extensive studies in larger sample size are needed to evaluate this hypothesis.

IN VIVO ASSESSMENT OF THE TOXICITY OF HUMAN LIMBUS-DERIVED STROMAL/MESENCHYMAL STEM CELLS WITH OR WITHOUT ALGINATE ENCAPSULATION FOR CLINICAL USE

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Purpose: To assess the ocular and systemic toxicity of human limbus-derived stromal/mesenchymal stem cells (hLMSCs) with or without alginate encapsulation as per Indian FDA guidelines.

Methods: The hLMSCs were obtained from human cadaveric corneoscleral rims and cultivated in a CGMP setting using established protocols. Cells with (En+ hLMSCs) or without (En- hLMSCs) alginate encapsulation were used for the animal experiments. The study involved 3 groups of 6 New Zealand white rabbits each, which underwent corneal wounding followed by treatment with sham (G1), En- hLMSCs (G2), and En+ hLMSCs cells (G3). Ophthalmic assessment including intraocular pressure, blood investigations and inflammatory markers (IL-6, TNF-, IgE) expression in serum and tears were assessed on days 1, 7, 14, 21, and day 28. At the end of 28 days, the animals were sacrificed, and the organs were subjected to histopathological examination.

Results: No rabbits were lost during the study. Ophthalmic examination showed no significant difference in IOP, corneal clarity and conjunctival congestion between the three groups at every time point. Haematological parameters were comparable between the three groups. The inflammatory markers in tear and serum (TNF-α and IL-6) were not significantly elevated in the groups receiving En+/En- hLMSCs. Histological examination did not show any abnormality in the ocular and the corneal tissue and the viscera.

Conclusions: The results of the study show that hLMSCs do not cause any local or systemic toxicity in recipients, implying that these cells are safe for clinical use and their efficacy can be assessed in human clinical trials.

PREVENTION OF CORNEAL SCAR FORMATION BY USING EXTRACELLULAR MATRIX HYDROGEL FOR CORNEAL REGENERATION

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Purpose: Corneal blindness is estimated to affect 4.9 million people globally and is 4th major cause of blindness. Despite recent advancements in surgical strategies there is unmet need for clinically feasible material and the shortage of high-quality donor tissue is still in existence. An approach to this problem is tissue engineered cell based corneal substitute. This study is to evaluate the potential utility of ECM hydrogel derived from cadaveric cornea for corneal tissue regeneration.

Methods: Cadaveric cornea rejected for corneal transplantation were decellularized, lyophilized for ECM hydrogel preparation. Biochemical and physical properties of the prepared ECM hydrogel was analysed. Cell viability, migration, proliferative and metabolic activity of the encapsulated keratocytes in ECM hydrogel were assessed.

Results: The DNA content of native and decellularized tissue was 1033 ± 99.7 ng/mg
and 29.9 ± 2.45 ng/mg respectively. The sGAGs and total collagen retained after decellularization was 80.9 ± 8.44 and 33.4 ± 4.7%. The percentage of proliferating cells was 85.8 ± 2.42% after 24 h of encapsulation and reduced to 12 ± 0.77% by day 14. Live–dead assay showed majority of keratocytes were viable. MTT assay showed a significant increase in the cell metabolic activity until day 14. Analysis of cell migration showed more than 75% of cells were migrating at a gel height between 200 and 250 µm from the bottom, through the ECM hydrogel.

Conclusions: Our *in vitro* study has shown that the prepared ECM hydrogel has simple formulation procedure, biocompatible and a promising material for preventing blindness due to scar formation.

INVESTIGATION OF CRYSTALLIN GENE MUTATIONS IN PERSISTENT FETAL VASCULATURE DISEASE

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Purpose: PFV (Persistent Fetal Vasculature) is a rare congenital ocular disorder caused by incomplete regression of hyaloid artery. Crystallin proteins constitute approximately a third of the mass of the human lens, enabling it to have a high refractive index and optical transparency. Therefore, the present study aims to understand the role of crystallin gene mutations in the vasculature development leading to PFV in humans.

Methods: Fifty patients were divided into three groups: i) PFV(n=11) (ii) PFV with cataract (n=14), (iii) congenital cataract (n=25, internal control). The exons of all eleven crystallin genes (*CRYAA, CRYAB, CRYBA1, CRYBA4, CRYBB1, CRYBB2, CRYBB3, CRYGC, CRYGS, CRYGB, CRYGD*) were amplified using Polymerase Chain Reaction in the patients and examined by automated DNA sequencing. The PCR products were examined for mutations using bioinformatic tools.

Results: Crystallin mutation analysis revealed thirty-four population variants in patients and internal controls. *CRYAA* (rs872331), *CRYAB* (rs4252582), *CRYBA4* (rs5761637), *CRYBB1* (rs147206089, rs4049504), *CRYBB3* (rs13055430) and *CRYGD* (rs2242074, rs2305429) are eight of them reported to be associated with congenital cataract. The Minor Allele Frequency of all detected variants were comparable with normal control databases 1000G and IndiGen.

Conclusions: In this study, no pathogenic crystallin mutations were to be correlated to PHPV phenotypes. Considering that PHPV is a rare condition, one of the study's limitations was the small sample size. We expect that vascular regression process overlaps with multiple molecular signaling pathways during the embryonic process, thus a Whole Exome analysis with large sample size and family members will reveal more about PHPV pathogenesis.

CHARACTERIZATION OF EXTRACELLULAR VESICLES FROM RETINAL CELLS INFECTED WITH *PSEUDOMONAS AERUGINOSA*: APPLICATION IN BACTERIAL ENDOPHTHALMITIS

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Purpose: To characterize Extracellular vesicles (EVs), released by Retinal pigment epithelial (ARPE-19) cells challenged with *Pseudomonas aeruginosa* (PA).

Methods: EVs isolated from serum-free media of ARPE-19 challenged with PA by differential ultracentrifugation, was characterized by Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Fluorocet (System Biosciences) and western blotting for CD81. They also underwent liquid chromatography-mass spectrometry (LC-MS) analysis for protein identification.

Results: SEM and DLS analysis confirmed the presence of EVs and the absence of cellular contaminants. The results obtained from SEM showed their sphere-like shapes of around 468 nm (infected cells) and 100 nm (uninfected cells) in diameter. Through DLS, we found out that the mean diameter of the EVs isolated by ultracentrifugation was 99.5±7nm in cells infected with PA while the size of exosomes from uninfected cells was 85±12 nm. Quantification of EVs showed a high number of EVs in cells infected with PA in comparison to uninfected cells (PA = $1.1 \times 10^7 \pm 1560926$; Control = $4.9 \times 10^6 \pm 1121661$ for 500ng of EVs protein). The EVs isolated from PA infected cells were positive for CD81 and >800 different proteins were identified. Functional enrichment analysis linked the proteins to biological processes such as biological adhesion, cell communication, cell motility, metabolic process, complement pathway proteins and heat shock proteins.

Conclusions: EVs from retinal cells challenged with *P. aeruginosa* were larger in size compared to uninfected cells. The effective isolation of EVs will benefit the downstream analysis and clinical translation of EVs and determine their role in the pathology of bacterial endophthalmitis.

GENERATING RETINAL DYSTROPHY MODELS IN ZEBRAFISH

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Purpose: To knockout zebrafish *rd3 and abca4b* genes linked to retinal dystrophies and to study the effects of loss of function on retinal development and visual function.

Methods: CRISPR-Cas9 genome editing tool was used to create *rd3* and *abca4b* mutant models in zebrafish. spCas9 and sgRNA mix was injected into single cell stage fertilized embryos and the larvae are allowed to develop. Tail fin clips of juvenile fishes was used for DNA isolation and screening for founder fishes (F_0) with in-del mutations by PCR and Sanger sequencing. The founders were then backcrossed with *wt* animals to obtain F_1 heterozygotes (F_1). Interbreeding of F_1 resulted in F_2-*wt*, F_2-heterozygotes and F_2-null homozygotes (F_2). Retinal morphology of F_2 homozygous mutants was evaluated by immunohistology at 3 months, 6 months and 12 months of age.

Results: Retina of the null homozygous fish at 3 months showed underdeveloped cones with lamination defects in the outer nuclear layer. Both rod and cone degeneration were seen at later time points (6 and 12 months), with significant loss of UV and Blue single cones. Marked degeneration of cone subtypes resulted in severe visual defects and the mutant fishes were almost blind and lost their ability to see and catch their feeds.

Conclusion: *rd3* and *abca4b* knockout models of zebrafish has been successfully created by CRISPR/Cas9-based genome editing.

RPE HYPOXIA CELL CULTURE MODEL TO STUDY ANTI-ANGIOGENESIS THERAPEUTICS.

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Purpose: Vascular endothelial growth factor (VEGF) is a key mediator of angiogenesis. Anti-VEGF treatments are most effective in treatment of diabetic retinopathy, age related macular degeneration and corneal neovascularization. Human umbilical vein endothelial cells (HUVEC) respond greatly to hypoxia and are commonly used to study anti-angiogenesis studies under hypoxic conditions. Retinal pigmented epithelial (RPE) provide nutrients to photoreceptors and interior retina and subjected to hypoxic conditions under various pathological conditions. A little is known if RPE and how it responds to hypoxic conditions. Alternate models to study anti angiogenic studies in hypoxic conditions are also highly desired.

Methods: RPE cell line was kept under normal and hypoxic environmental conditions (1% O_2) for 24 and 48 hours. VEGF-A expression was measured by qPCR at mRNA level and Total VEGF protein by flow cytometer.

Results: RPE cells continuously produce VEGFA, however under hypoxic conditions a fold change of 2.54 and 9-fold increase in VEGF-A was observed under hypoxia as compared to normal conditions at 48hr. Total VEGF protein in the supernatant increased from 114 to 336 pg/ml (3-fold) with under normal conditions, however under hypoxia, VEGFA was nearly 8 times more after 48 hours (about 2600 pg/ml).

Conclusion: RPE cells showed significant upregulation of VEGF under hypoxic conditions. Thus, it may be used as an in vitro model to study anti-VEGF treatments under hypoxic conditions.

ASSESSMENT OF *IN VIVO* SURVIVAL AND SAFETY OF HUMAN INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED, RETINAL PIGMENTED EPITHELIAL (RPE) CELLS IN DYSTROPHIC RCS RAT EYES

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Purpose: To standardize sub-retinal cell injection procedures in dystrophic RCS rats and to evaluate *in vivo* survival and safety of human induced pluripotent stem cell-derived, retinal pigmented epithelial (hiPSC-RPE) cells.

Methods: Retinal dystrophic and albino RCS rats at 3-4 weeks of age (N=16) were immune suppressed by oral administration of cyclosporine from 2 days prior to injection and continued thereafter. iPSC-derived enriched RPE cells (5×10^5) in 3-5 µL saline were delivered into sub-retinal space of their right eye using a 28 gauge, blunt tipped Hamilton syringe via the trans-scleral route. A topical antibiotic was administered for 5 days post injection to avoid infections. At 1, 2, 4, 12 weeks and at 6 months, fundus imaging was performed, rats were sacrificed and the eyeballs were analyzed by IHC.

Results: The technique of cell injections into the sub-retinal space was standardized with minimal damage to the retinal vasculatures. The antibiotic regime was appropriate that no serious ocular infections noted. Pigmented hiPSC-RPE cell retention at the transplant site could be visualized by fundus evaluations. None of the animals developed any abnormal growth till 6 months. IHC evaluation of the experimental eyes confirmed cell survival in the sub-retinal space till 3 months and delayed retinal outer nuclear layer degeneration. Mature RPE cells maintained their cell morphology, pigmentation and expressed human-specific mitochondrial-antigen and an RPE-specific marker.

Conclusion: Transplanted hiPSC-RPE cells remained localized to the injection site, maintained their identity and did not proliferate *in vivo*, thus confirming their purity and safety for translational applications.

TEAR BACTERIAL MICROBIOME DYSBIOSIS IN THE DRY EYE DISEASE

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Purpose: Dry eye disease (DED) is a condition which affects the quality of tears and thus the ocular surface homeostasis. Bacterial microbiome plays an important role in the vicious cycle of dry eye disease. Thus the 'tear' microbiome was used to understand the bacterial microbiome dysbiosis in the DED patients compared to healthy.

Methods: Bacterial microbiome was generated from the DNA of tear samples of healthy (n=24) and DED (n=30) individuals. Sequencing of V3-V4 region of 16S rRNA gene was performed on the Illumina HiSeq2500 platform. Reads were processed in QIIME to assign the taxa. Statistical analysis of the healthy and DED microbiome was done in R to assess the alphadiversity and betadiversity indices. Significant changes between the healthy and dry eye disease cohorts were ascertained by Linear discriminant effective size analysis.

Results: Tear microbiome was generated in all the 54 healthy and DED individuals. Phylum *Actinobacteria* significantly increased in DED compared to healthy. 25 genera were identified in all the samples and genera *Lactobacillus* and *Bacillus* were predominantly present in both healthy and dry eye disease cohorts. Genera *Haemophilus, Pseudomonas* and unclassified bacteria significantly increased in DED patients compared to healthy. While genera *Cutibacterium* and *Paracoccus* significantly increased in healthy

Conclusions: The results of the study indicate significant changes in the bacterial diversity composition, in the phyla and genera in DED patients compared to healthy. The results also revealed significant increase in the abundance of the opportunistic pathogens in the DED cohort compared to healthy.

EVALUATION OF THE ROLE OF CONDITIONED MEDIUM SECRETOME AND EXOSOMES ROLE IN LACRIMAL GLAND REGENERATION

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Purpose: One of the promising approach for treating dry eye disease (DED) is through lacrimal gland cultures which have been demonstrated to retain secretory function ex-vivo. Therefore, this study aims to characterize the secretome of lacrimal gland cultures from conditioned medium(LM) and exosomes isolated from LM.

Methods: LG-cultures were isolated by an explant and enzymatic digestion techniques and the cultures conditioned medium(LM) secretome was investigated. The proteins from the cultures and normal Tears(TF) were evaluated by high-resolution accurate- mass spectrometry (MS- MALDI TOF). Exosomes from conditioned medium of LM were isolated using the differential ultracentrifugation method and resuspended in PBS and characterised using FESEM, TEM and DLS.

Results: The protein profiling identified various proteins in TF and LM of which 40% were common between TF and LM mainly immunoglobulin heavy variable, coronin 2B, SNARE-associated protein snapin, protein FAM110D etc. With the FESEM, TEM extracellular vesicles (>400nm), round uniform with unimodal size distribution was observed along with exosomes(150nm).The Dynamic light scattering(DLS) Intensity analysis plot showed that the size of exosomes around 100-150nm along with extracellular vesicles whose size was around 300-400 nm.

Conclusions: The conditioned media of lacrimal gland cultures shows 40% of similarity with tear proteins suggesting a promising potential of the secretome. The media also is rich in extracellular vesicles and exosomes which need to be evaluated further.

INHIBITORY ROLE OF TETRASPANIN PEPTIDES AGAINST BACTERIAL ADHESION TO THE CORNEAL EPITHELIUM FOR PREVENTING CORNEAL KERATITIS

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Purpose: Adhesion of bacteria to the extracellular domains of the tetraspanin transmembrane proteins of the plasma membrane is an essential step for initiation of the pathogenesis. However, blocking tetraspanin extracellular domains (EC2) with neutralizing antibodies or specific peptides could reduce the bacterial adhesion and prevent infection. Bacterial keratitis is a major cause of vision loss, hence prevention of bacterial attachment to the corneal epithelium using specific tetraspanin peptides might be helpful in reducing corneal infection.

Methods: We have examined the expression of CD9 tetraspanin transmembrane domains by human corneal epithelial (HCE) cells using immunofluorescence staining. Inhibition of culture proven clinical *Pseudomonas aeruginosa* (LVP3) isolates adhesion to the HCE cells by synthesized CD9 tetraspanin peptides was determined by co-culturing the peptides pretreated cells with bacteria. *In vivo* models were also used to evaluate the inhibitory activity of CD9 tetraspanin peptides against LVP3 adhesion to mice cornea.

Results: Immunofluorescence staining and flow cytometry studies have shown that HCE cells expressed CD9 tetraspanin transmembrane proteins. Adherence of LVP3 to HCE cells was reduced by pretreatment of cells with synthesized tetraspanin peptides. *In vivo* studies also showed that tetraspanin peptides pretreatment reduced the LVP3 adhesion to the mice cornea.

Conclusions: Thus, the present study demonstrates that the CD9 tetraspanin transmembrane domains plays a crucial role in bacterial adhesion to corneal epithelial cells and by blocking them with specific tetraspanin peptides could minimize the bacterial load on cornea.

ESTABILISHMENT OF A MURINE MODEL OF FUNGAL ENDOPHTHALMITIS: QUANTITATION OF INFECTION PARAMETERS

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Purpose: To develop a murine model of *Aspergillus fumigatus* (AF) and *Candida albicans* (CA) endophthalmitis and characterize the disease pathobiology.

Methods: Endophthalmitis was induced in C57BL/6 mice by intravitreal injection of spores of AF and CA strains. Disease progression was assessed by slit-lamp examination and clinical scoring followed by enucleation at 72hrs post-infection (pi). Enucleated eyes were used to estimate fungal burden and retinal tissue damage by hematoxylin and eosin, Grocott methenamine silver (GMS) and TUNEL staining. The level of inflammation was assessed by neutrophil infiltration and immunostaining [CD45, Myeloperoxidase (MPO)], while retinal stress was assessed by Glial Fibrillary Acidic Protein (GFAP) staining.

Results: Our data revealed that injection of 15,000 CFU/µl of AF and CA caused an increase in opacity, corneal haze as well as fungal burden upto 72hrs pi. CA infected eye mice showed severe infection with increased neutrophilic infiltration at 72hrs pi in comparison to AF infected mice eye. Histological analysis revealed heavy cellular infiltrates in the vitreous cavity as well as disruption of normal retinal architecture and increased retinal cell death, while GFAP staining indicated higher retinal stress along with more TUNEL positive cells. Both CD45 and MPO positive cells count was high in both AF and CA infected mice eye at 72 hours p.i. in comparison to 24 hours p.i.

Conclusion: Our results indicate that both AF and CA induced increased inflammatory responses in C57BL/6 mice. Our model will be a powerful tool in understanding the pathobiology of fungal endophthalmitis, and test therapeutic modalities.

ALTERATIONS IN THE OCULAR SURFACE FUNGAL MYCOBIOMES IN BACTERIAL KERATITIS PATIENTS

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Purpose: To characterize the ocular surface mycobiomes of healthy individuals (HC) and individuals with bacterial keratitis (BK), and to assess whether ocular surface mycobiome dysbiosis is prevalent in BK.

Methods: A total of 52 conjunctival swabs (HC.SW) were collected from both the eyes of 26 HC. Both conjunctival swabs (BK.SW, n=22) and corneal scrapings (BK.CR, n=22) were collected from microbiologically-proven BK patients. Genomic DNA was extracted and ITS2 region was amplified. The standard Illumina protocol was applied to generate fungal amplicon libraries and sequenced using Illumina HiSeq 2×250 bp chemistry. Quantitative Insights Into Microbial Ecology pipeline was used for downstream analysis and statistical analyses were performed in R.

Results: Of the 96 ocular samples processed, ITS2 fungal mycobiomes could be generated only from 66 samples (46 HC.SW, 16 BK.SW and 4 BK.CR). Ascomycota and Basidiomycota were detected in all the mycobiomes and minor phyla Mucoromycota and Mortierellomycota were significantly different between HC.SW and BK.SW mycobiomes. A total of 118 genera were identified and *Malassezia* and *Candida* were present in all the 66 ocular mycobiomes. Compared to the HC.SW mycobiomes, the abundance of 8 and 7 genera significantly decreased and 2 and 1 genera significantly increased in the BK.SW and BK.CR mycobiomes respectively. Few genera were significantly different between BK.SW and BK.CR. Heat map analysis and NMDS plots also clearly segregated HC.SW, BK.SW and BK.CR mycobiomes.

Conclusion: For the first time, our study demonstrated dysbiotic changes in the mycobiomes of the conjunctiva and cornea of BK patients.

HUMAN LACRIMAL GLAND MESENCHYMAL STEM CELLS: ROLE IN DRY EYE DISEASE

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Purpose: Mesenchymal stem cells (MSCs) from lacrimal glands (LGs) have been evaluated in murine models to treat dry eye disease (DED). We herein aim to identify, isolate and characterize the LG- MSCs from normal and DED patients.

Methods: Normal human LGs (n=6; mean age=54.6±19.6) from individuals undergoing LG debulking surgery and LG biopsies from patients with severe DED due to Stevens-Johnson syndrome (SJS; n=3) were cultured and p3 generation cells were characterized. Also, the differential expression of genes in the lacrimal gland tissues of normal and SJS patients were analyzed by q-PCR. Limbal derived stromal cells were used as controls.

Results: The normal LG cultures demonstrated spindle shaped cells in sheets and colonies similar to the limbal MSCs. The immunophenotyping of lacrimal and limbal cells revealed positive expression for CD105 (45.37%, 52.31%), CD73 (80.19%, 89.63%) and negative expression for CD45 (3.11%, 0.84%) and HLA-DR (4.27%, 5.93%) respectively. Trilineage differentiation into osteo, adipo and chondrogenic lineages were noted similar to control cells. In contrast to the normal LGs, the tissues from SJS patients showed no growth. However, upregulation of inflammation related genes i.e., IFN-β1(14-fold), CCL5 (5-fold), CCL2 (8-fold), Ro60 (4.5-fold) and downregulation of lactoferrin was noted in LGs of DED patients.

Conclusions: The study establishes the novel finding of stromal cells from human LG cultures with characteristic features of MSCs in terms of phenotype and trilineage differentiation. We also provide data for altered gene expression profile in DED patients which warrants further studies with more patient samples to explore its role in DED.

INVESTIGATING THE METABOLIC AND IMMUNE SENESCENCE CHANGES OF ACTIVATED MULLER GLIA WITH HIV1 TAT.

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Purpose: Comorbidities abound inspite of increased life expectancy of HIV patient with the advent of combined antiretroviral treatment (cART). HIV pathogenesis accelerates cellular senescence, chronic immune activation, inflammation and HIV associated neurocognitive disorders (HAND) in infected individuals. Our hypothesis suggests physiological changes in retinal cells during the course of HIV infection can lead to cellular senescence at the eye. Muller glia is predominant glia cells at the retina. The cytotoxic effect of HIV1 TAT shows by changes in ROS, Glucose transporter and changes in 8OHDG at the nucleus.

Methods: We detected Reactive Oxygen Species (ROS) (indicator of inflammation and infection), 8- OHDG ELISA (detects oxidative stress), Glut1 (involves in cellular metabolism) and CDKN2A (indicator of senescence) in HIV TAT activated Muller glia. Further at functional level of cells, 2-NBDG (fluorescent analogue of Glucose) was investigated to show metabolic activity.

Results: ROS experiment has shown significant changes ($p < 0.05$) on treatment with 100 and 400ng/ml HIV TAT protein. 2- NBDG showed increase in the uptake that signifies activated cells are metabolically more active compared to control cells. At RNA level, Glut1 and CDKN2A expression has shown significant changes with different concentration (100 and 400ng/ml) of HIV TAT at different time points (4, 8, 24 hour). Further to check oxidative stress at the nucleus, 8-OHDG ELISA signifies activated Muller glia are under stress compared to control samples.

Conclusions: Our study suggests that when cells are HIV TAT activated, they undergo through different metabolic and inflammatory pathways accelerated senescence.

MY JOURNEY IN EXECUTING THE "PROGRAMME SUPPORT FOR RESEARCH ON RETINOBLASTOMA"

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Purpose: Challenges are there in getting and executing a big research grant.

Methods: Grant was focused on aptamer therapeutics, DNAzymes, microRNA, tumor microenvironment, fatty acid synthase and identifying the ligands using bioinformatics, methylation and on proteomics and RNA sequencing.

Results: MiRNa profile of the retinoblastoma (RB) cells generated. let7 family of miRNAs are down regulated in RB. survivin and splice variants expressed in RB. RB cells targeted using miRNA mimics, antagomirs, DNAzyme and aptamer chimeras. Tumor microenvironment is present in RB and signaling pathways identified. Fatty acid synthase ligands identified. APC-2 gene was hypermethylated in RB samples. Reduced APC-2 lead to increased Wnt signalling pathway protein, β-catenin suggesting tumour suppressive role of APC-2 gene. Tumor proteomics and first phosphosignalling atlas of RB generated, developed a new computational approach called invariant differential expression analysis (iDEA) that has the potential to resolve this reproducibility issues. iDEA performs traditional DEA followed by a Boolean analysis-based filtering step. First coding and Non-coding RNA signatures in RB by RNA sequencing generated. Metabolic modelling and actual measurement by LCMS analysis identified dysregulated Lipid which can function in RB disease progression.

Conclusions: We had challenges in methylation data analysis and validation, aptamer miRNA had post conformational changes. We submitted the final report in September 2021. We published 32 papers and 5 PhD students and 20 project students benefitted from the grant.

GENETIC ASSOCIATION ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) WITH POLYPOIDAL CHOROIDAL VASCULOPATHY (PCV) IN INDIAN POPULATION

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Purpose: PCV is a complex ocular disorder that results in progressive and irreversible vision loss. Phenotypic character of PCV is the branching vascular network with polypoidal lesions in the choroidal layer. Genotypic association of SNPs has been shown in PCV patients of many ethnic groups. We analyzed the association of 5 different SNPs with PCV and determined their significance of association.

Methods: In total 103 PCV cases and 198 controls were recruited for this the study. Genomic DNA was extracted from peripheral blood from the individuals and genotype analysis was done using Sanger sequencing method for the following the 5 SNPs rs2217332 (*HERPUD1),* rs10490924 *(ARMS2)*, rs11200638 (*HTRA1*) and rs547154 & rs2242572 (*C2*). Chi square and other statistical tests were performed using SPSS V 23.0.

Results and conclusion: *HERPUD1* (rs2217332; P = 0.002), *ARMS2* (rs10490924; P = 0.000059), *HTRA1* (rs11200638; P = 0.02) showed different levels of association with PCV in Indian population. Statiscal analysis of genotypic association showed that *HERPUD1, ARMS2, HTRA1* are significantly involved with PCV in Indian population. Of the gene analysed *ARMS2* (rs11200638) is highly significantly associated with PCV compared to other genetic variants and this is the first report from Indian population. *ARMS2* gene association has also been shown in Korean population. Other genetic variants tested in this study showed different levels of significant association in different ethnic groups. However, genetic variants in C2 gene is not associated with PCV in any ethnic groups. This genotype analysis will facilitate better clinical diagnosis of PCV patients.

FLOW CYTOMETERY OF T REGULATORY CELLS IN PRESUMED TUBERCULAR UVEITIS(TBU)

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Purpose: To determine the pattern of T regulatory (T Reg) cells in Presumed Tubercular Uveitis.

Methods: A prospective study was done recruiting patients visiting uvea clinic with the diagnosis of TBU. 34 patients including age matched control group were recruited for the study. Patients were chosen based on 1. anatomical site of involvement of uveitis, Anterior, Intermediate, Posterior and Panuveitis. 2. Positive Mantoux 3. Positive Quantiferon TB Gold Test 4. Abnormal findings in High Resolution Computed Tomography (HRCT), of chest. Blood sample was drawn from the participants and PBMC were isolated by Density Gradient Centrifugation Method and analysis was done with Flow Cytometer. CD4+, CD25+ and FOXP3+ were used as hallmark of T reg cells. IL17A+, CD45RA+ and CD45RO+ was included, suggestive of active inflammation. CD45RA+ and CD45RO+ define naïve and memory T cell responders, respectively.

Results: There were 19 males and 15 females. The mean age of the patient was 32.94 ± 6.49. There was increase in IL17A+ cells confirming active uveitis condition. Presumed TBU patients also showed a decrease in naive marker CD45RA+ and increase in memory marker CD45RO+. There was a increase in T reg cells in Tubercular uveitis, P value was (0.007) by applying paired t test.

Conclusion: Our study showed that Treg markers CD4+, CD25+ and FOXP3+ were higher in presumed TBU patients. IL17/ T reg cells ratio is increased in TBU patients and may be used for the presumptive diagnosis of TBU along with the existing clinical parameters.

KERATINIZATION PROCESS IN LID MARGINS OF CHRONIC STEVENS-JOHNSON SYNDROME PATIENTS: STUDY OF CYTOKERATIN PROFILE AND KERATINOCYTE GENE EXPRESSION.

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Purpose: To study the cytokeratin profile and keratinization-related genes expression in keratinized lid margins of chronic Stevens-Johnson syndrome (SJS) patients.

Methods: Twenty-four eyelid margins from chronic SJS patients and normal eyelid margins (n=7) were evaluated using immunofluorescence staining (CK10, CK1, FLG, CK19) and quantitative PCR (KGF, EGF, PTEN, HBEGF, TGFα, TGFß, TGM1). Eyelid margins were divided into cutaneous epithelium i.e., from eyelash root to meibomian glands opening (normally keratinized), and marginal conjunctiva (LMC; normally non-keratinized), and were studied separately.

Results: Cytokeratin 1/10, filaggrin were expressed in keratinized conjunctiva similar to the cutaneous epithelium of the normal eyelid. Expression of cytokeratin 19 was confined to the basal epithelial layer of LMC in SJS compared to full-thickness expression in normal LMC. Increased expression of PTEN ($p \le 0.0002$) and TGFß ($p \le 0.01$) was observed in the cutaneous epithelium of SJS patients compared to normal eyelid cutaneous epithelium. LMC of SJS patients showed increased TGM1 ($p \le 0.002$), PTEN ($p \le 0.002$), EGF (0.001), TGFα ($p \le 0.04$), TGFß ($p \le 0.002$) expression compared to normal LMC. No differences were observed [TGM1 ($p \le 0.06$), PTEN ($p \le 0.15$), EGF ($p \le 0.5$), KGF ($p \le 0.6$), TGFα ($p \le 0.2$), TGFß ($p \le 0.6$)] between cutaneous epithelium and LMC of SJS patients.

Conclusions: In lid margins of SJS, there is an increased expression of keratinization-related genes compared to normal lid margin. Keratinized LMC shares similar cytokeratin profile and keratinization genes expression as seen in cutaneous epithelium of SJS patients, indicating the possibility of the cutaneous epithelium as a source for keratinized LMC.

ANALYSIS OF EPIGENETIC MODIFICATIONS IN LEBER'S HEREDITARY OPTIC NEUROPATHY (LHON)

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Purpose: Leber's Hereditary Optic Neuropathy (LHON) is the common mitochondrial inherited disease which leads to vision loss. The three primary mutations G3460A (MT-ND1), G11778A (MT-ND4) and T14484C (MT-ND6) are the major cause of LHON. Apart from genetic modifications, epigenetics plays a major role in disease pathogenesis. Epigenetic modifications in the nuclear genome might have role in the assembly and functioning of complex 1 in mitochondria. The present study aims to check the histone modifications in the nuclear gene *NDUFS4* and its role in complex 1 dysfunction.

Methods: Blood samples were collected from LHON patients and controls after obtaining the informed consent. The study has been approved by the institutional ethics review committee. From the blood samples PBMCs were isolated and chromatin immunoprecipitation (ChIP) was carried out using antibodies for both activation (H3K9Ac, H3K27Ac and H3K4Me3) and repressive marks (H3K9Me2 and H3K27Me3). The NDUFS4 expression was analyzed using qPCR technique.

Results: *NDUFS4* promoter and other exonic regions shows histone modifications (Methylation and Acetylation) while comparing the LHON and controls. These histone modifications in *NDUFS4* indicates epigenetic control over LHON progression.

Conclusion: Our results depict that nuclear gene (NDUFS4) might have an influence on mitochondrial genome particularly in complex 1 region. Further studies are under progress to understand the epigenetic influence on mitochondrial complex 1 which is associated with LHON.

GENETIC ASSOCIATION OF RS220057 AND RS220060 OF *ZEB1* WITH FUCHS ENDOTHELIAL CORNEAL DYSTROPHY IN INDIAN POPULATION

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Purpose: Fuchs endothelial corneal dystrophy (FECD) is a progressive, hereditary, multifactorial eye disease that affects the inner most layer of the cornea, the endothelium. Advanced stages of FECD are characterized by corneal edema, loss of transparency, and apoptosis of corneal endothelial cells. It is one of the most frequent (16.6%) reasons for Penetrating Keratoplasty in India**.** It has been reported that different mutations in the transcription factor zinc finger E-box binding homeobox 1 (*ZEB1*) cause late onset FECD in both familial and sporadic population- based studies. Gupta *et al.,* 2015 has found positive association of intronic variant of *ZEB1*, rs20060 with FECD in the northern Indian population.[1] Also rs220057, and rs220060 is genetically associated with FECD in multi-generational Chinese familial study.[2] So, we intended to investigate the genetic association of rs220057 and rs220060 with FECD in the East Indian population to check for any functional role of ZEB1 on FECD.

Methods: Blood samples were collected from age and gender-matched 205 controls and 88 FECD patients after a detailed assessment via specular microscopy. Genomic DNA was extracted and genotyping was done by Sanger's sequencing method. The associations of rs220057 and rs220060 polymorphisms were computed by Chi-squared test.

Results: Both the variants, rs220057 and rs220060 did not show significant association with FECD, either allele or genotypic level.

Conclusion: rs220057 and rs220060 have no major contributing role for FECD pathogenesis in our population. Because ZEB1 is upregulated in FECD so the role of other genetic and epigenetic contributing factors should be checked to understand the cause and effect of ZEB1 upregulation in case of disease progression.

IDENTIFICATION OF A MUTATION IN A FAMILIAL CASE OF CONGENITAL HEREDITARY ENDOTHELIAL DYSTROPHY (CHED) FROM INDIA AND UNDERSTANDING ITS FUNCTIONALITY VIA COMPUTATIONAL ANALYSIS

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Purpose: Corneal endothelial dystrophies are the prime cause of blindness among corneal dystrophies and the major indications of corneal transplants. *SLC4A11* mutations, has been known to cause its pathogenicity. Mutation spectrum and database of *SLC4A11* is necessary for the future gene therapy or gene editing based therapeutic interventions. The purpose of this study is to identify the underlying mutation in *SLC4A11* in a familial case of CHED.

Methods: Sanger's sequencing was performed for all 19 exons of *SLC4A11* in the family comprising 3 affected, one unaffected siblings and asymptomatic parents. The segregation of identified mutation was checked in family and 20 controls were analysed. Further *in-silico* analysis was performed using homology-based modeling (Modeller10.1).

Results: A novel homozygous C to G DNA-nucleotide substitution was identified in all three affected cases at cDNA 1514 position resulting in a change of amino acid Serine to Tryptophan at 489th position (p.Ser489Trp). Both parents were found heterogynous showing their carrier status. p.Ser489Trp mutation was absent in unaffected child (U1) and healthy 20 control analyzed. The *in-silico* analysis confirmed the pathogenicity and homology-based modelling showed increase in hydrogen bond interactions around the mutated residue.

Conclusions: The identified *SLC4A11,* c.1514 TCG>TGG (p.Ser489Trp) novel mutation confirms the clinical diagnosis of autosomal recessive familial case of CHED and verified its pathogenicity in-silico. This position can be considered as a hot-spot region for *SLC4A11* mutation as there are two previous reports causing CHED at the same region and it need to be further validated in more controls.

PROTEOMIC FINGERPRINTING OF AQUEOUS HUMOR IN RAT MODEL OF OCULAR INFLAMMATION

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Purpose: Blood ocular barriers maintaining the homeostasis of eye breach during ocular inflammation. Ocular inflammation leads to high risk for sight-threatening complications and vision loss. The aqueous humor present in eye could serve as surrogate medium for predicting ocular inflammation developmental stages or treatment response/strategies. Therefore, the aim of this study was to develop a strategy for complete fingerprinting of proteins in aqueous humor during ocular inflammation by a single analytical platform.

Methods: Wistar rats of either sex (n=6) were used for this study after ethical clearance. Endotoxin was administered at the dose of 200 µg/100µL into the hind paw to establish an experimental model of ocular inflammation and saline (100µL) was injected in control animals. Aqueocentesis was performed post 24 hrs inflammation and samples were subjected for analysis using high resolution mass spectrometer.

Results: Analysis of aqueous humor proteome in inflammatory conditions revealed significantly higher number of proteins identified in LPS challenged rats (519) as compared to saline challenged rats (88). Various classes of proteins viz. heat shock protein, apolipoprotein, angiotensinogen, β-crystallin, complement factors etc., pertaining to inflammatory and other biological pathways were found to be overexpressed in case of inflammation as compared to the control group.

Conclusion: The results of this study have shown a sensitive method was able to generate data for in depth analysis of aqueous humor proteome in ocular inflammatory conditions. The observed enrichment of proteins from this study reveals the may aqueous humor proteome analysis may be used as an approach for predicting developmental stage of inflammatory conditions and response to therapy.

AURORA KINASE B INHIBITION ENHANCES THE EFFICACY OF CHEMOTHERAPY DRUG CARBOPLATIN AGAINST RETINOBLASTOMA CELLS

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Purpose: To investigate the combination activity of Aurora kinase B (AURKB) inhibitor barasertib and chemotherapy drug carboplatin in Retinoblastoma (RB) cell lines.

Methods: The IC50 concentrations of Barasertib and Carboplatin were determined in RB cells by treating them with a series of drug concentrations and subsequent plotting of standard curves. The synergy between AURKB inhibition and Carboplatin was deciphered according to the method proposed by Chou and Talalay. The data was analyzed using Compusyn software and drug concentrations showing a Combination Index (CI) score of < 1 were taken as synergistic. Cell cycle analysis and percentage apoptosis were determined in RB cells after treatment with a combination of Barasertib and Carboplatin and compared to treatment with each drug in isolation. The levels of pro-apoptotic and anti-apoptotic proteins were determined using immunoblotting.

Results: The combination study showed that Barasertib and Carboplatin synergistically inhibited RB cell growth. Additionally, RB cells were more significantly arrested at the G2M phase of the cell cycle and there was a greater increase in percentage apoptosis when compared to treatment with each drug alone.

Conclusion: Overall, our data shows that Barasertib and Carboplatin can function synergistically in inhibiting RB tumor cell growth.

FUSARIUM PROTEASES IN AN IN VITRO CONDITION AND EX VIVO CORNEAL EXPLANT INFECTION.

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Purpose: Fusarium keratitis is a devastating corneal infection and studies on virulence factors will aid in understanding the pathogenesis. To measure the expression of extracellular proteases in an in vitro condition and an ex vivo corneal explant infection model.

Methods: Four isolates of Fusarium solani species complex (MTCC, Cs1, CSH3 and Cc50) were maintained in the lab by subculturing in Potato Dextrose Agar. Azocasein assay was carried out to measure the protease activity in in vitro growth conditions and from the infected cornea from the ex vivo corneal model at various time points. Real time PCR was done to measure the gene expression of seven protease genes (C7Z0E6, C7ZFW9, C7Z7U2, C7ZNV5, C7YY94, C7Z7Y4, C7YQJ2, C7YVF3)

Results: The specific activity in all isolates was highest at 8th day. Maximum specific activity was observed in Cs1 followed by CSH3 and Cc50. The relative gene expression of C7YY94, C7Z7U2 and C7Z6W1 was maximum in all FSSC isolates in an in vitro condition. In an ex vivo infection condition, among all seven expressed genes, C7Z6W1 showed highest expression followed by C7Z7U2 and C7YQJ2. Among all 3 isolates, the maximum gene expression was observed in Cs1 and minimum gene expression was seen in CSH3.

Conclusions: Clinical isolates of FSSC produce varying amounts of proteases and differ in specific activity and gene expression in both conditions (in vitro and ex vivo). Further, detailed exoproteome studies are warranted.

NETWORK PHARMACOLOGY AND IN VITRO ANALYSIS OF SILIBININ AGAINST TGFB2-INDUCED LENS EPITHELIAL CELL MIGRATION AND EPITHELIAL-MESENCHYME TRANSITION

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Purpose: Growth factor induced lens epithelial cell (LEC) migration and epithelial-mesenchyme transition (EMT) are the key processes involved in pathogenesis of posterior capsular opacification (PCO). Silibinin, a natural flavonolignan confers therapeutic effects to variety of cells via regulation of signaling pathways. In this study, the potential therapeutic action of silibinin on PCO was explored by network pharmacology, molecular docking and *in vitro* verification using HLE B-3 cells.

Methods: Target fishing, construction of protein-protein interaction (PPI) network, GO analysis, KEGG analysis and molecular docking were performed to decipher the underlying pharmacological mechanism of silibinin for the prevention of PCO. MTT assay, wound healing assay, immunofluorescence, western blotting, and qRT PCR analysis were employed to evaluate inhibitory capacity of silibinin against TGFβ2-induced cell migration and EMT in vitro.

Results: From the interaction data and PPI network analysis 41 targets of silibinin were related to PCO. GO and KEGG enrichment analysis showed that targets were highly correlated with peptidyl-serine phosphorylation, positive regulation of mesenchymal cell proliferation and PI3K-Akt signaling. Silibinin reduced the viability of LECs, inhibited the wound healing capacity of LECs, and suppressed alteration in the EMT markers viz., cytoskeletal proteins, cell adhesion markers, extracellular matrix molecules, and transcription factors. AKT1 and CTNNB1 were identified to be the key hubs of the network. *In vitro* experiments verified that silibinin decreased the levels of phosphorylated AKT, PDK1, PTEN, c-Raf and GSK3β in TGFβ-stimulated cells. The effect of silibinin treatment on phosphorylated Akt resembled that of PI3K inhibitor LY294002.

Conclusion: Results suggest that silibinin can suppress LEC migration and EMT most likely by inhibiting the activation of PI3K-Akt signaling otherwise induced by TGFβ2. Silibinin might be a good candidate for PCO prevention.

DIFFERENTIAL TRANSCRIPTOME AND HISTOLOGICAL ANALYSIS OF METHICILLIN-RESISTANT AND SUSCEPTIBILE *STAPHYLOCOCCUS AUREUS* ENDOPHTHALMITIS IN A MOUSE MODEL

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Purpose: To compare the histopathological features and whole transcriptome of Methicillin resistant *(MRSA)* and Methicillin-susceptible (*MSSA*) *Staphylococcus aureus* in a murine model of endophthalmitis.

Methods: *MRSA* and *MSSA* endophthalmitis was induced in C57BL/6 mice and disease progression was scored clinically and histologically at 24 hours p.i. Retinal changes were monitored by H&E, CD45, MPO and GFAP staining followed by retinal cell death evaluation. Whole Transcriptome was analysed using the SuperPrint G3 Mouse Gene Expression v2 chip. Differential gene expression analysis (Limma package, R) was done followed by enrichment of pathways (KEGG database).

Results: Increased corneal haze, diminished vitreous clarity and red reflex was observed in *MRSA* infected mice eye compared to *MSSA* ($p= 0.04$). Histological assessment also corroborated with increased disease severity in *MRSA* ($p = 0.02$). Although *MRSA* infected eye displayed higher CD45+ cells and greater GFAP intensity, the difference was not statistically significant. However, higher retinal cell death was found to be associated with the *MRSA* infection ($p= 0.007$). Our study also revealed that MRSA infection induces changes in host transcriptome *(FC= 1.5, p= 0.05)*, revealing the involvement of several interleukins (IL-11,15,10,1ra), chemokines (CCL-11, CXCL-1), Interferon receptors, MMPs, Neruopilin2 (NRP-2), Ubiquitin associated peptidase and apoptotic ligands. ErbB signalling, JAK-STAT, adipocytokine and Ras signalling were the top divergently enriched pathways.

Conclusions: Our study confirms the differential host immune response triggered by MRSA infection in the eye. Our study may help to elucidate the mechanisms of pathogenesis and to identify additional candidate drug targets for the treatment of MRSA endophthalmitis.

ANTI-VEGF MORPHOLINOS AS AN EFFECTIVE APPROACH TO INHIBIT HYPOXIC INDUCED VEGF IN VITRO

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Purpose: Vascular endothelial growth factor (VEGF) is a vital mediator of pathological angiogenesis in retinal diseases like Diabetic Retinopathy and Age related Macular Degeneration. Anti-VEGF therapeutics have been most successful approach to treat such eye conditions. Recent studies highlight the need for smaller and more potent anti VEGF therapeutic molecules. We aim to study the effect of anti-VEGF morpholinos on the expression of VEGF inside Retinal Pigment Epithelium (RPE). RPE cells express pathological VEGF constitutively with significant upregulation under hypoxic conditions serving as alternate model to HUVEC cells to study anti-VEGF inhibition *in vitro*. The morpholinos were targeted against VEGFA intron-exon junctions pre mRNAs.

Methods: Anti-VEGF morpholinos were transfected inside RPE cell line via neon transfection system and kept under hypoxic environmental conditions (1% O_2) for 48 hours. VEGF-A inhibition was measured by qPCR at mRNA level using Taqman probes.

Results: Overall, the expression of VEGFA was inhibited upto 90% as compared to control oligos.

Conclusion: Results show that anti-VEGFA morpholinos are very effective in inhibiting hypoxic induced VEGFA expression in vitro and may be a promising Anti-VEGF therapeutic strategy.

IN SEARCH OF POTENTIAL MARKERS TO UNDERSTAND THE PATHOPHYSIOLOGY OF ALLERGIC EYE DISEASES

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Purpose: The increasing global burden of allergic eye diseases is a serious concern. A large proportion of children and young adults get affected, however, accurate diagnosis is often challenged by lack of specific markers and overlapping anterior ocular surface disorders including infectious keratitis and dry eye disease. Therefore, the identification of novel objectively measurable biomarkers is essential for early diagnosis and efficient management of allergic eye diseases.

Methods: This study was approved by the Institutional Ethics Committee (LEC 10-19-366). Following informed consent, tear samples were collected from allergic conjunctivitis patients (<21 years of age) using Schirmer's strip. Total proteins were isolated and extracted from tear samples using different combination of buffers. 10% SDS-PAGE was used to segregate the extracted proteins whereas Western blotting was performed to study their expression. Cytokine array (#ARY022B, R&D Systems) was used to analyse tear cytokines. To study the expression of candidate genes, total RNA was isolated from tears (#SKTOPU-100, Eurogenentec), reverse transcribed (#18091050, ThermoFisher Scientific) and genes of interest were amplified using PCR. Impression cytology was used to detect presence of inflammatory cells in ocular surface epithelia.

Results: We observed differential regulation of tear cytokines viz., IL-1ra; IL-8; IP-10, CXCL-10, MCP-1, CXCL10, MIF, MMP-9, TFF3 and vitamin D binding protein in allergic conjunctivitis patients, compared to healthy controls. Impression cytology revealed presence of neutrophils, eosinophil and lymphocytes.

Conclusions: Our results suggest involvement of tear cytokines and inflammatory cells in allergic conjunctivitis patients. Further studies are required to identify specific biomarkers of allergic eye diseases.

NANOCRYSTAL FORMULATION FOR POORLY WATER-SOLUBLE DRUG

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Purposeb Amphotericin B (AB) is used for treating fungal keratitis. Being a BCS class IV drug, the water solubility of AB is poor, which poses formulation challenges. We hypothesize, nanocrystals (NC) could aid better solubility and permeability by reducing particle size (PS) and increasing surface area.

Methods: NC were prepared by anti-solvent precipitation method. Tocopherol Poly (ethylene glycol) 1000 Succinate and poloxamer P407 was used as a stabilizer and surfactant, respectively. The preparation method was optimized for product and process parameters. The developed NC were characterized for PS, zeta potential, drug loading, thermal analysis by Differential Scanning Calorimetry (DSC), Scanning electron microscopy (SEM) and *In-vitro* drug release. The loading efficiency and *in-vitro* drug release was determined by High Pressure Liquid Chromatography.

Results: AB-NC were successfully developed and characterized using different techniques. The optimized NC formulation was clear, the particle size was found to be 96.11±2.04 nm, with a polydispersity index (PDI) of 0.427±0.02 and zeta potential of -23.5±-0.51 mV. DSC results showed the absence of crystalline drug peaks in nanocrystals formulation, which indicates the amorphous nature of drug. The drug loading of the optimized formulation was found to be 62.73±2.7 %. The *in-vitro* cumulative drug release was found to be 93.83±5.03% in 48 hours.

Conclusions: The present study concludes the formation of amorphous AB-NC using DSC and SEM studies. NC formulation showed sustained release of AB. Further *ex-vivo* corneal permeation and *in-vitro* toxicity studies are going on for the optimized formulation.

ROLE OF S100A12 IN *STREPTOCOCCUS PNEUMONIAE* KERATITIS

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Purpose: Microbial keratitis is the spectrum of ocular infectious diseases that affects the cornea and accounts for one of the major causes for vision impairment and corneal blindness globally (Ezisi, 2018). The most common causative gram positive bacteria is*Streptococcus spp.* which falls under the medium priority of antibiotic resistant(AR) bacterial species (WHO-PPL report, 2016-2017). Antimicrobial peptides, a part of the host innate immunity, are the most potent alternative to AR bacteria. S100A12 is a calcium binding host-defense protein. It has been shown to have antimicrobial effects on various microbes by acting as damage associated molecular pattern and initiate a pro-inflammatory immune response or by starving the pathogens by nutritional immunity.

Methods: The effect of S100A12 against *S. pneumoniae* growth was studied by doing a colony forming unit assay. The expression of S100A12 in *S. pneumoniae* infections was checked in corneal tissues of keratitis patient as well as *in vitro* using human corneal epithelial cells (HCEC) by immunofluorescence assays.

Results: We found increased S100A12 expression in corneal tissues of patients with *S. pneumoniae* keratitis. Increased expression of S100A12 was observed in HCEC in response to *S. pneumoniae* infection. S100A12 was also found to be effective in inhibiting the growth of *S. pneumoniae in vitro.*

Conclusions: S100A12 is thus likely to play a role in *S. pneumoniae* keratitis and is effective in inhibiting the growth of *S. pneumoniae*. These initial results suggest more studies that will be helpful to develop S100A12 as an alternative therapeutic intervention against *S. pneumoniae.*

WHOLE MITOGENOME/EXOME SEQUENCING OF LHON PATIENTS UNCOVERS MUTATIONS IN MITO-NUCLEAR GENES ASSOCIATED WITH OXPHOS COMPLEX I IMPAIRMENT

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Purpose: Leber's hereditary optic neuropathy (LHON) is a mitochondrial disorder that primarily affects retinoganglion cells due to defective production of oxidative phosphorylation complex I (OXPHOS complex I) and causes irreversible vision loss. Most often 95% of the proband harbor primary mitochondrial DNA mutation in OXPHOS complex I causes bioenergetic failure. The electron transport complex I subunits were encoded by both mitochondria and nuclear genome. Hence mutations in nuclear genes encoding the OXPHOS complex I have also been linked in the modulation of phenotypic expression via mitochondrial complex I DNA nuclear type. Thus, in this study, 30 LHON probands were subjected to both whole mtDNA sequencing and whole exome sequencing (WES) in order to identify nuclear modifier genes and the mitochondrial gene associated with the disease.

Methods: Sanger sequencing and WES were performed to characterize the whole mitochondria and nuclear genome of the LHON patients.

Results: Whole mt genome and WES results displayed 30% of the proband possess primary mt DNA mutation, 1% of the proband carry LHON associated mt DNA mutation, 10% of the individual harbor other mtDNA SNP, 22.3% of the proband comprise both LHON associated mt DNA and nuclear gene mutations, 23.3% of the proband encompass only nuclear gene mutation and the remaining 13.3% of individual harbors no mutation in both genomes

Conclusions: Recently, LHON cases are tested negative for mtDNA mutation that follows autosomal recessive inheritance. As a result, these atypical cases must be subjected to whole genome sequencing/WES in order to identify the appropriate mito-nuclear genes associated with the disease.

MICROBES OF THE HUMAN EYE: ANTIMICROBIAL RESISTANCE AND BIOFILM FORMATION

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Purpose: The ocular surface harbours different types of microorganisms. These microbes under conditions of trauma, could cause ocular diseases which could be resolved by using antimicrobials. At times, these microbes become resistant to antibiotics which could be attributed to their ability to form a biofilm. Thus, understanding biofilm formation and AMR is crucial in treating ocular infections. The purpose of this study was to understand the correlation between antimicrobial resistance (AMR) and biofilm formation in ocular bacteria.

Methods: AMR was monitored as per the guidelines of Clinical & Laboratory Standards Institute and biofilm formation by several methods including confocal and scanning electron microscopy.

Results: Ocular *Candida albicans* (CA)*, Staphylococcus aureus* (SA), *taphylococcus epidermidis* (SE) and *Escherichia coli* (EC) exhibited resistance to several antimicrobial compounds and were also positive for biofilm formation. Microbes were 100 times more resistant to antimicrobial agents in the biofilm than in planktonic phase. Biofilm thickness as monitored by confocal microscopy increased from 4 h (adhesion phase) and peaked at 72-96 h in EC, SA, SE and CA. Copious amounts of exopolymeric substances were produced in the biofilm phase. Scanning Electron Microscopy results confirmed biofilm formation in SA and SE on polystyrene plates and cadaveric cornea. Biofilm formation occurred in 4 stages: attachment phase, microcolony formation, maturation and dispersal phase. The temporal sequence of these phases varied between the isolates.

Conclusions: Biofilm formation and AMR were positively correlated. Temporal dynamics of biofilm formation was observed in ocular pathogens.

WHOLE GENOME SEQUENCING OF SOUTH INDIAN CONSANGUINEOUS FAMILIES FOR TYPE 2 DIABETIC RETINOPATHY

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Purpose: Diabetic Retinopathy (DR) is a microvascular sight-threatening complication of type 2 diabetes. In this abstract, we present the Whole Genome Sequencing (WGS) of endogamous families with type 2 diabetes and DR with aim of identifying novel variants associated with DR pathogenesis.

Methodology: We collected two large families from Tamil Nadu and assessed their phenotypes based on clinical and imaging criteria. WGS was performed for 31 samples at Medgenome, Bangalore. The samples consist of 6 DR patients, 11 diabetic and 14 healthy controls. The WGS data was subject to data cleaning and genetic relationships between individuals were reconstructed using KING. In an effort to find long stretches of homozygosity by descent (>1Mb), Automap was used for identifying Regions of Homozygosity (ROH). Variants called in the WGS data were classified using ANNOVAR, using mutation filters as well as CADD score for exonic, non-synonymous SNPs. We are in the process of analyzing coding variants that show homozygosity in affected individuals and have deleterious potential.

Results and conclusion: The QC report for WGS showed significant alignment (>99%) and genome coverage (>90%). We analysed the data and identified a number of exonic, non-synonymous SNPs which appear to be deleterious. A variant in SATL1 was identified as deleterious with a CADD score of 43; this gene is significantly expressed in the retina. Also, genes associated with deleterious SNPs were found to be enriched in AMPK and Metformin pathways which are associated with diabetes. We present our discussion based on the results of the WGS data analysis.

IDENTIFICATION OF NEW PROTEIN MARKERS IN SUB PROTEOME OF HEALTHY HUMAN EYE

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Purpose: The structural hierarchy of the retina is critical for visual function, which is due to specialized protein functions. A detailed subproteomic analysis of normal human eye may therefore provide a dataset for new investigations into the physiology of retinal functions and pathophysiology of ocular diseases.

Methods: Proteins were extracted from sclera, choroid, retina and RPE of 6 healthy donor eyes and fractionated into peptides by trypsin digestion. Whole proteome was analyzed using mass spectrometry followed by bioinformatic analysis using DAVID.

Results: We identified 1437, 1348, 2094 and 1316 proteins in sclera, choroid, retina and RPE with FDR of <1% for protein. 260 proteins were found common in all the tissues. We found 728 proteins unique (highly abundant) to retina involved primarily in neural machinery, transport, visual perception, calcium binding etc. 20 proteins unique to choroid involved in transport of nutrients and cytoskeleton organization. We identified 180 proteins unique to sclera, majorly involved in extracellular matrix organization, cell-cell adhesion and complement activation. Amongst 71 unique proteins of RPE, many were involved in fatty-acid oxidation and protection of retina, visual perception, cellular homeostasis and transport.

Conclusions: Discovery and characterization of the new proteins whose biological function is not known particularly in ocular physiology our comprehension of the cell specific signalling networks in the retina. These observations will have implications in the pathogenesis of retinal diseases and discovering novel therapies.

ASSESSMENT OF RETINAL GANGLION CELL FUNCTIONS AND ITS CLINICAL GENETIC CORRELATION IN PRIMARY CONGENITAL GLAUCOMA

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Purpose: Primary congenital glaucoma (PCG) is an autosomal recessive disease that occurs due to developmental defects of anterior chamber angle, thereby resulting in limited aqueous humor outflow and corresponding loss of retinal ganglion cells. Photopic negative response (PhNR) measures retinal ganglion cell activity, which is reduced in glaucoma. This study aimed to identify association of PhNR and clinical-genetic parameters on the basis of severity of PCG.

Methods: The PhNR characteristics on full field electroretinogram were recorded for 128 eyes of clinically well characterized PCG cases (n=64). Probands were categorized into mild, moderate and severe stages of glaucoma based on the clinical parameters including intraocular pressure (IOP), corneal diameter (CD), and cup to disc ratio (CDR). PhNR (amplitude and implicit time) were compared between all three groups. Test of correlation was done between PhNR characteristics and IOP, CD and CDR in the background of candidate genes mutation.

Results: The PhNR amplitude and implicit time significantly differed when compared between mild vs moderate (p=0.01, p=0.02) and mild vs severe (p=0.02, p=0.06) PCG probands. Moderate degree of correlation was noted between CDR and PhNR amplitude at the same visit in mild (r=0.3) and moderate (r=0.33) PCG probands, respectively. PCG patients harbouring mutations in *CYP1B1, LTBP2, TEK, MYOC, CRYBB1* and *CRYGB* exhibited reduced PhNR functions compared to those without mutations.

Conclusion: This study indicated reduced retinal ganglion cell response in moderate and severe PCG cases compared to mild ones. Reduced PhNR responses in cases harbouring mutations could provide additional insights into PCG pathogenesis.

LOSS OF ADULT STEM CELLS IN CATARACTOUS HUMAN ANTERIOR LENS EPITHELIUM

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Purpose: We have earlier confirmed the presence of stem cells (SOX-2$^+$ CX-43$^-$) in the central zone of normal human anterior lens epithelium. This study aims to understand the nature of these stem cells in cataractous lens.

Methods: Donor lens of both normal and cataract were obtained from excised globes (n=15, age 20-85 years). Whole mounts of the anterior lens epithelium were immunostained for the expression of stem cell marker SOX2, and differentiated cell marker Connexin-43 (Cx-43). Further the cataract epithelium was characterized for apoptotic cell marker TUNEL. Images were acquired and analyzed using Leica SP8 confocal microscope.

Results: The adult stem cells cells (1.89±1.47%) positive for SOX-2 and negative for Cx-43 were present only in the central zone of the anterior lens epithelium in normal lens (n=3). However, no such nuclear expression of SOX-2$^+$ cells was observed in the lens epithelium from cataractous donors (n=5). Nuclear blebbing was observed in 39.8±13.1% of cells in the central zone but not in other zones and such cells were confirmed to be apoptotic by the expression of TUNEL (n=3). Among the cells with nuclear blebs, 11.8±4.1% were negative for Cx-.43.

Conclusion: Absence of SOX-2$^+$ stem cells and presence of apoptotic cells in the central zone of cataractous lens epithelium indicates that pathological changes to stem cells are associated with cataract formation. Further studies are essential to understand the age-related molecular changes in the stem cells of the anterior lens epithelium of cataractous lens.

ROLE OF GENES INVOLVED IN AUTOPHAGY IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

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Purpose: Diabetic retinopathy (DR) is a neurovascular complication of diabetes (DM) causing the loss of ganglion cells in the retina. Impaired autophagy triggered by dysfunctional retinal pigment epithelium or microglia could be associated with increased apoptotic cell death. Earlier, our lab demonstrated aberrant microglial activation leading to inflammation and angiogenesis in DR pathogenesis. In the present study we aimed to further explore the role of microglial-mediated autophagy genes in DR.

Methodology: The study was approved by the institutional review board (IRB) of LVPEI, Hyderabad, India. Peripheral blood samples from patients (DM, PDR & NPDR) and controls (n=50); cadaveric retinas from diabetic and non-diabetic donors (n=10) and epiretinal membranes (ERM, n=10) from DR cases and controls were collected and RNA was isolated. Quantitative expression of genes involved in autophagy were performed. $\Lambda\Lambda$CT was compared across different categories and significance estimated using a student t-test.

Result: The expression of *TREM2, an* autophagy-associated gene was significantly (p-value = 0.001) upregulated in all categories as compared to control (NDM and/or NDM/No-DR), while other autophagy genes *LDLR* and *LC3-II* were upregulated in only PDR vs controls. However, their expression were significantly downregulated in cadaveric retina from diabetic donors and ERM from severe DR as compared to controls.

Conclusion: Increased expression of *TREM2* gene in blood samples of diabetic individuals with/without DR confirms a strong role of microglia in disease pathogenesis. The downregulation of the autophagy genes in the retina of DR needs to be explored for their potential role in DR pathogenesis.

DETAILED INVESTIGATION ON THE ROLE OF LIPID METABOLIZING ENZYMES IN RETINOPATHY OF PREMATURITY PATHOGENESIS.

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Purpose: Extremely preterm infants are at risk of developing retinopathy of prematurity (ROP) that causes impaired vision or blindness. ROP progression is characterized by neovascularization and neuroinflammation in the retina. Lipid metabolism is significantly altered in neovascularization, inflammation and neurodegeneration however its role in regulation of ROP has not been explored much. We therefore, aimed to explore the contributions of altered lipid metabolism in ROP pathogenesis.

Methods: Blood, vitreous humor (VH) and fibrovascular membrane (FVM) samples were collected from preterm infants with ROP and No-ROP. Quantitative PCR was performed for comparing gene expression of lipid metabolizing enzymes, angiogenesis and apoptotic genes among cases and controls. Activity for lipid metabolizing enzymes was assessed by measuring their metabolites in VH by LC-MS/MS. Further confirmation of significantly deregulated lipid metabolizing enzyme was performed in FVM by immunohistochemistry.

Results: Most of the lipid metabolizing enzymes genes such as *CYP1B1, CYP2C8, COX2,* and *ALOX15* were upregulated while *EPHX2* responsible for conversion of epoxy fatty acids into diol fatty acids was significantly (<0.05) down regulated. The metabolites derived from abundantly expressed enzymes (blood) were found to be upregulated while metabolite from *EPHX2* did not show any change. Moreover, Ephx2 was not seen in the glial cells present in FVM of ROP subjects. Angiogenic gene *VEGF165/189*, *Notch1* and *APH1B*, apoptotic genes *Caspase3/8* were also significantly (<0.05) upregulated.

Conclusions: Our result suggests that lipid metabolism has a potential role in ROP pathogenesis. A reduced *EPHX2* activity and expression seems to cause abnormal angiogenesis via *Notch1* upregulation.

A CASE OF ANIRIDIA WITH RARE MUTATION IN *PAX6* GENE IN AN INDIAN FAMILY

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Purpose: Aniridia is a rare pan-ocular genetic disorder that might affect different parts of the eye. Depending on the type of mutation and genes involved, it renders a challenging visual prognosis for different individuals. The purpose of this study is to report a rare mutation in the PAX 6 gene and its clinical characteristics in a case of familial aniridia.

Methods: A 21-year-old female came for pre-conceptional genetic counselling eager to prevent the disease from passing on to future generations. Clinical features of complete aniridia, aniridia associated keratopathy (right eye grade 3 and left eye grade 2), increased corneal thickness, superior lens subluxation, nystagmus, and optic neuropathy. She had a family history of similar clinical presentation for her mother, sister and half-brother.

Results: On genetic evaluation by NGS-technology, a heterozygous mutation (c.1268A>T; p.*423L) was identified in *PAX6* gene; a gene known to cause aniridia in autosomal dominant pattern. Sanger sequencing of the variant confirmed absence of this mutation in her unaffected maternal uncle and presence in her affected sister.

Conclusions: We were able to successfully deduce the pathogenicity of the variant by segregation analysis and computational evidence suggesting a deleterious effect on gene product, thus offering appropriate prenatal diagnosis. This mutation is extremely rare and has not been previously reported in the Indian population. We describe the specific phenotype associated with the mutation and discuss with the few reported in literature.

GUT MYCOBIOME DYSBIOSIS DURING FOUR MONTH FOLLOW-UP IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Purpose: To study the gut mycobiome changes in streptozotocin-induced diabetic rats and rats with retinal changes

Methods: Diabetes was induced by intraperitoneal streptozotocin injection and the animals were monitored for a period of 4 months. Progression of retinal changes due to prolonged diabetes was assessed by retinal histopathology. DNA from faecal pellets was used for fungal microbiome generation and metagenomes were analyzed by QIIME and R.

Results: Compared to the control rats, dysbiosis was observed in the gut mycobiome of diabetic rats and diabetic rats with retinal changes both at the phylum and genus levels. Heat maps generated with differentially abundant genera showed that the control and diabetic rat microbiomes as well as control and diabetic rats with retinal changes formed distinct clusters. NMDS plot depicting the β-diversity, separated the control rat microbiome from the microbiomes of diabetic and diabetic rats with retinal changes. However, the microbiomes of diabetic rats and diabetic rats with retinal changes overlapped with each other.

Conclusions: The present study demonstrated the dysbiosis in the gut mycobiomes of diabetic rats and diabetic rats with retinal changes both at the phyla and genera levels, in comparison with the control rats. Similar studies on gut mycobiomes may help in identifying specific fungi associated with retinal changes during prolonged diabetes and develop novel therapies for arresting the initiation and/or progression of diabetes induced retinal changes.

MOLECULAR REGULATORS OF RETINOBLASTOMA TUMOR PROGRESSION

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Purpose: Inactivation of *RB1* gene is the known genetic lesion in Retinoblastoma (RB) initiation. Although multiple molecular alterations at genomic and transcript level were documented in RB tumor progression, regulators of these alterations are largely unknown. As these molecular events can be influenced epigenetically, we have checked the differential expression of miRNAs to investigate their possible role in regulating the cancer pathways involved in the RB tumor progression.

Methods: RNA Samples isolated from 7 RB Tumor and 3 normal pediatric retinal tissues were used for miRNA sequencing using NEB Next Multiplex Small RNA Library Prep kit. Total RNAseq was also done with same samples using NEB Ultra RNA-Seq Library Prep kit to check the altered gene expression. Analysis of miRNA and RNAseq data was performed using an in-house pipeline

Results: Data analysis identified 246 differentially expressed miRNAs based on the relative abundance. Further, it was narrowed down to 11 miRNAs (6 downregulated and 5 upregulated) based on their biological relevance. The target genes of these miRNAs were found to be involved in vital cancer pathways like TGF beta signaling pathway,chemokine signaling pathway, Focal adhesion, Ras signaling pathway, MAPK signaling pathway etc. implying their involvement in cell growth and migration.

Conclusion: Our study identified the key microRNAs that regulate the major pathways involved in cell growth and migration. Functional validation of these microRNAs would confirm their role in tumor progression of RB and derive the path towards their use as therapeutic biomarkers.

EVALUATING THE COMBINED EFFECT OF OCULAR UV EXPOSURE WITH GENETIC PREDISPOSITION AND OTHER ENVIRONMENTAL FACTORS IN ETIOPATHOGENESIS FOR PSEUDOEXFOLIATION SYNDROME IN INDIAN POPULATION

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Purpose: The major objective of the study was to evaluate the combined effect of ocular UV exposure and genetic predisposition/environmental factors as key causative factors for Pseudoexfoliation Syndrome (XFS) in Indian population.

Methods: Measurement of ocular UV exposure was verified by ascorbic acid concentration in aqueous humour and alkaline comet assay of surgically excised Tenon's tissue. Single nucleotide polymorphisms (SNP) analysis was carried out for known genetic loci of *LOXL1, POMP* and *TMEM1* genes associated with XFS. Logistic regression analysis was performed to evaluate the combined effect of ocular UV exposure and genetic predisposition to XFS. The combined effect of ocular UV exposure and other environmental factors was verified by gene expression analysis for extracellular matrix (ECM) markers in patient-derived Tenon fibroblasts from high and low-risk UV exposed individuals exposed to oxidative stress, hypoxia or inflammation.

Results: Analysis of aqueous humor samples form 61 XFS and 70 control samples revealed significantly lower ascorbic acid concentration in XFS patients compared to controls, with no significant changes in comet tail length from corresponding Tenon's fibroblasts. Genetic predisposition to XFS was observed in SNPs rs3825942, rs41435250, rs8818 (*LOXL1)* and rs3737528 (*POMP*). No significant changes could be observed in XFS patients in correlation to the levels of UV exposure in cells exposed to other environmental factors.

Conclusion: Ascorbic acid in the aqueous humor of the eye plays critical role as UV radiation quencher. More work to be elicited on genetic and environmental interaction to clearly explain the relationship between XFS pathology and UV exposure.

EXTENSIVE META-ANALYSIS OF GENE EXPRESSION DATASETS WITHIN RETINAL SAMPLES OF DIABETIC RETINOPATHY

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Purpose: Diabetic Retinopathy (DR) is a major ocular disease in patients with persistent hyperglycemia. Despite the attempts to establish a link between these conditions, the angiogenesis causing visual impairment on prognosis of early non proliferative diabetic retinopathy (NPDR) to late proliferative diabetic retinopathy (PDR) are yet to be characterized.

Methods: An extensive gene expression meta-analysis of two independent publicly available microarray data of the two stages in DR were conducted to identify shared gene expression signatures and overlapping biological processes. Using INMEX bioinformatics tool, significantly regulated (up and down) genes between PDR and NPDR were compared and analyzed using the combine effect size method.

Results and Conclusions: This identified a total of 7,935 differentially expressed genes (DEGs) and the heatmap of differentially expressed genes was created by the visualization tools of INVEX. INMEX based meta-analysis identified Retinol Binding Protein 3 (RBP3) and Peripherin 2 (PRPH2) found to be the top upregulated genes. Interestingly, the study has generated a novel database of candidate markers, involved in retinal angiogenesis of NPDR and PDR, which might aid in the identification of prognostic biomarkers pertaining to vision loss.

MECHANOTRANSDUCTION AND RETROTRANSPOSONS IN AGE-RELATED MACULAR DEGENERATION

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Purpose: Geographic Atrophy (GA), an untreatable form of age-related macular degeneration (AMD), is characterized initially by sub-RPE deposits followed by varying degrees of RPE, photoreceptor, and choriocapillaris dropout. Decades of research have focused on identifying the biochemical components of these sub-RPE deposits, and targeting individual components of these deposits has been a prominent focus of developing investigational treatments. However, the effect of the mechanical changes caused by these deposits on RPE homeostasis has not been well studied. The aim of this study is to determine how mechanical effects exerted by sub-RPE deposits modulate RPE homeostasis in GA.

Methods: To simulate the mechanical effects of sub-RPE deposits, primary human RPE (hRPE) cells were seeded on plates with variable substrate stiffness. RPE cells were treated with small-interfering RNA against integrin YAP or TAZ. Subretinal injections were performed in C57BL/6J, YAP$^{f/f}$, TAZ$^{f/f}$, *Casp1*$^{-/-}$, and *NLRP3*$^{-/-}$ mice and assessed by fundus photography, Optical Coherence tomography (OCT) and Zonula occludens-1 (ZO-1) staining.

Results: IHC, RT-PCR and western blotting revealed decreased TAZ expression in the RPE of human donor eyes with GA compared to control eyes. DICER1 and TAZ protein expression was decreased in hRPE plated on softer substrates. Conditional RPE specific knockout of YAP and TAZ induced spontaneous RPE degeneration.

Conclusions: This study provides new evidence to suggest a paradigm shift in our understanding of the pathogenesis of GA by deciphering a role for the mechanical effects induced by sub-RPE deposits on the RPE thereby identifying potential novel therapeutic strategies.

MUTATIONAL ANALYSIS OF *RB1* GENE IN 34 RETINOBLASTOMA (RB) PATIENTS FROM NORTH INDIA

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Purpose: The purpose of the study is to establish genotype-phenotype correlation, identify unique mutations in North Indian population, and deduce functional significance of identified mutations in RB.

Methods: This is a retrospective study review of RB patients from Jan 2019 that presented at Dr Shroff's Charity Eye Hospital and underwent gene analysis. EDTA blood samples was collected for all probands and *RB1* gene analysis for point mutations by next generation sequencing was carried out to detect presence of changes in *RB1* gene. When no sequencing variation was identified, deletion/ duplication analysis in *RB1* by multiple-ligation probe analysis was carried out for all bilateral cases and unilateral cases <2 years age.

Results: Germline mutation detection rate for bilateral cases was 70% (9/13) with mean age at diagnosis of 14 months and 9% (2/21) for unilateral cases with mean age of diagnosis at 31 months. 5 novel mutations, c.772_776del, c.1649T>G, c.1536_1537del, c.211A>T and c.184C>T were identified for our patients in *RB1*. Among the hereditary cases, unique phenotypic observations as low penetrance (LP) in presence of mis-sense mutations (2 cases) was observed with skipped generation and no familial unilateral cases were present in our cohort.

Conclusions: Our genotype- phenotype correlation analysis is consistent with previously reported literature with a unique case of unidentified hereditary RB family. We postulate this might be due to chromosomal rearrangements, molecular changes in deep-introns, splice-sites or promoter region, which were not analyzed.

ANALYSIS OF GENETIC POLYMORPHISM FOR AGE-RELATED MACULAR DEGENERATION (AMD) IN NORTH-INDIAN POPULATION AND ITS ASSOCIATED PHENOTYPIC CHARACTERISTICS

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Purpose: Age related macular degeneration (AMD) is strongly linked with polymorphism in arms-2, htra-1 and cfh in world-wide studies. However, polymorphism in these genes varies in different geographical locations and populations. Especially, locus of interest are arms-2 (rs10490924), htra-1(rs11200638) and cfh (rs1061170). Our study is aimed to estimate the prevalence of arms-2, htra-1 and cfh polymorphism and its association with clinical phenotypic characteristics in AMD patients of north Indian region.

Methods: AMD patients were enrolled in the study after written patient consent, grouped into ARED 1-4. Blood samples were collected, Single Nucleotide Polymorphism (SNP) assay for arms-2 (rs10490924), htra-1(rs11200638) and cfh (rs1061170) was performed using TaqMan PCR assay. Phenotypic characterization was recorded using Fundus Photography and Optical Coherence Tomography. Statistical analysis was performed within various groups using IBM SPSS Statistics Software.

Results: We enrolled 104 AMD and 54 control subjects. Frequency distribution analysis indicated that AMD occurs irrelevant of gender ($p>0.05$). ARED-4 (advanced AMD) had strong linkage with arms2 (81.9%), htra1 (81.9%) and cfh (83.3%) risk alleles ($p<0.005$). Further, statistical analysis showed strong association of risk alleles with clinical phenotype such as – drusens, serous detachment, RPE atrophy, disciform scars and CNVM in ARED-4 patients ($p<0.0001$).

Conclusion: Our study reports polymorphism in arms-2, htra-1 and cfh is strongly correlated with advanced AMD in north Indian population. Further, these risk alleles are strongly associated with typical clinical findings in ARED-4 patients.

WHOLE EXOME SEQUENCING ASSOCIATES NOVEL SPLICE-SITE MUTATION IN *IMPG2* GENE WITH FAMILIAL STARGARDT-LIKE JUVENILE MACULAR DYSTROPHY

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Purpose: To delineate disease causing mutations in a north Indian family diagnosed with Stargardt-like juvenile macular dystrophy by whole exome sequencing analysis.

Methods: Whole exome sequencing was performed using peripheral blood genomic DNA samples of affected individuals and the carrier. The Agilent SureSelect Human All Exon V5 Kit was used for enrichment of coding exons and flanking intronic sequences followed by sequencing on Illumina HiSeq 2000/2500 platform. After data analysis the potential pathogenic variants were screened employing segregation analysis and Sanger sequencing. *In silico* prediction tools and splicing reporter minigene assay was utilized to access the pathogenic potential of the variant that co-segregated with Stargardt-like juvenile macular dystrophy phenotype in the affected family members.

Results: We identified a novel splice-site variant NC_000003.11(NM_016247.3): c.1239+1G>T, co-segregating in the affected members in the gene Interphotoreceptor Matrix Proteoglycan 2 (*IMPG2*). The identified variant is present immediately after exon 11; therefore, it is predicted to disrupt wild-type donor site and to affect splicing of *IMPG2* transcripts. We confirmed the splice-site changes in the *IMPG2* transcripts using minigene functional assay.

Conclusions: This is the first study to report a novel variant in Interphotoreceptor Matrix Proteoglycan 2 (*IMPG2*) that is linked with Stargardt-like juvenile macular dystrophy. Ours is the foremost report to provide the description of *IMPG2* mutation from India. Therefore, our study will dissect the genetic etiology of Stargardt-like juvenile macular dystrophy in India.

THE TEAR BACTERIAL MICROBIOME OF THE HEALTHY HUMAN EYES

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Purpose: Bacterial microbiome of the ocular surface plays an important role in understanding the health and disease state of the eyes. Conjunctival swabs (CS) were the major source of sampling; however collecting CS from the diseased eyes is difficult and painful. Therefore 'tears' which is a less invasive approach to collect was used to establish the bacterial microbiome in the healthy eyes.

Methods: Bacterial microbiome was generated from the DNA of tear samples (n=24) of healthy individuals. Sequencing of V3-V4 region of 16S rRNA gene was performed on the Illumina HiSeq2500 platform. Reads were processed in QIIME to assign the taxa. Statistical analysis of the tear and the CS microbiome was done in R to assess the alphadiversity and betadiversity indices. Significant changes between the tear and CS cohorts were ascertained by Linear discriminant effective size analysis

Results: Tear microbiome was generated in all the 24 healthy eyes. Seven out of the top 10 predominant bacterial genera remained same in both tear and conjunctival swab microbiomes, which include genera such as Staphylococcus, Streptococcus, Acinetobacter, Escherichia-Shigella, Cutibacterium, Micrococcus and Finegoldia. Lefse analysis indicated significant increase of 9 genera in tear microbiome compared to conjunctival microbiome. Where as 29 genera were significantly abundant in CS microbiome.

Conclusions: The results of the study indicate that the predominant bacterial genera remained same in both conjunctiva and tears. However Tear and CS microbiomes had significant differences in the bacterial genera indicating that tear film microbiome and the conjuctival microbiome are different.

COMMON MUTATIONS IN *SAG* AND *GRK1* GENE IN OGUCHI DISEASE PATIENTS FROM INDIA

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Background: Congenital Stationary Night Blindness (CSNB) is a group of genetically and clinically heterogeneous non-progressive retinal disorder. CSNB exists as two variant forms (Oguchi disease and fundus albipunctatus) with distinctive fundus abnormalities and are inherited as an autosomal recessive trait. We report here the molecular genetic analysis in Oguchi disease patients from India.

Methodology: Seventeen patients with Oguchi disease characterized by ophthalmic examinations including electroretinogram (ERG), color vision test, optical coherence tomography (OCT), fundus auto fluorescence (FAF). Targeted re-sequencing using next generation sequencing (NGS) on Illumina MiSeq platform was performed for all the CSNB candidate genes and thus identified variants were validated by Sanger sequencing followed by segregation analysis, control screening.

Results: Pathogenic variants were identified in total of 11 patients in 38% (6/16) *SAG* and 31% (5/16) in *GRK1* genes. Two common mutations, p.R292X and p.Asp537Valfs*7 was observed in exon 11 and 7 of *SAG* (N=5) and *GRK1* (N=4) genes respectively. The probands had mild myopia with normal color vision typical ERG findings with tapetal reflex.

Conclusion: Exon 11 in *SAG* and exon 7 in *GRK1* could be considered as mutational hotspot regions for CSNB gene. The putative role of novel genes in CSNB in Indian patients is also suggested in N=5 (~31%) patients with Oguchi cases. The data are of potential value in framing a cost effective and rapid strategy in screening Oguchi disease for our population.

TARGETED NEXT-GENERATION SEQUENCING IN THE MICROBIAL DIAGNOSIS OF POST-OPERATIVE ENDOPHTHALMITIS

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Purpose: To evaluate the feasibility and advantage of targeted next-generation sequencing (NGS) in improving diagnostic yield in infectious endophthalmitis.

Methods: Vitreous fluids collected from consecutive patients with clinical diagnosis of post-operative infectious endophthalmitis between April 2019 to April 2021 were analyzed by both traditional and NGS techniques. Conventional tests included aerobic and anaerobic culture on commercially available culture plates and further analysis using Vitek 2. NGS technology consisted of total DNA extraction using Qiagen mini kit and V3–V4 regions of the bacterial 16S rRNA gene and amplification of ITS 4 regions of the fungal genome by PCR and deep sequenced on an Illumina HiSeq 2500 machine. Paired reads were curated, taxonomically labeled, and filtered.

Results: Of 61 patients with clinical endophthalmitis, 21 (34.4 %) were culture positive (Bacterial-19, Fungal-2). Targeted NGS could be performed in 40 vitreous samples only; it could not be done in others due to lack of sample/microbial genome. NGS diagnosed the presence of microbes in 40/61 (83%) patients-Bacterial-30, Fungal-8, and mixed infections- 2. The difference was statistically significant *(p=0.0005).* There was a fair (Cohen's k=0.24) agreement between culture and NGS for culture-positive cases (16 of 21). In culture-negative vitreous samples, NGS identified predominantly S*taphylococcus sp (n=6)* and *Fusarium sp (n=4).* Additionally, NGS detected polymicrobial infections in many culture-negative samples (17/19; 89.5%) and few culture-positive samples (9/21; 42.8%).

Conclusions: NGS is a novel technology for identifying pathogens in postoperative endophthalmitis. Currently, cost is one of the important deterrents for routine use.

SUMOYLATION OF RD3 AND ITS EFFECTS ON INTRACELLULAR TRAFFICKING AND SUB-NUCLEAR LOCALIZATION

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Purpose: LCA12 is a severe form of autosomal recessive disorder that affects young children and is caused by mutations in *RD3* gene. RD3 plays an important role in the intracellular trafficking of guanylate cyclase from inner to outer segments of photoreceptors. Apart from its cytosolic co-localization along with guanylate cyclase, RD3 is known to enter the nucleus and localize to discrete sub-nuclear domains. This study aims to elucidate the unknown functions of RD3 in the nucleus.

Methods: The *RD3* gene was amplified from retinal cDNA and cloned into retroviral vectors. Sites of post-translational modifications on RD3 was predicted using NetPhos3.0, GPS sumo etc. The sumoylation consensus sites were altered by site-directed mutagenesis and the effects of mutation on RD3 sub-cellular localization was assessed by ectopic expression and immunocytochemistry in HeLa cells.

Results: Ectopically expressed RD3 localized to distinct nuclear domains, where it absolutely co-localized with SUMO-1. Also, the nuclear RD3 domains partially co-localized with different sub-nuclear compartments that are enriched with sumoylated proteins such as PML and coiled bodies. Bioinformatics analysis revealed two consensus sumoylation sites towards the C-terminal region of RD3 protein. Mutant RD3 with disrupted sumoylation consensus sites could still enter the nucleus, organized into discrete punctas and co-localized with SUMO-1, but not with Coilin.

Conclusions: The results suggest that RD3 undergoes sumoylation and also interacts with sumoylated proteins within nuclear sub-domains such as the PML bodies and coiled bodies. While the nuclear transport of RD3 is sumoylation independent, its interaction with other nucleolar proteins requires its SUMO modification.

EXOME SEQUENCING AND ANALYSIS OF RARE CODING VARIANTS IN A PRIMARYANGLE CLOSURE GLAUCOMA FAMILY WITH AUTOSOMAL DOMINANT INHERITANCE

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Purpose: Primary Angle Closure Glaucoma (PACG) is more prevalent in Asian countries and strong positive family history, ethnicity, gender being identified as majorrisk factors. Next Generation sequencing based strategies have identified rare variantsin the etiology of complex diseases.

Methods: Whole Exome Sequencing wasperformed (Illumina HiSeq platform) for the trios (affected mother, daughter and unaffected son) in a PACG family with autosomal dominant inheritance. Average of Q30 score was used as a cutoff to remove low quality bases and the variants with quality score ≥ 50 were considered for annotation. The annotated variants using VariMAT pipe line were filtered by applying stringent criteria that included (i) zygosity state (autosomal dominant model) (ii) MAF (<1%) [ExAC and 1000 Genome Browser] (iii) variant type (iv) *in silico* prediction on its pathogenicity and ranked by Varelect tool(an online tool for variant prioritization based on phenotype). The rare coding variants in genes/loci that were earlier reported were analysed in addition to the other loci to identify the candidate genes for PACG. The prioritized variants were further validatedand co-segregated in the family members by Sanger sequencing.

Results andConclusions: A total of 90647 variants were obtained per sample after annotation, of which 221 variants were prioritized. Further analysis of these variants in Varelect ranked ten rare novel coding variants with high scores. These genes were either associated with ocular quantitative traits (*NOS3, COL1A1, POU6F2, LRP1, CAPN2*) or indirectly with glaucoma (*PDE6B, CDH23, BAZ2B, PXDN, ZSWIM6*) and the co- segregation analysis results are discussed.

CLINICAL REASSESSMENTS AND WHOLE-EXOME SEQUENCING UNCOVER NOVEL *BEST1* MUTATION ASSOCIATED WITH BESTROPHINOPATHY PHENOTYPE

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Purpose: The diagnosis of retinal dystrophies can be challenging due to the spectrum of protean phenotypic manifestations. This study employed trio-whole exome sequencing (trio-WES) to unveil the genetic cause of an inherited retinal disorder in three generations of a family.

Methods: Proband's initial ophthalmic examinations was performed in the year 2016. WES was performed on a proband-parent trio to identify causative mutation followed by Sanger validation, segregation analysis, sequence and structure-based computational analysis to assess its pathogenicity. Based on the genetic findings, detailed clinical reassessments were performed in year 2020 for the proband and available family members.

Results: WES revealed a novel homozygous *BEST1* mutation c.G310A (p.D104N) in the proband and heterozygous for the parents, indicating autosomal recessive inheritance. Segregation analysis showed heterozygous mutation in maternal grandfather and normal genotype for younger brother and maternal grandmother. Moreover, the structure-based analysis revealed the mutation p.D104N in the cytoplasmic domain, causing structural hindrance by altering hydrogen bonds and destabilizing the BEST1 protein structure. Proband's clinical assessment was consistent with autosomal recessive bestrophinopathy (ARB) phenotype. Additionally, characteristic absent light rise and decreased light peak-to-dark trough ratio (LP:DT) was observed bilaterally in EOG.

Conclusions: Our study demonstrates the utility of WES and clinical re-evaluations in establishing the precise diagnosis of autosomal recessive bestrophinopathy associated with a novel mutation, thus expanding the *BEST1* related mutation spectrum.

SILK FIBROIN MEMBRANES FOR THE CULTURE OF HUMAN CORNEAL ENDOTHELIUM

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Purpose: Tissue engineering is directed towards mimicking native extracellular environment to induce favourable cellular behaviour. This study aimed to compare expression of extracellular-matrix (ECM) proteins by cells on silk films with native tissue, and also to understand the cytochemistry behind cell attachment to the substrate.

Methods: Primary human corneal endothelial (HCE) cells were seeded on silk films of Philosamia ricini(PR), Antheraea assamensis(AA) and on decellularized human Descemet's membrane obtained from donor corneas. Cells were fixed at 48hours, 7days, 15days and 30days and expression of collagen 4, collagen 8, fibronectin and laminin was determined using immunostaining and western blot. Adhesion of HCE to the silk films were determined by distribution of integrin molecules ($\alpha2,\alpha3,\alpha5,\alpha v,\beta1,\beta3,\beta6$) at the gene and protein levels using PCR and immunostaining, respectively. Distribution of focal adhesions (FA) were also quantified using paxillin at similar time points.

Results: HCE cells cultured on both AA and PR showed positive expression for collagen 4,8, fibronectin and laminin. $\alpha2,\alpha3,\alpha5,\alpha v,\beta1,\beta3,\beta6$ integrins were expressed by cells on silk films, which was comparable to native-tissue. The FA's of 48hrs were significantly larger($95\mu M\pm6.5$) and located towards the periphery of cells compared to that of 30 days ($47.8\mu M\pm7.8$), when it was a monolayer of cells with more punctate like FA's located all over the cells. Similar punctate like FA's were also observed in case of native tissue.

Conclusion: Our data suggests that both AA and PR are able to closely mimic the extracellular environment of native tissue to support the growth of HCE cells.

EFFECTS OF *RB1* INACTIVATION ON RETINAL DIFFERENTIATION OF IPSCS

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Purpose: *RB1* plays an important role in eye development and its loss of function is associated with retinoblastoma. Here, we aim to generate *RB1* mutant iPSCs models to study the effects of loss-of-function on retinal development and maturation.

Methods: Adipose-derived mesenchymal cells from familial retinoblastoma patient were reprogrammed to iPSCs using episomal constructs. Isogenic $RB1^{-/-}$iPSC lines were generated by transfecting CRISPR-Cas9 vector carrying gRNA targeting exon18 of *RB1*. Mutant lines were characterized by RT-PCR, immunofluorescence, western blotting and karyotyping. Lines were differentiated into retinal lineage using established protocols.

Results: A patient-derived mesenchymal cells harboring nonsense mutation in exon18 of *RB1* ($RB1^{+/-}$) was reprogrammed into iPSCs. Three isogenic $RB1^{-/-}$ iPSC lines were generated by CRISPR editing of a normal iPSC line. All mutant lines maintained their stemness, expressed lineage-specific markers upon differentiation and embryoid body formation and maintained their genomic integrity. The lines were differentiated towards retinal lineage, where $RB1^{+/-}$line formed normal eye-fields and 3D optic cups in suspension, while the efficiency of eye-field formation was significantly reduced in $RB1^{-/-}$ mutants. All mutants retained their ability to differentiate into early retinal precursors.

Conclusion: *RB1* mutant iPSC models have been successfully generated wherein, the loss of *RB1* significantly affected normal eye field formation, neuro-retinal differentiation and lamination *in vitro.*

GENERATION AND VALIDATION OF ANIMAL MODEL OF LIMBAL STEM CELL DEFICIENCY USING MECHANICAL DEBRIDEMENT

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Purpose: Limbal stem cell deficiency (LSCD) marked by corneal epithelial defect, neovascularizationand conjunctivalization is a leading cause of corneal blindness. A simple, reproducible and sustainable method for generation of animal models of LSCD is necessary for assessment of safety and efficacy of new therapeutic modalities. The aim of the study is to generate and validate limbal stem cell deficiency in animal model using mechanical debridement withAlger brush II rotating burr.

Methods: Complete corneal epithelial debridement was performed in New Zealand White Rabbit (n=10) using the Alger brush-II 1.0-mm round-ended burr from limbus to limbus after 360° peritomy and recession of the conjunctiva. Rabbits were observed for vascularization and extent of epithelial defect using photograph, slit lamp, fluorescein staining and anterior segment optical coherence tomography post debridement.

Results: In 7/10 (70%) of the eyes showed superficial neovascularization with different grades after 4 weeks. In 3/10 (30%) the neovascularization reached the central cornea. All eyes showed epithelial defect up to 3 weeks as evident by fluorescein staining. In 7/10 (70%) epithelial defect was observed even after 5 weeks of debridement.

Conclusions: Mechanical debridement using alger brush-II 1.0-mm round-ended burr is an effective method for the establishment of LSCD in New Zealand White Rabbits with variable LSCD features. Further optimization using deeper (2.5 mm) round ended burr may help in betterLSCD generation in rabbits.

ADULT STEM CELLS FOR HUMAN RETINAL PIGMENT EPITHELIUM ARE PRESENT IN ITS PERIPHERAL REGION

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Purpose: The presence of stem cells (SCs) in human Retinal Pigment Epithelium (RPE) has been demonstrated only upon culturing. This study aims to identify the location of adult SCs in human RPE.

Methods: RPE was demarcated into three equal concentric regions with optic nerve head as centre: central, equatorial and peripheral region. Flatmounts of RPE were immunostained for the proliferating cell marker, Ki67 and images were acquired using confocal microscope. Five fields per region were analysed for calculating the percentage of proliferating cells, cell size and density. Functional analysis for adult SCs, sphere formation assay and clonal analysis, was carried out using RPE cells isolated from the three regions. The spheres were immunostained for Ki67, SC markers- KLF4, CMYC, SSEA4 and RPE specific marker-RPE65.

Results: The RPE cells in the periphery were larger in size (23.6±2.7µm) and less dense (1700±282cells/mm^2) compared to the other regions (size, density: equatorial 16.5±0.9µm, 2815±442cells/mm^2; central 13.9±0.6µm, 3597±626cells/mm^2). Proliferating cells (Ki67$^+$) were restricted to the peripheral RPE (0.147±0.003%). Further, peripheral RPE cells alone had the sphere forming ability (1.185±0.41%) and clonal ability (20±2.35%). Expression of RPE65 and Ki67 in spheres indicated that the spheres were formed by proliferating RPE cells and the expression of SC markers indicated the presence of SCs.

Conclusion: The adult SCs for human RPE were identified to be located in its peripheral region by their high proliferative potential- sphere forming and clonal ability. This basic research will help to understand the status of these SCs with ageing and in retinal degenerative diseases.

CLINICAL SCIENCES

MOLECULAR DIAGNOSIS OF RHINO-ORBITO-CEREBRAL MUCORMYCOSIS FROM ORBITAL TISSUE SAMPLES

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Purpose: To evaluate the ITS2 - PCR-based technique for the diagnosis of mucormycosis from fresh tissue specimens in patients diagnosed with rhino-orbito-cerebral-mucormycosis (ROCM) post COVID-19.

Methods: Eleven cases of ROCM were included in the study seen between June – August 2021. One half of the sample (orbital mass / necrotic material) was processed for routine microbiological work up by direct microscopy (calcofluor white stain) and culture for bacteria and fungus and the other half was processed for PCR. Total DNA was extracted using the Qiagen mini kit and the ITS 2 region of the fungal genome were amplified by PCR and PCR products were sequenced.

Results: All patients included in the study were male with a mean age of 51 ± 13.18 years. ROCM cases were confirmed in 5/11 cases by the demonstration of aseptate ribbon-like hyphae under direct microscopy though culture was positive for Mucorales in only 2/11 cases and identified as *Rhizopus* sp.. In comparison, ITS2 PCR confirmed mucormycosis in 5/11 cases and included four microscopy positive samples and one sample which was negative by smear and culture examination. Direct sequencing confirmed the presence of Mucorales in these tissues.

Conclusions: PCR amplification of ITS2 region is a reliable and simple method of early diagnosis of mucormycosis directly from tissue samples.

EFFECTIVENESS AND FUTURE IMPLICATIONS OF COVID-SAFETY GUIDELINES FOR MANAGING ROP DURING COVID-19 PANDEMIC. THE INDIAN TWIN CITIES ROP STUDY REPORT NUMBER 10

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Purpose: To evaluate the effectiveness and future implications of COVID-safety guidelines for managing ROP. Design: A prospective study conducted at LV Prasad Eye Institute, Hyderabad from beginning of lockdown in India from March 23,2020 till the end of first phase of lockdown on May 29, 2020 as imposed by the Telangana state and the central government of India.

Methods: We evaluated 200 prematurely born infants (<34 weeks of Gestational age) using the new safety guideline protocols developed in conjunction with the Indian ROP society for care during COVID times.

Results: New guidelines were implemented in 106 (53%) infants who were low- risk while 94 (47%) infants with high risk were followed up as per the old guidelines. Out of the 106 infants (212 eyes) managed by new guidelines; good outcome (group 1) was seen in 102 (96.2%) infants. 27 of the 102 infants had some form of ROP and 5 of these infants needed treatment. None of the low-risk babies with no detachment at presentation managed by new guidelines required surgery later (group 2). Two (1.9%) infants came with retinal detachment at presentation and underwent successful surgery (Group 3) and two infants (1.9%) were lost to follow up.

Conclusions: New risk stratification COVID Pandemic ROP guidelines were an efficient and safe strategy in managing low risk ROP babies. Proper planned scientific strategy, team work and with support of enabling legislation by the Government of India, played a critical role in saving newborns from going blind from ROP during the pandemic.

Effectiveness Of A Virtual Reality Simulation-Based Training Curriculum For Manual Small Incision Cataract Surgery Among Novice Surgeons: A Randomized Controlled Trial.

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Purpose: To determine the effect of a novel simulation-based training curriculum for scleral tunnel construction in manual small incision cataract surgery (MSICS) compared with traditional training.

Methods: In this randomized control trial, participants with minimal or no prior experience with MSICS; were assigned either to simulation-based training, the Experimental Group (EG), or conventional training, the Control Group (CG). Residents in EG were trained to perform scleral tunnel construction using a simulation-based curriculum (HelpMeSee Eye Surgery Simulator), while residents in CG followed their institution-specific curriculum before progressing to live surgery. Surgical videos of the first 20 attempts at tunnel construction were reviewed. The primary outcome was the total number of any of 9 pre-specified errors. Three raters randomly selected from a pool of 10 masked video reviewers made observations to record incident errors.

Results: On average, the total number of errors was 9.25 (95% confidence interval [CI] 0 to 18.95) in the EG and 17.56 (95% CI 6.63 to 28.49) in the CG (P = 0.05); the number of major errors was 4.86 (95% CI 0.13 to 9.59) in the EG and 10.09 (95% CI 4.76 to 15.41) in the CG (P = 0.02); and the number of minor errors was 4.39 (95% CI 0 to 9.75) in the EG and 7.47 (95% CI 1.43 to 13.51) in the CG (P = 0.16).

Conclusions: Novice surgeons trained using the novel simulation-based curriculum performed fewer errors in their first 20 attempts at tunnel construction compared to those trained with a conventional curriculum.

OCULAR TOXOPLASMOSIS IN IMMUNOCOMPROMISED PATIENTS: CLINICAL FEATURES AND VISUAL OUTCOMES

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Purpose: Toxoplasmosis is an opportunistic infection of eye that affects patients receiving chemotherapeutic or immunosuppressive drugs. Ocular Toxoplasmosis (OT) among Immunocompromised hosts is very sparsely reported in medical literature and herein we report the clinical features ,visual outcomes and management of OT in Immunocompromised Asian Indian patients.

Methods: Retrospective analysis of data records between January 2006 to February 2019

Results: We report 36 eyes of 27 patients. Average age of presentation was 40 years with male preponderance (Male n=17, Female n=9). 23 patients were known HIV positive and 3 patients were diagnosed after the ophthalmic evaluation. Unilateral presentation was more in compare to bilateral presentation (unilateral n= 17, bilateral n= 06) Systemic tuberculosis was another systemic co morbidity seen in two patients. Anterior segment involvement was seen in form of anterior uveitis 68.5% of the eyes (n=24, 68.5%). Posterior segment involvement was noted to be equal in form of unifocal and multifocal presentation, (unifocal n= 18, 51.4% and multifocal in n= 17, 48.5%). Majority of the eyes had Primary activation (n= 27, 77% and Reactivation: n= 08, 23%).

Conclusions: Posterior pole multifocal lesions, bilateral lesions with atypical presentation should alert the clinician for HIV ocular toxoplasmosis. Limitations include retrospective study.

INTRASTROMAL FLUID DRAINAGE COUPLED WITH DESCEMETOPEXY IN AN UNUSUAL CASE OF RECURRENT CORNEAL HYDROPS IN DOWNS SYNDROME

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Purpose: We present a rare case of a child having recurrence of acute hydrops in Downs syndrome.

Methods: Acute corneal hydrops in keratoconus is caused due to rupture of Descemet's membrane and subsequent influx of aqueous into the corneal stroma. Persistent eye rubbing has been a feature of Downs syndrome and has been implicated in the pathogenesis of keratoconus. In our patient, ASOCT helped us in determining the morphology of the tear, which was in the form of shelve/flap.

Results: Shelf like DM break was an unusual morphologic presentation on ASOCT. The factors which might have led to the recurrence of acute corneal hydrops in this child would be persistent eye rubbing and failure of detection of shelve like DM break on ASOCT in the initially clinical setting due to severe corneal edema.

Conclusions: Preventing eye rubbing in a child with Downs syndrome is a great challenge but prevention of eye rubbing seems to be the only modifiable risk factor in such children. Surgical intervention should be aimed at closing the Descemet's membrane break by Descemetopexy with venting incisions for fluid drainage in severe hydrops. ASOCT features of hydrops gives us an insight into the clinical management of patients with acute corneal hydrops.

COVID-19 VACCINE UPTAKE IN PATIENTS WITH OCULAR SURFACE DISEASES IN INDIA

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Purpose: To describe the COVID-19 vaccine uptake in patients with ocular surface disease at a tertiary eye care center in India.

Methods: This questionnaire based study included patients aged 45 years and above diagnosed with an ocular surface disease related to an auto-immune pathophysiology such as sjogren's syndrome, stevens johnson syndrome (SJS), ocular cicatricial pemphigoid (OCP) and cicatrizing conjunctivitis. A follow up survey was performed 3 months later among the patients who did not receive the vaccine.

Results: Overall, there were 364 respondents who responded to the survey. The majority were female (68.41%) patients. The average age of the patients was 58.5±9.35 years. Half of the patients had a diagnosis of sjogren's syndrome (50.27%) followed by patients with stevens johnson syndrome (21.98%). Just about half of the patients (49.73%) had received the COVID-19 vaccine and the majority received covishield (42.86%) vaccine. The majority of the patients (60.22%) received only a single dose of the vaccine and had cited non availability of the vaccine (49.18%) as a reason for the same. There were 133 respondents who responded to the follow up survey. More than half of the respondents (57.89%) had still not received the vaccine and the majority (73.21%) who received it had taken only a single dose. Lower vaccination uptake rates were seen among patients with OCP (78.57%) and cicatrizing conjunctivitis (60%) at the last follow up.

Conclusions: Half the patients with ocular surface disease related to auto-immune pathophysiology had not received the COVID-19 vaccine. The vaccine shortage was a major reason for the same. Patients should be encouraged to receive the vaccine pro-actively to protect everyone from the future waves of the COVID-19 pandemic.

PHOTODYNAMIC ANTIMICROBIAL THERAPY WITH ROSE BENGAL AS EARLY ADJUVANT THERAPY FOR FUNGAL KERATITIS: CLINICAL PILOT TRIAL WITH INVITRO CORRELATION

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Purpose: To report the treatment outcome with Photodynamic Antimicrobial Therapy (PDAT) with Rose Bengal (RB) as an adjuvant in patients with fungal keratitis with *in vitro* correlation.

Methods: Patients with confirmed fungal keratitis underwent PDAT-RB and were prescribed topical natamycin 5% drops hourly and oral ketoconazole 200 mg twice a day. This was performed by applying rose bengal (0.1%) to the de-epithelialized cornea for 30 minutes, followed by irradiation with a 6 mW/cm^2 custom-made green LED source for 15 minutes (5.4 J/cm^2).

Results: Following informed consent, seven patients (male-5, female-2, mean age 47.7 years) with fungal keratitis were recruited. There were 3 cases each of *Fusarium* and *Aspergillus flavus* and 1 case of *Acremonium* sp. The average vertical and horizontal diameters of the corneal infiltrate were 4.12+/- 0.55 and 3.99+/- 1.19 mm, respectively. The average depth of corneal involvement was 283 +/- 75.27μ as measured by anterior segment OCT. Clinical resolution was achieved in the cases with *Fusarium* keratitis with an average time of 39 days. Three cases of *A. flavus* and a single patient with *Acremonium* keratitis worsened and needed therapeutic keratoplasty (TPK) for resolution. PDAT-RB produced clear inhibition of *Fusarium and Acremonium* sp. with no effect on the growth of *A. flavus.*

Conclusions: While the *in vitro* and *in vivo* results of PDAT-RB matched for *Fusarium species* and *Aspergillus flavus* keratitis being favourable in the former and non-favourable in the latter, these results were discrepant in *Acremonium* sp.

FLICKER INDUCED RETINAL VASODILATION USING HAND-HELD ELECTRORETINOGRAM AND INFRARED IMAGING

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Purpose: To present flicker induced retinal vasodilation (FRV) using a novel method

Methods: Pupils of a healthy subject were dilated using tropicamide. He was made to sit in the laboratory comfortably, and blood pressure was recorded and documented to be stable till 15 minutes. The subject had refrained from exercise, beverage and food for the past 2 hours before imaging was initiated. Baseline retinal fundus images were acquired with infrared imaging. The 2 eyes of the subjects were sequentially exposed to light flickers at 30 Hz using standard protocols for 20 seconds with hand-held ERG device. Immediately following the flicker exposure, an infrared video was recorded for 30 seconds. This cycle was repeated thrice.

Results: As compared to baseline, the retinal vessels were noted to dilate first, followed by rapid constriction over 0-10 seconds. This was phenomenon was documented using still imaging, video and by performing measurements using calipers.

Conclusions: Combination of ERG and fundus imaging is an alternative method for performing the FRV test. Following validation, the test can be employed in basic retinal laboratories for studying the functions of the neurovascular unit in healthy and diseased eyes.

MOLECULAR DETECTION OF *TOXOPLASMA GONDII* IN OCULAR SAMPLES

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Purpose: Ocular toxoplasmosis (OT), caused by *Toxoplasma gondii* (*T. gondii*) through congenital or acquired routes results in necrotizing retinitis with vasculitis and destruction of the retina. PCR based detection is a rapid and reliable tool in early diagnosis of atypical or clinically challenging cases of OT.

Methods: Nested PCR targeting *RE* gene was standardized and applied directly on clinical specimens for the detection of *T.gondii* and compared with the previously standardized nested PCR targeting *B1* gene (data already available)

Results: Among 40 ocular samples, 7 were positive for both *B1* gene & *RE* gene (Retinochoroiditis -3, Toxo retinitis -3, Pan uvetitis -1), 14 were positive for *RE* gene and negative for *B1* gene (Retinochoroiditis -6, Anterior uvetitis -4, one each from Viral retinitis , Toxoplasma retinitis, Intermediate uveitis. The positivity of *RE* gene and *B1* gene were 21 (52.5%) and 7 (17.5%) respectively from suspected OT. From the present study we could able to pick up 21 more positive samples from OT suspected patients.

Conclusions: Our study shows that PCR targeting *RE* gene is more sensitive than *B1* gene in detecting *T. gondii d*irectly from clinical specimens. The higher positivity of *RE* gene could be due to more copies of the repetitive gene (200 to 300 copies) present in *T. gondii* genome than *B1* gene (35 copies). Further, the significance of *RE* gene over *B1* gene would be established by applying the standardized nested PCR technique on more number of clinical specimens to study statistical significance.

ORBITAL MENINGIOMA IN ASIAN INDIANS: A RETROSPECTIVE STUDY OF 56 SPECIMENS

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Purpose: To examine the cohort of intraorbital meningiomas based on the current World Health Organization (WHO) classification and evaluate demographics and histopathology of orbital meningiomas.

Methods: Retrospective study of 56 cases of orbital meningioma

Results: The mean age at presentation was 39 years (median, 39.5 years; range, 3 to 71 years). Three tumors arose in children. Mean duration of clinical symptoms was 20.4 months (median, 8.7 months; range, 0.5 months to 10 years). There were 22 (39%) males and 34 (61%) females. All lesions were unilateral. Most common clinical presentation was proptosis (n=51; 91%). Clinico-radiological diagnoses included sphenoid wing meningioma (n = 17; 30%), optic nerve sheath meningioma (n = 15; 27%), fungal granuloma in (n = 4; 7%), and orbital metastasis (n=3; 5.4%) cases. The most common histopathological subtype was meninigothelial meningioma (n = 44; 79%). Most of the tumors were WHO grade 1 (n = 52; 93%).Four tumors (7%) were atypical meningioma of grade II with nuclear atypia and significant mitotic figures. Psammomatous calcification was noted in 20 (36%) tumors.

Conclusions: Orbital meningioma is a rare tumor of orbit and more commonly arises in middle-aged females. Proptosis is the most common symptom. Histopathologically, meningiothelial meningioma of WHO grade 1 is frequently found in Asian Indians.

CLINICAL AND MICROBIOLOGICAL CHARACTERISTICS OF 12 CASES OF RHINO-ORBITAL MUCORMYCOSIS

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Purpose: To describe the clinical and microbiological features of patients with rhino-orbital mucormycosis(ROM) seen at LV Prasad Eye Institute between May 2021 and July 2021.

Methods: Diagnosed clinically and radiologically, blind or endoscopically collected nasal swabs or orbital mass tissues were obtained from 12 cases with ROM. Smears were stained with 10% potassium hydroxide with 0.1% calcofluor white and examined under fluorescence microscope. The samples were inoculated on blood (5% sheep blood) agar and potato dextrose agar. The former was incubated at 37°C and the latter at 27°C for 1-2 weeks. Mucorales grown were identified morphologically by lactophenol cotton blue mount and tested by microbroth dilution method for antifungal susceptibility against natamycin(N), amphotericin B(AB), caspofungin(C), posaconazole(P), ketoconazole(K) and voriconazole(V). Clinical information was obtained from electronic medical record.

Results: The age of the patients ranged from 27 to 75 years and majority (8/12) were male. Ten patients were diabetic and 9 had recovered from COVID-19. Seven patients had history of hospitalisation during COVID-19 infection and 5 had received steroid. Nine out of 12 fungal isolates were identified as *Rhizopus arrhizus* and one each isolate was *R.azygosporus*, *Mucor species* and *Unidentified mucorale.* All isolates were susceptible to N and AB with MIC less than 8µg/ml. The susceptibility to posaconazole was high with MIC <2µg/ml for 10/12 (83.33%) isolates. MIC of other drugs was variable.

Conclusions : *Rhizopus arrhizus* is predominantly associated with ROM and most isolates are susceptible to amphotericin B, natamycin and posaconazole.

MICROBIOLOGICAL PROFILE OF CANALICULITIS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERNS: A 11-YEAR REVIEW AT A REFERRAL EYE CARE CENTRE

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Purpose: To analyze the microbiological profile and *in vitro* antibiotic susceptibility patterns of bacterial isolates in canaliculitis, an infection of the lacrimal drainage system of the eye.

Methods: The laboratory records of patients presenting with canaliculitis from whom specimens were obtained for microbiological investigations at our tertiary eye care centre in South India from January 2010 to December 2020, were reviewed.

Results: A total of 138 canalicular pus samples were collected from 120 patients and submitted for microbiological studies during the study period. A total of 191 micro-organisms were isolated from 122 culture-positive specimens. The micro-organisms isolated were predominantly aerobic Gram-positive bacteria (83.03%), with Coagulase-negative Staphylococci (31.41%), *Corynebacterium* spp (15.18%), *Staphylococcus aureus* (10.47%) and Viridans *Streptococci* (9.42%) accounting for a majority of the isolates. *Actinomyces spp* (6.28%) was the most common anaerobic bacterium isolated. Our study revealed several bacteria not previously associated with canaliculitis namely *Ottowia* spp*, Elizabethkingia meningoseptica, Aeromonas salmonicida, Capnocytophaga ochracea and Campylobacter gracilis.* Polymicrobial aetiology was observed in 37.7% of culture-positive samples. Analysis of antibiotic susceptibility patterns of the isolates revealed a high proportion of Gram-positive bacteria susceptible to chloramphenicol (92.78%) compared to fluoroquinolones including ciprofloxacin (75.96%), norfloxacin (62.79%) and gatifloxacin (61.66%).

Conclusions: This study represents the largest series of canaliculitis reporting the microbiological profile and antibiotic susceptibilities of the isolated micro-organisms, till date. Gram-positive bacteria accounted for a majority of isolates, predominated by *Staphylococcus* spp. The increasing resistance of Gram-positive bacteria to fluoroquinolones warrants antibiotic treatment in canaliculitis is based on *in vitro* antimicrobial susceptibility patterns.

POLYMERASE CHAIN REACTION ASSAY TO DIFFERENTIATE MUCORALES FROM NON-MUCORALES IN POST COVID-19 RHINO‑ORBITAL FUNGAL INFECTIONS

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Purpose: This study was designed to differentiate aseptate mucorales from other septate fungi with PCR assay for an early diagnosis of suggested of rhino-orbital-cerebral mucormycosis (ROCM) infection.

Methods: A set of six fungal isolates (3 *Rhizopus oryzae*, 1 *Asperigillus flavus, 1 Asperigillus niger*, 1 *Candidida albicans*) were included in the study. Genomic DNA was isolated from all 6 isolates using QIAamp DNA Mini Kits (Qiagen) following the manufacturer's instructions. PCR was done with the genomic DNA to amplify a portion of 28S large subunit of RNA gene. The amplified PCR products were run in agarose gel and subsequently sequenced with Sanger sequencing with forward and reverse primers. The obtained nucleotide sequences from all fungi were matched with homologous sequences in the GenBank using the NCBI BLAST (NCBI) program. The analytical sensitivity and specificity of the primers was analysed by PCR assay.

Results: An intense band of ~340 bp amplicon size was obtained from genomic DNA of *Rhizopus oryzae*. In contrast, relatively smaller ~259bp amplicon size was obtained from genomic DNA of *Asperigillus flavus, Asperigillus niger* and *Candidida albicans* with this PCR assay.

Conclusions: In conclusion, our PCR based assay holds promise as a rapid diagnostic tool in differentiating between mucorales, non-mucorales and *Pythium* in a single PCR reaction. Further, this assay will be able to confirm presence of mucorales and non-mucorales in cases with mixed infection which is difficult in culture owing to suppression of growth of one fungal species by the other.

POROSITY OF OCULAR PROSTHESIS FABRICATED BY THREE DIFFERENT TECHNIQUES OF POLYMERIZATION

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Purpose: To compare the effects of three polymerization heat treatment techniques on the porosity of polymethyl methacrylate (PMMA) used in customised ocular prosthesis (COP).

Methods: A total of 30 COP's were included for evaluation following polymerization. The three different techniques used included, polymerized by water bath (Group A) at 90°C for 35 minutes, microwave (Group B) at 250°C for 15 minutes and pressure cooker (Group C) at 240°C for 20 minutes. The central back surface of the COP was scanned using an optical coherence tomography machine (OCT) for porosities. The image was edited to cancel the noise and ImageJ was used to covert the image to a binary format. The amount of porosity was calculated by using ImageJ software. Data analysis was done with one way ANOVA.

Results: The mean porosity value was 27.25±8.38, 29.13 ±7.63 and 32.38±8.68 (p 0.06) for the water bath, microwave and pressure cooker polymerization techniques. Box plot graph showed that the water bath technique led to less porosity compared to microwave and pressure cooker techniques, however this value did not reach statistical significance. The Q-Q plot graph showed that as the prosthesis thickness increases, the porosity decreases.

Conclusions: The result shows that there is no significant difference in the amount of porosity produced in a custom ocular prosthesis fabricated by using a water bath, a microwave or a pressure cooker for polymerization. Microwave and pressure cooker method of polymerization can use an alternative to the water bath technique which is more expensive.

ESTABLISHING AND CHARACTERIZING MOUSE CORNEAL ALKALI INJURY MODEL FOR PRE-CLINICAL STUDIES

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Purpose: Corneal ulceration is the common cause of vision loss. Corneal ulceration is characterized by: fibrosis, extracellular matrix deposition (ECM), angiogenesis and inflammation. This study highlights the ophthalmological, and histopathological changes throughout the progression of alkali injury induced corneal ulceration in mouse cornea.

Methods: c57bl/6 mice were used for the study. 2 mm wide and 50μm deep scar was made followed by application of 0.75N NaOH and irrigating with normal saline. Animals were imaged using the ophthalmological parameters; i) OCT, ii) Slit lamp and iii) Densitometry over the period of 15 days. At 15th day mice were sacrificed and eyes were enucleated for histology and histopathology.

Results: Clinical parameters were used to evaluate and grade the scars into nebular, macular and leucomatous. Slit lamp revealed re-epithelization of the wound in first 2-3 days followed by incidences of epithelial defects, inflammation, and opacification. Neovascularization was observed after 10th days of alkali burn. OCT: pachymetry and raster scans revealed extensive edema and thickening of the central cornea. Densitometry images revealed stable scar formation. Histology and histopathology supported the ophthalmological evaluation.

Conclusions: These results demonstrate dynamic changes in the process of corneal ulceration post alkali burn. Using the advance clinical techniques of OCT and densitometry we captured the temporal changes during corneal ulceration in mice. Based on our findings we suggest that it is important to closely monitor the ophthalmological parameters while establishing the mice ulceration model for pre-clinical study.

EVALUATION OF SERUM ANGIOTENSIN CONVERTING ENZYME AND LYMPHOPENIA IN PRESUMED SARCOID UVEITIS

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Purpose: To evaluate the diagnostic efficacy of elevated serum angiotensin converting enzyme (sACE) and lymphopenia in presumed sarcoid uveitis.

Methods: A single centre retrospective study was conducted on a cohort of 755 adult patients with uveitis between January 2019 and June 2020. Demographic, clinical and laboratory data were retrieved from our hospital database. Measurements of serum Angiotensin converting enzyme (sACE) and lymphocyte counts were analysed.

Results: The mean (SD) age of the study group was 41 ± 13 years range (18 – 83); 58% patients were males and 42% patients were females. Presumed Sarcoid uveitis was diagnosed in 50 (7%) patients, presumed tubercular uveitis in 222(29.4%), and other group of uveitic entities noted in 483 (64%). The mean age in the presumed sarcoid uveitis group was 42 ± 11 years while in presumed tubercular uveitis was 40 ± 12.9 years. Intermediate and posterior uveitis were the most common anatomical diagnosis in presumed sarcoid uveitis (59% and 20%, respectively) and in presumed tubercular uveitis (46% and 38%, respectively). Elevated sACE was noted in 78% of presumed sarcoid uveitis and 46% in presumed tubercular uveitis. The combination of high serum angiotensin converting enzyme and lymphopenia was noted in 17% in presumed sarcoid uveitis and 9.7% in presumed tubercular uveitis.

Conclusions: Our study indicates that the sACE seems to have better sensitivity in presumed sarcoid and presumed tubercular uveitis group. Elevated sACE was characterise of presumed sarcoid uveitis than presumed tubercular uveitis.
Lymphopenia has not been found to be diagnostic biomarker of presumed sarcoid uveitis.

BACTERIOLOGICAL PROFILE OF ANAEROBIC FLORA ISOLATED FROM CONJUNCTIVA: A ONE YEAR REVIEW AT A REFERRAL EYE CARE CENTER

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Purpose: Anaerobic flora of the ocular surface are not very well-characterized, as they are fastidious, slow growing and strenuous to isolate in culture. The present study aims to investigate the prevalence and abundance of anaerobic bacterial flora in the conjunctiva of patients scheduled to undergo ocular surgery over a one year period.

Methods: The study included 1999 consecutive pre-operative conjunctival swabs of patients visiting a tertiary eye care center in South India from January 2019 to December 2019. The conjunctival swabs were subjected to routine microbiological analysis which included isolation of anaerobic bacteria by conventional culture methods. Cultures for anaerobic bacteria were incubated upto 12 days. If anaerobic growth was observed, the colonies were isolated and identified either by conventional techniques and PCR based DNA sequencing.

Results: Of the 1999 conjunctival swabs, 471 (23.5%) specimens grew obligate anaerobes. *Cutibacterium acnes* was the most prevalent anaerobe, accounting for 95% (452/471) of the anaerobic isolates. The other 5% of the anaerobic isolates included *C. avidum* (n=10), *C. granulosum* (n=5), *C. humerusii* (n=3) and *Leptotrichia trevisanii* (n=1).Semi-quantitative culture revealed that anaerobes were present predominantly in the range of 10-100 CFU/swab (86.4%), with mean culture time-to-positivity of 6.23 days. The overall male: female ratio of the samples in our study was 1.74. Interestingly, males showed a 2.5-fold higher growth of anaerobes compared to females.

Conclusions: This large study adds important information on the composition, diversity and time to culture positivity of anaerobes isolated from the conjunctival sac using culture-dependent methods.

PHENOTYPIC VARIATIONS IN SIBLINGS WITH OCULOCUTANEOUS ALBINISM

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Purpose: To describe the phenotypic variations in ocular features of siblings presenting with oculocutaneous albinism and to compare the similarity and differences in their clinical features.

Methods: Electronic medical records of consecutive siblings diagnosed with albinism that presented from January 2016 to December 2020 were screened to identify affected siblings. 42 sibling pairs were identified and variations in their phenotypic characteristics were studied.

Results: A wide variation in the clinical features amongst the siblings with the same genetic type of albinism was observed. A difference of > 4 snellen lines was observed in n=18 pairs (42.85%). Compound hypermetropic astigmatism was the commonest refractive error. Of the 42 pairs, the refractive status was observed to be dissimilar or different in 34 pairs (80.95%). Although individually strabismus and abnormal head posture was observed in one third to one fourth individual patients, both siblings with a similar strabismus was seen in n=7 (16.67%) and with abnormal head posture was seen in n=5 (13.33%). Nystagmus was the most consistent finding, it was of a similar nature (horizontal jerk or pendular form) in 65% sibling pairs and of a dissimilar nature in 35% pairs.

Conclusions: A wide variation in the phenotypic presentation was observed in siblings with oculocutaneous albinism. While advising families, we thus cannot predict if a younger sibling born with albinism may be similarly affected in terms of severity of the condition and the need for supportive and rehabilitative services.

EFFECT OF STRESS MANAGEMENT IN CENTRAL SEROUS CHORIORETINOPATHY

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Purpose: To determine the effect of stress management in Central Serous Chorioretinopathy (CSCR) patients.

Methods: 30 CSCR patients aged between 25-55 years with active disease were included in this prospective experimental study and their perceived stress score and vision-related quality of life were assessed with Cohen's Perceived Stress Scale and National Eye Institute Visual Functioning Questionnaire 25, pre and post 1-month use of a mobile-based stress management app, Virtual Hope Box. Correlation between the perceived stress score, foveal thickness, subfoveal choroidal thickness, visual acuity, spherical equivalent and visual functioning questionnaire were studied.

Results: There was a significant improvement in the perceived stress level and vision-related quality of life post-1-month usage of mobile-based stress management app. The foveal thickness of the affected eye showed a significant positive correlation with driving and the spherical equivalent of the unaffected eye correlated positively with the peripheral vision. Perceived stress score negatively correlated with mental health and dependency. The subfoveal choroidal thickness of the affected eye showed a significant positive correlation with colour vision. There was no significant correlation between the other parameters tested.

Conclusions: The stress levels of the CSCR patients can be reduced and their vision-related quality of life can be improved through mobile-based stress management apps.

ONE DONOR CORNEA FOR TWO PATIENTS – A NOVEL DMEK MARKING TECHNIQUE THAT ENABLES A DONOR CORNEA TO BE USED FOR BOTH DMEK AND DALK OF SAME SIZE

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Purpose: To overcome the donor corneal shortage during pandemic we tried a novel technique of marking the DMEK graft while preparing it and preserved the rest of the stroma to be used for DALK for another patient.

Methods: The initial DMEK graft preparation is the same as routine up to the slow separation of Descemet's to a three fourth fold. The folded Descemet's with the stromal side exposed is gently dried at one spot and marked with a suitable marker stained with gentian blue pen. Then the Descemet's is unfolded and punched to 8 mm with eventual complete separation manually producing two graft of 8 mm size each, the Descemet's for DMEK and rest of the stroma for DALK.

Results: We used 3 donor cornea for 6 patients who were kept under close follow up for initial 10 days and reviewed after 1 month. One DMEK patient needed single air injection post op, the rest had uneventful recovery. One DALK patient with traumatic central corneal scar had tractional retinal detachment so had no visual benefit but all the others had good recovery.

Conclusions: This simple technique will be useful to corneal surgeons during this pandemic donor shortage.

CLINICAL PROFILE OF PSEUDOEPITHELOMATOUS HYPERPLASIA ASSOCIATED WITH CHRONIC VERNAL KERATOCONJUNCTIVITIS

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Purpose: To describe the clinical profile of pseudoepitheliomatous hyperplasia (PEH) of the ocular surface associated with chronic vernal keratoconjunctivitis (VKC).

Methods: This is a retrospective study including 31 eyes of 27 patients with PEH secondary to VKC presenting to our tertiary eyecare centre from 2016 to 2020.

Results: PEH associated with VKC was more common in males(19/27; 70.4%) compared to females(8/27; 29.6%). Mean age at diagnosis of PEH was 25.7+/- 13.79 years. Median duration of ocular allergy was 3(IQR: 1.5-5) years. The lesion was unilateral in 24 (85.1%) and bilateral in four(14.8%) patients. Nodular lesion over the ocular surface was the most common presenting complain (19/31, 70.4%). All lesions were perilimbal, and the median basal diameter of the lesion was 5 (IQR: 4.5-6) mm. Feeder vessel was noted in 70.9% (22/31) of the eyes and pigmentation over the lesion in 80.6% (25/31). Anterior segment optical coherence tomography (AS-OCT) was documented in 28 eyes and features included irregular hyperreflective epithelium noted in 75%(21/28), epithelial dipping in 46.4%(13/28), and subepithelial hyperreflective lesion with posterior shadowing in 32.1%(9/28) of the cases. Histopathology confirmed the diagnosis of PEH in all cases. Following anti-allergic therapy, reduction in size of the lesion was noted in 84% (26/31) and only symptomatic relief was noted in 16% (5/31) eyes.

Conclusions: Ocular surface PEH is a manifestation of chronic VKC that is a close mimicker of OSSN. Detailed history-taking, ocular examination and AS-OCT characteristics can help in establishing diagnosis and avoid interventions like excision biopsies in classic cases.

RETINITIS PIGMENTOSA IN LAURENCE-MOON-BARDET-BIEDL SYNDROME IN INDIA: ELECTRONIC MEDICAL RECORDS DRIVEN BIG DATA ANALYTICS

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Purpose: To describe the clinical presentation and demographic distribution of retinitis pigmentosa (RP) in Laurence-Moon-Bardet-Biedl (LMBB) syndrome patients.

Methods: This is a Cross-sectional observational hospital-based study. 244 patients with RP in LMBB syndrome presenting to our hospital network between March 2012 and October 2020 were included. Electronic medical record database was used for data retrieval.

Results: There were 244 patients in total, with hospital-based prevalence rate of 0.010% or 1,000/100,000 population. The mean and median age of patients was 15.22 ± 7.56 and 14 (IQR: 10-18.5) years respectively with majority being in the age group of 11-20 years (133/244 patients; 54.50%). Males were more commonly affected 164 patients (67.21%) and majority (182 patients; 74.59%) were students. All 244 patients (100%) complained of defective central vision at presentation. More than one-fourth of the patients had severe visual impairment to blindness at presentation. Prominent retinal feature at presentation was diffuse or widespread retinal pigment epithelial degeneration in all patients.

Conclusion: Patients with RP in LMBB syndrome present mainly in first to second decade of life with severe visual acuity impairment to blindness early in life. It is important to rule out LMBB syndrome in early onset RP with central visual acuity impairment. On the other hand, all patients diagnosed or suspected with LMBB syndrome systemic features at physician clinic should also be referred for ophthalmic evaluation, low vision assessment and rehabilitation.

LONG TERM OUTCOMES OF TRANSCUTANEOUS NON-IMAGE GUIDED BLEOMYCIN SCLEROTHERAPY IN CONJUNCTIVAL LYMPHATIC MALFORMATIONS: A PROTOCOL-BASED MANAGEMENT IN 16 EYES

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Purpose: To study the efficacy and the long-term outcomes of treating conjunctival lymphatic malformations with bleomycin sclerotherapy.

Methods: A retrospective interventional study of 16 eyes treated between December 2014 and December 2019. Regression of the lesion was assessed as less than 25% regression in size (poor), 25-50% regression (fair), 50-75 % regression (good), more than 75% regression (excellent) and complete regression of the lesion.

Results: The mean age at treatment was 18 ± 13.09 (15 years, 3 to 59 years). Isolated conjunctival presentation was seen in 4, palpebral-conjunctival presentation in 2 and orbital-palpebral-conjunctival presentation in 10 eyes with a mean of 3.32 ± 5.29 clock hours (4, 2-9 clock hours) of conjunctival involvement. The average cumulative units of bleomycin injected per patient were 3 ± 1.5IU (3, 1-6IU). The average number of treatment sessions required were 1.6 ± 0.7 (median 2, range 1–3). The average does of bleomycin injected per session was 1.8 ± 0.3IU (medial 2 IU, range 1-2 IU) Excellent response to bleomycin sclerotherapy was seen in 11 (69%) cases. Significant residual lesion and recurrence was noted in one case each. Adverse reactions noted were moderate grade of inflammation in 5 (31%) and limited extraocular motility in 2 eyes (13%). There was a sustained tumour regression over a mean follow-up duration of 13.25 ± 11.36 months (11, 2-38 months).

Conclusions: Excellent response with minimal residual lesion can be obtained in 86% of conjunctival VLMs with bleomycin sclerotherapy. When recurrent, repeat bleomycin sclerotherapy offers excellent outcomes.

EARLY AGEING CHANGES AMONG THE HIV POSITIVE INDIVIDUALS

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Purpose: HIV-infected individuals have an increased risk of age-related morbidity despite antiretroviral treatment (ART)**.** HIV infection favours a chronic low state of inflammation and the impact of this in HIV positive individuals eyee have not been explored well. The purpose of this study was to elucidate which structure of the eye has the ageingchanges at the earliest in the HIV positive subjects.

Methods: We have investigated in a cohort of HIV positive (Group A and B) and HIV negative patients (Group C) presenting with age related maculopathy, functional ocular parameters. The HIV positive individuals were further divided in to two groups based on the age all the subjects underwent detailed ophthalmic evaluation.

Results: 22 eye of eleven subjects with HIV positive (7 in Group A and 4 in group B) and 14 eyes of 7 subjects from HIV negative individuals were included in the study. The ocular parameters were comparable between HIV positive and negative individuals. The central corneal thickness, Corneal endothelial cell density, Lens densitometry, Central macular thickness, retinal volume, vessel and perfusion density, Central Choroidal thickness were increased in group A and B compared to group C. Hexagonality of the endothelial cell were decreased in group A and B compared to group C. Ganglion cell layer thickness decreased in group A and B compared to group C

Conclusion: An early ageing change happens for HIV positive individuals in many structures of the eye.

EFFECTS OF NEEDLE ASSISTED RADIOFREQUENCY ABLATION ON HUMAN EYELASH: A HISTOPATHOLOGICAL AND MORPHOMETRIC STUDY

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Purpose: To analyze the histopathological changes in human eyelash following electroepilation using radiofrequency (RF) cautery.

Methods: RF current was applied to the eyelash root in human eyelids (from exenteration or eyelid shortening procedures) at a pre-determined depth of 3mm excision a-priori, and processed for routine histology. Studied parameters were the extent of necrosis, involvement of the eyelash bulb, and type of bulb damage (partial or whole necrosis). Also, seven patients of chronic cicatrizing conjunctivitis with trichiasis (n=4) and distichiasis (n=3) underwent electroepilation using the above RF protocol.

Results: Twelve eyelid specimens of seven patients (mean age 40 years; 5 upper eyelids, 7 lower eyelids) were evaluated histologically. Majority of specimens (92%) showed coagulative necrosis in the eyelash bulb, follicle, orbicularis oculi, adjacent nerves, and blood vessels. The mean depth of necrosis was 2.12 ± 0.63 mm. Of 12, eleven eyelash bulbs showed necrotic changes with only partial bulb involvement in 55% of eyelids. The horizontal extent of coagulative necrosis was not uniform across the lash track, and the majority showed a wider area of damage in the lower segment. Of 7, all patients with trichiasis (4/4) showed no recurrence of lash misdirection whereas 66% of distichiasis patients (2/3) showed recurrent lash growth with one sitting of electroepilation applied at 3mm.

Conclusions: Electroepilation guided by RF current produces variable necrotic changes in the eyelash root, leaving portions of intact bulbs in half of the eyelids. When RF is applied at 3mm depth, distichiatic eyelashes often show recurrence.

COMPARATIVE EVALUATION OF REFRACTIVE OUTCOMES BETWEEN SILICONE OIL FILLED EYES AND EYES AFTER SILICONE OIL REMOVAL IN PATIENTS UNDERGOING CATARACT SURGERY IN A TERTIARY EYE CARE CENTRE.

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Purpose: To compare the axial length (AL) and refractive outcomes in silicone oil filled eyes and eyes after removal of silicone oil in patients undergoing cataract surgery and comparing the results postoperatively.

Methods: The study recruited a total of 223 eyes that had undergone vitreoretinal procedure for retinal detachment from April 2018 to November 2020. We divided them into two groups based on the mode of surgery. 186 (83.4%) eyes in Group 1 underwent cataract surgery along with silicone oil removal in the same sitting and 37 eyes (16.59%) in group 2 underwent cataract surgery after silicone oil removal depending on the grade of cataractous changes.

Results: Mean axial length and IOL power were 24.11mm ($\pm$ 2.29) and 18.97D ($\pm$ 4.3) in Group 1 and 23.69mm ($\pm$ 2.54) and 19.51D ($\pm$ 4.57) in Group 2 respectively. 92 eyes from Group 1 and 15 eyes Group 2 developed visual acuity better than 0.3 logMar post-operatively. Although P values of axial length and spherical equivalent did not show any noticeable change in the operated eyes among the groups, yet visual acuity among the groups were statistically significant (P=0.0001). Moreover, mean axial length of operated and non-operated eyes in Group 1 was also statistically significant (P=0.01).

Conclusions: Postoperative refractive outcome needs a closed observation and better technique like swept source optical coherence tomography and low-viscosity silicone oil as axial length can be measured appropriately in silicone oil filled eyes.

CLINICAL FEATURES AND SURVIVAL OUTCOMES OF OCULAR ADNEXAL LYMPHOMA IN SOUTH INDIAN COHORT

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Purpose: Ocular Adnexal Lymphoma (OAL) is the most common neoplasm of the orbit. There is no clear correlation of clinical features with survival outcome in South Indian OAL patients. This study is focused to assess clinicopathological features and their effect on treatment outcomes and survival patterns.

Methods: The clinical and pathological details of 112 OAL patients were collected for 8 years (2012-19). Survival rates were analysed to predict the risk factors and correlate them with poor prognosis.

Results: The median age of presentation was 57 years (Range: 32-81 years). All of them were Non-Hodgkin's lymphoma with 98% B-cell origin and only 2% with T cell origin. Complete details about the treatment outcome and survival data were available only in 79 patients. The median progression free survival was 24 months. Complete remission was observed in 53% and disease recurrence in 20% at the last follow-up. Disease dissemination was observed in 23% of patients with a median overall survival of 56 months. The overall and disease specific survival for 3 and 5-year was 49% and 32% respectively. Disease specific survival was found to be better in patients under 55 years of age (p= 0.0329) among the OAL patients.

Conclusions: Prognosis of OAL was influenced largely by age and possibly by disease relapse, dissemination and secondary lymphoma. Histologically, anaplasia and diffuse large cells were commonly seen in patients with poor survival. A long-term follow- up is warranted for understanding the importance of these risk factors in survival outcome of OAL patients.

RETROSPECTIVE STUDY OF DEMOGRAPHIC PROFILE, CLINICAL CHARACTERISTICS AND OUTCOMES OF DSAEK GRAFT REJECTION

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Purpose: To study the demographic profile and clinical characteristics of DSAEK graft rejection at a tertiary eye care facility.

Methods: The data of all patients with DSAEK graft rejection from May 2012 to February 2019 was retrieved from the medical record database and only those patients with minimum of 1 year follow up were included in the study. Data on demographic details, indication for DSAEK, associated ocular co-morbidities, clinical presentation, and the graft recovery was analysed.

Results: 61 patients (61 eyes) with DSAEK graft rejection were included in this study. Mean value of age(years) of study subjects was 52.44 ± 19.8 with median(25th-75th percentile) of 58(37.5-68). 68.52% of patients were males and 31.48% of patients were females. 24.59% were treated for bullous keratopathy and 42.62% were treated for failed grafts.64.81% were associated with ocular co-morbidities with secondary glaucoma being the most common among them (22.59%) Signs of graft rejection at the initial visit were edema (57.41%),KP(40.74%) and endothelial rejection line (3.7%). In present study, in 33.33% of patients, time point of 1st Rejection was 0-<6 months followed by >2 years (25.93%) and >1 year to 2 years (22.22%).55(74.55%) grafts recovered completely among which 11 required systemic steroids.

Conclusions: Immunological graft rejection is an important postoperative complication after DSEK. Pre-existing glaucoma was most common ocular morbidity among patient presenting with graft rejection. Graft rejection in EK being less severe than PK and most of them recovered with topical steroids.

CLINICAL PROFILE OF PATIENTS WITH UVEITIC GLAUCOMA

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Purpose: To assess the clinical profile and determinants of progression in patients of uveitic glaucoma

Methods: A retrospective, observational case series was done at a tertiary eye care center in north India, where the EMR (Electronic medical record) data of all patients diagnosed with uveitic glaucoma and had any visit between Jan 2019 – June 2021 were included. Only those patients who had a comprehensive glaucoma evaluation at all visits were included in the study. Patients with prior diagnosis of primary glaucoma or other coexisting cause of secondary glaucoma were excluded from the study. The clinical profile included visual status, intraocular pressure (IOP), optic nerve head assessment, sequential anterior chamber angle changes, frequency of inflammatory episodes and eventual need of surgical intervention. The data was analyzed using SPSS 21 software with a significant p value of <0.05

Results: A record of 96 patients of uveitic glaucoma was analyzed. On gonioscopy both open angle and closed angle variants were observed, but IOP control was better in patients with open angles at presentation. Low vision in the patients was attributed to both uncontrolled intraocular inflammation and glaucomatous progression. Patients uncontrolled on medical management eventually required surgical intervention (Trabeculectomy/ tube implant) for IOP control

Conclusions: Glaucoma is a common and potentially blinding complication of uveitis. There is vicious cycle of intraocular inflammation and IOP rise. An adequate management of both uveitis and glaucoma is required. IOP control is better achieved with quelling of intraocular inflammation

PRESENTATION, ETIOLOGY AND OUTCOMES OF CORNEAL ULCERATION IN SJOGREN'S SYNDROME

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Purpose: To report the clinical course of corneal ulceration/perforation in patients with Sjogren's syndrome.

Methods: A retrospective descriptive study of patients diagnosed with Sjogren's syndrome (primary and secondary) and corneal ulceration over the past 8 years at a tertiary eye care network. Assessed parameters were demographics, clinical details, microbiological profile, types of intervention, and their outcomes.

Results: Forty-six eyes of 44 patients (11 males; mean age,50 years) had corneal ulceration (28 eyes) or perforation (18 eyes) at the time of presentation. The mean Schirmer I values were 3.7 ± 3.5 mm. Of 46 eyes, 38 had sterile ulceration/perforation, and the rest 8 showed microorganisms on microscopy or culture. The location was peripheral in 63% of eyes, and it was the first presenting sign of Sjogren's syndrome in 26% of patients. Twenty-eight eyes with ulceration required medical management alone in 15 eyes, soft contact lens and isobutyl cyanoacrylate in 12 eyes, and amniotic membrane grafting in one eye. Four eyes with ulceration worsened and required penetrating keratoplasty (n=2) and amniotic membrane grafting (n=2). Corneal perforations were successfully managed with isobutyl cyanoacrylate patch and bandage contact lens (BCL; n=15), corneal patch graft (n=2), and multi-layered amniotic membrane grafting (n=1). The average time taken for ulcers to heal was 49 days over a mean follow-up duration of 10 months.

Conclusions: Corneal ulceration or perforation in Sjogren's syndrome is often sterile and can be a presenting sign of undiagnosed SS. These patients usually respond to intensive medical therapy and BCL and isobutyl cyanoacrylate patch application.

CLINICAL PRESENTATIONS, MICROBIOLOGY, TREATMENT AND FACTORS AFFECTING OUTCOMES IN LENS ABSCESS WITH CONCURRENT ENDOPHTHALMITIS

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Purpose: To report the clinical presentations, microbiology, treatment and factors affecting outcomes in lens abscess with concurrent endophthalmitis

Methods: Retrospective, consecutive, non-comparative case series. All cases presenting to our center with lens abscess with concurrent endophthalmitis from 2016 to 2021 were included. The data analysis included patient demography, clinical presentations, interventions, and final treatment outcomes. The main outcome measures were the final visual acuity and the globe salvation. The factors determining better visual and/or anatomical outcomes were analyzed.

Results: One hundred and two eyes of 102 patients were included. Males constituted 71.6% of recruits. All cases were following open globe injury. Age at presentation was 30.47±19.51 (median 31). Duration of symptoms at presentation was 5.59±14.36 days (median 0.5). Presenting vision in logMAR was noted to be 3.02±0.74 (median 3.5). Corneal tear or an infiltrate was noted in 59.8% of cases and 61.8% cases were culture positive. Culture revealed 33 different organisms with *Staphyococcus epidermidis* being commonest (11.11%). Final vision in logMAR was 2.1±1.32 (median 2.8) and the improvement was significant (p<0.0001). In a multivariate logistic regression analysis, female gender (OR 7.91, p=0.007), initial vitrectomy instead of biopsy (OR 11.72, p=0.0009), negative culture (OR 14.28, p=0.0004) were factors determining favourable anatomic outcome. Cornea having absence of infiltrate on presentation (OR 25, p=0.004) and initial vitrectomy instead of biopsy (OR 21.96, p<0.0001) were factors determining favourable functional outcome.

Conclusions: Lens abscess occurs in the setting of open globe injury. Prompt vitrectomy leads to a significant globe salvage and better functional outcomes.

OUTCOMES OF NOVEL APPROACH OF COMBINED SCLERAL IMBRICATION AND ENCIRCLAGE/BUCKLE FOR COMPLEX CHILDHOOD ONSET RETINAL DETACHMENTS

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Purpose: To report outcomes of a novel technique of combined buckle with modified scleral imbrication in complex childhood onset retinal detachments. There was varying proportion of rhegmatogenous, exudative and tractional components.

Methods: Retrospective analysis of 24 operated eyes is reported. The surgical technique included modified scleral imbrication and encircling band with or without additional buckle, cryopexy, subretinal fluid drainage, laser or paracentesis.

Results: Age at surgery ranged from 3 months to 14 years (mean 6 years). Diagnosis included ROP (17 eyes); FEVR (4 eyes) and miscellaneous (3 eyes). Indications included rhegmatogenous with tractional and/or exudative component (13 eyes); progressive tractional detachment (7 eyes); tractional with exudative detachment (two eyes) and rhegmatogenous with abnormal vitreous adhesions (2 eyes). Retina showed complete attachment (with or without dry folds) and no progression of disease in 19 eyes (79%). Of five eyes with incomplete resolution, one had successful vitrectomy surgery with oil, two eyes were deemed to be inoperable as disease was already too advanced at initial presentation and two eyes had only partial reattachment. Postoperative visual acuity was stable or improved in six older children where it could be recorded. No postoperative complication was noted at mean follow-up of 5 months.

Conclusions: Every eye operated by this technique was extremely challenging. The novel technique provided a new way of managing these complex childhood onset retinal detachments successfully with a single surgery, that otherwise would have required multiple vitreoretinal procedures using silicone oil, often with variable and unpredictable outcomes. Long-term outcomes are awaited.

SEVERE CICATRICIAL ENTROPION REPAIR USING MUCOUS MEMBRANE GRAFT: OUTCOMES IN CICATRIZING CONJUNCTIVITIS AND STUDY OF HISTOPATHOLOGICAL CHANGES

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Purpose: To report a novel technique for addressing primary and recurrent cicatricial entropion using mucous membrane graft.

Methods: Twenty-five eyelids of twenty-four patients (50±21 years; 16 females) with severe cicatricial entropion (18 upper and seven lower eyelids) had surgical correction using ALR and labial mucosal grafting, spacing the ciliary margin away from the lid margin and reconstruction of the lid margin and posterior lamella. Histopathology of excised lid margins (n=6) was evaluated for degree of inflammation and fibrosis using immunohistochemistry.

Results: The indications for surgery included Stevens-Johnson syndrome (17), chemical injury (5), ocular cicatricial pemphigoid (2), and post-surgical scarring in congenital distichiasis with lymphedema (1). Thirteen cases were of recurrent entropion, and the rest were primary cicatricial entropion. Entropion resolved in all but four eyelids (84% success rate) with residual focal trichiatic lashes, which were successfully managed with radiofrequency ablation in one patient and lash everting suture in another patient. All patients reported a reduction in ocular discomfort and ocular staining with one-line improvement in visual acuity in 57% of patients at a median follow-up duration of 11 months (range, 3–36 months). Histopathology of excised scarred lid margins revealed diffuse subepithelial fibrosis with conjunctival squamous metaplasia, orbicularis atrophy, and presence of CD20 & few CD3 positive lymphocytes.

Conclusions: The use of labial mucosa and anterior lamellar recession achieves a reasonable success rate in primary and recurrent cicatricial entropion. There is ongoing chronic inflammation in the lid margins of cicatrizing conjunctivitis patients with entropion.

DEMOGRAPHIC PROFILE AND CLINICAL CHARACTERISTICS OF PATIENTS PRESENTING WITH ACUTE OCULAR CHEMICAL BURN

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Purpose: To study the demographic profile and clinical characteristics of patients with acute ocular chemical burn (OCB) presenting to a tertiary eye care facility.

Methods: The data of all patients with OCB presenting between January 2016 to May 2020 was retrieved from the medical record database and only those patients who presented within one month of chemical burns were included in this study. Data on demographic details, clinical presentation, grading of chemical injury as per Roper Hall and Dua's classification and management advised was analysed.

Results: 944 patients (1342 eyes) with acute chemical burns were included in this study. Median age of patients presenting with acute OCB was 28.5 $\pm$ 14.36 years (Range: 7months-87 years) with male: female ratio of 5:1. Workplace injuries constituted 61.5% (n=815) while domestic accidents at home constituted 34% (n=450) of the total cases. Only 147 patients (15.5%) patients presented within 24 hours of injury. Nearly half the eyes (40.4%, n=548) had alkali injury, with lime being the most common causative agent in adults and children. Most ocular injuries were low grade chemical injuries (Grade 1,2,3 Roper Hall and Dua's classification) involving 90.28% eyes (n=976). Majority eyes required only medical management (85%; n=1143 eyes) while amniotic membrane transplantation was required for 12.6% eyes (n=170).

Conclusions: Acute ocular chemical burns are more common in male patients in the younger age group. Most ocular chemical injuries are accidental. The importance of personal protective equipment at the workplace should be emphasised.

EFFICACY OF COLISTIN AND AMPHOTERICIN B ADDITION IN CORNEAL STORAGE SOLUTION

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Purpose: To assess the efficacy of addition of Polymyxin E (Colistin) and Amphotericin B in corneal storage solution (MK medium).

Methods: A standard broth microdilution test and a checkerboard assay were performed for 5 multi-drug resistant (MDR) clinical strains of *P. aeruginosa* and 5 clinical strains of Methicillin-Resistant *S. aureus* (MRSA) against Colistin and Gentamycin. Similar tests were performed to test the efficacy of Amphotericin B along with Gentamycin against 5 clinical strains of *C. albicans* and the Minimum Inhibitory Concentration (MIC) and the Fractional Inhibitory Concentration Index (FICI) were calculated.

Results: The mean FICI for a combination of Colistin and Gentamycin is $\sum$FICI = 3.90 and $\sum$FICI = 2.17 for *P. aeruginosa* and *S. aureus* respectively and the mean FICI for a combination of Amphotericin B and Gentamycin is $\sum$FICI = 0.93 for *C. albicans* suggesting that Colistin - Gentamycin combination has an additive effect (p=0.00001) on *P. aeruginosa* and *S. aureus* and Amphotericin B - Gentamycin combination has an additive effect (p=1) on *C. albicans*.

Conclusions: The addition of Colistin and Amphotericin B may have an inhibitory effect on Bacterial and Fungal contamination respectively and could potentially be used as an additive in corneal storage media.

SURGICAL MANAGEMENT OF UNILATERAL PARTIAL LIMBAL STEM CELL DEFICIENCY: CONJUNCTIVAL AUTOGRAFTS VERSUS SIMPLE LIMBAL EPITHELIAL TRANSPLANTATION

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Purpose: To evaluate the clinical outcomes of conjunctival autograft (CAG) versus simple limbal epithelial transplant (SLET) for management of unilateral partial limbal stem cell deficiency (LSCD)

Methods: This retrospective, comparative, interventional case series evaluated 30 eyes of 30 patients with unilateral partial LSCD. After corneal pannus dissection, 17 patients underwent CAG where graft was harvested from ipsilateral or contralateral eye while 13 patients underwent SLET where limbal biopsy was harvested from contralateral eye. The primary outcome measure was anatomical success in the form of restoration of a completely epithelised, stable, and avascular corneal surface at last follow-up.

Results: Both groups were comparable in terms of age at time of surgery, pre-operative best-corrected visual acuity, median duration since injury, number of clock hours of limbus involved, and number of previous surgeries performed. The most common etiology for LSCD was chemical burns in both groups. The median duration of post-operative follow-up was 5.6 months [interquartile range [(IQR): 3.6-15.1] in the CAG group versus 6.2 months (IQR: 4.5-12.2) in the SLET group (p=0.75). The anatomical success rates were 86.5 ± 8.9 % in the CAG group and 28.3 ± 13.7 % in the SLET group at final follow-up visit (p = 0.025). Most failures in both groups occurred within the first eight months after surgery.

Conclusions: For eyes with partial LSCD secondary to chemical burns, CAG is a safe and effective method for restoring the corneal epithelium. Limbal transplantation may not be necessary for the treatment of partial LSCD.

PHENOTYPES AND TREATMENT OUTCOMES OF A NOVEL PROTOCOL OF EARLY DELIVERY AND RETINAL SCREENING SOON AFTER BIRTH, IN SIBLINGS OF FAMILIAL EXUDATIVE VITREORETINOPATHY (FEVR) PROBANDS

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Purpose: To report phenotypes and outcomes of a novel protocol of early delivery and Retinal screening, in siblings of FEVR probands.

Methods: 12 families of FEVR were informed the risk of disease in next offspring. A clinical protocol to detect FEVR at the earliest in these newborns included ocular fetal imaging, elective delivery around 37 weeks of gestation and retinal examination soon after birth. Eight of the 12 babies followed the protocol. Neonatal FEVR was managed primarily using laser and additional surgery if needed.

Results: All 12 Probands were legally blind/ had significant low vision. Eight compliant newborns were screened between day one and day 20 of birth. Three had normal examination and five had bilateral FEVR warranting immediate treatment. Eight eyes with retinal new vessels had primary laser but all needed vitrectomy for progressive retinal traction within next 4 weeks. Anatomical and visual outcomes were good in all these eyes. Two eyes of one baby with congenital vitreous haemorrhage and tractional detachment needed primary surgery of which one eye progressed to phthisis. The other eye needed surgery for secondary glaucoma and resulted in low vision status. Three of four babies who came beyond 2 months of birth presented with irreversible bilateral total retinal detachment and permanent blindness. One had normal fundus

Conclusions: Delivery earlier by 3-4 weeks of the expected date and immediate retinal management provided a window of opportunity to treat a rapidly progressive blinding disease of newborns. Our protocol will need validation with larger cohorts.

BENT HAPTIC TECHNIQUE FOR SCLERAL FIXATION OF INTRAOCULAR LENS IN APHAKIC EYES WITHOUT CAPSULAR SUPPORT: DESCRIPTION OF A NEW TECHNIQUE

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Purpose: To study the efficacy and safety of a modified trans-scleral intraocular lens (IOL) fixation technique in aphakic eyes.

Methods: The lens haptic was bent and tucked in a scleral pocket 1.5 mm from limbus to improve the lens stability. This prospective study included 43 surgeries performed by a single surgeon. The data were analysed for stability and position of IOL, refractive changes, best-corrected vision and associated complications.

Results: Mean age of the subjects was 53.8 ± 18.5yrs (range 6-81yrs). Surgical aphakia (58.14%) was the most common cause. The corrected distance visual acuity improved significantly at 6 weeks (p=0.0003). The mean residual spectacle correction was $+0.74 \pm 1.2$D spherical equivalent (cylinder -1.6 ± 1.5D at 84 ± 50^0) at the last follow up (24.35 ± 6.71wks). Lens tilt on ultrasound biomicroscopy (kappa 0.762; p<0.001) and the IOL centration (kappa 0.411; p=0.001), assessed by two independent masked observers were satisfactory. Transient post-operative vitreous hemorrhage was the most common complication (46.5%). Cellular deposits on IOL surface (18.6%), cystoid macular edema (11.6%), subconjunctival haptic exposure (4.66%) and haptic slippage (2.33%) were the other complications.

Conclusion: This method of trans-scleral IOL fixation is an effective rescue procedure for eyes with deficient capsular support.

COMPARISON OF TOPICAL NEPAFENAC 0.3% MONOTHERAPY VS A COMBINATION OF LOTEPREDNOL AND NEPFENAC 0.1% IN CONTROLLING POST-OPERATIVE INFLAMMATION AFTER PHACOEMULSIFICATION.

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Purpose: To compare the post-operative efficacy of nepafenac 0.3% alone vs a combination of loteprednol and nepafenac 0.1 % in controlling post-operative inflammation after uneventful phacoemulsification.

Methods: This prospective study included 500 patients with senile cataract who underwent uncomplicated phacoemulsification with foldable intraocular lens implantation by a single surgeon. Pre-operative evaluation included visual acuity assessment, non-contact tonometry, slit lamp evaluation and indirect ophthalmoscopy. Patients in the Nsaids monotherapy group received Nepafenac 0.3% in tapering doses and patients in the topical steroids+Nsaids group received Loteprednol in tapering doses along with Nepafenac 0.1% eye drops. Post operatively patients were reviewed on day 10 and day 45 when visual acuity, conjunctival congestion, cornea and anterior chamber reaction were assessed by two independent observers. Optical coherence tomography of the macula was done on 45th post-operative day to assess macular volume and central macular thickness.

Results: Each group included 250 patients. There was no statistical significance observed in visual acuity, conjunctival congestion or anterior chamber reaction at day 10 and day 45 between the two groups. Analysis of OCT macular volume and cystoid macular edema also did not show any statistical significance between the two groups. Compliance was better in the topical Nsaids monotherapy group compared to the topical steroids + Nsaids group.

Conclusions: No differences were found between Nepafenac 0.3 % monotherapy and combination of nepafenac 0.1% and steroid group in controlling post-operative inflammation after cataract surgery.

CORRELATION OF THE SURFACE AND DEPTH IMAGING METRICS OF CORNEAL SCARS WITH THE FINAL VISUAL OUTCOME

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Purpose: To test the correlation of surface parameters vs depth & density of corneal stromal scars in affecting visual outcome.

Methods: This was a prospective study including 19 patients with healed unilateral ulcerative keratitis. Uncorrected visual acuity(UCVA), high resolution anterior segment optical coherence tomography, Scheimpflug imaging was documented in both eyes and contact lens corrected visual acuity (CLVA) in eyes with corneal scarring. 2 arms of vision independent in-vivo imaging metrics: 1) Scar Surface parameters - Higher order aberrations (HOA) (aberration coefficient, Zernickes polynomials Z0-Z4values) and changes in corneal topography 2) Depth and Density parameters- depth(microns), percent change in corneal thickness, corneal densitometry and epithelial : stromal reflectivity ratio were investigated. Spearmans coefficient and multivariate regression analysis models were used to find correlation between these factors and final UCVA,CLVA.

Results: Mean logMAR UCVA ,CLVA and depth of corneal scar in eyes with corneal scarring was 0.76,0.28 and 158 microns. Surface parameters like Aberration coefficient, Z4 quadrafoil, Z4 secondary astigmatism and depth parameter of percent change in corneal thickness had correlation with UCVA (0.75,-0.58 ,-0.52 0.49 Spearmans coefficient), while only the Aberration coefficient showed correlation to the final CLVA (0.78). Other depth parameters like densitometry, epithelial stromal reflectivity, depth of corneal scar did not correlate with vision. Multivariate regression analysis showed only Aberration coefficient to be associated significantly to UCVA (p=0.004 Coefficient +/- SE 0.19+/-0.05).

Conclusions: In corneal stromal scarring, deranged surface parameters like higher order aberrations affect the final visual outcome more than depth, density, keratometry and reflectivity of the scar.

REPEATABILITY AND RELIABILITY OF DIFFERENT DRY EYE DISEASE DIAGNOSTIC PLATFORMS IN TEAR FILM EVALUATION

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Purpose: To assess the repeatability and reliability of different commercially available diagnostic platforms in the objective assessment of tear film parameters in healthy individuals without any dry eye disease.

Methods: Thirty healthy individuals (N=60 eyes) had their tear film parameters (Lipid layer thickness (LLT), Tear meniscus height (TMH), Non-invasive tear break up time (NIBUT)) assessed by two observers on two different occasions. The three instruments that were used are- LipiView® II, IDRA ocular surface analyzer and Oculus keratograph 5M. Bland-Altman analysis and mixed effects model with maximum likelihood estimation were used to calculate intra- and interobserver variability between the instruments.

Results: There were no significant intraobserver differences noted in LLT values measured with Lipiview, NIBUT using Oculus & IDRA, and Oculus TMH values. Between two observers, there were significant differences in LLT measurements (mean difference of 6.60; p = 0.002) and TMH measurements (mean difference of 0.03; p = 0.0001), obtained using IDRA but not for Oculus or Lipiview. Between instruments, all the measurements (LLT, NIBUT and TMH) were significantly different (p<0.0001 for LLT; p= 0.002 for TMH; p<0.0001 for NIBUT).

Conclusions: No two dry eye diagnostic platforms can be used interchangeably for the evaluation of tear film. The NIBUT and LLT measurements using Oculus and Lipiview are more reliable than IDRA, and Oculus is more reliable than IDRA for TMH assessment.

FACTOR IN A RISK MODEL FOR PSEUDOEXFOLIATION SYNDROME - A HOSPITAL BASED STUDY

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Purpose: To assess the impact of ocular UV exposure as a major factor as risk for pseudoexfoliation syndrome.

Methods: Subjects above 18 years with and without pseudoexfoliation syndrome were included in study. A standardized questionnaire was administered to assess the lifetime UV exposure. Conjunctival ultraviolet autofluorescence (CUVAF) photography was used to detect the cellular changes with exposure to UV. Ascorbic acid concentration was measured by BCA technique and comet assay was done to detect the DNA damage. SNP analysis was done for known genetic loci by PCR method. Predictive risk analysis was performed to estimate the potential risk factors.

Results: There were 274 controls and 130 cases with PXF. Regression showed lifetime UV exposure (OR:1.12, 95% CI: 1.00 - 1.25, p-value 0.05), CUVAF damage (OR: 1.03, 95% CI: 1.01 - 1.06, p-value 0.01), outdoor work (OR:1.84, 95% CI: 1.11 - 3.03, p-value: 0.017) and no usage of spectacles (OR: 1.62, 95% CI: 1.00 - 2.63, p-value 0.05) to be significantly associated with the development of XFS. Low ascorbic acid concentration was noted in cases with XFS. Genetic predisposition showed, SNPs rs3737528, rs41435250 and rs8818 with OR >1.0 suggesting a high risk for XFS development. Predictive risk analysis showed age in years, CUVAF damage and the place of residence (Rural) to be associated with XFS.

Conclusion: There is a known genetic association for XFS pathogenesis, this study identified the environmental factors like the lifetime ocular UV exposure due to outdoor work to be significantly associated with risk of XFS pathogenesis.

OPTOMETRY
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PUBLIC HEALTH

LARGER ON-PATHWAY DEFICITS IN ROD-DOMINATED DISEASE THAN CONE-DOMINATED DISEASE

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Purpose: ON-pathway deficits are associated with rod system dysfunction. Therefore, ON pathway function can potentially act as a surrogate marker of the rod system. Here, we have used a tablet-based application (iOS) called 'EyeSpeed' to assess the ON and OFF pathway deficits in patients with known retinal diseases.

Methods: 34 patients (24male, 10 female) participated in the study. The patients were diagnosed with either rod-dominated disease [n=21; retinitis pigmentosa (19), rod monochromatism (2)] or cone-dominated disease [n = 13; Stargardt's disease (6) or cone dystrophy (7)] based on the fundus findings, history and/or electroretinographic findings. The participants were aged between 10- 61 years. The inclusion criteria were that near vision was N24 or better and absence of any other ocular disease. The participants task to identify number of black / white targets embedded in a binary noisy background. Outcome variables are reaction time, accuracy and performance index [accuracy*(1/reaction time)].

Results: The mean difference in reaction times (dark - light) for rod-dominated disease [Mean (SEM): -1.94s (0.37) was significantly higher than cone-dominated disease [[-0.62s (0.22)]; unpaired t-test, p=0.019]. However, mean differences between dark and light targets in accuracy and performance index were not significantly different between the two groups.

Conclusion: ON pathway deficits in rod-dominated disease is prominent compared to the OFF-pathway deficits.

META ANALYSIS ON CONTACT LENS DROPOUTS

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Purpose: Contact lenses are used for optical or therapeutic purposes. Although, it offers many advantages over eyeglasses, still dropouts are reported. The purpose of this review is to know the reasons for dropouts among contact lens wearers.

Methods: Two authors independently searched Cochrane library, PubMed and Web of science for articles which contain any data on the dropouts rates and reasons among contact lens users. Search was made on all kinds of contact lenses. Articles were screened based on their title, then abstract and finally based on full text. PRISMA flow chart was used to show the number of articles included and excluded at each stage of screening. Newcastle Ottawa scale (NOS) grading is used for quality assessment and Prometa3 is used for statistical analysis.

Results: Out of 298 articles screened, 28 were found to be relevant. Amongst11 articles focused on dropout rates and rest on compliance levels. NOS grading showed moderate quality for grading of articles. As the heterogeneity was considerable ($I^2 > 50$), random effects model have been used for forest plots. Various dropout reasons have been noted and forest plots have been used, showingfavorstowards non-compliance. Likewise, funnel plots have been used to look for publication bias.

Conclusions: Lack of proactive recommendation by eye care practitioners and improper care and regimen are two commonly seen factors among contact lens wearers who dropout. Correct patient selection and pre-screening is a key element in addressing both these issues.

INTEROCULAR CONTRAST BALANCING PARTIALLY IMPROVES STEREOACUITY IN KERATOCONUS

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Purpose: In keratoconus, dissimilar degradation image quality between two eyes is associated with suppression of weaker eye and poor stereoacuity. This study evaluated whether stereoacuity may be improved by balancing interocular retinal image contrasts to weaken suppression.

Methods: Stereoacuity of 33 subjects (age: 16 to 33years) with asymmetric keratoconus was tested in randomized order at: 1) baseline with equal interocular stimulus (~100%), 2) their individual "contrast balance point" in the stronger eye (with less severity) and 100% contrast in the weaker eye and 3) contrast levels biased either in favour of the stronger or weaker eye (20% above or below the balance point, respectively). Contrast balance point was estimated using binocular rivalry paradigm (Marella et al., 2021) and stereoacuity was measured with random dot stimuli, both using standard psychophysical techniques.

Results: Median (25th-75th IQR) stereoacuity was 748.8arc sec (288.7-1248.7arc sec) at baseline and improved to 419arc sec (96.9-860.1arc sec) in the contrast balanced condition (~37.5% improvement in stereoacuity; p<0.001), independent of the baseline stereoacuity or the subject's balance point (r=0.1, for both). Biasing contrast in favour of either eye resulted in a deterioration of stereoacuity, relative to the contrast balanced condition (p<0.001). Contrast bias toward the weaker eye produced greater loss of stereoacuity than bias toward the stronger eye (881.3 arc sec (268.0-1654 arc sec) and 502.6arc sec (184-1118.6arc sec), respectively (p=0.002)).

Conclusion: Contrast balancing produces a partial improvement in stereoacuity in keratoconus. Other optical and neural factors need to be considered for explaining the loss of stereoacuity in keratoconus.

ASSESSMENT OF MACULAR VISUAL FUNCTIONS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA SYNDROME

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Purpose: The study assesses the macular visual functions in subjects with and without risk for Obstructive Sleep Apnoea (OSAS).

Methods: Prospective Case-Control study, subjects were classified as cases with presence of snoring and controls in absence of it. Cases were administered with standardized Berlin Questionnaire and were grouped as high risk and low risk based on their scores. Colour discrimination (FM 100 Hue test), Contrast sensitivity (Pelli-Robson) and photo stress recovery test was measured following comprehensive eye examination. Body mass index (BMI) and neck circumference was also measured.

Results: There were 20 cases and 17 controls. There was no significant difference (p=0.334) in age between high risk cases (45.1±7.1), low risk cases (47.5±5.2) and controls (42.1±10.1). There was significant difference in BMI (pvalue=0.001) among high risk cases (30.88±6.22), Low risk (25.40±2.91) and controls (24.16±3.42), and also for Neck circumference (High risk=39.59±3.52), (Low risk=37.86±1.77), (Controls=36.82±2.62), (pvalue=0.035). The total error score in colour discrimination was significantly different among High risk (112±84.17), Low risk (74±43.98) and Controls (56±39.43) (pvalue=0.04). Contrast sensitivity did not show any difference between groups (pvalue=0.897). Similarly, visual acuity and photo stress recovery test did not show any difference between the groups (pvalue=0.959 and pvalue=0.324 respectively). There was a positive correlation (r=0.590, pvalue=<0.001) between BMI and neck circumference.

Conclusions: Cases showed deficits in colour discrimination when compared with controls. Obesity and snoring is a risk factor for OSAS. Thus, individuals with this condition must undergo detailed ophthalmic examination with colour discrimination test and must be evaluated in sleep laboratory.

ASSESSMENT OF MUSCULOSKELETAL AND SLEEP DISORDER ALONG WITH VISUAL AND OCULAR PROFILE AMONG DRIVERS

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Purpose To assess visual and ocular profile along with sleep and musculoskeletal disorder among drivers.

Methods Prospective observational study among drivers above 21 years of age, with minimum 2 years of experience were recruited. Comprehensive eye examination was conducted. The drivers who were in need of spectacles were dispensed. Compliance was assessed after 3 months from the date of spectacle distribution through telephonic conversation. Drivers with ocular abnormalities were referred to tertiary eye care centre. The standardized questionnaire was used to understand about sleep patterns and musculoskeletal disorder among drivers.

Results The study included 111 drivers with mean age of 44 years (SD: 9). Average years of experience and hours of driving was found to be 16.47 years (SD: 9) and 10 hours (SD: 3.22) respectively. About 89 (80.18%) drivers had uncorrected refractive error and was dispensed with spectacles of which 91.6% were compliant. Ocular surface disorders were pingecula (24), pterygium (3) and early lens changes (37). About 7 were referred for further evaluation due to posterior segment abnormalities. Prevalence of musculoskeletal disorder was about 69.36%. Out of 78(72%) drivers, 19(18%) had subthereshold insomnia, 9(8%) had moderate insomnia and 2(2%) had severe insomnia. Work related MSD was strongly associated with distance of driving (OR: 1.009, p value: 0.030) and sleep score (OR: 1.282, p value: 0.008).

Conclusions 80.18% of drivers were dispensed with glasses for refractive error. Prevalence of musculoskeletal disorder and insomnia was found to be 69.3% and 38.4%, respectively.

AWARENESS OF CATARACT AND GLAUCOMA IN TWO RURAL DISTRICTS OF TELANGANA, INDIA

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Purpose: To determine the level of awareness of cataract and glaucoma and identify the determinants of awareness in two rural districts of Telangana, India.

Methods: A population-based cross-sectional study was conducted using the Rapid Assessment of Visual Impairment (RAVI) methodology in Khammam and Warangal districts. A validated questionnaire was administered to participants aged ≥40 years to assess the level of awareness of cataract and glaucoma.

Results: The awareness questionnaire was administered to 3,273 participants of whom 1,433 (43.8%) were men, 1,985 (60.6%) of them had no education, and 1,645 (50.3%) were from Khammam district. In total, 2539/3273 (77.6%) participants reported awareness of cataract. Awareness of cataract was higher in Khammam compared to that in Warangal (84.4% versus 70.6%; $p<0.01$). Only 41/3,273 participants were aware of glaucoma. Awareness of glaucoma was also higher in Khammam (1.88% versus 0.61%; $p<0.01$). Younger age groups, men, any level of education, and residing in Khammam were factors associated with awareness of cataract. People only having any level of education and residing in Khammam were associated with awareness of glaucoma.

Conclusion: Awareness of cataract was higher, but awareness of glaucoma was very poor. There is a need to spread awareness about these potentially blinding conditions. Moving forward, this can be a critical step in developing a preventive eye care strategy to achieve universal eye health in India

OCULAR DIMENSIONAL CHANGES IN PRIMARY ANGLE-CLOSURE SUBJECTS

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Purpose: To evaluate ocular dimensional changes in normals and primary angle-closure subjects using IOL master.

Methods: In a prospective study, 30 subjects were recruited in each normal, PACS, PAC, and PACG group respectively. Clinical parameters such as Axial length (AXL), Central corneal thickness (CCT), Anterior chamber depth (ACD), Lens thickness (LT), and Average keratometry values (Avg K) were measured using the IOL master 700. One-way ANOVA was performed for the comparison of clinical parameters between the groups.

Results: A total of 120 eyes were analysed, there were 60 males and 60 females. Mean (SD) age for normals was 54.1(9.2) years and primary angle-closure subjects were 58.6(8.8) years ($p>0.05$). Mean (SD) ACD (in mm) of normals was 3.23(0.43), PAC was 2.8(0.40), PACS was 2.7(0.46) and PACG was 2.9(0.30). Mean (SD) LT (in mm) of normals was 4.3(0.46), PAC was 4.7(0.46), PACS was 4.73(0.45) and PACG was 4.53(0.50). ACD and LT showed a statistically significant difference between normals, PACS, PAC, and PACG (One way ANOVA, $p<0.05$). ACD showed a statistically significant difference in PACS, PAC, and PACG when compared to normals ($p<0.01$). LT showed a statistically significant difference in PACS and PAC when compared to normals ($p<0.01$) using the Post hoc Bonferroni test.

Conclusions: This study shows that there is a difference in ACD and LT between normals and primary angle-closure subjects. It is inferred that the angle-closure group should be observed for structural dimensional changes over the period to prepone the need for peripheral iridotomy and further management.

OCULAR SUN EXPOSURE DAMAGE AND ITS ASSOCIATION WITH PTERYGIUM AND CATARACT AMONG SALT PAN WORKERS

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Purpose: To assess the ocular sunexpoure damage among the salt pan workers as part of theoutdoor occupation.

Methods: Cross-sectional study where the subjects underwent comprehensive eye examination including conjunctival ultraviolet autofluorescence (CUVAF) imaging, a biomarker for conjunctival Ultraviolet (UV) damage as part of occupational sun exposure. The area of CUVAF will be traced with "ImageJ" software and will be converted to mm^2. The cumulative lifetime UV dose/exposure was arrived based on the location and nature of the occupational task. The images were analysed to find the association betweenthe area of damage, cumulative lifetime UV dose and ocular morbidities.

Results: About 48 saltpan workers (21 male and 27 female)with mean age 48±8.7 years were included. The median area of conjunctival UV damage in the right and left eye was 8.24[14.75]$mm^2$and 13.18[13.75]mm^2respectively. The mean cumulative lifetime UV dosewas 1.83±0.93. The risk of pterygium was associated with CUVAF scores (OR 1.06, 95% 1.00-1.14), and for cataract (OR 1.201, 95% 1.06-1.34) evenafter adjusting for age, gender and years of experience.No statistically significant difference in the area of conjunctival damage were noted between the gender,occupational task, use of personal protective equipment.

Conclusion: The salt panworkers are constantly exposed to reflected UV in their workplace. The CUVAF can be used to estimate the conjunctival UV damage and associated ocular morbidities among outdoor workers.

GLOBALLY PREVALENT GLAUCOMA DETECTION GUIDELINES AND PROTOCOLS FOR EYE CARE PROFESSIONALS: A REVIEW

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Purpose: Early accurate diagnosis with timely referral is mandate to detect and prevent glaucomatous damage. Glaucoma guidelines provide a holistic approach to eye care practitioners in clinical diagnosis and intervention. The study aims to review the prevalent guidelines, protocols, schemes for glaucoma diagnosis and referral worldwide.

Methods: Online literature search was conducted in PubMed with the use of relevant MeSH terms. Articles representative of glaucoma guidelines, diagnostic protocols, referral schemes, practice patterns for eye care professionals in various countries published between 2009-2021 were included into the study.

Results: After filtering and reviewing of 227 articles, 36 relevant articles were found to be apposite for the review. American Academy of Ophthalmology guidelines (US), Asia Pacific Glaucoma Guidelines- SEAGIG (India), Canadian Ophthalmological Society glaucoma clinical practice guidelines (Canada), German Ophthalmological Society guidelines (Germany), National Health and Medical Research Council guidelines (Australia & New Zealand), National Institute of Clinical Excellence (United Kingdom), Scottish Intercollegiate Guidelines Network guidelines (Scotland) were found to be implemented as glaucoma clinical practice protocols in different nations. Community refinement of glaucoma through schemes/ referral pathways were widely prevalent in UK, resulting in reduction of false positive referrals. The most preferred and habitual diagnostic techniques among the established guidelines were IOP measurement (Goldmann Applanation Tonometry), Optic disc evaluation (Slit lamp fundoscopy), Visual field examination (White on white Standard Automated Perimetry), Gonioscopy & Central corneal thickness measurement.

Conclusion: Although there are multitudinous glaucoma clinical practice and referral guidelines, firm conformance to these guidelines is imperative to potentially detect the insidious disease.

WHY DO CHILDREN WITH AMBLYOPIA TAKE LONGER TO SEARCH?

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Purpose: Even when viewing with the dominant eye, individuals with amblyopia took longer in a conjunction visual search task. We investigated if this will be the case for searching in real world images as well.

Methods: Real world images along with a search target were displayed on a computer for participants to search. Eye movements were tracked with Eyelink 1000 Eyetracker. Children (6-16 years of age) in control group (n=14) had monocular visual acuity 20/25 or better and cases (n=23) had anisometropic amblyopia, with best corrected visual acuity in the amblyopic eye between 20/100 to 20/40 and dominant eye 20/25 or better. Three viewing conditions were randomized: dominant eye, non-dominant eye and binocular viewing. Trials with correct response, reaction time, average saccade count and saccade amplitude along with interest area's (target locations) dwell time, regression saccades and fixation count were analyzed.

Results: Regardless of binocular viewing or dominant eye viewing children with amblyopia took longer time (p=0.041) to search and made more number of saccades (p=0.007), when compared to the controls. Additionally, their saccade amplitude was smaller, with more fixations (p=0.017) and longer dwelling (p=0.041) in the target interest areas.

Conclusions: In spite of having good visual acuity children with amblyopia take longer time to search in real world images. The need to make smaller saccades and having to fixate longer indicates a deficiency in how the image is sampled and information is assimilated. This could arise from higher order cortical deficits or reduced visual span in children with amblyopia.

EVALUATION OF CHORIOCAPILLARIS FLOW DEFICITS IN EYES WITH POLYPOIDAL CHOROIDAL VASCULOPATHY, FELLOW UNAFFECTED EYES AND AGE-MATCHED HEALTHY EYES.

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Body of the abstract (250 words):

Purpose: To evaluate the choriocapillaris flow deficits (CCFD) on Swept-Source Optical Coherence Tomography Angiography (SS-OCTA) in eyes with unilateral polypoidal choroidal vasculopathy (PCV) (group 1), fellow unaffected eyes (group 2), and to compare them with age-matched healthy controls (group 3).

Methods: This is a prospective, cross sectional and observational study. Using the SS-OCTA, the CC (Choriocapillaris) slab was segmented from the structural OCT (Optical Coherence Tomography) and the corresponding flow map was multiplied after signal compensation. The resultant image was evaluated for CCFD in equidistant squares measuring 1x1 mm, 1.5x1.5 mm, 2x2 mm, 2.5x2.5 mm, 3x3 mm and 6x6 mm centered on the fovea.

Results: The percentage of flow deficits were significantly increased (One way ANOVA, $p = 0.003$ and $p = 0.049$) in group 1 when compared to group 2 and 3. In the multiple pairwise comparison using post hoc Bonferroni, CCFD of 1mm in group 1 and 2 ($p=0.019$), group 1 and 3 ($p = 0.003$) and CCFD of 1.5mm in group 1 and 3 ($p =0.044$) were statistically significant. Correlation analysis showed no significant correlation between CCFD, age, BCVA (Best corrected visual acuity), FT (foveal thickness) and SFCT (Subfoveal choroidal thickness). Linear regression analysis showed that the CCFD was negatively correlated with the distance from the foveal center in group 1 ($\beta=-0.613$, $p = 0.046$).

Conclusion: Eyes with PCV demonstrated a significant flow impairment in the choriocapillaris layer as compared to the fellow unaffected eyes and age-matched healthy eyes.

RETINAL SHAPE DIFFERENCES IN ISOMETROPES AND ANISOMETROPES

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Purpose: Considering the differences in biomechanical, structural, and optical characteristics of the fellow eyes of anisometropes, this study aimed to investigate if there are differences in retinal shape between the eyes of isometropes and anisometropes.

Methods: A total of 28 isometropes (mean ±SD spherical equivalent low myopic eye: -3.2±2.0D; high myopic eye: -3.5±2.0D) and 17 anisometropes (-2.5±2.3D; -4.6±2.3D) were included in this prospective experimental study. Central and peripheral eye lengths were obtained after pupil dilation using the Lenstar LS 900 along the horizontal retinal meridian out to ±30 degrees, in 10-degree steps. Retinal coordinates were estimated using peripheral eye lengths and ray tracing to determine retinal shapes in terms of vertex radius of curvature (R_v), and asphericity (Q).

Results: R_v was smaller in high myopic eyes of both isometropes (mean±SEM: 12.2±0.5mm vs. 11.8±0.4mm; p=0.20) and anisometropes (10.8±0.5mm vs. 10.1±0.5mm; p=0.10). High myopic eyes of anisometropes had significantly smaller R_v (10.1±0.5mm vs. 11.8±0.4mm, p=0.01) and more prolate shape compared to that of isometropes (-1.0±0.2, vs. -0.2±0.2, p<0.001). High myopic eyes of anisometropes had significantly smaller R_v and more prolate shape compared to the isometropic eyes of same refraction (Rv: 10.4±0.7mm vs. 12.2±0.4mm, p=0.03; Q: -1.0±0.3 vs. -0.0±0.2, p=0.009), but such a trend was not seen with low myopic eyes (p>0.10).

Conclusions: Anisometropes had steeper and prolate retina along the horizontal meridian compared to isometropic counterparts with same refractive error. This difference in the retinal surface shapes in isometropes and anisometropes might play a role in myopiogenesis.

A NOVEL VIRTUAL SURGERY SIMULATION PLATFORM TO PREDICT POSTOPERATIVE CORNEAL STIFFNESS BEFORE REFRACTIVE SURGERY

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Purpose: To develop a finite element method (FEM) and Artificial Intelligence (AI) based platform for predicting post laser vision correction (LVC) surgery corneal stiffness (kc(mean)) using preoperative data.

Methods: Platform (called AcuSimX™) took preoperative Corvis-ST and Pentacam (OCULUS Optikgerate Gmbh, Germany) data to compute the preoperative in-vivo corneal material property coefficients. Material coefficients and planned surgical parameters were then used to predict kc(mean) using a surgery-specific predictive FEM model and an AI (Orange AI, University of Ljubljana, Slovenia) incorporating postoperative measurement uncertainties. AI was build using 529 eyes from 529 patients (Narayana Nethralaya, India; Sankara Nethralaya, India; Humanitas Clinical and Research Center, Italy). The platform was further tested using 7 eyes that developed ectasia after LVC. A nomogram was derived to find ectasia risk at the preoperative stage. Predictability of the kc(mean) was assessed using mean absolute error (MAE), and intraclass correlation coefficient (ICC) relative to in-vivo postoperative measurement.

Results: MAE and ICC were 6.24N/m and 0.84 [95% CI: 0.80-0.84], respectively for the training cohort (60% of the total data). Similarly in the test cohort (40% of the total data), these were 6.47N/m and 0.84 [0.78-0.89], respectively. Predicted kc(mean) was statistically similar to the in-vivo postoperative measurement ($p > 0.05$) in ectasia eyes. The nomogram was able to separate all the 7 ectasia eyes from 529 normal eyes.

Conclusion: A novel virtual surgery simulation platform was developed and an effective ectasia risk identification nomogram was proposed. AcuSimX™ was an easy platform that can be used by clinicians in their clinics.

INFANTS GET BETTER AT GAZING WITH INCREASE IN AGE

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Purpose: It is known that infant's saccadic reaction time reduces with age. We investigated if the same trend exists for gaze (eye/head) reaction time to stimuli presented with the Pediatric Perimeter (PediPeri), an in-house built device to measure infant's visual fields.

Methods: Infants (age: 3 to 8 months) were placed in supine position inside the PediPeri dome. Two types of static stimuli namely, Meridian (one LED light strip) and Quadrant (two adjacent LED light strips were illuminated) were presented. Total of 8 Meridians and 4 Quadrant stimuli were displayed at a peripheral angular extent of 30° to 90°. Video recordings of infant's gaze were analysed.

Results: A total of 705 infants were recruited (3 months [n=166], 4 months [n=109], 5 months [n=55], 6 months [n=125], 7 months [n=140], and 8 months [n=110]). Median reaction time for both the stimuli showed a reduction with increase in age (Kruskal-Wallis test, p<0.05). Meridian stimuli were significantly (Wilcoxon Signed Rank test, p≤0.05) longer than the Quadrant stimuli within all the age groups.

Conclusions: In accordance to previous studies, we observed that the gaze reaction time also decreases with age. The PediPeri device can be used to quantify these gaze reaction time in infants. The disparity observed with longer reaction time to the Meridian stimuli when compared to the Quadrant stimuli could be due to the lower saliency of the former stimuli.

ANTI DYSPHOTOPSIA LENS - A NOVEL INTRAOCULARLENS DESIGN TO PREVENT DYSPHTOPSIA POST CATARACT SURGERY

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Purpose: To design and evaluate a new intraocular lens to prevent negative dysphotopsia in the form temporal crescentric shadow post cataract surgery

Methods: Most accepted theory being gap between iris and optic leading to a grey shaded area formed between ray missing IOL and ray refracted by IOL causing a temporal crescentric shadow.our concept was to give normal crystalline lens profile to IOL optic to eliminate gap between IOL and iris which simulates reverse optic capture or better.The new IOL is of hyperbola shape where optic comes in contact with pupil margin after implantation in the capsular bag there by eliminating the space that light rays can pass through and produce negative dysphotopsia.The lens was implanted in 56 patients in whom the other operated eye had negative dysphotopsia.

Results: None of the 56 patients(at 1 year) complained of any dysphotopsia in which ADL lens was implanted

Conclusion: This study suggests that new IOL design is effective in eliminating negative dysphotopsia

VARIATIONS IN RETINAL IMAGE QUALITY WITH DIFFERENT CONTACT LENS DESIGNS IN KERATOCONUS – INSIGHTS FROM COMPUTATIONAL ANALYSIS

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Purpose: Improvement of visual functions in keratoconus is partial and dependent on the contact lens (CL) design, vis-à-vis, non-keratoconic controls. This study provides the optical basis for these observations by identifying Zernike wavefront aberration coefficients that dictate image quality (IQ) with different CL designs in keratoconus.

Methods: Through-focus curves for the LogNS IQ metric were constructed from the native wavefront aberrations of controls (n=10 eyes; 20 – 21yrs) and from those of keratoconic cases with conventional RGP, Rose K2®, Miniscleral and Kerasoft® CL wear (n=15 eyes; 20 – 28yrs) over 3mm pupil diameter. The impact of specific Zernike wavefront aberration terms on peak IQ was assessed by re-constructing the through focus curves of controls with selected Zernike wavefront coefficients from cases.

Results: Peak IQ was similar for conventional RGP, Rose K2 and Miniscleral CL's (p>0.05, for all) but was significantly poorer with Kerasoft CL (p<0.01, for all), all relative to controls (p<0.01, for all). Peak IQ of controls deteriorated to the level of CL wear in keratoconus in the presence of astigmatism and it was in-between controls and CL wear in the presence of coma, across all four designs. All other Zernike terms did not impact IQ significantly.

Conclusions: Image quality improves only partially with CL wear in keratoconus, relative to controls. RGP, Rose K2 and Miniscleral CL's produce similar and greater improvement in IQ than Kerasoft CL. The partial improvement of IQ in keratoconus appears to depend on the magnitude of astigmatism and coma left as residual errors with CL wear.

CHARACTERISTICS OF OCULOMOTOR FUNCTION AMONG SPORTS CONCUSSED ATHLETES

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Purpose: To compare the oculomotor function between sports concussed athletes and aged matched controls.

Methods: Thirty mild concussed athletes were recruited and compared with aged matched controls. All the participants underwent a comprehensive ocular assessment followed by oculomotor assessment which included tests for accommodation, vergence, eye movements and reading parameters.

Results: Three categories of oculomotor-based deficits were found: convergence insufficiency (40%), accommodative insufficiency (25%), and oculomotor-based reading dysfunctions (20%). A statistically significant reduction in the mean ± SD of the following parameters was noted in concussed athletes v/s controls:- binocular accommodative amplitude: 7.13 ± 1.59 v/s 15.35 ± 2.95 ($p<0.001$), convergence amplitude: 14.23 ± 5.00 v/s 5.65 ± 0.90 ($p<0.001$), positive fusional vergence for distance: 21.17 ± 8.97 v/s 31.32 ± 6.23 ($p<0.001$), vergence facility: 6.47 ± 1.47 v/s 11.84 ± 1.00 ($p<0.001$), accommodative facility : 7.10 ± 4.57 v/s 11.67 ± 1.83 ($p<0.001$),reading speed: 66.97 ± 17.82 v/s 144.13 ± 24.45 ($p=0.03$) and DEM ratio: 1.40 ± 0.19 v/s 1.17 ± 0.06 ($p<0.001$).

Conclusions: Concussions caused by sports have a considerable impact on oculomotor parameters. These findings have substantial therapeutic implications in terms of establishing a periodic screening programme for athletes so that essential therapy can be provided for a better outcome.

DOES TACROLIMUS CUTANEOUS APPLICATION OVER LID HELP?

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Purpose: To evaluate the role of cutaneous 0.1% tacrolimus in the management of chronic vernal keratoconjunctivitis.

Methods: It was a prospective, longitudinal, non-comparative, open label, clinical study of 40 patients (80 eyes) with moderate to severe, active chronic vernal keratoconjunctivitis (Grade 3 or 4 on Bonini scale). Patients <8years, mild disease, active infection and associated ocular disorders were excluded. Patients were started on twice daily cutaneous application of tacrolimus 0.1% over skin of upper eyelid along with standard therapy [anti-allergic & steroid therapy] for 3 months. Study parameters included severity, ocular surface evaluation [conjunctival hyperemia, FTBUT, Schirmer's, staining score, OSDI, lipid layer imaging (Lipiview)], & corneal tomography. Disease clinical severity change was assessed by clinical severity score & composite assessment score (CAS) devised and 5-5-5 exacerbation grading at 3 months follow-up (FU). The patients were graded on clinical scores based on presenting signs and symptoms and the potency of the topical steroid necessitated for treatment.

Results: In 40 patients (mean age 11.52±3.96), 85% had palpebral, 15% limbal & 5% mixed form of VKC. Significant change in CSS [12.12±1.44 to 1.7±1.36 (p= 0.0001) (Wilcoxon signed-rank test)] and CAS [14.52±2.30 to 1.99±1.9 (p=0.0001) Wilcoxon signed-rank test)] was noted.

Conclusions: Treatment with topical tacrolimus was successful in 97.5% (78 eyes) of patients as proved by the change in clinical scores Itching was the first symptom to be relieved at four weeks. Topical therapy with tacrolimus applied cutaneously over eyelid skin is a viable alternative in chronic vernal keratoconjunctivitis.

CHOROIDAL THICKNESS IN THE FELLOW EYES OF RESOLVED CENTRAL SEROUS CHORIORETINOPATHY

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Purpose: To measure the choroidal thickness of the clinically unaffected contralateral eyes in patients with Central Serous Chorioretinopathy (CSCR) during disease activity and after resolution.

Methods: Prospective cross-sectional study conducted over a year. The study sample comprised 50 patients with CSCR and 50 controls of emmetropic individuals.

Results: Mean age of the patients was 38.8 years (SD ±6.52). Foveal thickness (FT), macular thickness (MT) and sub foveal choroidal thickness (SFCT) were measured in both eyes at the acute and resolved stage of CSCR in the fellow eyes. There was significant difference of SFCT in the affected eye group between acute stage and after resolution ($p < 0.00001$). SFCT was thickened in the fellow eyes in acute stage of CSCR (mean 354.23 micron, SD ±30.02) and reduced after the resolution (mean 340.74 micron, SD ±29.76) but did not touch the baseline thickness (mean 322.15 micron, SD ± 23.21) $p=0.009$.

Conclusions: The eyes of CSCR (affected eyes) showed significant reduction of SFCT however did not come to the normal range after resolution of fluid. Unaffected eyes also showed reduced SFCT after CSCR was resolved in the affected eye but not as controlled group. It hints change in auto regulation of choroidal flow partially due to bilateral choroidal dysfunction theory even persist after resolution of fluid in the affected eyes.

STRUCTURAL ARRANGEMENT OF COLLAGEN FIBRILS IN POST-LASIK ECTASIA AND HEALTHY POST-LASIK CORNEAS

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Purpose: To study the changes in collagen fibril structures in post-LASIK ectasia (PLE) and compare with healthy post-LASIK corneas eyes using custom built ultrahigh resolution polarization sensitive (PS) – OCT.

Methods: 15 PLE eyes, 20 healthy post-LASIK eyes and 50 normal (un-operated) eyes were imaged to analyse the collagen fibril structure with PS-OCT. Enface maps representing corneal fibril distribution based on polarization sensitive information were generated using phase retardation (PR) information from the posterior cornea.

Results: Enface maps of the normal eyes showed preferential arrangement of collagen fibril structures. PLE eyes showed abrupt changes in PR enface maps as compared to healthy post-LASIK eyes. PR enface maps of healthy post-LASIK eyes closer to normal (un-operated) eyes. Zonal analyses of PR values showed higher retardation in PLE eyes in regions of higher curvatures.

Conclusions: PLE eyes showed abrupt changes in corneal fibrillar arrangement, possibly indicating loss of corneal integrity. Posterior corneal surface enfaces maps derived from PS-OCT can be used to track changes in collagen fibril arrangement in post-refractive surgeries aiding in early ectasia detection. PS-OCT can also help in identifying corneas with abnormal morphology/fibrillar arrangement prior to the surgery, which could be overlooked otherwise.

EVALUATION OF CORNEAL FIBRIL DISTRIBUTION IN THIN AND ASYMMETRIC KERATOCONUS CORNEAS USING ULTRAHIGH RESOLUTION POLARIZATION SENSITIVE OCT

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Purpose: To evaluate phase retardation (PR) distributions across healthy, thin corneas, asymmetric and bilateral KC.

Methods: Fourty eyes from 20 subjects divided across four groups, healthy corneas (group 1; n=10 eyes), thin corneas with no clinical disease (group 2; n=10 eyes), asymmetric KC (one eye had KC and the other appeared tomographically healthy) (group 3; n=5 eyes) and clinical KC (group 4; n=15 eyes). All subjects underwent imaging on our PS-OCT, MS-39 (CSO, Firenze, Italy) and biomechanical assessment with the Corvis-ST (OCULUS Optikgerate Gmbh, Wetzlar, Germany) along with routine clinical examination. Using PS-OCT, phase retardation (PR) was derived and zonal analysis was performed in annular regions. From MS-39, aberrations were analysed with Zernike polynomials. Epithelium Zernike indices and total corneal thickness were also computed. From Corvis-ST, the CBI (Corvis biomechanical index), TBI (total biomechanical index) and BAD-D (Belin-Ambrosio overall deviation index) were analysed.

Results: PR enface maps of healthy corneas showed least retardation in the corneal apex and increased radially outwards towards the periphery of the cornea. Group 1, 2 and 3 had similar curvatures and only the CBI, TBI and BAD-D were similar ($p>0.05$). The PR distributions clearly showed that the eyes in groups 1, 2 and 3 had a normal corneal distribution unlike group 4 eyes ($p<0.05$), despite group 3 following common definitions of sub-clinical disease and forme fruste KC.

Conclusion: Analysis of PR distributions may contribute in effectively minimizing the erroneous classifications between healthy and diseased corneas and assist in early corneal disease diagnostics.

REFRACTIVE ERROR SHIFT IN VARIOUS LENS STATUS POST VITREO-RETINAL SURGERY: EFFECT OF SILICONE OIL TAMPONADE AND SILICONE OIL REMOVAL

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Purpose : To evaluate surgically induced refractive error shift following silicone oil tamponade (SOT) and silicone oil removal (SOR) in different lens status post-vitreoretinal surgery.

Methods : This retrospective analysis included 150 eyes of different lens statuses, phakic (n=50), pseudophakic (n=50) and aphakic (n=50) who had undergone pars plana vitrectomy (PPV) with SOT and SOR between January 2017 and December 2020 was performed. Spherical equivalent refraction with SOT and post-SOR was evaluated after a month of each surgery.

Results : A total of 146 patients were included. The median age was 46 (Inter-Quartile range 32 to 58) years including all three groups. The mean spherical equivalent (SE) ± standard deviation (SD) with SOT in phakic eyes was 4.00±0.65 diopters (D) and post-SOR was -2.85±0.60D (p<0.001). In pseudophakic eyes, the mean SE ± SD with SOT was 3.25±0.54D and post-SOR was -0.65±0.56D (p≤0.002). Similarly, in aphakic eyes, the mean SE ± SD with SOT was 2.75±0.60D and post-SOR was 6.84±0.59D (p<0.001). On multivariate analysis, significant predictors for refraction post-SOR in phakic eyes was the mean SE with SOT (P<0.001), for pseudophakic eyes the diagnosis of rhegmatogenous retinal detachment (RRD) (p=0.01) and the mean SE with SOT (p=0.02). Also, in aphakic eyes the SE with SOT is the only predictor for SOR refraction (p<0.001).

Conclusions : The mean SE refraction with SOT is an important predictor for refraction post-SOR in all three lens statuses whereas the diagnosis of RRD may favor the SOR refraction in pseudophakic eyes.

COMPARISON OF PEDIATRIC PERIMETER VIDEO ANALYSIS BETWEEN NAÏVE EXAMINER AND STANDARD EXAMINER

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Purpose: Pediatric Perimeter is a device to measure infant's visual field and gaze (eye/head response) reaction time (RT). Videos recorded from this device are analyzed offline to study the infant's gaze. Here we validated the video analysis of a naïve examiner to an experienced examiner.

Methods: From 100 tested infants, 17 (mean age: 6.78±2.04 months, 154 video clips) were randomly selected. The video data were post processed by an algorithm that selected stimuli onset and offset segments and split it into frames. A naïve examiner (non-clinician) analyzed these frames, previously analyzed by a gold-standard examiner (optometrist with experience of analyzing over 100 infants). The analysis involved identifying a valid response (true positive) and rejecting an invalid response (true negative) of an infant's gaze to a light stimulus. Additionally, the RT of the infant to look at the stimuli in valid responses were also calculated.

Results: The sensitivity, specificity, positive and negative predictive values of the naïve examiner were 69%, 85%, 78% and 77% respectively. The mean difference (12±42ms) in RT was minimal and a strong agreement was observed (intra-correlation coefficient =0.98) between the two examiners.

Conclusion: The higher specificity indicates that it is easier for a naïve examiner to determine an invalid response than a valid response in an infant. Hence, more training is needed to identify valid responses. The strong correlation between the naïve and standard examiner shows discrete frames presented at the acquisition camera frame rate, allows the naïve examiner to accurately identify the infant's gaze onset frame.

SPECTRAL COMPOSITION OF AN AMBIENT LIGHT VARIES WITH DIFFERENT LOCATIONS AND TIME OF A DAY: AN IMPACT ON MYOPIA

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Purpose: We aimed to investigate the variation in visible electromagnetic spectral composition of an ambient light in different a) outdoor/indoor locations, b) time of a day, and c) seasons.

Methods: The spectral irradiance (SPD) was recorded using a hand-held spectrometer in three outdoor ('open playground-OPG', 'under shade of big tree-UST' and 'canopy-CAN'), and three indoor locations ('room with multiple large windows-RMW', 'closed room-C-Room', and 'closed corridor-C-Corr') across five different time points (3-hours intervals between 6:30 to 18:30 clock-hours) during summer and non-summer season. The distribution of SPD across spectrum was further assessed as short (380-500nm), middle (505-565nm) and long wavelength (625-780nm).

Results: The overall median (Q1, Q3) SPD (W/nm/m^2) across three outdoor locations [0.11(0.09, 0.12)] was 153 times higher than that of indoor locations [0.0007(0.0001, 0.001)]. Among outdoor locations, SPD was always recorded highest in the OPG [0.27(0.21, 0.28)], followed by UST [0.083(0.074, 0.09)] and CAN [0.014(0.012, 0.015)] irrespective of different time points in a day, whereas, in indoor locations, RMW always recorded highest SPD [0.023(0.015,0.028)]. The overall median SPD was highest in the mid-day [0.19(0.17, 0.21)], which gradually decreased during morning and evening hours, with significant differences in SPDs between seasons [0.096(0.08, 0.10) vs. 0.084(0.071, 0.094), P<0.01]. SPD and percentage composition of middle wavelength was significantly (P<0.01) higher than short and long wavelength in all the six locations (mean difference of 5-13%/4-8% against shorter/longer wavelengths respectively at outdoors).

Conclusion: Spectral irradiance varied with different locations, time of a day, and seasons. Further studies are warranted to understand implications of such variation across locations in myopiogenesis and control.

DETERIORATION OF VISUAL ACUITY DEPENDS ON THE FREQUENCY AND AMPLITUDE OF TIME-VARYING OPTICAL DEFOCUS

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Purpose: Optical defocus of a given magnitude deteriorates visual acuity. However, the impact of temporally-varying optical defocus on visual acuity remains unknown. This study systematically assessed changes in high-contrast visual acuity to various amplitude and frequency combinations of sinusoidally-varying temporal optical defoci.

Methods: Post-cycloplegic visual acuity of 13 emmetropic adults (20 – 26yrs) were determined for optical defoci of different amplitude (0.25D, 0.5D, 1D and 2D) and frequency (0.25Hz, 0.5Hz, 1Hz and 2Hz)combinations induced using a focus tunable lens. Visual acuity was calculated as the 50% correct response of psychometric functions derives for 300ms presentation of individual Sloan optotypes. Empirical results were compared with an ideal observer model, incorporating optical and neural filters, neural noise and a cross-correlation decision operator, to determine putative decision strategies dictating visual acuity with temporal blur.

Results: Visual acuity deteriorated with both amplitude and frequency of temporal defocus. This deterioration was greater for lower than higher temporal frequencies, across all defocus amplitudes tested ($p < 0.001$). Overall, these losses were smaller than equivalent amplitudes of static defocus ($p < 0.001$). These trends were well-described by model simulations incorporating an averaging of defocus over the target presentation. Alternate strategies involving detection of least defocus or shorter averaging durations individually did not match the empirical results.

Conclusions: Visual acuity loss with temporally-varying defocus increases with the amplitude of the sinusoidal signal, more so for lower than higher frequencies. These results may be explained by the temporal averaging of defocus signal over the entire target presentation duration.

INFLUENCE OF RETINAL NERVE FIBRE LAYER LOSS ON BINOCULAR VISUAL FIELD SENSITIVITY

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Purpose: To evaluate the pattern of relationship between the loss of Retinal Nerve Fibre Layer (RNFL), and Ganglion Cell Layer (GCL) with Binocular Visual Field (BVF) sensitivity.

Methods: Optical Coherence Tomography (OCT) and Humphrey Field Analyzer (HFA, SITA Standard 24-2) reports from glaucoma patients (two subsequent visits) were extracted. Eyes were categorised as better or worse using perimetry indices. From the Monocular VF (MVF) reports, Integrated Visual Fields (IVF) Mean Deviations (MD) were generated using the binocular summation model. A structural to functional comparison was performed by considering the percentage of RNFL and GCL thinning along with the decline in BVF sensitivities. An agreement analysis was done for the RNFL thinning and BVF sensitivity loss.

Results: 12 glaucomatous eyes from 6 patients with a median age (IQR) of 60 (28) years were included. The percentage of change in MVF - MD was found to be in accordance with the change in RNFL and GCL thinning whereas this was not apparent in BVF- MD. Moderate agreement was found between the location of better eye's RNFL loss and corresponding monocular VF defect (K=0.54, p<0.05) whereas no agreement was noted between the worse eye's RNFL loss and BVF defect.

Conclusions: Agreement between better eye's structural loss and BVF defect can be attributed to the compensatory behavior by the better eye for the defects in the contralateral eye during binocular viewing conditions. Further studies might be beneficial to have comprehensive analysis and substantial conclusion with these preliminary findings.

FEASIBILITY OF CONTACT LENS SERVICES IN RURAL SECONDARY CENTRES OF EYE HEALTH PYRAMID

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Purpose: To estimate the proportion of new patients (aged <40 years) visiting rural secondary eye centres (SCs) of L V Prasad Eye Institute who can be potential contact lens (CL) users.

Methods: After IRB approval, electronic medical records of 5540 new patients (47.32% female) seen between October to December 2019 (pre-COVID) and 5323 patients (45.20% female) seen between October to December 2020 (during COVID pandemic) across five SCs located in Mudhole, Paloncha, Dhulipalla, Rajgangpur, and Berhampur were reviewed retrospectively. The potential CL users were categorized based on the purpose: 1) correcting refractive errors, 2) cosmetic, 3) functional (for ectatic conditions where spectacles may not provide the best visual outcomes), and 4) combination – different purpose in both eyes.

Results: Overall, 25.73% of new patients seen across 5 SCs in 2019 and 22.97% of patients seen in 2020 were identified to be potential CL users. Among 5 SCs, Berhampur showed the highest proportion of potential CL users in both the period; 33.48% in 2019 and 27.90% in 2020. During the COVID pandemic, there was a significant decrease in the proportion of children across all centres (mean difference 8.03%, p<0.0001); however overall numbers of potential CL users were similar in 2019 and 2020. In most of the patients (96.66%) indication for CL wear was their refractive errors. However, a small proportion of patients may need CLs for cosmetic (1.32%), functional (1.66%), and combination (0.34%).

Conclusion: Overall, one in four new patients may potentially be offered contact lens services in rural secondary centres.

OBJECTIVE PHOTOSENSITIVITY LUMINANCE IN HEALTHY HUMAN EYES ASSESSED USING AN AUTOMATED OCULAR PHOTOSENSITIVITY ANALYZER: A STEP TOWARDS TRANSLATION OF A CLINICAL TOOL FOR ASSESSING PHOTOPHOBIA

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Purpose: To evaluate objective photosensitivity luminance in healthy eyes, thereby providing a normative dataset that will lead to a better understanding of diseases causing photophobia.

Methods: It was a prospective cross-sectional study. Emmetropes whose visual acuity was better than 0.18 logMAR (6/9) with no other ocular abnormality were included. Headache Impact Test-6 questionnaire and visual light sensitivity questionnaire were administered. Objective photosensitivity luminance was assessed manually by evaluating the videos recorded using infrared camera and noting down the intensity of light at the first squeezing reflex.

Results: A total of 75 normal subjects (age range, 7-71 years) were included. Median age was 32.7 years (inter-quartile range, 20.3-47.9 years). Forty subjects (53.3%) were males. Median Headache Impact Test score was 38 (inter-quartile range, 36-42) and visual light sensitivity questionnaire score was 11 (inter-quartile range, 8-15). Mean (standard deviation) right eye, left eye and binocular objective photosensitivity luminance was 3.25 (0.55), 3.35 (0.47) and 3.15 (0.52) loglux respectively. Age was positively correlated with only binocular objective photosensitivity luminance and there was no correlation between age and right eye or left eye objective photosensitivity luminance.

Conclusions: The study characterized, for the first time, objective photosensitivity luminance and established a normative dataset. The data would help in understanding the pathophysiology of the diseases causing photophobia, monitoring the disease progression and thereby evaluating the treatment modalities.

POPULATION BASED ASSESSMENT OF EFFECTIVE CATARACT SURGICAL COVERAGE IN TELANGANA IN SOUTH INDIA

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Purpose: Cataract is the leading cause of vision loss in India. Effective Cataract Surgical Coverage (eCSC) is proposed as an indicator to assess universal eye health coverage in a given region. We report on eCSE in four districts in Telangana in South India.

Methods: A population-based study using Rapid Assessment of Visual Impairment (RAVI) was conducted among individuals aged ≥40 years in four districts of Warangal, Adilabad, Khammam, and Mahbubnagar in Telangana during 2014 & 2017. The study teams visited households and conducted eye assessments which included visual acuity measurement, anterior segment examination including lens examination, and distance direct ophthalmoscopy. A structured questionnaire was used to document the history of cataract surgery. *Effective cataract surgical coverage* (eCSC) was calculated as the number of people with operated cataract and a good outcome (presenting visual acuity 6/18 or better) as a proportion of those having operable plus operated for cataract.

Results: In total, 11,238 participants are examined from four districts. The mean age (standard deviation) of the participants was 54.1±11.2 years; 6137 (54.6%) participants were women. Overall, eCSC was 42.1%. It ranged from a least of 38.2% in Mahbubnagar, 38.9% in Warangal, 44.9% in Adilabad, and the highest of 47.3% in Khammam district.

Conclusions: Effective Cataract surgical coverage varied across the four districts in Telangana. There is a significant unmet need for cataract surgery in all four districts in Telangana. As cataract is an avoidable cause of vision impairment, strategies are needed to meet the large unmet need in the region.

THE EVALUATION OF STAGING SYSTEMS FOR VISUAL FIELD DAMAGE CLASSIFICATION IN GLAUCOMA

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Purpose: To compare categorical systems for classifying the severity of glaucomatous Visual Field Defects (VFDs).

Methods: This cross-sectional study included 400 Visual Field (VF) reports from Humphrey Field Analyzer (HFA). Three investigators categorized these reports using the: (a) Hodapp Anderson Parrish (HAP), (b) enhanced Glaucoma Severity Staging (eGSS), (c) Advance Glaucoma Intervention Study (AGIS), and (d) Collaborative Initial Glaucoma Treatment Study (CIGTS) systems. The agreement between each of the systems' categorization of VFD into normal versus defective stages was evaluated (kappa analysis). Since AGIS, CIGTS and eGSS have a difference in the number of stages, we combined some stages to form 'consolidated stages'. The VF global indices such as Mean Deviation (MD), Pattern Standard Deviation (PSD), and Visual Field Index (VFI) were also compared across the systems (Kruskal–Wallis).

Results: The percentage (n) of fields staged as normal with HAP, AGIS, eGSS, and CIGTS were 25% (99), 25% (99), 21% (85), and 16% (63), respectively. Agreement between the systems in classifying a VF into severity stages was substantial between HAP and eGSS (K = 0.75), whereas the rest of the pair-wise agreement ranged between 0.67 to 0.51 (substantial to moderate). Comparison of global indices across the systems showed no statistically significant difference in any of the severity stages except for MD in the normal.no defect category (p < 0.001).

Conclusions: The CIGTS staged abnormal visual fields more severely than eGSS and AGIS. Among the four staging systems, the eGSS seemed to similarly categorize the glaucomatous VFDs like HAP system.

HEALTH AND VISION RELATED QUALITY OF LIFE AMONG DIABETICS AND NON-DIABETIC INDIVIDUALS

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Purpose: Diabetes is a multisystemic vascular disorder potentially affecting physical, mental, emotional and social aspects of health. This study aims to assess the self-reported health and vision related quality of life in diabetic and non-diabetic individuals using questionnaire.

Methods: Health related Quality of life (HRQoL) was assessed using the Rand SF36 questionnaires. The National Eye Institute Visual Function Questionnaire 25 (NEI VFQ 25) was used to assess the Vision related Quality of life (VRQoL).The questionnaires were administered online as a google form, to participants over 30 years of age. Email reminders were sent to non-responding participants and they were encouraged to further share the form to their contacts.

Results: Over a period of 4 weeks, 143participants (mean age: 36.5 ± 16 years) responded to the questionnaires. Out of 143 participants, 41 were excluded from study for not satisfying inclusion criteria. Around 64% of the subjects (N=66) were non-diabetic and only 35% (N=36) had diabetes. Most of the diabetic participants (35.2%, N=36) had less than 5 years duration of diabetes. The HRQoL was significantly less ($p<0.05$) in diabetics had (56.7 ± 9.6) compared to non-diabetic individuals (60.5 ± 9). In addition to general health scores, physical and emotional health was significantly decreased in those with diabetes (Mean score difference: 20 ± 2, $p<0.01$). The mean VRQOL score was 86.3 ± 10.3 among non-diabetics and 89.1 ± 7.6 among diabetic individuals.

Conclusions: Decreased scores in diabetic individuals indicate a tendency for decreased functionality, especially in association with longer duration of diabetes, compared to non-diabetic counter parts.

COMPARISON OF FULL FIELD FLASH ELECTRORETINOGRAM BETWEEN TABLETOP AND HANDHELD DEVICE BY CONTACT LENS ELECTRODE

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Purpose: To compare each waveform amplitude on full field flash electroretinogram (FERG) between tabletop and handheld device by Burian Allen (B-A) contact lens electrode.

Methods: A cross sectional study conducted at L V Prasad Eye Institute. International society for clinical electrophysiology of vision (ISCEV) guided standard reference was followed for each test protocol of FERG procedure on healthy individuals. Dry eyes, intolerant to rigid contact lens or insufficient palpebral aperture height were excluded from the study. Both tabletop (Metrovision) and handheld (LKC) systems were used on a same day using B-A electrode. Ten recordings were averaged for each test protocol and noise level of < 2uv was taken for comparison. All the tests were done monocularly.

Results: 50 eyes of 25 healthy individuals of mean age of 25.63 years (SD $\pm$ 4.96) were recruited in the study. All biphasic wave amplitudes from each scotopic and photopic phase showed statistically significant test result between the two systems except photopic 3.0 flicker response (P=0.06). Amplitude difference was more distinguishable in scotopic phase and predominantly more obvious for b waves (scotopic 0.01, difference of around 190uV). There was no noticeable difference seen on implicit time. Time difference between tabletop and handheld was around six minutes.

Conclusions: Noise level with B-A electrode is much less. Although both the systems are good at picking up wave responses using contact lens electrode, yet tabletop system gives better test results and handheld takes less chair time.

STEREOLITHOGRAPHY BASED 3D-PRINTED MICROFLUIDIC DEVICE TO DEVELOP NANOMICELLES FOR OCULAR DRUG DELIVERY

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Purpose: To develop a portable and automated thermal management platform with a unique microfluidic device to prepare dexamethasone (dex)-loaded nanomicelles for ocular drug delivery.

Methods: Tocopheryl Polyethylene Glycol Succinate (TPGS) and poloxamer-407 were used to prepare micelles in microfluidic device. Solution-A (dexamethasone) and Solution-B (TPGS+ Poloxamer), prepared in solvent, were injected into the microfluidic device through the automatic-syringe pump injector (1ml/min). Both the solutions were mixed in serpentine channel mixer before reaching the reservoir. Thin-layer was formed after solvent evaporation, which was rehydrated with phosphate-buffered-saline for preparation of micelles. Prepared micelles were characterized for their particle size, polydispersity index (PDI), and zeta-potential using dynamic-light scattering techniques, whereas, entrapment efficiency and drug-loading was measured using high-performance liquid chromatography. Ex-vivo permeation studies were performed using goat cornea in Franz-diffusion apparatus and calculated the apparent permeability coefficient. Hen egg test-chorioallantoic membrane (HET-CAM) study was performed to evaluate the toxicity of dex-loaded micelles.

Results: Prepared micelles showed mean particle size of 8.72 ± 1.39nm with PDI of 0.2, and zeta-potential of -15.23 ± 0.11mV. Entrapment efficiency and drug-loading of prepared micelles was found to be 66.79 ± 10.14% and 1.01 ± 0.31%, respectively. In ex-vivo permeation studies, the amount of drug permeated through cornea was found to be 0.14158 µg/cm^2. HET-CAM scoring was found to be zero for micelles, which indicates no toxicity.

Conclusions: Dex-loaded micelles were successfully prepared using stereolithography based 3D-printed microfluidic device which is automated, cost-effective and consumes minimum volume as compared to traditional method.

COMPARISON OF COLORIMETRIC PROPERTIES OF NON-STANDARDIZED ISHIHARA PSEUDOISOCHROMATIC PLATES AND STANDARD ISHIHARA PSEUDOISOCHROMATIC PLATES

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Purpose: Color vision charts that mimic the properties of Ishihara's pseudoisochromatic plates(PIP) are available in the market. The colorimetric properties and clinical validity of these charts are not clearly known. As a prequel, the study aims to compare the colorimetric difference between non-standardized Ishiharaand standard Ishihara PIP.

Methods: The study was conducted between 2020-2021 with a non-standardized PIP procured from local source(PIP1) and a gold standard version (2016 Kanehara & Co., Ltd., Tokyo, Japan, PIP2). Totally 36 dots from each type of plate were selected for analysis based on their size and color through visual inspection. The colorimetric values were measured with a spectrophotometer(Spectra-Scan PR-655). The measured values were analysed with CIE L*a*b* color space. Lightness(L*), red-green(a*), blue-yellow(b*) values, color difference(ΔE) and Weber's contrast between the background and digit were calculated.

Result: The L* values of the PIP1 were found to be higher while the a* and b* values were found to be lower when compared to PIP2 indicating that the charts are more saturated, shifted towards green and blue hues. The color difference of the dots in the PIP1 varied significantly when compared to the PIP2[ΔE-transformation plate (11.70 to 33.98), ΔE-vanishing plate(22.90 to 66.50), ΔE-hidden plate(21.71 to 59.78), and ΔE-diagnostic plate(15.67 to 46.34)]. Weber's contrast for the vanishing plate of the PIP1 was found to be -0.16.

Conclusion: The non-standard PIP had different color properties when compared to the standard version. This has a strong implication in the diagnosis of the condition ina clinical setup.

BARRIERS TO UPTAKE OF EYECARE SERVICES: BASELINE FINDINGS OF AKIVIDU VISUAL IMPAIRMENT STUDY (AVIS)

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Purpose: To report on barriers for uptake of eyecare services in Akividu region in south Indian state of Andhra Pradesh.

Methods: Population-based cross-sectional survey was conducted using Rapid Assessment of Vision Impairment (RAVI) protocol. Multi-stage random sampling method was used to enroll subjects. After clinical examination, the participants with vision impairment were asked on the reasons for not seeking eye care services was asked and documented. This was an open-ended question and the response was marked to one of the listed 13 responses. If the responses were did not match with the list, then documented in verbatim. VI defined as presenting visual acuity (PVA) worse than 6/18 in the better eye.

Results: In total, 2587 participants were examined. The mean (sd) age of the participants was 55.1± 11.3 years; 54.3% were women and 47.3% had no formal education. Prevalence of VI was 13.8% (95% CI: 12.5% to 15.2%). The major barriers for not seeking eye care includes, aware of the problem but can manage (42.3%), no one to accompany (12.2%) and fear of losing eyesight (8.6%).Person related barriers were the significant barriers for not seeking eye care in females compared to males (p<0.05).

Conclusions: Overall, person related barriers such as manage with present vision were the major barrier for seeking eye care. Health promotion activities should include behavior change techniques to address the personal barriers for uptake of eye care services.

BINOCULAR SUMMATION OF LUMINANCE FLICKER: EFFECT OF INTEROCULAR DIFFERENCE IN RETINAL ILLUMINANCE

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Purpose: Binocular summation in the spatial domain fails with increasing interocular differences in retinal illuminance.The purpose of this study was to investigate whether differences in retinal illuminance between the two eyes had a similar effect on binocular summation of luminance flicker.

Methods: onocular and binocular flicker modulation thresholds (FMTs) for cone-enhanced stimuli were measured using the *Flicker Plus* test in the fovea and at four parafoveal locations (5°)using adaptive staircases in 8 observers with normal vision (24–28yrs).A baseline retinal illuminance of 930Td (for a 7 mm pupil diameter)and 7 levels of interocular differences in retinal illuminance (in the range 10 to 80% of baseline)were produced using appropriate neutral density filters over one eye.Binocular summation ratios (BSR's)were calculated as the ratio of the monocular threshold in the best eye to the binocular threshold.

Results: Foveal and parafoveal FMTs increased monotonically with a reduction in retinal illuminance in one eye (for monocular viewing)and with interocular differences in retinal illuminance(for binocular viewing).This change was statistically significant for retinal illuminances <460Td in the eye with the neural density filter (p=0.001).Binocular FMT's(2.28 – 4.28%)were significantly smaller than the corresponding monocular FMT's(4.04 – 7.62%)for all retinal illuminances (p=0.001).The resultant BSRs were significantly larger for interocular differences in retinal illuminance between 10 and 50% (mean ± 1SD: 1.70 ± 0.21) than those ≥ 60% (1.26 ± 0.04).

Conclusions: Binocular summation of luminance flicker appears relatively immune to interocular differences in retinal illuminance.The extent of binocular summation does, however, reduce with increasing interocular differences in retinal illuminance.

CHANGE IN DYNAMIC VISUAL ACUITY WITH AND WITHOUT WATER-INDUCED BLUR

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Purpose: Change detection and depth judgment are factors affecting navigation with water-induced blur such as driving on a rainy day or during deep water diving. In this study, we investigated changes in Dynamic Visual Acuity (DVA) with and without water-induced blur (WIB) in adult human eyes.

Methods: DVA was measured using DYNVA© software for 20 young participants. A Palomar ring-disc target was moved in 4 trajectories (left-right, down-up, right-left & up-down) and 10 different velocities (0.06 to 0.74 m/s), on a Display++ monitor placed at 2m from the participants. For each velocity the smallest target for which the subject identifies the direction of motion correctly was measured with and without WIB. WIB was simulated by splashing water using water sprinkler against a transparent glass tank placed in front of the monitor. The average DVA for 5 repeated measurements was obtained for each target velocity.

Results: On an average across subjects, directions and velocities, the decimal DVA without WIB was 0.12 ± 0.01 was significantly ($p<0.001$) better than decimal DVA with WIB 0.08 ± 0.01. For both with and without WIB condition, a strong and significant negative correlation was found between target velocity and decimal DVA ($r=-0.77$, $p<0.001$). The mean DVA was significantly lower for vertical directions than the horizontal directions ($p<0.01$) for both with and without WIB.

Conclusions: Our results imply that the blur produced as a result of water significantly decreases DVA across all target velocities, which could have significant implications on navigation in environmental conditions with water-induced blur.

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