ARVO International Chapter Affiliate

ARVO-INDIA

Indian Eye Research Group

30th Annual Meeting of Indian Eye Research Group

Evolving Dimensions in Eye Research

27-29 September, 2024

All India Institute of Medical Sciences
New Delhi

Program Book

Organizers

Prof. M. Srinivas

Prof. J. S. Titiyal

Prof. Radhika Tandon

Prof. Namrata Sharma

Prof. Tanuj Dada

Prof. Rohit Saxena

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Pre-Conference Workshop cum Symposium

27th September 2024

ARVO-INDIA

Indian Eye Research Group

30th Annual Meeting of Indian Eye Research Group

"Advanced Techniques to Augment Innovations in Vision Sciences"

Symposium cum Workshop

Program Schedule

Venue: LT-6, 6th Floor, Dr. R.P Centre, AIIMS **Date**: 27th September 2024 (Friday)

Time	Topic	Speaker
08:30-09:00	Registration	
09:00-09:10	Welcome address	Prof. Nabanita Halder
09:10-09:20	Inaugural address	Prof. Tanuj Dada
09:20-09:30	Highlights of Symposium	Prof. T. Velpandian
09:30-11:00 **Session 1**	**Cutting Edge Techniques in Ocular Imaging**	
	Moderator: Prof. Thirumurthy Velpandian	
09:30-10:00	Photoreceptor Cell Loss Is Associated with Müller Cell Vulnerability in Aging Human Retina	Prof. Tapas C Nag All India Institute of Medical Sciences, New Delhi
10:00-10:30	Advancing Ophthalmic Research: Unveiling Retinal Lipid Dynamics and Therapeutic Targets with iMScope™QT ***(Live from Lab)***	Dr. Naatasha Isahak Shimadzu (Asia Pacific) Pte. Ltd.
10:30-11:00	Group photo and coffee break	
11:00-12:00 **Session 2**	**Artificial intelligence and innovative devices**	
	Moderator: Prof. Rohit Saxena	
11:00-11:30	Leveraging Artificial intelligence for Early Detection and Management of Juvenile Open-Angle Glaucoma ***(Live from Lab)***	Prof. Dinesh Gupta International Centre for Genetic Engineering and Biotechnology, New Delhi
11:30-12:00	Applying Eye tracking Technology in Managing Amblyopia	Dr. Premnandhini Satgunam L V Prasad Eye Institute, Hyderabad
12:00-13:00 **Session 3**	**Advanced and alternative approaches in Vision Sciences**	
	Moderator: Dr. P. Sundaresan	
12:00-12:30	Advancing Corneal Regeneration: Bioprinting with Biomimetic Decellularized Cornea Matrix Hydrogel	Prof. Falguni Pati Indian Institute Technology, Hyderabad
12:30-13:00	Vision sciences (*Netravigyan*) in Ayurveda	Prof. Rama Jayasundar All India institute of Medical Sciences, New Delhi
13:00-14:00	Lunch	

14:00-15:00	**Translational and Complementary techniques in Eye Research**	
Session 4	*Moderator: Dr. Subhabrata Chakrabarti*	
14:00-14:30	Multiplex Assays in Ocular Research: LESS IS MORE	Dr. Shiva Balasubramanium ImmunitasBio Pvt Ltd., Bangalore
14:30-15:00	Genomics and Microfluidics: Approaches to Understanding AMR	Prof. Devarshi Gajjar The Maharaja Sayajirao University of Baroda, Vadodara
15:00-16:00	**Newer exploratory Models in Experimental Eye Research**	
Session 5	*Moderator: Prof. N. Angayarkanni*	
15:00-15:30	Navigating the translational route of therapeutic gene editing in resource limited settings	Prof. Debojyoti Chakraborty CSIR Institute of Genomics and Integrative Biology, New Delhi
15:30-16:00	Zooming into retinal architecture at single cell resolution	Dr. R. Sharada Sankara Nethralaya, Chennai
16:00-17:00	**Drug Development and Toxicity: Escalating innovations from Bench to Bedside**	
Session 6	*Moderator: Dr. Inderjeet Kaur*	
16:00-16:30	Polymeric patch: A platform technology to deliver ocular therapeutics.	Prof. Nirmal Jayabalan The Birla Institute of Technology and Science (Pilani), Hyderabad
16:30-17:00	Challenges in Retinal Organoids and Potential Advancements	Dr. Anwar Azad P Aravind Medical Research Foundation, Madurai
17:00-17:10	Concluding Session	Prof. T. Velpandian All India Institute of Medical Sciences, New Delhi
17:10-17:30	High Tea	

Symposium Speaker

Prof. Tapas C Nag

Professor,
Department of Anatomy,
All India Institute of Medical Sciences,
New Delhi, India
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Photoreceptor cell loss is associated with Müller cell vulnerability in aging human retina

Müller cells (MC), a type of macroglia, maintain retinal homeostasis by removing extracellular K+ and glutamate and support neurons in normal physiological activities. Photoreceptor cell loss (PCL) is prominent in aging human retina. It is unknown if they are unable to regulate PCL with aging. Since MC handle many metabolic functions for retinal neurons, it is hypothesised that with aging, they are likely to undergo changes (e.g., oxidative stress, OS) due to continual, overburdened activities. This study examined aging changes in 22 postmortem human retinas (age: 35-98 years) by light and electron microscopy and immunohistochemistry. Observations revealed structural alterations in MC with aging, due to enhanced lipid peroxidation. There was increased lipid peroxidation and nitrative stress in photoreceptors that they invest. Localisation with markers of MC (glutamine synthetase) and OS (4-hydroxy 2 nonenal and 3-nitrotyrosine) suggested increased OS in MC and their surroundings, which might impair the proper functioning of both cellular compartments. MC attempted to protect them from OS via proliferation of smooth endoplasmic reticulum. They accumulated lipids, lipofuscin and autophagosomes, implicating their reduced phagocytic potential and autophagy with aging. Also, autophagosomes were accumulated in photoreceptor synapses, suggesting defective autophagy may lead to their vulnerability. With aging, MC undergo OS, which is a major problem for maintaining photoreceptor cell integrity in aging.

Symposium Speaker

Dr. Naatasha Isahak

Product Management, Analytical & Measuring Instruments Division,
EXPERT Centre, Shimadzu Asia Pacific Pte. Ltd.
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Advancing Ophthalmic Research: Unveiling Retinal Lipid Dynamics and Therapeutic Targets with iMScope™QT

MALDI Imaging, or Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging, is a crucial tool in clinical research, allowing for precise spatial mapping of biomolecules within tissue samples without the need for labeling or staining. The iMScope™ QT, an advanced platform combining high-resolution optical microscopy with MALDI mass spectrometry imaging (MSI), is transforming ophthalmic research by enabling detailed visualization of molecular distributions in ocular tissues. Recently, the iMScope™ QT was employed to study the effects of traumatic optic nerve injury (TONI) on retinal phospholipid composition, revealing significant changes in lipid distribution linked to retinal ganglion cell death and Müller glial cell activation. These findings underscore the iMScope™ QT's essential role in ophthalmology, providing insights into retinal lipid dynamics and identifying therapeutic targets to preserve vision. Its versatility extends to studying various eye diseases, such as age-related macular degeneration, diabetic retinopathy, and glaucoma. The iMScope™ QT's ability to operate in both positive and negative ion modes enhances its capacity to detect and analyze a wide range of biomolecules, making it a valuable asset for advancing diagnostics and treatment strategies in ophthalmology.

Symposium Speaker

Prof. Dinesh Gupta

Group Leader, Translational Bioinformatics Group
International Centre for Genetic Engineering and Biotechnology,
New Delhi, India
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Leveraging Artificial Intelligence for Early Detection and Management of Open-Angle Glaucoma

Open-Angle Glaucoma (OAG), characterized by an open angle of the eye anterior chamber, is a chronic, progressive and irreversible optic neuropathy that can lead to irreversible vision loss if not detected and managed early. The challenges of early diagnosis often result in delayed treatment and poor prognoses. This talk explores how Artificial Intelligence (AI) is transforming the landscape of OAG detection and management. The talk will discuss the integration of AI-driven applications, including machine learning algorithms and deep learning models, in identifying subtle clinical OAG indicators that conventional methods or even human eyes may miss. Emphasis will be placed on the use of AI for enhancing diagnostic accuracy, predicting disease progression, and personalizing treatment plans, thereby optimizing patient outcomes. The talk will describe our recent research on i) classifying juvenile-onset OAG (JOAG) using clustering, ii) deep learning models to identify angle dysgenesis using ASOCT images, and iii) machine learning models to predict long-term trabeculectomy outcomes in JOAG. Following the talk, a demonstration of few applications will also be presented. The talk will also highlight the practical applications of AI in real-world clinical settings and address the ethical and practical considerations of implementing these technologies in ophthalmology. By leveraging AI, we can improve the timeliness and effectiveness of interventions in OAG, ultimately preserving vision and quality of life for affected patients.

This session will interest clinicians, researchers, and healthcare technologists committed to advancing glaucoma care through innovative AI solutions.

Symposium Speaker

Dr. Premnandhini Satgunam

Scientist,
Brien Holden Institute of Optometry and Vision Sciences,
LV Prasad Eye Institute, Kallam Anji Reddy Campus,
Hyderabad, India
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Applying Eye tracking Technology in Managing Amblyopia

Amblyopia is a neurodevelopmental disorder of the binocular visual system. Clinically it is characterized by a reduction in visual acuity, despite best optical correction (in cases of ametropia) or despite best aligned visual direction (in cases of strabismus) and absence of any ocular pathology. The main hallmark for amblyopia is visual acuity reduction of 2 lines or more in the amblyopic eye in comparison with the "good" fellow eye. Patching has remained the gold standard treatment for amblyopia. Monocular visual acuity improvement has remained the main outcome measure of success for the treatment. This talk would show evidence for deficiencies in the visual system of amblyopia that extend beyond visual acuity and to the so-called good eye. Understanding amblyopia through eye movement studies gives newer insights to the understanding of this pathology. Treatments beyond patching will also be discussed. Specifically, treatments aimed at breaking the suppression mechanism of amblyopia will be stressed. Fixational eye movement stability computed from BCEA (bivariate contour ellipse area) shows promise to be a marker for monitoring improvement in amblyopia.

Symposium Speaker

Prof. Falguni Pati

Associate Professor and Head,
Department of Biomedical Engineering,
IIT Kharagpur, India
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Advancing Corneal Regeneration: Bioprinting with Biomimetic Decellularized Cornea Matrix Hydrogel

Vision impairment affects over 1.3 billion people worldwide, and corneal opacity is a major cause. This condition often results from infections, chemical burns, or accidents, and current treatments have their limits. Recent advances in corneal tissue engineering, like cell-based and matrix-based methods, show promise but still face challenges in fully regenerating the cornea. In this talk, I'll introduce a new approach to corneal repair using an advanced bioprinting technique with a decellularized cornea matrix (DCM) hydrogel. Our DCM hydrogel is created through a special process that keeps essential extracellular matrix (ECM) components while removing cellular debris. We use this hydrogel to bioprint both the corneal stroma and epithelium, making a big step forward in tissue engineering. Our method has shown impressive results: high cell viability, effective cell movement, and maintenance of keratocyte function. The bioprinted tissues have well-organized structures and strong tissue-specific gene expression. Importantly, the DCM hydrogel helps prevent scar tissue formation, which is a major challenge in corneal repair. This new bioprinting technology could greatly improve the treatment of corneal issues, including injuries and scars, and reduce the need for donor corneal tissues.

Symposium Speaker

Prof. Rama Jayasundar

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Ayurvedic approach to Ocular health

The world is going through an unprecedented and extraordinary health scenario. Unprecedented because ill health has become a fact of life and many diseases are vying with each other to take the top slot as a serious health hazard. Extraordinary because despite acquiring nuanced details about human biology and availability of sophisticated technologies to study the most subtle structures in the body, diseases are on the increase. At the same time, there is also a growing interest in alternative and holistic approaches to the management of health and disease. All these inevitably brings into focus ayurveda, one of the longest unbroken healthcare systems in the world. One of the eight main branches of Ayurveda is devoted to eye care and management of eye disorders. This talk will articulate the ayurvedic approach to ocular health.

Symposium Speaker

Dr. Sivasankar Baalasubramanian

Founder & CEO,
ImmunitasBio Private Limited,
Bangalore, India
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Multiplex Assays in Ocular Research: LESS IS MORE

Multiplex assays enable simultaneous measurement of multiple biomarkers from small-volume samples, addressing a critical challenge in ocular research where sample availability is often limited. These assays are highly valuable for biomarker discovery, pharmacokinetic studies, and understanding inflammatory responses in diseases like age-related macular degeneration (AMD), diabetic retinopathy, and glaucoma. The advantages of multiplex assays include high sensitivity, cost and time efficiency, and reduced sample volume requirements, making them ideal for analyzing delicate ocular tissues such as aqueous humor and tear fluid. However, challenges like standardization, validation, and complex data interpretation need to be addressed to maximize their potential. Overall, multiplex assays offer a robust solution for comprehensive biomarker analysis in ocular research, enhancing our understanding of ocular diseases and their treatment.

Symposium Speaker

Prof. Devarshi Gajjar

Professor,
Dept of Microbiology and Biotechnology,
Maharaja Sayajirao University of Baroda,
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Genomics and Microfluidics: Approaches to Understanding AMR

Antimicrobial resistance (AMR) is a global health crisis and both fungal and bacterial pathogens have become less susceptible to all available drugs. Due to sequencing technology improvements, microbial whole-genome sequencing (WGS) has become a central tool to understand and possibly control AMR. Genomic analysis of two such pathogens 1) Pan drug-resistant (PDR) *Klebsiella pneumoniae* and 2) Azole resistant - *Fusarium Solani* (causing keratitis) will be discussed. Pan drug-resistant (PDR) *Klebsiella pneumoniae* has become increasingly common due to the convergence of virulence and resistance plasmids. With the help of Illumina and Nanopore sequencing, we characterized the ST147 lineage with the unusual presence of multiple copies of blaNDM, blaOxa etc both on chromosome and plasmid. *Fusarium* spp. is one of the most common keratitis-causing fungus in India but the WGS data is scarce. With the help of Illumina sequencing, we characterized azole resistance which showed the presence of multiple mutations (Indels and SNPs) in CYP51A in all four *Fusarium* isolates. The genomic data provides a fundamental understanding of the AMR genetic makeup which needs to be validated using a compact, easy and convenient system. Microfluidics has emerged as a powerful and very promising platform used to study AMR and innovations in microfluidics have developed point of care testing. Microfluidics provides us with dynamic and single cell level information, high through put screening, rapid, cost-effective analysis with minimal sample use, polymicrobial interactions etc. Some of the latest antimicrobial susceptibility testing strategies for detecting AMR genes are Hybridization Chain Reaction (HCR), Recombinase Polymerase Amplification (RPA) etc. To summarize, genomics and microfluidics are two precision advanced tools in the fight against AMR.

Symposium Speaker

Prof. Debojyoti Chakraborty

Principal Scientist & Assistant Professor,
CSIR- Institute of Genomics and Integrative Biology,
New Delhi, India
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Navigating the translational route of therapeutic gene editing in resource limited settings

Genome and transcriptome editing toolboxes are expanding at an extremely rapid pace but the road to transformative clinical translation is limited by multiple factors ranging from comprehensively profiling the safety of these advanced technologies to developing strategies for equitable accessibility options for patients from diverse backgrounds. Our work addresses both these aspects through protein engineering of CRISPR effectors and their derivatives to developing pipelines for taking lab level discoveries to the clinic through coordinated programs with multiple stakeholders in the patient communities, academia, government and industry. Through focused disease associated gene therapy strategies, we are gradually developing and improving first generation platform technologies to make them affordable and accessible to patients suffering from inherited disorders.

Symposium Speaker

Dr. Sharada Ramasubramanyan

Senior Principal Scientist and In-Charge,
RS Mehta Jain Department of Biochemistry and Cell Biology,
Vision Research Foundation, Sankara Nethralaya,
Chennai, India
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Zooming into retinal architecture at single cell resolution

The neurosensory retina is a highly specialized tissue of the eye with a convoluted architecture of diverse cell types and their anatomical circuits makes it difficult to separate and study them. A deep understanding of the intricate molecular signalling and knowledge of gene expression profile in each of the individual neural retinal cell types is important in understanding the pathophysiology for vision threatening diseases such as Diabetic Retinopathy (DR), Retinal Vein Occlusion (RVO), and Age-related Macular Degeneration (AMD). Single-cell (sc) sequencing has revolutionized ophthalmic research in the last few years and many groups have adapted the technology to accelerate molecular research in eye samples, especially to address dynamic changes in gene or protein expression between healthy and diseased-state. In this presentation, the workflow of single cell RNA sequencing would be briefly discussed and will focus on highlighting the utilization of this technique in decoding the retinal architecture at a single cell resolution. The talk would also include discussions on our recent attempt in performing sc-RNA sequencing (sc-RNA seq) in retina of diabetic donor eyes and emphasize some of the practical concerns in terms of sample preparation.

Symposium Speaker

Prof. Nirmal Jayabalan

Associate Professor,
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Polymeric patch: A platform technology to deliver ocular therapeutics

Though eye drops are convenient formulation to treat anterior segment eye diseases, but they have several limitations, such as precorneal clearance, poor bioavailability, etc. Thus, it necessitates frequent administration, making it less patient compliant. Additionally, many drugs have poor aqueous stability, and eye drops containing preservatives are known to cause ocular toxicity. To overcome these challenges, we have developed polymeric patches with different biomaterials, shapes, and patterns for delivering ocular therapeutics with improved therapeutic outcomes. They were fabricated using conventional solvent-casting and advanced techniques like 3D printing. A corneal patch platform was developed to enhance the corneal residence and stability of ocular therapeutics. Further, to improve the ocular residence and permeation compared to eye drops, a conjunctival patch platform was developed. Moreover, the dry nature of the patch allows the development of a preservative-free formulation that can reduce the incidence of toxicity. The polymeric patch platform fabricated using 3D printing is easy to reproduce, preservative free, and has scope for personalization; thus, it could be a potential patient-friendly alternative to eye drops. However, more studies are required to determine the dosage regimen, packaging, and biocompatibility of polymeric patches for their translation into clinics.

Symposium Speaker

Dr. Anwar Azad Palakkan

Department of Immunology & Stem Cell Biology,
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Challenges in Retinal Organoids and Potential Advancements

Recent advancements in organoid technology have revolutionized our understanding of organ development and disease. Organoids, which are three-dimensional miniaturized versions of organs grown in vitro, offer an unprecedented opportunity to model complex biological processes and study various diseases in a controlled environment. However, despite the tremendous progress in this field, organoids—including retinal organoids—still face significant limitations. These challenges include poor structural organization, incomplete maturation, insufficient functional integration, and limited vascularization, all of which hinder their full potential as models for human biology and disease.

In this presentation, I will share insights from my work on renal organoids, where we have successfully addressed similar challenges by refining various aspects of the organoid development process. By applying strategies from renal organoid research—such as optimized differentiation protocols, enhanced vascularization, and improved tissue organization—we can potentially overcome these limitations in retinal organoids, thereby improving their fidelity as models for human eye diseases. Such advancements could pave the way for more accurate and effective treatments for retinal disorders and contribute significantly to the field of regenerative medicine.

Program Schedule

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ARVO-INDIA

Indian Eye Research Group

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Program Schedule- Day 1

ARVO-INDIA 2024 MEETING			
Day 1 - 28th September 2024			
Venue: JLN Auditorium			
Time	**Event**	**Speaker**	**Title**
09:00-09:30	Inauguration	Prof. J. S Titiyal	Welcome Address
		Prof. M. Srinivas	Inaugural Address
		Prof. Thirumurthy Velpandian	About ARVO INDIA & Meeting Overview
Session 1		***Chair:*** *Prof. Devarshi Gajjar, Prof. Karnika Saigal*	
09:30-09:50	**Keynote lecture-1**	***Prof. Radhika Tandon*** *All India Institute of Medical Sciences, New Delhi*	*Research Perspectives in Ophthalmology: current status and way forward*
09:50-10:00	Free paper-1	**Swagata Ghosh** *Aravind Medical Research Foundation, Madurai*	*Corneal transcriptomic signatures in Fusarium keratitis patients with distinct disease trajectory: deciphering protective and pathogenic host-response*
10.00-10.10	Free paper-2	**Lakshminarayanan Gowtham** *LVPEI, Hyderabad*	*Efficacy of Miltefosine in Ex vivo Human Corneal Model of Acanthamoeba Keratitis using Clinical Isolates*
10.10-10.20	Free paper-3	**Pinal Trivedi** *The Maharaja Sayajirao University of Baroda, Vadodara*	*Impact of drug and steroid therapies on Fusarium keratitis in an ex vivo caprine cornea model*
10.20-10.30	Free paper-4	**Saumya Srivastava** *All India Institute of Medical Sciences, New Delhi*	*Development of lateral flow strip test for the diagnosis of acanthamoeba keratitis*
10.30-11.00	Group Photo		
11.00-11.30	High Tea		
Session 2		***Chair:*** *Prof. Nabanita Halder, Dr. Nirmal Jayabalan*	
11.30-12:15	**Bireswar Chakrabarti Oration**	**Prof. Uday Kompella** *University of Colorado Denver Anschutz Medical Campus, CO, USA*	*Ocular drug and gene delivery*
12:15-12:25	Free paper-5	**Sai Shreya Cheruvu** *Birla Institute of Technology Sciences, Pilani*	*Light responsive in-situ hydrogel: an injectable depot platform for intravitreal drug delivery*

12:25-12:35	Free paper-6	**Bhaskar Sharma** *All India Institute of Medical Sciences, New Delhi*	*Impact of immunocompetent cells in regulating age-related choroidal capillary changes in rat*
12:35-12:45	Free paper-7	**Tapas K Roy** *All India Institute of Medical Sciences, New Delhi*	*Functional evaluation of peptide transporter using pharmacological tools after their topical & intravenous administrations in rabbits*
12:45-12:55	Free paper-8	**Manthan S Hiremath** *Birla Institute of Technology Sciences, Pilani*	*Pre-clinical ocular pharmacokinetics, safety, and efficacy of topical trpv1 antagonist ser 114 for ocular pain*
13:00-14:00	Lunch		
14:00-15:00	Poster Session-I		
Session-3		***Chair:*** *Prof. Rohit Saxena, Dr. Premnandhini Satgunam*	
15:00-15:20	**Keynote lecture-2**	**Dr. Srinivasan Senthilkumari** *Aravind Medical Research Foundation, Madurai*	*MicroRNA - based Therapeutics for Steroid-induced Ocular hypertension/ Glaucoma–Insights from Ex vivo Studies of Human Eyes*
15:20-15:40	Free paper-9	**Disha Singh** *LVPEI, Hyderabad*	*Production of GMP grade limbal stromal stem cells and hydrogel for clinical use*
15:40-15:50	Free paper-10	**Divya** *PGIMER, Chandigarh*	*Proteomic insights into outer blood-retinal barrier disruption: the role of hypoxia and hyperglycaemia in diabetic macular edema*
15:50-16:00	Free paper-11	**Nisha Sinha** *Dr. Shroffs Charity Eye Hospital, Delhi*	*Transcription factor klf4 as a modulator of cellular senescence in trabecular meshwork cells*
Session-4		***Chair:*** *Prof. K Dharmalingam; Prof. T. Velpandian*	
16:00-16:45	**VN Reddy Oration**	**Prof. N. Angayarkanni** *Medical Research Foundation, Sankara Nethralaya, Chennai*	*Homocysteine and Paraoxonase: A Dual Force in Ocular Pathology Dynamics*
16:45-17:30	**Symposium on HRMS using OrbiTrap Technology** (Thermo India Pvt. Ltd.)	**Prof. K. Dharmalingam** *Aravind Medical Research Foundation, Madurai*	*Proteomics of Ocular Extracellular Vesicles*
		Prof. T. Velpandian *All India Institute of Medical Sciences, New Delhi*	*Ocular Pharmaco-omics for drug discovery*
		Dr. Inderjeet Kaur *LVPEI, Hyderabad*	*Proteomics of Vitreous in AMD-Analysing key pathways*
17:30-18:30	iQuest/Board Meeting		
19:30 onwards	Gala Dinner (Faculty Club)		

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Program Schedule- Day 2

ARVO-INDIA 2024 MEETING			
Day 2 - 29th September 2024			
Venue: JLN Auditorium			
Time	**Event**	**Speaker**	**Title**
Session 5		***Chair:*** *Dr. G. Kumaramanickavel; Dr. Subhabrata Chakrabarti*	
09:00-09:50	**D. Balasubramanian Oration**	**Dr. P. Sundaresan** *Aravind Medical Research Foundation, Madurai*	*Molecular Genetics in Ocular diseases: Laneway for Personalised Medicine*
09:50-10.00	Free paper-12	**Samir Bera** *LVPEI, Hyderabad*	*The involvement of crystallin gene variants and genotype phenotype correlation in PCG pathogenesis*
10.00-10:10	Free paper-13	**Krishna Haridas** *ONGC, Vision Research Foundation Chennai*	*Discovery of fusion transcript as a diagnostic biomarker for uveitis: an inflammatory eye disease*
10:10-10:20	Free paper-14	**Daipayan Banerjee** *Aravind Medical Research Foundation, Madurai*	*Epigenetic Alternation is Pterygium: Genome-wide methylation profiling reveals potential oncogenic pathways*
10:20-10:30	Free paper-15	**Anshu Yadav** *MDU, Rohtak*	*Association of interleukin-8 polymorphisms with age-related macular degeneration in north Indian patients*
10:00-11:00	High Tea		
Session 6		***Chair:*** *Prof. T. Velpandian, Prof. N Angayarkanni*	
11:00-11:45	**S.S. Badrinath Oration**	**Prof. Ramanjit Sihota** *Shroff Eye Centre, Delhi*	*Experiential understanding of Primary angle closure disease*
11:45-11.55	Free paper-16	**Pranav K Pandey** *All India Institute of Medical Sciences, New Delhi*	*Bioengineered human corneal graft preparation strategy for extended utilization of human donor corneas: an in vitro study*
11.55-12.05	Free paper-17	**Ramkailash Gujar** *Dr. Shroffs Charity Eye Hospital, Delhi*	*Deep learning-based detection of ocular surface squamous neoplasia from ocular surface images*

12.05-12.15	Free paper-18	**Gorati Vani** *LVPEI, Hyderabad*	*Correlating the ocular surface changes associated with visual display terminal users*
12:15-13:15	Poster Session-II		
13:15-14:00	Lunch		
Session-7		***Chair:*** *Dr. Namrata Sharma, Dr. Inderjeet Kaur*	
14:00-14:20	Keynote Lecture 3	**Dr. Anil Tiwari** *Dr. Shroffs Charity Eye Hospital, Delhi*	*Clear Sight, Bold Vision: Harnessing Emerging Expertise to Advance Ophthalmic Innovation*
14:20-14:30	Free paper-19	**Karuvel Kannan Sarawathi** *Aravind Medical Research Foundation, Madurai*	*Genomic and transcriptomic profiling identifies distinct molecular signatures in recurrent ocular adnexal b cell lymphoma*
14:30-14:40	Free paper-20	**Aman Verma** *Dr. Shroffs Charity Eye Hospital, Delhi*	*Assessing reactive oxygen species-related genes role in the aggressiveness of ocular surface squamous neoplasia (OSSN)*
14:40-14:50	Free paper-21	**Jyotirmayee Talapatra** *LVPEI, Bhubaneswar*	*Evaluating the functional significance of TET1 in human retinoblastoma*
14:50-15:00	Free paper-22	**Bilal Ahmed** *All India Institute of Medical Sciences, New Delhi*	*Glutathione peroxidase-4 as a biomarker in retinoblastoma: association with pathological parameters and patient outcome*
15:00-15:30	Concluding Session		
15:30-15:35	Vote of thanks		
15:35-16:00	High Tea		

Orations and Keynote Lectures

Bireswar Chakrabarti Oration 2024

Prof. Uday Kompella

Professor of Pharmaceutical Sciences, Ophthalmology, and Bioengineering
Co-director, Colorado Centre for Nanomedicine and Nanosafety,
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Ocular drug and gene delivery

Delivery of therapeutic agents to the eye is challenging due to multiple static and dynamic barriers, with the challenges escalating drastically for macromolecules. Delivery of small molecules is also inefficient, with about 5% or less of the applied drug reaching the anterior segment of the eye. To date, small molecules cannot be delivered to the back of the eye with an eye drop, to effectively treat retinal diseases. Our research focused on novel delivery systems, including nanomedicines, and alternative routes of administration such as transscleral and suprachoroidal routes for drug delivery to the eye. Additionally, we developed pharmacokinetic/mathematical models to predict drug delivery to the eye. In the field of gene therapy, we have explored non-viral as well as viral vectors such as empty viral capsids for gene delivery. This presentation will discuss some of the key findings in our journey to understand and advance ocular drug and gene delivery. The delivery systems to be highlighted include polymeric nanoparticles, viral vectors, implants, and contact lenses.

SS Badrinath Oration 2024

Prof. Ramanjit Sihota

Senior Consultant (Former Head of Glaucoma Unit, Dr. RP Centre, AIIMS)
Shroff Eye Centre, New Delhi, India
Email: rjsihota@gmail.com

Experiential understanding of Primary angle closure disease

Primary angle closure disease was very sparsely researched till about 30 years ago, and since then there has been a gradually increasing understanding. Our research since 1990 has been at the forefront of decoding Primary angle closure disease - anatomically with the latest imaging techniques available, subtyping and classifying all aspects of the disease, histopathology and electron microscopic work on the trabecular meshwork, physiological studies to determine why an anatomical variation leads to raised intraocular pressure, biochemical changes associated with PACD, genetic studies looking at why only certain eyes with similar anatomy develop glaucomatous optic neuropathy and finally studies on the long term management and prognosis of each subtype of primary angle closure disease.

D Balasubramanian Oration 2024

Dr. P. Sundaresan

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Molecular Genetics in Ocular diseases: Laneway for Personalised Medicine

Many types of eye disease can be inheritable and most cases of blindness in infants are caused by inherited eye diseases such as cataract, retinal degeneration, glaucoma and eye malformations. In adults, leading causes of blindness are age-related macular degeneration, cataract and glaucoma. Among the 19,000 human genes, more than 600 are associated with ocular disorders. Genetics in ocular disorders are becoming increasingly important for an accurate molecular diagnosis and for the development of novel genotype –specific treatments. For the past two decades, we extensively studied the molecular genetics of various eye diseases in Indian population and identified candidate genes for Congenital Hereditary Endothelial Dystrophy (CHED) Primary Angle Closure Glaucoma (PACG) and Pseudo exfoliation (PXF) using Next Generation Sequencing technology. We have performed gene expression studies and functional analysis using Zebra fish model for many eye diseases. In addition, we also provide quality gene testing for some of the inherited eye disorders. Currently our research focuses on the leading cause of inherited retinal dystrophies and understanding the molecular mechanism underlying the disease pathogenesis. Our research laboratory offers genetic counselling based on moral and ethical values to provide possible solutions for people facing risk. Furthermore, our endeavour aims to construct a gene registry of the visual system specific to ethnicity. During the oration, I will discuss in detail how to translate the basic research from bench to bedside.

VN Reddy Oration 2024

Prof. Angayarkanni Narayanasamy

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Homocysteine and Paraoxonase: A Dual Force in Ocular Pathology Dynamics

Homocysteine (Hcy), a reactive thiol by-product of the cellular methylation is formed from methionine and accumulates upon metabolic dysregulation. It acts as a double-edged sword: as a pro-oxidant altering cellular redox status and in homocysteinylation via its cyclic ester, homocysteine thiolactone (HCTL) forming protein adducts (N-homocysteinylated or S-homocysteinylated proteins) paving way for several pathologies associated with hyperhomocysteinemia (HHcy). Serum Paraoxonase-1 (PON1), a major thiolactonase enzyme sitting on the HDL fraction of circulating lipoproteins as well as the cellular Paraoxonase, PON2 can prevent this modification attributing to the protection. This act and counteract is explored in the anterior and posterior ocular pathologies.

Keynote Lecture 1

Prof Radhika Tandon

Professor of Ophthalmology,
Dr Rajendra Prasad Centre for Ophthalmic Sciences,
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Research Perspectives in Ophthalmology: current status and way forward

As with all fields of medicine, Research in Ophthalmology has made significant strides in recent years, advancing our understanding and treatment of a wide range of eye diseases in leaps and bounds. The lecture will review the current state of ophthalmic research in India and the world, highlighting key breakthroughs and emerging trends that are shaping the future of vision care. A major focus will be on innovations in disease management, including gene therapies, implants, and stem cell treatments, which offer new hope for conditions that were hitherto deemed untreatable. Additionally, advances in artificial intelligence (AI) and big data analytics are not only transforming diagnostics, but also providing a scope for precision medicine personalized treatment plans. AI-powered tools are now being used to detect a plethora of diseases at earlier stages, improving accessibility for millions of patients and providing a platform for equipoise in eye care. The role of telemedicine in improving access to eye care in remote and underserved regions and providing integrated health care service delivery modalities. Despite these advancements, several challenges remain. The talk will discuss current gaps in knowledge, such as the need for more precise biomarkers for early disease detection, the ongoing quest for neuroprotective therapies, cost effective service delivery models, research funding to support research and development in orphan diseases. Another critical area is the integration of ophthalmic research with broader systemic health studies, particularly in understanding the ocular manifestations of systemic diseases like diabetes and hypertension, ageing, dementia and developmental disorders. Looking ahead, collaboration between clinical and translational research will be key in overcoming these challenges. By

fostering interdisciplinary approaches and leveraging cutting-edge technologies, ophthalmology is poised to make further leaps in patient care and treatment. The presentation will provide a comprehensive overview of the current research landscape in India and the world and propose strategies for addressing future challenges, with the goal of providing affordable eye health care to all and improving outcomes for patients with ocular diseases.

Keynote Lecture 2

Dr. S. Senthilkumari

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MicroRNA-based Therapeutics for Steroid-induced Ocular hypertension /Glaucoma–Insights from *Ex vivo* Studies of Human Eyes

Steroid therapy is the mainstay in the management of systemic and ocular autoimmune and inflammatory diseases. Long-term use of these steroids can cause elevated IOP termed as steroid-induced ocular hypertension (SI-OHT) and if left untreated, it can lead to secondary open angle glaucoma or SIG. There have been limited studies investigating the genetic basis of the steroid response. In some patients, glucocorticoid receptor gene mutations might influence the IOP elevation response, but the evidence for this is not clearly understood. Even though POAG and SIG share similar clinical presentations, the molecular and genetic mechanisms responsible for the differential GC responsiveness in susceptible individuals are not well understood. MicroRNAs (miRNAs) are small non-coding RNAs, which regulate gene expression either by mRNA degradation or translational repression. They are detected in most of the biological fluids including glaucoma-affected tissues/fluids such as aqueous humor, tears and retina where they are preserved in extracellular vesicles, or bound to carrier proteins thereby providing a remarkable stability to miRNAs. These properties make miRNAs suitable bio-markers for many diseases including ocular diseases.

Given that no genetic markers allow for the identification of GC responders, our lab has utilized the human organ-cultured anterior segment (HOCAS), an *ex vivo* model system to determine the GC responsiveness of the donor eyes for studies into unraveling the miRNA(s) role in steroid response. In this talk, our experiences in understanding the role of miRNAs in GC responsiveness and the feasibility of developing miRNA-based therapeutics for the management of steroid-induced OHT/glaucoma will be discussed.

Keynote Lecture 3

Dr. Anil Tiwari

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ECM Remodelling and Immune Response in Vernal Keratoconjunctivitis (VKC) Pathophysiology

Vernal Keratoconjunctivitis (VKC) is a chronic, allergic inflammatory disease of the ocular surface. This study explores the role of extracellular matrix (ECM) remodelling and immune response in VKC, focusing on the upregulation of key molecular markers and the potential impact of medication in reversing the disease's pathophysiology. Impression cytology samples from VKC patients' conjunctiva were collected of four controls, five unmedicated patients and seven medicated patients. Post RNA extraction, these samples were sent for transcriptomic profiling. Differentially expressed genes related to ECM remodelling and immune cell infiltration were identified. Validation was performed using qRT-PCR on impression cytology, immunohistochemistry (IHC) on tissue biopsies, and zymography on tears from active VKC patients. The analysis revealed significant upregulation of Tenascin C, Fibronectin, MMP15, MMP12, and MMP2, indicating active ECM remodelling and an ongoing inflammatory response in VKC. These molecules play crucial roles in tissue remodelling, fibrosis, and immune cell regulation within the conjunctival stroma, highlighting their importance in VKC pathogenesis. This study emphasizes the role of ECM remodelling and immune cell infiltration in VKC, focusing on the upregulated expression of Tenascin C, Fibronectin, MMP15, MMP12, and MMP2. The findings suggest that targeted therapies may help downregulate these molecules, offering a potential approach to reverse or reduce VKC's pathological effects.

Proteomics Symposium Speaker

Prof. Inderjeet Kaur
Prof. Brien Holden Eye Research Centre,
L V Prasad Eye Institute,
Hyderabad, India.
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Proteomics of vitreous humor in age-related macular degeneration- Analyzing key pathways

Age-related macular degeneration (AMD) is a known inflammatory and proangiogenic retinol-choroidal disease. This study aimed to analyze the vitreous proteome for the identification of key pathways in disease pathogenesis. Plasma and vitreous humor (VH) samples were collected during partial vitrectomy for cataract management from AMD patients (n=58 & 3 respectively) and controls (n=60 & 3). VH global proteome profiling was performed among patients and controls (n=3 each) by LC-MS/MS and novel and differentially regulated proteins were identified using MaxQuant and pathway analysis was done by GO, Reactome and IPA. Data was analyzed using appropriate statistical tools and further correlated with clinical outcome. Identified extracellular matrix (ECM) and other inflammatory proteins were validated in the plasma by multiplex ELISA. We identified a total of 758 different proteins with at least two unique peptides for AMD and 902 for controls. 38 of these were significantly dysregulated among patients and controls with a fold change of $\geq$1.5, of which 23 were upregulated and 15 downregulated. 15 novel ocular proteins were identified. Besides ECM and complement, the top significantly dysregulated proteins (*p-value*: ≤0.05) included those involved in lenticular and non-lenticular functions (CRYAA, CRYAB, CRYBA1) angiogenesis (COL18A1, HSPG2), apoptosis (DKK3, TGFB2), and cell movement of endothelial cells (CDH2). Multiplex Elisa confirmed a significant (p-value <0.05) upregulation of alternate complement pathway and ECM proteins in AMD patients. The identified AMD-associated proteins/pathways provide a further insight into disease pathogenesis. However, their role in predictive testing and therapeutics for the disease is warranted.

Proteomics Symposium Speaker

Prof. K. Dharmalingam

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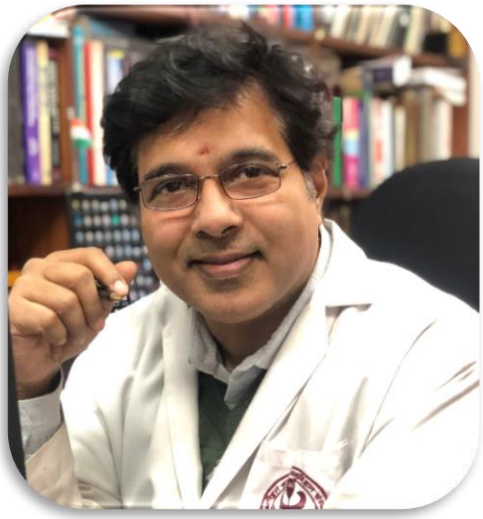
Proteomics of Ocular Extracellular Vesicles

Extracellular vesicles are non-replicating lipid bilayer nanovesicles produced by all cells. EVs have been gaining importance since they play a central role in the fundamental aspects of living systems. Further, their impairment leads to altered immune response and disease. In the context of eye diseases, particularly fungal infections, we are exploring the role of EVs in disease onset, progression, and immune modulation. In this talk, I will present the recent data from our lab exploring the proteome of EVs from both the host and the fungus. The use of Mass Spectrometry will be highlighted during the talk.

Proteomics Symposium Speaker



Prof. Thirumurthy Velpandian

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Exploring Multi-Omics Dimensions with Mass Spectrometry: Cutting-Edge Discoveries from Our Bioanalytical Lab

High-resolution mass spectrometry (HRMS) is a powerful tool for comprehensive OMICS analyses, enabling detailed insights into the complex pathophysiology of ocular diseases, even from micro-volume samples. Using an Orbitrap Fusion Tribrid mass spectrometer (ThermoFisher Scientific), we conducted two experiments: (1) metabolomic, proteomic, and lipidomic profiling of aqueous humor and plasma from patients with primary open-angle glaucoma (POAG), primary angle-closure glaucoma (PACG), and cataract controls; and (2) pharmacoproteomic analysis of aqueous humor from endotoxin-induced uveitis (EIU) rat models treated with topical dapsone or prednisolone. The glaucoma study identified novel pharmacological targets, specifically monoamine interactions with trace amine-associated receptors (TAAR), which play a role in intraocular pressure regulation. Proteomic analysis uncovered unique O-glycosylated proteins (mucins), dysregulated lipoproteins, and proteins linked to Wnt signaling and fibrotic pathways. Lipidomic data revealed specific phosphocholines and sphingomyelins as potential glaucoma biomarkers. In the uveitis model, pharmacoproteomics demonstrated that dapsone exhibited anti-inflammatory effects comparable to prednisolone, suggesting its potential as an alternative therapeutic option for uveitis, without the side effect of increased intraocular pressure.

These findings broaden our understanding of glaucoma and uveitis mechanisms, offering pathways for future therapeutic innovations.

Abstracts
(Free papers)

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Indian Eye Research Group

30th Annual Meeting of Indian Eye Research Group

Free Papers

Abstract ID	Name of Presenter
FP-01	Swagata Ghosh
FP-02	Lakshminarayanan Gowtham
FP-03	Pinal Trivedi
FP-04	Saumya Srivastava
FP-05	Sai Shreya Cheruvu
FP-06	Bhaskar Sharma
FP-07	Tapas Kumar Roy
FP-08	Manthan S Hiremath
FP-09	Disha Singh
FP-10	Divya Ranga
FP-11	Nisha Sinha
FP-12	Samir Bera
FP-13	Krishna Haridas
FP-14	Daipayan Banerjee
FP-15	Anshu Yadav
FP-16	Pranav K Pandey
FP-17	Ramkailash Gujar
FP-18	Gorati Vani
FP-19	Karuvel Kannan Saraswathi
FP-20	Aman Verma
FP-21	Jyotirmayee Talapatra
FP-22	Bilal Ahmed

FP-01

Corneal transcriptomic signatures in fusarium keratitis patients with distinct disease trajectory: deciphering protective and pathogenic host-response

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Purpose: To identify host-response factors that contribute to favourable or unfavourable treatment outcome in Fusarium keratitis patients.

Methods: Corneal swab and epithelial scrapings are collected from clinically identified fungal keratitis patients and grouped into non-severe (mild-moderate) and severe categories based on ulcer size, depth and status of inflammation. Following pathogen identification Fusarium keratitis patients are followed throughout treatment to further categorize them into healed and not-healed (Total Penetrating Keratoplasty-TPK) cases. Three replicates of pooled total RNA (3-4 patients each) from each category are used for mRNA-sequencing along with donor corneal RNA as healthy controls. Unsupervised hierarchical clustering and differential gene expression analysis relative to healthy control and non-severe patients with healed outcome are used to identify potential protective and pathogenic changes. Quantitative RT-PCR analyses are underway for validation in individual patients.

Results: We identified potentially protective gene-expression changes in the non-severe patients with healed outcome that are either lacking or not-significant in patients requiring TPK, irrespective of whether they presented non-severe or severe symptoms. On the other hand, we found potentially pathogenic changes in the gene-expression specifically in our TPK categories that were absent in both non-severe and severe patients who healed with treatment. Enriched in the protective gene list are previously unknown players of corneal host-response which include potential regulators of innate immunity and inflammation. In addition, metabolic genes with potential role in drug metabolism and tissue homeostasis and regulators of innate and adaptive immunity are implicated in unfavourable changes.

Conclusions: Our data identifies distinct gene-expression changes in the host tissue associating with specific disease trajectory.

FP-02

Efficacy of Miltefosine in Ex vivo Human Corneal Model of *Acanthamoeba* Keratitis using Clinical Isolates

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Purpose: The US Food and Drug Administration (USFDA) granted miltefosine orphan drug designation in 2016 for treating Acanthamoeba keratitis. This study evaluates miltefosine's in vitro and ex vivo efficacy against clinical isolates of Acanthamoeba keratitis and its safety profile to rationalize its localized ocular application.

Methods: *Acanthamoeba* spp. isolated from corneal scrapings of keratitis patients (n=17) were cultured axenically, genotyped, and tested for miltefosine's minimal cysticidal and trophozoicidal concentrations (MCC and MTC). Safer concentrations of miltefosine were determined using human corneal epithelial (HCE) cells at four incubation points. *A. castellanii* (T4) trophozoites and cysts were challenged on confluent monolayers of HCE and *ex vivo* human corneal models in the presence and absence of miltefosine for 24hrs. Cytopathic effects and changes in the corneal morphology were evaluated using microscopy and histopathology analysis.

Results: The majority of *Acanthamoeba* isolates tested were T4 genotypes (82.3%). MTC90 and MCC90 of miltefosine were 0.125 and 4mg/mL respectively. Miltefosine was found safe on HCE at 0.0625 and 0.125mg/mL for 4 and 0.25hrs, respectively. Microscopical findings showed *A. castellanii* trophozoites destroyed the cellular structures of HCE within 24hrs without miltefosine. Drug pre-treatment prevented the initiation of infection at both the tested concentrations (0.0625 and 0.125mg/mL). The histopathological analysis confirmed that miltefosine pre-treatment and post-treatment effectively prevented the infection spread.

Conclusions: Miltefosine was effective against *Acanthamoeba* trophozoites and cysts *in vitro* and *ex vivo*, with >30-fold higher cidal concentration for cysts compared to trophozoites. At the effective trophozoicidal concentration, the drug was safe for HCEs and *ex vivo* corneal tissue.

FP-03

Impact of drug and steroid therapies on fusarium keratitis in an ex vivo caprine cornea model

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Purpose: *Fusarium* keratitis is a corneal infection. *Fusarium* is less susceptible to available antifungals. It is important to understand immunological properties of cornea during *Fusarium* keratitis. Current study features an ex vivo caprine cornea infection model to study *Fusarium* keratitis.

Methods: The established ex vivo explant model was used to test efficacy of antifungal drugs (Voriconazole, Posaconazole, Natamycin & Amphotericin B) and steroids (Dexamethasone Betamethasone, Fluoromethalone), Lupeol, and Gallic acid, followed by H&E staining and cytokine profiling. The levels of cytokines/chemokine such as IL-1ß, IL-17, IL-6, IFN-γ, TGF-ß, IL-4, IL-10 and MCP-1 were analysed by ELISA in uninfected and infected corneas.

Results: Corticosteroids increased fungal invasion compared to untreated control corneas and certain combinations of antifungals and corticosteroids affect corneal fungal invasion differently. For instance, the combination of Posaconazole with dexamethasone, betamethasone, or Fluoromethalone, as well as the combination of Voriconazole with the same corticosteroids, appear to reduce the extent of fungal invasion into cornea. In contrast, other combinations of antifungal and corticosteroid treatments seem to worsen the fungal penetration into corneal tissue. Lupeol can increase immune response and decrease corneal fungal infections when used with antifungals. our study suggests an inverse relationship between concentration of Gallic acid and extent of fungal invasion in cornea.

Conclusions: An overabundance of pro-inflammatory cytokines may result in an excessive inflammatory response and harm to tissues. However, completely inhibiting pro-inflammatory cytokines may potentially compromise immune system. This study may contribute to development of an immunotherapy for *Fusarium* keratitis, which might provide a powerful therapeutic.

FP-04

Development of lateral flow strip test for the diagnosis of *Acanthamoeba* keratitis

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Purpose: Acanthamoeba keratitis (AK) is a severe eye infection caused by *Acanthamoeba castellanii*, often misdiagnosed due to symptom similarity with other infections. Current diagnostic methods lack rapid and reliable options. This study focuses on developing a lateral flow-based strip test using a polyclonal antibody against the Acanthamoeba mannose-binding protein (MBP1) for effective, point-of-care diagnosis of AK.

Methods: Recombinant MBP (rMBP) from *A. castellanii* was conjugated with gold nanoparticles to form antibody-gold conjugates. These conjugates were coated onto a glass fiber matrix, and lateral flow strips were prepared on nitrocellulose membranes with an 8 µm pore size. Test lines were printed using a patented device, and the membrane was blocked with 5% non-fat milk. The strips, assembled on a PVC backing sheet with a sample application pad, antibody-gold conjugated pad, NC membrane, and absorption pad, were tested with *A. castellanii* antigens, allowing sample migration upwards.

Results: After 15 minutes of antigen migration, a colour signal appeared on the strip. The result was interpreted based on the colour intensity of the test line, indicating a positive result when the colour was visible.

Conclusions: The results demonstrate that MBP1 has significant potential as a diagnostic marker and supports the development of a rapid diagnostic assay based on lateral flow technology.

Acknowledgement: We thank ICMR New Delhi for the Research Associate fellowship provided to Dr Saumya Srivastava (File no. 5/3/8/30/ITR-F/2020), which supported this work.

FP-05

Light responsive in-situ hydrogel: an injectable depot platform for intravitreal drug delivery.

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Purpose: Non-VEGF pathways, such as the renin-angiotensin-aldosterone system (RAAS), show promise as therapeutic targets for retinal diseases. Elevated renin levels in diabetic retinopathy (DR) are linked to retinal angiogenesis and inflammation. Spironolactone (SPL) has demonstrated the potential to reduce angiogenesis. Therefore, a long-acting SPL-loaded stimuli-responsive hydrogel system has been developed to address the increasing non-responder population to anti-VEGF therapy for DR.

Methods: The methacrylated hyaluronic acid (MeHA) was synthesised. Spironolactone-loaded light responsive hydrogel (SPL-LRH) was formulated using MeHA (1.5%), Irgacure 2959 (0.05%) and SPL (4%). The formulation's release kinetics, and in vitro safety potential were investigated. In vivo residence was evaluated using a near-infrared (NIR)-imaging system. Safety was evaluated in rats using histology, while the therapeutic efficacy was studied in streptozotocin-induced diabetic rat model.

Results: Methacrylation of HA was confirmed by Proton-Nuclear Magnetic Resonance (1H NMR) and Fourier Transform Infrared spectroscopy (FTIR). UV crosslinking at 365 nm resulted in the sustained in vitro release of SPL over 90 days. The NIR-imaging showed intravitreal hydrogel depot resided in vitreous for >2 months. The hydrogel was found to be safe in retinal pigment epithelial cells. SPL-LRH depot effectively reduced retinal inflammation, blood-retinal-barrier breakage, and glial cell activation in diabetic rats.

Conclusions: SPL-LRH was found to be a safe, efficacious long-acting delivery system to manage DR. This approach may reduce the frequency of intravitreal administration and could be an alternative or an adjunct therapy to the anti-VEGF. This injectable platform technology can also be explored to deliver therapeutics for other chronic ocular diseases.

FP-06

Impact of immunocompetent cells in regulating age-related choroidal capillary changes in rat

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Purpose: Aging and age-related ocular diseases, including age-related macular degeneration (AMD), are associated with significant choroidal capillary loss, leading to reduced nutrient and oxygen supply to the outer retina, and resulting in photoreceptor and retinal pigment epithelium (RPE) loss. This study aimed to investigate the phenotypes of immunocompetent cells involved in choroidal capillary changes linked to aging.

Methods: Sixteen-month-old Wistar rats were used to examine age-related changes in the choroid through histochemistry, ELISA, Western blotting, immunohistochemistry, and transmission electron microscopy (TEM).

Results: Aged rats exhibited vacuolated endothelium, detached electron-dense pericytes, and disrupted basal lamina, in contrast to controls with intact endothelium and pericytes. There was a significant increase in inflammatory markers such as CD68, TLR4, and NLRP3 in the choroidal homogenates of aged rats. Additionally, an increase in Iba-1 positive microglia, macrophages, monocytes, and mast cells was observed along the capillaries. Aged rats also showed a higher frequency of mast cell degranulation, correlated with increased permeability of the aging choroidal vessels. Whole-mounted choroid analysis revealed distinct distribution patterns of mast cells and macrophages along the capillary walls, while elevated CX3CR1 levels indicated the onset of inflammatory changes, triggering microglial activation and migration towards the choriocapillaris.

Conclusions: This study reveals significant structural and inflammatory changes in the aging choroid, characterized by increased microglia, macrophages, and mast cell degranulation. These findings suggest that chronic inflammation in aging contributes to increased vascular permeability and microglial activation, highlighting potential therapeutic targets for AMD and diabetic choroidopathy.

FP-07

Functional evaluation of peptide transporter using pharmacological tools after their topical & intravenous administrations in rabbits

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Purpose: Increasing number of peptide-based therapies holds potential for developing new drugs for ocular diseases, making it crucial to understand peptide transporters in ocular tissues to optimize PK/PD.

Methods: To determine the substrate specificity, transcorneal penetration of glycylsarcosine (glysar) was evaluated at different concentrations and pH levels. To assess the role of peptide transporters, rabbits (n=4) were divided into five groups: 1) Gly-sar 2) Polymyxin B 3) Gly-sar with losartan pretreatment 4) Polymyxin B with losartan pretreatment and 5) Polymyxin B with Gly-sar pretreatment. After topical instillation & intravenous administrations of gly-sar, polymyxin B and losartan, tear and aqueous humor (AH) were collected at 15, 30, 60 and 120 mins. The drug levels were analysed using LC-MS/MS.

Results: Topically applied glysar and polymyxin B showed a maximum AH concentration of 0.06 and 0.450 nmol/mL at 30 and 15 min, respectively. Upon blockage of transcorneal peptide transporters, the concentration of glysar and polymyxin B reduced to 0.006 nmol/mL ($p<0.05$) and 0.414 nmol/mL ($p<0.05$), respectively. The AUC of blocker pre-treatment group was significantly lower compared with control group (1.82 times). Tear kinetics of glysar (1.4614 µmol/mL) showed a rapid decline at 30 min up to 1 hr followed by a gradual precorneal elimination. After blocker pre-treatment, the precorneal residence of glysar (1.97 nmol/mL) decreased significantly ($p<0.001$). However, after intravenous administration of Gly-sar (1.4614 µmol/mL), its concentration in AH of blocker pre-treated group was reduced to 0.031 nmol/mL ($p<0.01$) and in tear increased to 0.06 nmol/mL ($p<0.05$).

Conclusions: For the first time, our data shows the functional importance and involvement of peptide transporters in the ocular disposition of peptide substrate like Gly-sar which is majorly transported by PEPT2.

Acknowledgement: We thank ICMR for providing Senior Research Fellowship to Mr. Tapas Kumar Roy (F.No. 45/10/2022-PHA/BMS).

FP-08

Pre-clinical ocular pharmacokinetics, safety, and efficacy of topical TRPV1 antagonist SER114 for ocular pain

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Purpose: Ocular pain is a common patient complaint recorded in eye clinics across the globe. Its onset is associated with various causes like eye injury, trauma, and post-surgical complications. Currently, there is no marketed treatment for ocular pain; moreover, the existing pain management techniques have limited efficacy and are associated with adverse effects. Transient Receptor Potential Vanilloid 1 (TRPV1) expressed on the cornea is reported to play an essential role in pain perception. In this study, we have evaluated the potential of SER114, a novel TRPV1 antagonist, for treating ocular pain.

Methods: A nanoemulsion-based topical ophthalmic formulation of SER114 was developed. The safety of the developed formulation was evaluated using cell viability assay and Draize test. Its efficacy in mitigating pain was assessed by hypertonic eye wipe test. Further, single-dose pharmacokinetic and tissue distribution studies were conducted to understand the biodistribution of SER114.

Results: The SER114 formulation was biocompatible with the Human Corneal Epithelial cells. The Draize test indicated that the developed formulation was safe and well tolerated in the eye. SER114 effectively reduced ocular pain compared to untreated control without compromising corneal sensitivity. Pharmacokinetic studies showed that SER114 was bioavailable at the target site (cornea) for up to 12 hours upon single dose administration with minimal systemic exposure.

Conclusions: The results from this pre-clinical study indicate that SER114 eye drops could be a promising solution to treat ocular pain. However, further multiple-dose efficacy and pharmacokinetic studies are required to understand the potential of SER 114 in long term ocular pain management.

FP-09

Production of GMP grade limbal stromal stem cells and hydrogel for clinical use

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Purpose: Corneal transplantation is the primary treatment approach for corneal haze and scars; however, the primary challenge is the frequent failure of donor corneas to meet clinical standards. The aim of this study is to quantify and qualify the GMP grade tissues as alternatives to traditional corneal transplants in pre-clinical and clinical settings.

Methods: Limbal rims from non- transplant grade donor corneas, selected based on specific criteria (such as age, cause of death, and tissue expiry) were excised, fragmented, and digested with collagenase IV. The resulting cells were cultured and expanded through three passages before being harvested and cryopreserved. Meanwhile, cadaveric corneal buttons (n=50) were minced, washed, and decellularized. These decellularized tissues were freeze-dried and milled into a DCM powder, which was then converted into a hydrogel. A clinical formulation combining both the cultured cells and the hydrogel is prepared for therapeutic application.

Results: The DCM passed the sterility test and the pH-adjusted hydrogel (pH 7.0- 7.8) met the acceptance criteria with no CFU in bioburden and 5 mg/mL with retained collagen-GAG and no DNA content. It was then incubated to allow solidification. Revived cryopreserved cells were then introduced to the solidified hydrogel, which was incubated for 16 hours. Subsequent live/dead assay and confocal imaging confirmed that the cells were viable (≥ 70%).

Conclusions: The results demonstrate that the hydrogel effectively supports stromal stem cell proliferation, which may aid in corneal regeneration and potentially mitigate corneal scarring post-trauma.

FP-10

Proteomic insights into outer blood-retinal barrier disruption: the role of hypoxia and hyperglycaemia in diabetic macular edema

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Purpose: Hypoxia and hyperglycaemia are key contributors to diabetic macular edema (DME). The retina features two distinct blood-retinal barriers—an outer and inner—essential for maintaining immunological privilege in the eye. The outer blood retinal barrier (oBRB) is established by retinal pigment epithelium (RPE) cells, which are linked together by tight junctions. This study aims to investigate the alterations in molecular content and pathways in RPE cells exposed to hypoxia and hyperglycaemia.

Methods: In order to examine the molecular environment of oBRB, RPE cells were cultured under normal glucose concentration of 5.5mM (20% O2), glycaemic-25 mM for 5 days and hypoxia (1% O2) for 24 hours. The proteins extracted from cell pellets were subjected to enzymatic digestion, quantification and analysis using shotgun LC MS/MS. Proteome Discoverer 2.0 used for identification of proteins. The proteins were further analysed with MetaboAnalyst 6.0, Reactome, string etc.

Results: 5000 proteins, 4081 with high FDR, 1,658 statistically significant within three groups. STRING analysis showed IL-12 signalling in hypoxia and hyperglycemia, linked to oxidative stress, hemostasis, and ROS. IL-18 and IL-1 elevated in hypoxia and hyperglycemia, connected to Pyroptosis, inflammasome, and the Parthanatos pathway contributes to ROS-induced cell death. Hypoxia elevated YAP1, altering Hippo signalling and angiogenesis. Stress-upregulated antioxidant proteins included SOD, Peroxiredoxin-1, and cysteine- and glycine-rich proteins.

Conclusions: The study identifies key pathways and protein interactions that cause inflammation, oxidative damage, and cell death in RPE hypoxia and hyperglycaemia. IL-12 signalling, pyroptosis, and YAP1 influence have significance in angiogenesis, suggesting potential targets for therapeutic intervention in DME.

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FP-11

Transcription factor KLF4 as a modulator of cellular senescence in trabecular meshwork cells

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Purpose: This study investigates the role of Krüppel-like factor 4 (KLF4) in regulating the senescence and proliferation of glaucomatous trabecular meshwork (TM) cells. This research aims to advance our understanding of glaucomatous pathophysiology and explore potential therapeutic strategies for managing glaucoma through the modulation of TM cell function.

Methods: Trabecular Meshwork (TM) cells were isolated from the human cadaveric corneoscleral rim and treated with dexamethasone to characterize TM. TM cells were treated with transforming growth factor-beta (TGF-β;10 ng/ml) for 24 hours and analysed for the expression of KLF4, senescence (p27, p16, p53), and proliferation markers (cyclin D1 and PCNA) by qRT-PCR and immunofluorescence.

Results: TGF beta-treated cells showed differentiated morphology similar to senescent cells. The same was validated at transcript and protein levels too. Further, TGF-B decreased the expression of KLF4 while increasing the expression of p27, p16, and p53 and decreased the expression of cyclin D1 and PCNA.

Conclusions: This is the first report highlighting the role of transcription factor KLF4 in TM senescence. Increased expression of KLF4 along with the key cell cycle mediators promoted TM cell senescence and therefore the glaucomatous-like changes.

FP-12

The involvement of crystallin gene variants and genotype phenotype correlation in PCG pathogenesis

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Purpose: Primary congenital glaucoma (PCG) is a developmental disease which is largely attributed to the mutations in the CYP1B1 along with other genes involved in anterior segment dysgenesis (ASD). Crystallins are potential candidates that regulate transcription factors associated with ASDs. As, ASD and PCG exhibit genotypic and phenotypic similarity, we aimed to understand the involvement of crystallins in PCG.

Methods: Targeted DNA sequencing was performed on PCG cases (n=371) and ethnically matched controls (n=705) using customized gene panel consisting of 11 crystallins and other ASD and glaucoma-associated genes on an Ion Torrent platform using the Ion Ampliseq chemistry. Raw data was analysed using GATK and VarSeq after aligning with hg19 sequences. Genotype-phenotype correlation was assessed based on RGC functions captured through PhNR (Photopic Negative Response) in patients categorized based on the presence and absence of mutations and severity.

Results: Ten novel heterozygous variants (p. Ser45Gly [CRYAA], p. Arg22Cys [CRYAB], p.Arg214Gln [CRYBB1], p.Tyr198His [CRYBB1], p.Ser10Trp [CRYBB1] p. Met1Val and p.Glu177Lys [CRYBB3], p. Gln13His [CRYGD], p. Arg174Cys [CRYGS]) and one splice site variant c.253-1G>C [CRYGB] were observed. Eight variants were completely absent in the controls and rarely present in the1000Genome and GnomAD data bases. Two of these heterozygous variants cooccurred with the heterozygous CYP1B1 variants indicating potential digenic interactions. Strong correlation was observed between Cup-to-Disc ratios and PhNR amplitude among cases harbouring mutations (r=0.99).

Conclusions: Presence of rare variants in Crystallins genes suggest their potential involvements in PCG, which is further supported by the diminished RGC response of these patients' harbouring mutations.

FP-13

Discovery of fusion transcript as a diagnostic biomarker for uveitis: an inflammatory eye disease

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Purpose: A fusion transcript is the fusion of few exons form two genes; such fusion transcripts exist in cancer and inflammatory diseases. The fusion transcripts are potential biomarkers for diagnosis. Fusion gene transcripts have never been discovered in uveitis. Behçet's disease (BD), a subtype of uveitis, is an inflammatory disease and we presumed that fusion transcripts may be generated in the uveitis patients. We made serious attempts to discover fusion transcript in BD patients.

Methods: PBMCs were isolated from blood samples BD cases and controls and RNA was extracted following Trizol method. Total RNA sequencing for case and control was done using Illumina system. The chimeric RNA seq BAM files were used as input to Arriba (Arriba: https://github.com/suhrig/arriba) using default parameters. This tool predicted significant putative fusion transcripts. We validated one of the predicted fusion transcripts using Sanger Sequencing.

Results: We validated one of the fusion transcripts, a fusion of two adjacent genes (part of 28th exon of ATP9A gene and 2nd exon of NFATC2 gene) in chromosome 20. These two genes were associated with inflammatory response. The predicted fusion transcript showed 419 bp (ATP9A ;309bp and NFATC2 ;110bp). Following PCR amplification and Sanger sequencing of the fusion transcript was validated; the fusion product was identical to predicted size and mapped appropriately to the two fusion genes confirming its authenticity.

Conclusions: This is the first report on fusion transcripts in Uveitis. This fusion transcript is specific to BD cases. It is a potential genetic biomarker for diagnosis with clinical implications.

Epigenetic Alterations in Pterygium: Genome-Wide Methylation Profiling Reveals Potential Oncogenic Pathways

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Purpose: Pterygium is a highly prevalent, progressive conjunctival eye disease and is characterized by wing-shaped conjunctival fibrovascular overgrowth. Persistent sunlight exposure is a causative factor for this ocular surface disease and mostly affects people working outdoors. Surgical excision remains the only treatment option. While chronic sun exposure causes changes in the epigenetics of the skin and is a causative factor for skin cancer, the role of epigenetic alterations associated with pterygium is largely unexplored. This study aims to understand the contribution of genome-wide methylation alterations in pterygium pathogenesis.

Methods: Pterygium conjunctival tissue samples were obtained from patients during pterygium excision surgery. Conjunctival tissues from patients with cataracts served as comparative control. DNA was isolated and methylation chip analysis was performed using Illumina Infinium Epic v2 methylation 935k array (Medgenome). The raw data were analysed using the ChAMP R program and gene enrichment analysis was done using SRplot tools.

Results: 116 hypomethylated and 283 hypermethylated CpG sites were identified in conjunctival tissue from pterygium patients ($\Delta\beta > |0.1|$ and $P < 0.01$). The distribution of these sites mainly covered the gene body and the gene promoter region. Several genes are associated with skin cancer, pancreatic cancer, human colorectal cancer, gastric cancer and multiple tumorigenesis. Gene ontology showed that the biological function of these genes is mainly involved in histone methylation, cell-cell adhesion, regulation of GTPase activity, regulation of transforming growth factor beta production, and biological regulation.

Conclusions: This study sheds novel insights into the role of dysregulated methylation status in pterygium pathogenesis.

FP-15

Association of interleukin-8 polymorphisms with Age-related Macular Degeneration in North Indian patients

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Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss among the elderly. The inflammatory cytokine interleukin-8 (IL-8) has been implicated in AMD pathogenesis. This study investigates the association of IL-8 polymorphisms (rs4073, rs2227306, rs2227543) with AMD in a North Indian cohort.

Methods: The study included 100 AMD patients and 100 healthy subjects. Serum IL-8 levels were measured using an enzyme-linked immunosorbent assay (ELISA). DNA was extracted from peripheral blood using the Phenol-chloroform-isoamyl alcohol (PCI) method, and genotyping was performed using a polymerase chain reaction (PCR) method. Statistical analysis was performed using IBM SPSS Statistics 22.0.

Results: Serum IL-8 levels showed no significant differences between AMD patients and controls (p=0.65), or between control and dry AMD (p=0.39), control and wet AMD (p=0.59), and dry AMD and wet AMD (p=0.55). For rs4073, the A allele was associated with an increased risk of AMD (OR: 2.50, 95% CI: 1.08-5.78, p=0.0071). The recessive model showed a significant association with the AA genotype conferring a higher risk (OR: 3.00, 95% CI: 1.43-6.29, p=0.0026). The over dominant model revealed a protective effect of the heterozygous genotype compared to the homozygous genotypes (OR: 0.52, 95% CI: 0.30-0.92, p=0.023). Linkage disequilibrium (LD) analysis revealed moderate LD between rs4073 and rs2227306 (D' = 0.5133) and between rs2227306 and rs2227543 (D' = 0.559). Haplotype analysis identified eight haplotypes, with TCC being the most frequent. Notably, the TTT and TTC haplotypes were significantly associated with a decreased risk of AMD (OR: 0.14, 95% CI: 0.02 0.79, p=0.027; OR: 0.21, 95% CI: 0.06-0.73, p=0.016).

Conclusions: This study underscores the potential role of IL-8 polymorphisms in AMD susceptibility and highlights the significance of genetic screening in understanding the genetic basis of AMD in the North Indian population. Further studies with larger sample sizes are warranted to validate these findings and explore the underlying mechanisms.

Bioengineered human corneal graft preparation strategy for extended utilization of human donor corneas: an *in vitro* study

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Purpose: The study aimed to explore the feasibility and validate the preparation strategy for bioengineered decellularized cross-linked human corneal grafts (BCHCG) in vitro, using human corneas deemed not suitable for surgery (NSFS).

Methods: NSFS human corneas were selected, cut with a corneal trephine, and then decellularized using Triton X 100 in a perfusion bioreactor. They were cross-linked with chondroitin sulfate and stored in glycerol. The bioengineered corneas were assessed for structural integrity and immunological compatibility using Fourier-transform Infrared (FTIR) spectroscopy, DNA quantification, and oxidative mechanism analysis in THP-1 cells, measuring reactive oxygen species (ROS), nitric oxide (NO), and lipid peroxidation. Results were compared with native corneas.

Results: Three NSFS human donor corneas were divided into three groups: Native Cornea (NC), Decellularized Cornea (DC), and DC cross-linked with chondroitin sulfate (DC + CS) for BCHCG development. FTIR analysis confirmed complete decellularization in the DC and DC+CS groups, evidenced by the absence of plasma membrane stretching (2800-3100 cm^{-1}), which was present in the NC group. DNA quantification showed 4.76 ng of DNA per mg in NC, with no DNA detected in the DC and DC+CS groups. Microscopy confirmed cell absence in the DC and DC+CS groups. ROS release was significantly lower ($p<0.001$) in DC+CS and NC compared to DC, while NO release was significantly higher in NC ($p<0.001$).

Conclusions: The study validated the development of BCHCG from NSFS corneas, preserving collagen matrix integrity and decellularization, with promising translational potential for long-term storage and transport, advancing clinical application efforts for corneal transplantation in India.

FP-17

Deep Learning-Based Detection of Ocular Surface Squamous Neoplasia from Ocular Surface Images

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Purpose: Ocular Surface squamous neoplasia (OSSN) is a broad entity encompassing a spectrum of squamous neoplasms of conjunctiva and cornea. This study aims to explore the utility of Artificial Intelligence (AI) models in detecting OSSN from slit lamp (SL) images.

Methods: This is a retrospective observational study. Slit lamp (SL) images of OSSN disease, non-OSSN ocular surface lesions and normal ocular surfaces were collected (2013-2023). Images with minimum resolution of 1024 x 1024 pixels, under diffuse illumination were included. Data was divided into training, validation and test sets (70:10:20). In binary classification, Deep learning (DL) algorithms were applied on OSSN and Non-OSSN images and in ternary classification, DL algorithms were used on OSSN, Non-OSSN and normal images. The results of three DL algorithms were then compared.

Results: 159 images in OSSN group, 184 in non-OSSN group and 269 normal images were included. Data augmentation was performed to increase and balance the data. For binary classification, accuracy in OSSN detection for MobileNet, Xception and DenseNet was 86.8%, 81.9% and 89.6%, while for ternary classification, these values were 79.2%, 80% and 78.3% respectively. MobileNet and Xception both had a sensitivity of 89.8% for OSSN screening in binary classification, while Xception had the highest sensitivity (77.1%) for OSSN screening in ternary classification.

Conclusions: In the present study, AI model showed good performance in image based OSSN detection. AI models may provide a promising tool for OSSN screening in primary health care centres and for teleconsultation from remote areas in the future.

FP-18

Correlating the ocular surface changes associated with visual display terminal users

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Purpose: To understand the effect of prolong screen time usage in meibomian gland dysfunction individuals.

Methods: In this study, the Visual display terminal (VDT) users were categorised into with meibomian gland dysfunction (Group1, n=20) and without (Group2, n=20) meibomian gland dysfunction (MGD). Tear samples were collected from the participants by using a sterile glass capillary tube. Two questionnaires were used in the study. The dry eye symptoms were assessed by Ocular Surface Density Index (OSDI) score and the exposure to visual display terminals was understood by the questionnaire for Computer Vision Syndrome Score (CVS Score). Tear film parameters (NIKBUT and Meibography) was performed in a non-invasive method using Keratograph. Bacteria were cultured and identified from tear samples. Statistical significance was calculated using student's t test.

Results: The collected tear sample was used for isolating the bacteria on blood agar plates (24 - 72h at 37°C). The bacterial isolates showed haemolysis more in MGD group when compared to non-MGD group. Significant difference in the NIKBUT value and CVS scores is recorded in the Group - 1 (12.09, 2) compared to Group - 2 (6.3, 15.94) ($p<0.03$). Meibography and OSDI scores were significantly different between the Group - 1 (0, 1.92) and Group - 2 (2, 26.14) A positive correlation between the NIKBUT value, bacterial diversity and the CVS scores.

Conclusions: The results of the study indicate that individuals with meibomian gland dysfunction have high risk of ocular infections with VDT usage.

FP-19

Genomic and transcriptomic profiling identifies distinct molecular signatures in recurrent Ocular Adnexal B cell Lymphoma

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Purpose: Ocular Adnexal B Cell Lymphoma (OABL) is the most common orbital neoplasm in adults with disease recurrence posing a major challenge in treatment. Identifying molecular drivers linked to the lymphoma pathology is crucial for developing effective prognostic markers. Thus, the present study aims to uncover the molecular factors associated with OABL recurrence.

Methods: Whole exome sequencing (n=19) and transcriptome profiling (n=13) in tumors were performed and correlated with the clinical-pathological features. Pathogenic somatic mutations and copy number alterations were identified through stringent filtering criteria. Genes with significantly altered expression (log2 fold change >2 or <-2 and p-value<0.05) were identified through transcriptomic analysis followed by gene enrichment using enrichR.

Results: Frequent mutations were identified in genes of NFκB and chromatin modifier pathways, including TNFAIP3 (21%), KMT2D (21%), EP300 (16%), and MYD88 (10%). ROS1 (proto-oncogene), is a novel recurrently mutated gene and found in 21% of patients. Copy number alterations revealed the deletion of 6q23.3 (26%), CDKN2A (16%), TP53 (16%) and the gain of MALT1 (10%). Significantly, TNFAIP3 and ROS1 mutations/deletions were prevalent in patients with recurrence and lymph node dissemination. Expression data showed the dysregulation of NFκB signalling in recurrent cases compared to those in remission.

Conclusions: Our multiomic analysis reveals dysregulation of NFκB and chromatin modifiers signalling pathways, providing insights for newer, effective and targeted treatment of OABL.

FP-20

Assessing reactive oxygen species-related genes role in the aggressiveness of Ocular Surface Squamous Neoplasia (OSSN)

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Purpose: Ocular Surface Squamous Neoplasia (OSSN) is an ocular malignancy with a broad spectrum from dysplasia to invasive squamous cell carcinoma. Several studies have been done to understand the role of ROS and hypoxia in cancer but it is not elucidated in OSSN. Our study aims to identify potential biomarkers through transcriptomic analysis, which helps in early detection and tailored therapy.

Methods: RNA was extracted from OSSN tumor samples and healthy conjunctival tissues, and then transcriptomic analysis was performed. Data was analyzed to identify differentially expressed genes (DEGs). The Significant DEGs were further analysed with the cancer association and then validated by qPCR on OSSN patient samples.

Results: Our analysis revealed the significance of differentially expressed genes associated with cancer. Our study identified genes like PRDX1, PRDX2, PRDX4, and PRDX5 are involved in vital biological processes like cell survival, cell proliferation, Apoptosis, inflammatory response, and resistance to drugs. PRDX is known to play an important role in the progression and metastasis of tumor cells. They play a pivotal role in eliminating Reactive oxygen species and are also known to induce hypoxia. These specific genes can be used as putative markers for OSSN as we can see the significant differential expression in OSSN tumor samples compared to healthy conjunctiva samples.

Conclusions: This study provided promising molecular markers not only for OSSN but can also be used as a putative atlas to predict the aggressiveness of OSSN. Its detection can also help in predicting response to the therapy.

FP-21

Evaluating the functional significance of TET1 in human retinoblastoma

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Purpose: MYCN oncogene is overexpressed in retinoblastoma (RB) and could potentially regulate Ten eleven translocation (TET) gene expression, implying a role for TETs in retinoblastoma. TET proteins, which function as DNA demethylases, have gained prominence due to their dysregulation in cancers. The current study sought to investigate the functional significance of TET1 and its epigenetic role in retinoblastoma.

Methods: TET1 protein expression was evaluated in RB patient tissue specimens by immunohistochemistry. mRNA expression was determined in RB cell lines and patient specimens using RT-qPCR. TET1 was targeted in RB cells by genetic silencing using shRNAs and a small molecule inhibitor. Functional assays were performed in knockdown and inhibitor-treated cells. An in-silico promoter analysis of TET1 was performed to identify potential MYCN binding sites. The protein levels of TET1 were measured in MYCN knockdown RB cells by immunoblotting.

Results: TET1 was found to be overexpressed in patient specimens compared to morphologically uninvolved retina. TET1 silencing using shRNAs reduced cell survival, increased apoptosis and altered the cell cycle distribution. TET1 inhibition with small molecule inhibitor similarly resulted in reduced cell viability and enhanced apoptosis. Additionally, presence of MYCN binding sites on TET1 promoter region and reduction of TET1 protein levels in MYCN knockdown cells suggest a possible regulation of TET1 by MYCN.

Conclusions: Our findings indicate that TET1 is overexpressed in RB and its inhibition could be a potential therapeutic strategy. Further validation is required to fully understand the functional significance of TET1 in RB.

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FP-22

Glutathione peroxidase-4 as a biomarker in retinoblastoma: association with pathological parameters and patient outcome

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Purpose: Retinoblastoma (Rb) is the most common intraocular tumor in children. Glutathione peroxidase 4 (GPX4) reduces lipid peroxidation and prevents oxidative damage in cancer. GPX4 dysregulation is reported in multiple cancer types that indicates in regulation of tumorigenesis. It is a key regulator of ferroptosis and act as a potential target for modulating cell death in various cancer types. Our study aimed to investigate the expression of GPX4 in Rb cases and correlated with overall survival.

Methods: Gene expression of GPX4 was evaluated in 171 prospective Rb cases using quantitative real-time PCR (qRT-PCR). Protein expression was determined by immunohistochemistry (IHC) and validated by western blotting. Expression levels were correlated with clinicopathological parameters and patient outcome.

Results: There was a male preponderance in our study. In this cohort, mRNA expression of GPX4 was found to be upregulated in 66% Rb cases whereas protein expression was seen in 73% cases using IHC. Expression level of GPX4 was further validated using western blotting. In our study, expression of GPX4 was statistically significant with poor differentiation, massive choroidal invasion, and poor overall survival.

Conclusions: Our result suggests that upregulation of GPX4 in Rb cases might play a crucial role in suppressing the cell death by ferroptosis affecting the tumorigenesis in Rb. Future studies might give better insights in understanding the contribution of GPX4 as a potential biomarker in prognosis of retinoblastoma.

Abstracts
(Posters)

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Indian Eye Research Group

30th Annual Meeting of Indian Eye Research Group

Poster presentation: Day 1 (28th September 2024)

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PS-I-2	Manasi Tripathi	**PS-I-28**	Priyadarshini Sathe
PS-I-3	Riddhi Agarwal	**PS-I-29**	Alreeza Fernandes
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PS-I-22	Shridula Sankar	**PS-I-48**	Azima Fatima
PS-I-23	Tamizhmathy Mannangatty	**PS-I-49**	Bharti Sangwan
PS-I-24	Sandhya Kendre	**PS-I-50**	Naheed Arfin Borah
PS-I-25	Shruti Mhamane	**PS-I-51**	Gayatri Suresh
PS-I-26	Velmurugan Kailasam	**PS-I-52**	Jyoti Rajput

Abstract ID	Name of Presenter	Abstract ID	Name of Presenter
PS-I-53	Manasi Tripathi	**PS-I-57**	Keya Katare
PS-I-54	Devyani Bansal	**PS-I-58**	Shubhpreet Kaur
PS-I-55	Kashish Parnami	**PS-I-59**	Ramkishor Sah
PS-I-56	Ankita		

PS-I-01

Assessing the antifungal efficacy of corneal targeting peptide for management of *Fusarium* keratitis

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Purpose: Fungal keratitis is a major cause of corneal blindness worldwide. Standard topical therapies often yield suboptimal results due to poor penetration and the emergence of resistant fungal strains. Cell penetrating peptides (CPPs) with potent antifungal effect offer potential solutions. The present study evaluates the antifungal efficacy of a corneal-targeting peptide (CorTS 1), *in vitro* and *in-vivo*.

Methods: The antifungal and cell penetration efficacy of CorTS 1 was assessed *in- vitro* through the PI membrane damage assay and the internalization of fluorescently labelled peptides in *Fusarium dimerum* hyphae. Additionally, penetration and specificity in ocular tissue were studied using rabbit eyes along with evaluating the antifungal effectiveness of CorTS 1 in a mouse model of Fusarium keratitis.

Results: CorTS 1 successfully inhibited growth of *Fusarium dimerum* hyphae via modulation of membrane permeability. Furthermore, in a mouse model, the peptide showed better therapeutic outcomes than the drug of choice, 5% natamycin ophthalmic suspension. It also demonstrated effective penetration into rabbit cornea.

Conclusions: These findings suggest that biologically active CPPs represent a promising new treatment option for fungal keratitis.

PS-I-02

Estimation of tear film concentration of oral ciprofloxacin in patients with bacterial keratitis

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Purpose: To estimate the tear film concentration of oral ciprofloxacin in patients with bacterial keratitis and study the its correlation with various factors

Method: 104 eyes of 52 patients with bacterial keratitis were included in this study. Demographic details of patients were noted and general physical examination, ophthalmic evaluation and microbiological investigations were done. In addition to topical anti-microbial therapy, patients received oral Ciprofloxacin 500mg twice a day for 5 days. Tear samples were collected from infected and non-infected eyes respectively, 3 hours after the morning dose of day 5. These samples underwent liquid chromatography and mass spectrometry to estimate concentration of drug in tear film.

Result: Staphylococcus epidermidis was the most prevalent organism in our study (28.85%). Mean ulcer area was 28.46 ± 15.62 mm^2 (4 mm^2-72 mm^2), median was 24 mm^2. The mean concentration of ciprofloxacin in tear samples from the infected eyes was 824.47 ± 851.75 ng/ml (10.9 ng/ml - 3490 ng/ml) and in non-infected eyes was 504.92 ± 404.52 ng/ml (3.86 ng/ml - 1730 ng/ml). Mean concentration of ciprofloxacin in infected eyes was comparable to minimum inhibitory concentration (MIC) for Staphylococcus species. Drug concentration had a strong positive correlation with ulcer area (Spearman's rho = 0.957; p = 0.02) but a weak positive correlation with the presence of infection (Spearman's rho = 0.245; p = 0.08).

Conclusion: Oral ciprofloxacin reaches tear concentration similar to MIC of staphylococcus species. There is a strong correlation between the drug concentration in the tear film and the ulcer area.

PS-I-03

Antifungal potential of host defence peptide against *Candida albicans*

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Purpose: Antimicrobial peptides are envisaged as potential antimicrobial candidates that can help us to combat drug resistant pathogen. In this regard microbial keratitis is one of the diseases that needs immediate intervention considering the currently available therapies are either limited or ineffective. Recently, WHO has published a list which puts *Candida albicans* in the high priority pathogen group. Such a pathogen is known to form biofilms which is one of the resistance mechanisms that needs to be addressed on a priority basis.

Methods: The antifungal activity of the Host Defence Peptide (HDP) was ascertained by incubating the pathogen spores in the presence of the peptide for 24 hr and then proceeded with the Colony Forming Units (CFU/ml) method. The biofilm studies were performed using the standard Crystal Violet (CV) assay and electron microscopy. The time dependent antifungal activity of HDP was also determined using the CFU/ml method.

Results: In the time-kill curve, the HDP (100 µg/ml) was able to inhibit the growth of pathogen by 50% at 6 hr which further increased to 90% inhibition at 24 hr. Furthermore, more than 80% biofilm inhibition using the CV assay highlighted the antibiofilm activity of HDP. The membrane directed activity of HDP against the pathogen was established using Scanning Electron Microscopy (SEM). At higher magnification, the membrane distortion in the HDP treated sets when compared to the control was heightened which further strengthen our research findings for HDP as an efficient antifungal agent.

Conclusions: In a nutshell, the currently studied Host Defence Peptide (HDP) as an antifungal agent proposes a promising solution for the imminent threat of Anti Microbial Resistance (AMR).

PS-I-04

Infectious keratitis following accelerated corneal collagen cross-linking in keratoconus

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Purpose: The purpose of the study is to report the clinical profile, microbiology, treatment outcomes of patients with keratitis following accelerated corneal collagen cross-linking

Methods: The medical records of cases with keratoconus who underwent corneal collagen cross-linking were reviewed between the time period January 2021 to July 2024. Cases that developed keratitis were included in the study. Microbiology reports, clinical records and treatment outcomes were retrieved from the medical records.

Results: Of the 723 keratoconus eyes that underwent accelerated corneal collagen cross-linking at our centre, 13(1.79%) eyes developed keratitis. One case of keratitis had got its cross-linking from outside, was also included in the study(n=14). A total of 4 patients had associated Vernal keratoconjunctivitis. Among the infected cases 8 of them, had bacteria (gram positive cocci, *Staphylococcus spp.*) grown on in the culture or noted on gram stain. *Candida* was grown in fungal culture in one case. In one case, gram stain showed coccobacilli and KOH stain showed fungal filaments suggesting a mixed infection. One of them, had a re-infection which got healed in 4 days. The mean epithelial healing time was 17.8 days (range 2-45 days). The mean symptom to presentation days were 2.7 (range 1-5 days). None required a penetrating keratoplasty. A composite scale of education and occupation, modified Kuppuswamy scale was performed to assess socio-economic status.

Conclusion: Post CXL keratitis is a rare entity, predominantly associated with bacterial infections. Early identification, aggressive treatment with fortified antibiotics, microbiological investigations play an important role in improving the healing time.

PS-I-05

Molecular genotyping of *Acanthamoeba* species isolated from ocular specimens of patients with keratitis

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Purpose: Acanthamoeba Keratitis (AK) is an agonizing and sight-threatening ocular infection caused by *Acanthamoeba*, which is linked to traumatic corneal injuries and contact lens usage. There have been observed differences in geographical distribution, virulence, pathogenesis, and drug susceptibility profiles among various genotypes of *Acanthamoeba*. However, it remains uncertain whether such variations occur within sub-genotypes /species. The present study focus on the sub- genotypic level classification of *Acanthamoeba* isolates from corneal specimens in AK patients

Methods: A total of 32 isolates of *Acanthamoeba* between 2018 and 2024 were included in this study. Molecular genotyping of *Acanthamoeba* up to the sub-generic genotypic level was performed by PCR targeting 18S ribosomal ribonucleic acid subunit (Rns) sequence and phylogenetic tree analysis using MEGA X software.

Results: The study found that *A. castellanii* (38%) was the most predominant pathogen, followed by *A. culbertsoni (25%), A. polyphaga (13%), A.hatchetti (6%), A. triangularis (6%), A. jacobsi (6%), A. healyi (3%) and Acanthamoeba sp.(3%).* Four major genotypes were identified: T4 (72%), T10(19%), T12(3%), and T15 (6%). T4 genotype was further categorized into five sub genotypes: T4A, T4B, T4C, T4D, and T4F. The present study also identified five unique alleles in the diagnostic fragment region of *Acanthamoeba* isolates within T4 and one in the T15 genotype.

Conclusions: Our study identified *A. castellani* and the T4 genotype as the most common species and genotype in AK. To the best of our knowledge, this is the first study from India to report on *A. jacobsi* (T15 genotypes) which is a rare pathogen that causes AK.

PS-I-06

Streamlined approach to developing a *Pseudomonas aeruginosa* keratitis model in Swiss albino mice to assess clinical features

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Purpose: *Pseudomonas aeruginosa* keratitis is known for its rapid progression, antimicrobial resistance, and significant visual impairment. Treatment is challenging due to toxin production and antibiotic resistance. Animal models are essential for studying ocular infections and evaluating therapies, with Swiss albino mice being ideal due to their well-characterized immune systems and ocular anatomy. This study aims to develop a simplified model of *Pseudomonas aeruginosa* keratitis in Swiss albino mice to investigate corneal lesions and assess clinical features, thereby enhancing the understanding and treatment of this disease.

Methods: Four female Swiss albino mice, aged six to eight weeks, were housed under standard conditions. A handheld slit lamp was used to examine each mouse's eyes. One eye of each mouse was scratched with a fine needle and topically infected with a clinical strain of *Pseudomonas aeruginosa*, while the other eye served as a control. Clinical scores were recorded post-infection to evaluate disease severity, and slit lamp photography documented the disease response.

Results: A simplified experimental model of *Pseudomonas aeruginosa* keratitis was successfully developed in Swiss albino mice. The pathogen's presence was confirmed through culture, biochemical assays, and PCR methods. Disease severity increased over three days in all animals, demonstrating the model's effectiveness in replicating disease progression. Clinical features such as ulcer size, infiltrate depth, corneal edema, and conjunctival congestion were observed.

Conclusion: This simplified model of *Pseudomonas aeruginosa* keratitis in Swiss albino mice effectively replicates the disease. The consistent infection progression across subjects makes this model a valuable tool for studying ocular infections and developing new therapies.

PS-I-07

Identification of host cell-based protein diagnostic biomarker in vitreous fluid of tubercular uveitis patients

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Purpose: Although several diagnostic modalities are available for diagnosis of pulmonary TB, either conventional or Real time PCR (genomic DNA based) is used for the diagnosis of *Mycobacterium tuberculosis* (MTB) in vitreous fluid of clinically suspected tubercular uveitis (TBU) patients. DNA based diagnosis of MTB from vitreous fluid may give false negative result due to pauci-bacillary nature of bacteria. Always laboratory confirmation of the clinically suspected MTB patients is recommended to start an anti-tubercular therapy. This study was designed to find one/ two hyper expressed proteins produced by host cell during infection in the vitreous fluid of a TBU patient to consider as a potential diagnostic biomarker of TBU. This may increase the sensitivity and specificity of diagnosis

Methods: Vitreous sample collected from suspected TBU patients was diagnosed for MTB with RT PCR. Simultaneously, vitreous was collected from healthy controls and close differential of TBU i.e. Inflammatory uveitis patients. Protein content of vitreous fluid of each group of patients was measured with Bradford's method. Proteins IGKV1-17 and C8B which were reported recently from vitreous fluid of confirmed TBU patients were taken for studying differential protein expression among these 3 groups using western blotting and ELISA techniques.

Results: Proteins IGKV1-17 and C8B were found to over express by host cell of confirmed TBU patients compared to healthy controls and close differential inflammatory uveitis patients.

Conclusions: Proteins IGKV1-17 and C8B may be further considered as a potential diagnostic biomarker of MTB diagnosis in suspected TBU patients.

PS-I-08

Evolving antibiotic resistance patterns in *Staphylococcus* species isolated from conjunctiva of patients undergoing ocular surgery: insights from a decade of surveillance

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Purpose: To determine the antibiotic resistance trends of *Staphylococcus species* (Coagulase-negative Staphylococci-CNS and *Staphylococcus aureus*) isolated from conjunctiva of patients undergoing intra-ocular surgery.

Methods: In this retrospective study, data on pre-operative cultures of conjunctival swabs (19398 eyes of 14555 patients) from January 2014 - December 2023 at Sankara Nethralaya were collected. Bacterial isolates were identified using standard microbiological identification and antibiotic susceptibility testing methods.

Results: A total of 6345 bacteria were isolated, with CNS and *S. aureus* accounting for 50.4% and 5.9% of combined isolates, respectively. A comparison of trends in antibiotic susceptibility over two 5-year periods (2014-2018, 2019-2023) did not reveal any significant decrease in susceptibility of CNS to gentamicin (95% to 94%), tobramycin (96% to 94%) and cefazolin (98% to 96%). However, there was a significant reduction in susceptibility to ofloxacin (82% to 60%), gatifloxacin (76% to 61%), moxifloxacin (87% to 78%) and ciprofloxacin (72% to 68%). Similarly, *S. aureus* did not show any significant decrease in susceptibility to gentamicin (92% to 89%), tobramycin (94% to 92%) and cefazolin (95% to 93%) over the study periods. However, *S. aureus* displayed a significant decrease in susceptibility to ciprofloxacin (35% to 29%), moxifloxacin (55% to 36%), and ofloxacin (46% to 28%), while susceptibility to gatifloxacin continued to be low (25% to 27%).

Conclusions: Our studies indicate an increasing resistance of Staphylococci to fluoroquinolones, including fourth-generation fluoroquinolones (gatifloxacin and moxifloxacin), likely due to their rampant use in community practice. The study should prompt further dialogue on the choice of antibiotic prophylaxis during ophthalmic surgery.

PS-I-09

Identification of human corneal miRNAs in disease severity of *Psuedomonas aeruginosa* keratitis

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Purpose: *Pseudomonas aeruginosa* (PA) is one of the prominent bacterial pathogens causing keratitis and they are known to cause rapid and severe ulceration. miRNAs might contribute to disease pathogenesis in keratitis by altering gene expression during inflammation and wound healing. In this study, we propose to profile the human corneal miRNAs comprehensively in PA keratitis and identify the miRNAs associated with disease severity.

Methods: Corneal buttons were collected from poor outcome patients (n=3) after TPK surgery. Healthy corneas from cadavers were acquired from Aravind International Eye Bank, Madurai as controls. Corneal ulcer swabs were collected from good outcome patients at two stages namely, presentation (n=4) and review (n=4). Total RNA was isolated from the tissue and swabs using Trizol method. Small RNA sequencing was performed by outsourcing and the raw data was analysed with in-house bioinformatics pipeline. Target prediction and Function network analysis were performed with differentially expressed miRNA.

Results: 134 miRNAs were differentially expressed in poor outcome patients compared to cadaver controls. Further, 163 and 304 miRNAs were found to be differentially regulated in presentation and review of good outcome patients compared to controls. 7 miRNAs namely, miR-210-3p, miR-24-3p, miR-22-3p, miR-21-5p, miR-146a-5p, miR-146b-5p, miR-181a-5p were found to be upregulated during disease severity. While 2 miRNAs, miR-7e-5p and miR-29c-3p were downregulated during disease severity.

Conclusion: Comprehensive profiling of miRNA was achieved for the first time in PA keratitis. The shortlisted miRNAs may affect crucial pathways involved in disease pathogenesis thus leading to severity. Further functional studies on these miRNAs are warranted.

PS-I-10

Antifungal susceptibility profiles of *Fusarium* and *Aspergillus* flavus isolated from keratitis at a tertiary care centre in South India

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Purpose: *Fusarium* species and *Aspergillus flavus* comprise 60-70% of fungi causing keratitis in our setting. Here we studied the antifungal susceptibility of *Fusarium* spp. and *Aspergillus flavus* isolated from patients with keratitis presenting at our tertiary care centre.

Methods: In this prospective study, antifungal sensitivity testing (natamycin, amphotericin B, voriconazole) of fungi from 80 cases of culture positive fungal keratitis (48 *A. flavus* and 32 *Fusarium* spp.) by using Micro-broth dilution method was performed.

Results: Our antifungal susceptibility results indicated that natamycin had the lowest minimum inhibitory concentrations (MICs) against Fusarium (30/32 isolates had MIC <16 µg/ml, 2/32 had MIC > 32 µg/ml) with high MICs against *A. flavus* (46/48 isolates had MIC > 32 µg/ml and 2/48 had MIC of 32 µg/ml). Amphotericin B had low MICs against Fusarium (32/32 had MIC between 0.5-16 µg/ml) as well as against *A. flavus* (48/48 had MIC between 4-16 µg/ml). Voriconazole had the lowest MICs against *A. flavus* (48/48 isolates had MIC in the range of 0.5-2 µg/ml) and varying MICs against Fusarium spp. (18/32 isolates had MIC in the range of 0.5-16 µg/ml, 14/32 isolates had MIC > 32 µg/ml)

Conclusions: Fusarium and *A. flavus* demonstrated differential susceptibility to the anti-fungal agents tested, with *A. flavus* isolates showing low in-vitro susceptibility to natamycin, but high in-vitro susceptibility to voriconazole, while *Fusarium* isolates showed high in-vitro susceptibility to natamycin, but lower in-vitro susceptibility to voriconazole. With no single antifungal being universally effective against both fungi, antifungal therapy may need to be tailored depending on the pathogen identity.

PS-I-11

Characterizing the microbiome of the healthy human ocular surface at different sites of the eye

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Purpose: The purpose of the study is to understand the microbiome composition of the ocular surface in healthy individuals in tear film, conjunctiva and lid margin.

Methods: Bacterial microbiome was generated from the DNA of tear film (n=33), conjunctival swab (n=36) and lid margin (n=20) samples of the healthy individuals with a mean age of (33.3± 19), (44.5± 24), (29±12) respectively. The V3-V4 region of 16S rRNA gene was amplified and sequenced on the Illumina HiSeq2500 platform. Sequences generated were processed in QIIME to assign taxa. Further, statistical analysis was done to assess the alpha and beta diversity indices in R software. Wilcoxon signed rank test was used to analyze the significant changes.

Results: Significant differences were observed in the alpha diversity. PCoA showed distinct clusters of all the three groups indicating compositional differences. *Lactobacillus* is predominantly present in both Conjunctiva and tear samples. Significantly high abundance of *Corynebacterium* was observed in conjunctiva compared to other sites ($p<0.05$). Genera *Clostridium* and *Cutibacterium* are relatively high in tear film whereas genera *Staphylococcus* and *Mycobacterium* showed higher abundance in conjunctiva ($p<0.05$). Lid margin samples had higher abundance of *Pseudomonas* and *Acinetobacter* ($p<0.05$). Probiotic bacteria such as *Lactobacillus, Streptococcus, Escherichia-Shigella* and *Bacillus* are consistently present in more than 80% of the samples.

Conclusions: Conjunctiva, tear film and lid margin showed significant difference in the bacterial genera. However, genera *Acinetobacter, Corynebacterium, Staphylococcus, Streptococcus, Cutibacterium* and *Escherichia-Shigella* are found to be common to the ocular surface irrespective of the sample site.

PS-I-12

Investigated CXCL1 levels in HSV-1 keratitis patients to explore their role in spontaneous viral reactivation

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Purpose: To investigate the role of CXCL1 in HSV-1 keratitis by measuring its levels in the corneal epithelium and tear fluid of patients and correlating these levels with disease pathogenesis.

Methods: CXCL1 mRNA levels were quantified in the corneal epithelium of patients with HSV-1 keratitis, active disease and PRK (healthy individuals undergoing photorefractive keratectomy (PRK) who served as controls) using qPCR, while protein levels in tear were measured by ELISA. A subset of patients was followed until disease resolution and CXCL1 level was quantified. Additionally, levels of viral genes LAT and ICP27 were quantified by qPCR in this subset.

Results: Significantly higher CXCL1 mRNA levels were observed in the corneal epithelium and higher protein levels in the tear fluid during active keratitis episodes compared to PRK. Some patient was followed up till the viral infection is resolved. These patients were investigated for levels of viral genes. After correlating with CXCL1 level and viral genes it can be said that CXCL1 can be potential host marker for viral spontaneous reactivation.

Conclusion: The increased CXCL1 expression during active HSV-1 keratitis likely enhances the inflammatory response, contributing to recurrent episodes and potential corneal damage. Elevated CXCL1 levels could serve as a biomarker for predicting disease recurrence. Targeting CXCL1 could offer a novel therapeutic strategy to manage HSV-1 keratitis more effectively by reducing recurrence rates and minimizing corneal damage.

PS-I-13

Ocular *Nocardia* infections: *Nocardia amamiensis* keratitis and *Nocardia farcinica* endophthalmitis: case study

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Ocular manifestations of *Nocardia* spp. infection range from keratitis, uveitis, iritis, choroiditis, scleritis to retinal abscess or retinal detachment. Infection caused possess a challenging diagnosis, requiring targeted antimicrobial coverage. We report two cases of ocular *Nocardia* infections; keratitis caused by *Nocardia amamiensis* and endophthalmitis caused by *Nocardia farcinica*.

Case 1: A 22-year-old male; with 1-week history of ocular pain, redness, watering, photophobia and blurred vision in right eye following a foreign body (insect??) impacting the cornea. At presentation, visual acuity was 20/40 not improving with pinhole, slit-lamp examination revealed a paracentral corneal epithelial defect with conjunctival hyperaemia. Based on the clinical findings, empiric treatment included; eyedrop moxifloxacin 0.5% with natamycin 5%. Microbiological workup reported presence of *Nocardia amamiensis* confirmed using 16S rRNA sequencing. Targeted treatment was initiated, with topical fortified amikacin 5% along with oral co- trimoxazole 160mg/800mg. On follow up, epithelial defect healed completely in 3 weeks and at three months, there was complete resolution of infiltrates and a faint paracentral stromal scar.

Case 2: An immunocompetent years 60-year-old male; with complaints of ocular pain, redness, watering and poor gain of vision in right eye, following a manual small incision cataract surgery three days ago. Slit lamp examination revealed complete corneal melt with dense exudates present in anterior chamber. Ultrasound posterior segment showed mild to moderate amplitude spikes in the vitreous cavity. Presumptive diagnosis of post-surgical endophthalmitis with corneal melt was made and empiric treatment with vancomycin 5% and tobramycin 1.3% initiated. Patient underwent therapeutic penetrating keratoplasty, intra-operatively there was presence of white fluffy cotton balls filling the vitreous cavity. Patient received intravitreal injections of voriconazole, ceftazidime and vancomycin, along with systemic voriconazole. Microbiological workup revealed the isolate as *Nocardia farcinica* (16S rRNA sequencing). Accordingly, the treatment subsequently changed to topical fortified amikacin 5% along with oral co-trimoxazole160mg/800mg. One month post operatively, eye had started showing signs of atrophia bulbi, visual acuity inaccurate projection of rays, intraocular pressure digitally low, and the patient was pain free.

Conclusion: With the initiation of appropriate therapy in the form of amikacin and co-trimoxazole, *Nocardia amamiensis* keratitis resolved promptly. Nocardia endophthalmitis is a relatively uncommon form of endophthalmitis seen in clinical patients and mimics fungal endophthalmitis. Nocardia endophthalmitis tends to carry a poor prognosis. Delayed diagnosis of *Nocardia farcinica* endophthalmitis was associated with poor outcome. Thus, *Nocardia* spp.

are a cause of emerging ocular infections, high clinical suspicion with early and accurate diagnosis is essential for good visual recovery.

Prostanoid receptors modulation by ricinoleic acid: an evolving dynamic pathway in glaucoma

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Purpose: Prostaglandin analogues used in glaucoma therapy act on prostaglandin F (FP) receptor to enhance the uveoscleral outflow, however they do not have affinity for prostaglandin E3 (EP3) receptors which are extensively present in iris-ciliary body-trabecular meshwork. This study was conducted to assess the Ricinoleic acid (RA) dual agonism on FP and EP3 receptor.

Methods: The three-dimensional structure modeling and docking was performed for FP and E3 receptor with ricinoleic acid and their respective controls. To study interaction of RA with FP and EP3 receptor, calcium binding and cAMP release assays were performed in primary trabecular meshwork cells. The variable concentration of RA was compared to the positive and negative controls. The cells supernatants were assessed for the levels of PGF2alpha using ELISA.

Results: The in-silico study showed RA higher binding affinity for EP3 receptor as compared to its natural and synthetic ligand. The RA had less affinity for FP receptor compared to controls. In calcium release and cAMP assay, RA variable doses (0.5-50µM) were able to increase the release of calcium (ED50~3.37µM) and decrease the cAMP levels (ED50~5.6µM) in dose dependent manner. The RA mediated calcium release was inhibited by dual blockage of FP and EP3 receptors. The EP3 agonism by RA caused the comparable release of PGF2alpha in all groups, whereas the dose dependent increase was observed during the FP receptor blockage.

Conclusions: The study shows that RA had higher interaction with EP3 but comparable affinity for FP receptors. The release of prostaglandin by EP3 agonism might be responsible for its dual agonism. The compound can be tested further for its hypotensive properties in glaucoma.

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PS-I-15

Single and multidose ocular pharmacokinetics of topical posaconazole in humans

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Purpose: Evaluate the pharmacokinetics of single and multiple doses of topical posaconazole in humans.

Methods: Aqueous and tear samples were collected from 30 healthy volunteers and 60 cataract patients. For the precorneal drug clearance study, single-dose and multidose (1hourly and 2hourly) kinetics were analysed in tear samples from volunteers. To assess intraocular drug penetration, aqueous samples were collected from 60 cataract surgery patients at predetermined intervals after single dose and multidose administration of 1% posaconazole. Single dose kinetics involved sampling at 15,30,60, and 120 minutes post-application. For multidose kinetics, drug was administered five times with 1 hourly and 2 hourly intervals, and samples were collected 1 hour after last administration. Liquid Chromatography tandem mass spectrometry was used for analysis.

Results: Single dose of Posaconazole achieved mean aqueous concentration of 8.70±16.75ng/mL after 60 minutes. In the multidose kinetics, administering the drug at 1 hourly and 2 hourly intervals led to aqueous concentrations of 8.46±16.39ng/mL and 2.72±6.80ng/mL respectively. The tear drug concentrations of single dose kinetics at 10, 30, 60, and 120 minute post-application were 179.12±176.23g/mL, 144.43±227.03g/mL, 15.74±10.60g/mL, and 25.35±31.72g/mL, respectively. 1hourly and 2hourly dosing resulted in tear concentrations of 532.63±435.76g/mL and 567.59±768.17g/mL, respectively.

Conclusion: Posaconazole's tear concentration remains effective against fungal pathogens two hours post-administration, making it suitable against surface mycotic keratitis. However, its intraocular concentration not reaching the minimum inhibitory concentrations for most fungal species in single and multiple doses.

PS-I-16

Evaluation of dipyridamole eye drops for the treatment of corneal neovascularization: pharmacokinetics and ocular toxicity

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Purpose - The objective of presented study was to characterize and evaluate the antiangiogenic activity of developed dipyridamole (DYP) formulation in an experimental angiogenesis rat model. Further, the studies were performed to understand the ocular pharmacokinetics of DYP.

Methods - The formulations were prepared using various concentration of DYP (0.008% and 0.08%). The developed tested DYP formulations were subjected for clarity, appearance, pH, osmolarity and viscosity evaluation parameters. Alkali induced cautery eye of rats were treated with DYP. The images captured and quantified the blood vessel density using Aphelion® imaging software and percentage of fibrotic scar tissues was examined using Image-J® software. The evaluation of single and multi-dosing trans-corneal permeation was done using LCMS/MS with bevacizumab topical eye drop (1.25 mg/mL) as positive control.

Results - The low (0.008%) and high (0.08%) concentration of DYP exhibited most noticeable significant antiangiogenic activity by 58.83% and 79.15% respectively in experimental angiogenesis rat model. Single dosing of trans-corneal study showed that the lower concentration of DYP eye drops reached Cmax at 5 min, whereas high concentration of DYP eye drops reached the same at 10 min in aqueous humor. It revealed a 2.3-fold increase on higher concentration in the area under the concentration-time curves (AUC0–120min) in aqueous humor and 1.69-fold accumulation at 120 min in tears. In a multi-dose kinetics, the steady-state pharmacokinetic profile showed after double and triple dose administration in a day.

Conclusion - The stable DYP formulation was effective in antiangiogenic effect against cautery -induced neovascularization model. In the future, we expect that it serves the better therapeutic potential for corneal neovascularization (CNV) and other ocular disorders in humans.

PS-I-17

Novel organic cation transporter in blood tear barrier: Carrier of systemic drugs

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Purpose: The tear is considered an ultrafiltrate of plasma, indicating the movement of endogenous molecules across the Blood Tear Barrier (BTB) through selective carriers such as membrane transporters. We hypothesize that Novel Organic Cation Transporters (OCTN) in the BTB (lacrimal gland (LG) and conjunctiva) could shuttle cationic endogenous and exogenous (systemic drugs) molecules from blood to tear. OCTN is well-known to be expressed in conjunctiva but remains unexplored in the LG. The purpose of the current study was to delineate the functional role of OCTN in BTB.

Methods: OCTN expression in rabbit's LG was evaluated by polymerase chain reaction (PCR) and Western blotting. *In-vivo* tear kinetics was performed to understand OCTN function in BTB. L-carnitine (model OCTN substrate) was injected intravenously into rabbits (n=3) with or without topical pre-treatment with OCTN blockers (tetraethylammonium (TEA) and Quinidine). Tear was collected using Schirmer strips at various time points and analysed using Liquid chromatography-mass spectrometry.

Results: PCR and Western blotting confirmed the OCTN1 and OCTN2 expression in the LG with a relatively higher expression of OCTN2. A significant decrease in tear carnitine concentration was observed at 5 and 15 mins in TEA and Quinidine pre-treated groups compared to the control group ($p<0.01$). In the TEA and Quinidine pre-treated group, the AUC(0-2h) was 1.38-fold and 1.24-fold decreased compared to the control group.

Conclusions: The current study reported OCTN expression in the LG and established its functional importance in BTB. Further studies are ongoing to understand the entry of OCTN drugs substrates from systemic circulation to the eye.

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PS-I-18

Impact of oral administration of lactoferrin in post refractive surgery dry eye

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Purpose: To study the impact of oral administration of Lactoferrin in post refractive surgery dry eye.

Methods: This Randomized placebo-controlled trial enrolled 50 post refractive surgery patients. Patients were randomized in two groups Lactoferrin and Placebo. Patients take oral tablets twice a day before meals from the day of refractive surgery for two months. Clinical investigations were performed, including OSDI, NIBUT, Schirmer's test, Lipiview and OSA.

Result: Fifty participants with mean age 23.64 ± 2.76 years (54% females & 46% Males) were recruited and followed up after 1 month and 3 months post refractive surgery. Participants in Lactoferrin group had significant improvement in mean OSDI scores (p- value < 0.0001), average NIBUT (p-value < 0.0001) and in mean lipid layer thickness (p-value - 0.004) at 3 months.

Conclusion: Use of oral Lactoferrin tablet is a safe addition to post refractive surgery treatment. It also reduces the post refractive surgery dry eye symptoms and can be a reliable nutritional supplement for dry eyes.

PS-I-19

The anti-angiogenic role of angiotensin-converting enzyme inhibitor to mitigate diabetic retinopathy

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Purpose: The role of the ocular Renin-Angiotensin System (RAS) has been underexplored in mitigating diabetes-induced complications. The current study attempts to elucidate the mechanism of Angiotensin Converting Enzyme (ACE) inhibitor- Lisinopril in regulating the RAS components and angiogenesis factor in high glucose-exposed Human Retinal Endothelial Cells (HREC).

Methods: HRECs were cultured in an endothelial growth medium with high glucose condition (25 mM concentration). The tube formation and hen's egg test-chorioallantoic membrane assays were performed to evaluate the anti-angiogenic effect of lisinopril. The change in the expression levels of several molecular markers, including Vascular Endothelial Growth Factor A (VEGF-A), Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), Intercellular Adhesion Molecule 1 (ICAM-1), Hypoxia-Inducible Factor 1-alpha (HIF-α), and Angiotensin II Receptor Type 1 (AT1R) were quantified using real-time polymerase chain reaction.

Results: The ACE inhibitor- lisinopril, was safe from 10 nM to 2 µM with a % HRE cell viability of >80% in high glucose conditions. The high glucose-induced tube formation was significantly reduced (50 ± 5%) by lisinopril at the concentration of 1 µM. Furthermore, the lisinopril treatment modulated the expression of RAS and angiogenic markers.

Conclusions: The blockade of the ACE by lisinopril effectively downregulates the tube formation in the high glucose-exposed HRECs. This suggests the therapeutic modulation of the RAS signalling cascade could be an alternative to treat ocular angiogenesis associated with diabetic retinopathy.

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PS-I-20

Unveiling the mechanism of epidermal growth factor receptor inhibitor-induced ocular toxicity

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Purpose: Epidermal Growth Factor Receptors (EGFR) are known to regulate tight junction proteins, which, in turn, influence the integrity of corneal epithelial cell barrier functions and corneal homeostasis. But, EGFR inhibitors, used to treat certain cancers, are also known to cause ocular toxicity. We hypothesise EGFR inhibitors dysregulate the tight junction proteins responsible for maintaining corneal cell proliferation, differentiation, and migration, leading to ocular toxicity.

Methods: Human Corneal Epithelial (HCE) cells were treated with EGFR inhibitors (Erlotinib or Afatinib) for 24, 48, and 72 hr. Erlotinib was administered topically in the right eye of Wistar rats, thrice a day for four weeks. RNA was isolated from the HCE cells and cornea, followed by cDNA synthesis. Tight junction proteins (Claudin-1, Claudin-2, Claudin-3, Zona Occludens (ZO-1, ZO-2, ZO-3), and Occludin) gene expression was assessed using specific primers by Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR).

Result: RT-qPCR analysis showed a significant dysregulation of tight junction genes in HCE cells and corneas treated with EGFR inhibitors. Results showed a 5-10-, 3-5- and 1–3-fold increase in gene expression at 24, 48, and 72hr, respectively, in vitro and 1-2-fold increase in vivo compared to controls. Statistical data analysis was done using a one-tail unpaired t-test.

Conclusion: The in vitro and in vivo gene expression studies showed the upregulation of ZO, Claudin, and Occludin, which could be a potential underlying cause for EGFR inhibitors-induced ocular toxicity. However, further studies are needed to validate the role of tight junction proteins in ocular toxicity.

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PS-I-21

Biodegradable polymeric inserts for prolonged ocular residence of amphotericin in fungal keratitis

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Purpose: Fungal keratitis is a corneal infection currently treated with off-label lipid-based (Amphotericin B liposomes) eye drops. This treatment involves higher frequency of administration, leading to poor patient compliance. In order to improve the ocular residence of the drug, an Amphotericin B loaded conjunctival insert (Ampat_C) was prepared with a crescent shape.

Methods: Ampat_C was prepared with a combination of mucoadhesive polymers such as Hydroxypropyl methylcellulose and Chitosan (1:1) using solvent-casting method. The developed inserts were characterized using various techniques to evaluate its physiochemical and mechanical properties, swelling index, drug release and residence time.

Results: In-vitro drug release studies showed that nearly 75% of the Amphotericin B was released within 12 hours and more than 95% at 24 hours. The drug release pattern and the swelling behaviour of the insert suggest a diffusion-controlled delivery system. The hydrophilicity of the polymers, as indicated by the contact angle less than 90°, increased the interaction of the insert with the conjunctival sac leading to higher muco-adhesion. The developed insert showed a conjunctival residence time of more than 8 hours in rabbits.

Conclusion: Ampat_C showed a sustained release of Amphotericin B and improved drug residence time, which could reduce the dosing frequency of current topical eye drops. The developed patch was found be biocompatible. However, further pharmacokinetics and efficacy studies are required to determine the dosage regimen of Ampat_C.

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PS-I-22

An alternative for riboflavin eye drops – ribopat: riboflavin loaded polymeric ocular patch

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Purpose: Corneal crosslinking is a routine procedure used for treating keratoconus. However, it includes de-epithelialization, frequent administration of riboflavin eye drops, and long-term UV-A light exposure. To overcome the present challenges, we aim to fabricate a biodegradable riboflavin-loaded polymeric ocular patch (RIBOPAT)to reduce the frequency of administration and improve the corneal permeation of riboflavin.

Methods: RIBOPAT was prepared via solvent casting method after screening various polymers for their patch-forming ability. The fabricated RIBOPAT was evaluated for in vitro release, and mechanical properties like tensile strength and % elongation. The cell viability assay and the Draize test were performed to ensure the biocompatibility of the designed RIBOPAT. The in vivo residence time of RIBOPAT was also assessed to understand the dissolution time.

Results: RIBOPAT was developed using a combination of biocompatible polymers like sodium carboxymethylcellulose and κ- carrageenan (SCC-R), sodium alginate and κ-carrageenan (SAC-R) with 0.5mg/mL riboflavin. The SCC-R and SAC-R showed a 100% drug release in 1 hour. The tensile strength and % elongation of SCC-R and SAC-R was found to be 9259.2 and 1533.0 N/m2 and 49.98% and 48.25% respectively. SCC-R and SAC-R were proven to be safe and biocompatible, with an in vivo residence time of 15 and 18 minutes respectively.

Conclusion: RIBOPAT could be a potential alternative for riboflavin eye drops, in reducing the frequency of administration. Further, studies are required to evaluate RIBOPAT based corneal permeation and crosslinking procedure in keratoconus induced rabbit model.

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PS-I-23

HEMA-coated polymeric patch for sustained release of vancomycin to treat ocular infections

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Purpose: Vancomycin is a glycopeptide antibiotic, mainly used as an off-label eye drop to treat MRSA-resistant bacterial infections. These drops need to be administered more frequently (every 5-10 minutes for the first hour and hourly), which shows poor patient compliance. Hence, we aim to prepare a 2-hydroxyethyl methacrylate (HEMA) coated vancomycin-loaded polymeric patch for sustained release and to reduce the frequency of administration.

Methods: The polymeric patch was prepared by solvent casting method and uniformly coated with HEMA polymeric solution by UV crosslinking. Surface morphology, physicochemical, and mechanical properties of the patch were evaluated using various characterization techniques. In-vitro and In-vivo safety studies were performed to evaluate the toxicity of the developed formulation.

Results: The HEMA-coated patches were found to be more transparent and smoother than plain patches. SEM and AFM results confirmed the uniform and smooth surface of the HEMA-coated patch, which could help in controlling the burst release of vancomycin. In vitro drug release studies indicated that HEMA-coated patches could sustain (98%) the vancomycin release for 24 h. The safety studies prove no irritation or toxicity of the developed formulation.

Conclusions: The developed HEMA-coated polymeric patch could be a potential alternative delivery system for current topical eye drops. Also, it can be used as a platform delivery system to control the burst release. However, further in-vivo pharmacokinetics and efficacy studies need to be performed to evaluate the therapeutic potential of the formulation.

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PS-I-24

Investigating the role of microglia as a therapeutic target for retinal inflammation using network pharmacology

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Purpose: Inflammation in retinal diseases (such as Uveitis, Diabetic Retinopathy, and Age-related Macular Degeneration) is driven by immune cell infiltration, with microglia as primary responders. Curcumin has shown its potential in managing inflammation, but its effect on microglial activation is unclear. This study aims to explore potential mechanisms and targets of curcumin using network pharmacology.

Methods: Curcumin targets were screened using Swiss Target Prediction while microglial activation and retinal inflammation targets from GeneCards. Overlapping targets were analysed with Venny2.0. STRING database was used to build a Protein-Protein Interaction network. Hub genes were screened using Cytoscape, Gene Ontology (GO), and Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analyses were performed using ShinyGO.

Results: Through network data mining we identified 100 curcumin targets, 2563 microglial targets, and 7889 retinal inflammation targets, with 44 intersecting targets. Further screening resulted in the top 10 hub genes (EGFR, CSF1R, STAT3, AKT1, PTGS2, MMP9, TNF, APP, and RAF1) using Cytoscape analysis. KEGG analysis highlighted significant pathways such as AGE-RAGE signalling, and JAK-STATpathway while GO analysis detailed relevant biological processes and molecular functions.

Conclusions: Network pharmacology indicates that curcumin might influence microglia through key receptors such as CSF1R and EGFR, impacting pathways like AGE-RAGE and JAK-STAT. Further in vitro studies, including RT-qPCR and western blot analyses, are needed to verify these results.

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PS-I-25

Biomimetic polymeric patch for improved corneal residence

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Purpose: Corneal polymeric patches can potentially overcome the challenges associated with eye drops. However, the demand for improved corneal mucoadhesion and residence time still persists. In nature, rose petals exhibit hydrophobicity and strong adhesive properties predominantly due to the unique microstructures. Therefore, the current study attempts to fabricate a rose petal patterned biomimetic polymeric corneal patch using 3D printing to improve its mucoadhesion on the corneal surface.

Methods: Different biomimetic patterns inspired by rose petals were designed in Computer-Aided Design software, and molds were fabricated using 3D-printing stereolithography. Hemispheres (H), columns (C), pyramids (PY), and trigonal hexagons (THX) patterns were optimized through width, pitch, and height. Patterned patches of various polymeric combinations were prepared by replica molding technique. The patches were characterized for thickness, appearance, contact angle, and in-vivo residence time.

Results: The contact angles ≥90^o and contact angle hysteresis ≥ 20^o for 3D-printed molds were selected further, due to hydrophobicity and better adhesiveness. The polymeric patch fabricated using combination of xanthan gum and κ-carrageenan showed corneal residence time of 8 hours and 4 hours in THX and PY patterns, respectively, in comparison to the plain polymeric patch (30 minutes).

Conclusions: The developed THX and PY patterned patches showed a higher corneal residence time than the plain polymeric patches. This microfabrication-based technology can overcome the challenges associated with eye drops, like poor retention onto the ocular surface. Further, in-vitro and in-vivo studies are required to assess the biocompatibility of the developed biomimetic ocular patch.

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PS-I-26

3D-printed drug eluting conjunctival insert: A promising sustained delivery platform to deliver PDE-4 inhibitor for treating anterior uveitis

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Purpose: Anterior uveitis is the inflammation of the iris-ciliary body, which is majorly treated with conventional topical formulations. However, they have limitations like shorter residence time, high precorneal clearance and poor permeation. We have earlier demonstrated the potential of Apremilast (Phosphodiesterase-4, (PDE-4) to treat anterior uveitis (Sai et al ARVO-India, 2022). This study aims to prepare apremilast-loaded drug-eluting conjunctival insert to prolong the drug residence in the ocular surface, thereby reducing the dosing frequency and improve the treatment outcome.

Methods: The 3D printed drug-eluting conjunctival insert was prepared by UV crosslinking using 2-hydroxyethyl methacrylate (HEMA) along with crosslinkers and photocuring agents. The insert was characterized for physicochemical and mechanical properties. Efficacy study was performed using endotoxin induced uveitis rabbit model.

Results: The insert released (*in vitro*) the drug over 24 hours. In vitro and in vivo safety studies shows that the insert did not cause any irritation or toxicity which confirms it's biocompatibility. The contact angle study shows the hydrophilic interaction of the insert which indicates improved residence time in conjunctival sac. The in vivo efficacy study proves once a day administration of apremilast-loaded insert can reduce the infiltrated cells and protein in aqueous humour compared with the untreated group. Conclusions: The developed sustained-release drug eluting conjunctival insert could be used as an alternative for topical eye drops with once-a-day administration as compared to existing multiple dose formulation. The apremilast loaded insert was found to be safe and efficacious. However, further pharmacokinetics studies are required to determine the dosage regimen.

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PS-I-27

Artificial intelligence-driven transporter targeted approach to mitigate 5-fluorouracil-induced ocular toxicity

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Purpose: 5-Fluorouracil (5-FU) is a commonly used chemotherapeutic to treat colon, rectum, pancreas, and breast cancer. Studies have reported that 25-38% of patients undergoing systemic 5-FU therapy are affected by ocular toxicity and require cessation of therapy to manage these ocular complications. We hypothesize that Organic Anion Transporter-2 (OAT-2) expressed in the blood-tear barriers might be responsible for the uptake of 5-FU onto the ocular surface and cause ocular toxicity. Methods Artificial Intelligence (AI) and computational simulation were employed to understand the interaction of OAT-2 with 5-FU. The tear concentration of 5-FU in New Zealand white rabbits after intravenous administration (25 mg/kg) in the presence and absence of topical OAT-2 blockers were estimated using High-Performance Liquid Chromatography (HPLC). Further, a machine learning model was developed to predict novel OAT-2 inhibitors.

Results: Computational studies showed that 5-FU is a substrate of OAT-2. The pharmacokinetic study 5-FU showed that 5-FU entered the tear from systemic circulation. The tear concentration of 5-FU was reduced by 2.26 folds upon pre-treatment with OAT-2 inhibitors. Further, the AI model predicted novel OAT-2 inhibitors that could be used as topical therapy to minimize ocular toxicity.

Conclusions: OAT-2 transporter was found to play a vital role in the uptake of systemically administered 5-FU onto the ocular surface. The topical therapy with OAT-2 inhibitors reduced the accumulation of 5-FU and thus could be used as a potential therapy to mitigate ocular toxicity in cancer survivors undergoing 5-FU chemotherapy.

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PS-I-28

A mechanistic insight into the permeation of nanomicelles across the corneal barrier

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Purpose: Nanomicelles deliver water-insoluble drugs with improved permeation across the cornea. However, the mechanism through which the nanomicelles permeates across the cornea is not explored. Hence, this study aims to investigate the permeation mechanism of nanomicelles across the cornea.

Methods: Tocopheryl polyethylene glycol succinate (TPGS) was conjugated with Fluorescein isothiocyanate (FITC). Rhodamine (hydrophobic molecule) loaded FITC-conjugated TPGS (T-FITC) based nanomicelles were prepared by thin film hydration. The T-FITC nanomicelles uptake was evaluated in Statens Serum Institut Rabbit Cornea (SIRC) cells at 37 and 4°C. To understand the permeation mechanism across the cornea, T-FITC nanomicelles were administered topically to New Zealand rabbit's eye. At a pre-determined time point, SIRC cells or corneas were analysed for nanomicelles uptake/permeation using a confocal laser scanning microscopy.

Results: The uptake of T-FITC nanomicelles in SIRC cells was confirmed by the FITC signal which was high at 37°C compared to 4°C indicating the uptake via endocytosis. The FITC localization was higher in the corneal epithelium of T-FITC nanomicelles treated eyes compared to plain FITC treated eyes. The signals were also detected in the stroma and endothelium layer indicating the permeation of T-FITC nanomicelles across the corneal layers.

Conclusions: This study demonstrates that T-FITC nanomicelles permeate the cornea by endocytosis pathway. Further studies are needed to understand the specific endocytosis pathway for the permeation of nanomicelles across the cornea.

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PS-I-29

Ancient eye therapies- a promising scope for eye research

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Purpose: The results of a recent cross sectional, population-based survey study conducted across the country amongst persons of 50 years and above demonstrated that more than 1/4th persons are visually impaired in India. Ayurveda a 5000 years old Indian traditional system of medicine offers diverse knowledge in the field of netra rogas (eye diseases). It has elaboratively explained the diseases classification like shuklagata (conjunctival diseases), krishna-gata (corneal diseases), vartmagata (diseases of the eyelids), drishtigata (retinal diseases) along with their causative factors, treatment protocols and preventive aspects. Netra Kriyakalpa (eye therapies) such as Tarpana, Netra Seka, Pindi and Bidalaka are various procedures for eye ailments. Sushruta (considered as Father of Surgery) has clearly defined when and how to perform the eye therapies. If the patient is having acute inflammatory conditions, he has stated to avoid putting any herbal medications inside the eyes whereas it is safe to perform the therapies with closed eyelids. If the traditional knowledge of Ayurveda is used accurately it can serve for the betterment of our nation and entire world in the upcoming years.

Conclusion: Developing a bridge between the modern and indigenous medicine by keeping the arrays open for research ideas is the need of the hour. It's time to collectively work on a new era of research and together we can prevent eye ailments and serve for the betterment of our country and also help in globalising Ayurveda.

PS-I-30

Development of enhanced Sodium ascorbate eye drops for ocular emergency

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Purpose: Ascorbate eye drops are used in emergency management of corneal burn. Freshly ascorbate aqueous solution is highly unstable. The objective of present study is the development of a stable formulation of ascorbate by adding another antioxidant for its sterile reconstitution and use in clinics.

Methods: Approved chemical stabilizers were tested for their utility to increase the shelf life of ascorbate after its reconstitution. Three different types of formulations were prepared by adding different combination of antioxidant of 0.1% and 0.5% sodium metabisulphite (SM) with 10% sodium ascorbate in aseptic pharmaceutical drug dispensing facility. Samples were divided into two batches, room temperature (25 °C) and 4°C. Ascorbate content in the formulation were analysed on 0, 3 and 7 days using UHPLC. Osmolarity and pH were also recorded to monitor formulation stability.

Results: The use of 0.5% of sodium metabisulphite significantly increased the stability of 10% ascorbate up to day 3 at room temperature within pharmaceutically accepted limits. No significant decrease in pH and osmolarity were observed in the same indicating its utility in clinics.

Conclusion: This study clearly demonstrated that the shelf life of ascorbate can be enhanced based on the co-formulation compounds. The successfully developed reconstituted formulation was found to increase the shelf life up to 3 days by the incorporation of 0.5% of SM.

PS-I-31

Formulation and stability study of extemporaneous anidulafungin ophthalmic preparation

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Purpose-: Fungal Keratitis (FK) is a severe disease that can lead to vision loss. Voriconazole and caspofungin acetate, 0.5%, have been reported to be effective for FK. However, refractory cases with resistance to these drugs have been started to emerge. New antifungal agents such as semisynthetic echinocandin (anidulafungin), could be a promising alternative, however its topical formulation has not been standardized yet. Therefore, this study was conducted to understand the stability and efficacy profile of anidulafungin eye drops.

Methods-. The ophthalmic formulations of 0.5% anidulafungin eye drop was prepared and analysed for the quality and stability parameters at different day 0, 1, 3, 7, 14, 28 days (n=3). The eye drops were kept sealed until the day of analysis, either under refrigeration (at 4.0± 1.0°C) or (ii) at room temperature (25.0 ±1.0°C). The formulations were tested for the physical parameters of osmolality and pH. Further, the formulations were subjected to Anidulafungin content analysis using Ultra-High-Performance Liquid Chromatography.

Results- The ophthalmic formulations were found to be stable for 28 days, with a % drug concentration (94.6%± 0.64) and (89.9%± 3.5) at 4°C and 25 °C. The pH (4.4-4.47) and osmolarity (311.5-315.2 mOsm) of the ophthalmic solution remained within the pharmaceutical acceptance range.

Conclusion- The ophthalmic formulations of anidulafungin remained stable for 28 days under refrigerated condition but not at 25°C.

PS-I-32

Impact of pH and osmolarity on the safety of extemporaneously prepared eye drops: A comprehensive analysis

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Purpose- The study evaluated the physicochemical properties of extemporaneously prepared fortified eye drops, focusing on pH and osmolarity, to assess their alignment with safety guidelines and their potential contribution to corneal toxicity and abrasions.

Methods- Eye drops were compounded in the sterile facility at the pharmacy from different drug classes including immunosuppressants, antibiotics, corticosteroids, α-adrenergic agonists, mydriatics, antiseptics, miotics and β-blockers were selected. These drugs were prepared in typical concentrations for extemporaneous use and diluted with artificial tear, water for injection, normal saline or PVA following standard practices. pH and osmolarity were measured using a pH meter and osmometer. Mean values were calculated and compared to the recommended safety ranges (pH: 6.5-7.8; Osmolarity: 280-300 mOsm/kg).

Results- The pH for antimicrobials ranged from 2.75 to 6.78 with the median of 5.98, while the osmolarity was in wider range (11.66-440 mOsm/L) with median of 296.33 mOsm/L. The other formulations had pH range of 4.25-7.39 and osmolarity from 14.66-477.33 mOsm/L.

Conclusion- The deviation in physicochemical parameters from the standardised range may be responsible with the eyedrop instillation associated mild discomfort and redness. Further studies are required to substantiate the ocular side effects associated with the use of ophthalmic preparation with deviated physicochemical parameters.

PS-I-33

Association between plasma biocides levels and diabetic retinopathy

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Purpose- Emerging evidence suggests a link between biocides, and type 2 diabetes mellitus. This study aimed to quantify biocides levels in the plasma of patients with various stages of diabetic retinopathy (DR) and investigate a potential association between biocides exposure and DR progression.

Methods-Plasma samples were collected from patients with mild (n=10), moderate (n=12), and severe non-proliferative DR (NPDR, n=11) and proliferative DR (PDR, n=12), along with controls (n=13). The levels of eight common biocides (Metolachlor, Propachlor, Metribuzin, Atrazine, Dimethoate Imidacloprid, Thiobencarb, Pendimethalin) were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results- The study analyzed 8 biocides in the plasma of variable stages DR patients. Out of 8 biocides, 2 biocides (i.e. metolachlor and propachlor) were detected in the plasma of the study patients. The mean plasma concentration in PDR patients for metolachlor and propachlor was 0.18 ng/ml (range 0.179-0.203ng/ml) and 1.08 ng/ml (range 0.9-1.3 ng/ml).

Conclusion- This preliminary study found elevated biocides levels in DR patients, indicating a potential link between biocides exposure and DR progression. However, the further studies are required to validate the obtained results.

PS-I-34

Evaluation of azirine eye drops for the treatment of corneal neovascularization: an explorative study

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Purpose: The objective of presented study was to characterize and evaluate the antiangiogenic activity of developed Azirine (AZN) formulation in an experimental angiogenesis rat model.

Methods: The topical formulations were prepared using various concentration of Azirine (AZN) (0.001%, 0.01% and 0.1%). The developed AZN formulations were subjected for clarity, appearance, pH, osmolarity and viscosity evaluation parameters. The stability of the formulation was analyzed with UHPLC. Alkali cautery was induced using 75% $AgNO_3$ and 25% KNO_3 in eye of rats and were treated with AZN thrice for 6 days. Dypiridamole (0.008%) was used as the positive control. The images were captured on day 7 using slit lamp attachment of MICRON-III rodent imaging system. The corneal neovascularization area was assessed using grading system by three independent reviewers. The difference between the groups was considered significant at $p \leq 0.05$.

Results: The prepared ophthalmic formulation had pH (5.8-5.9) and osmolarity of (290-295 mOsm) within the acceptable pharmaceutical limits. In the cautery induced CNV model, the positive control showed 82% reduction in neovascular area ($p=0.005$) compared to control. The AZN 0.001% topical formulation could reduce angiogenesis upto 67% ($p=0.08$). While, as compared to the disease control, AZN 0.01% and 0.1% topical instillation showed significant reduction of neovascular area upto 89% ($p=0.05$) and 82% ($p=0.05$), respectively.

Conclusion: The stable AZN (0.001-0.1%) formulation was effective in antiangiogenic effect against cautery -induced neovascularization model. The topical formulation of AZN 0.01% can be explored further for its anti-angiogenic activity in corneal angiogenic condition.

Acknowledgement: This study is supported by the collaborative grant between CDRI-AIIMS, New Delhi.

PS-I-35

Fabrication of cornea mimetic lenticules: comparative analysis of 3D bioprinting and compression molding techniques

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Purpose: Bioengineered corneal lenticules are emerging as critical tools in addressing corneal defects which traditionally rely on donor transplants. Two powerful fabrication methods, 3D bioprinting and compression molding, offer promising alternatives with distinct advantages and challenges.

Methods: A comparative evaluation of both the fabrication techniques was done with similar bioink composition. Both the bioprinted and compression molded cornea lenticules were evaluated via physico-chemical characterization involving light transmittance, mechanical stability, swelling, optometry readouts like densitometry, keratometry and compatibility with human corneal stromal cells (hCSCs) and epithelial cells.

Results: The bioprinted lenticules exhibited robust mechanical properties (compressive modulus of 535.42 ± 29.05 kPa), high optical transparency (> 85%), excellent re-epithelialization, supporting corneal integration and regeneration, whereas compression molded lenticules achieved a compressive modulus of 400 kPa. Anterior Segment Optical Coherence Tomography (AS-OCT) imaging of these lenticules revealed opacity score of 18.6 with no hyper-reflectivity in comparison to 16.5 of native human cornea, thereby indicating a clear and transparent lenticule.

Conclusions: This study provides an in-depth understanding of both the facile fabrication strategies. Bioprinting excels in achieving precise structural fidelity and mechanical performance, whereas compression molding emphasizes rapid scalability, high throughput production. Both the methods offer a convenient patient specific customization options for surgeons, and the optimal choice between these techniques will hinge on the specific clinical requirements. Whether prioritizing detailed structural accuracy or efficient, large-scale production, addressing the pressing need for donor corneas and advancing the field of corneal regenerative medicine.

PS-I-36

Trabecular meshwork stem cell exosomes: identifying the cargo and functional efficacy for a cell-free therapy for glaucoma

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Purpose: To evaluate the efficacy of trabecular meshwork (TM) stem cell (SC)-derived exosomes on TM cell survival as well as proliferation, and to decipher the associated exosomal protein cargo by mass spectrometry.

Methods: TM and TMSC exosomes were isolated by ultracentrifugation and characterized. Uptake specificity in various cell types was determined using labelled exosomes. *In vitro* functional assays were carried out to evaluate the regenerative efficacy and antioxidant potential of the exosomes. To decipher the exosomal protein cargo mass spectrometry was performed.

Results: Exosomes from TM and TMSCs were within 30-200 nm size. Differential exosome uptake by TM cells, HLEB3, and 3T3 cell lines suggested varied surface components with maximum uptake of the TMSC exosomes by TM cells (74.6±16.9%). TMSC exosomes significantly enhanced TM cell migration (96.47±2.95%) and proliferation (Ki67-23.7±4.6%, BrdU-37.01±6.11%) compared to TM exosomes (migration- 79.34±9.26%, Ki67-12.13±0.88%, BrdU-22.26±4.59%). In addition, the TMSC exosome treatment reduced intracellular reactive oxygen species (iROS) by 9-fold and improved TM cell survival under chronic oxidative stress. Proteomic analysis revealed increased levels of proteins related to wound healing and antioxidants in TMSC exosomes which regulate TM cells through PI3K-AKT, TGF-beta, non-canonical Wnt, and mTOR signalling pathways.

Conclusions: This study demonstrated for the first time that the TMSC exosomes enhanced TM cell proliferation as well as migration *in vitro* and attenuated oxidative stress-induced cell death by reducing iROS. Further studies in animal models will pave the way for the potential application of TMSC exosomes in glaucoma treatment in the future.

PS-I-37

Effect of altered micronutrient transporters on placental functions and its association with risk of retinopathy in pre-term infants

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Purpose: The study is designed to understand the effect of micronutrient transporters on placental biology and how in-utero transfer of these events through the placenta to the developing fetus could affect the ROP progression.

Methods: Placenta were collected from six normotensive control women and twelve women delivering pre-term (six preterm without ROP and six with ROP). Infants born with gestational age ≤34 weeks and birth weight ≤1700g were included in the preterm group which were classified into ROP and non-ROP based on retinal evaluation. The gene expression of folate transporters (PCFT, RFC and FRα), vitamin B12 transporter (CD320), enzymes involved in folate & B12 metabolism (MS, MTHFR) and angiogenesis (VEGF-R, KDR) along with hypoxia (HIF-1α) was assessed in placenta by qRT-PCR.

Results: The study results demonstrates that the expression of folate transporters RFC, PCFT and FRα was decreased in pre-term placenta with ROP ($p<0.05$), however the expression of B12 transporter (CD320) was increased. We observed no change in the expression of methyl-tetrahydrofolate reductase (MTHFR); however, the expression of methionine synthase (MS) ($p < 0.05$) was increased with ROP progression. Hypoxia-inducible factor (HIF-1α) expression was found to be increased with ROP however we observed no change in the expression of VEGF-R and KDR. The expression of HIF-1α was positively correlated with birth weight.

Conclusion: The current study demonstrates deficient B12 status as evident from increased expression of B12 transporter, and its association with hypoxic induction in the pre-term placenta. This might result in fetal growth retardation in-utero and could be associated with increased risk of retinopathy.

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PS-I-38

Decline in YAP nuclear expression in lens epithelium of cataractous donor compared to healthy donor lens

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Purpose: Loss of Lens Epithelial Stem Cells (LESCs) in cataractous lens was demonstrated earlier by the absence of SOX2+ GJA1- cells and by the reduction of sphere-forming cells in central zone. Nuclear localization of Yes-associated protein (YAP) has been reported to be associated with stemness in various tissues. This study aims to analyse the expression of YAP in anterior human lens epithelium in normal and cataractous conditions.

Methods: Donor lenses of both normal (n=3) and cataract (n=3) were obtained from the eye banks of Aravind Eye Care System. The whole mount of anterior lens epithelium was immunostained for SOX2, YAP, and alpha-SMA. Sequential confocal images were acquired from one end to the other end of the epithelium and analysed.

Results: The nuclear expression of YAP was identified in 21.9±17.7% cells in central (C) zone and 15.3±17.3% cells in equatorial (E) zone of normal human lens epithelium. In cataractous condition a reduction in the nuclear YAP expression (C: 5.9±3.7%; E: 1.6±2.0%) was observed. While cells double positive for SOX2 and YAP were identified in normal lens (C: 9.0±3%; E: 7.1±6.9%), such cells were absent in cataractous lens epithelium. Additionally, the epithelial-to-mesenchymal transition marker alpha-SMA was expressed higher in cataractous lens compared to normal lens.

Conclusion: The absence of SOX2 positive cells, reduction in YAP positive cells, and increased alpha-SMA expression in cataractous lens provides additional proof for a probable role of stem cells in the development of cataract.

PS-I-39

Proteomic analysis of extracellular vesicles from saprophytic and clinical isolates of *Aspergillus flavus*: a comparative study

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Purpose: Characterizing the *A. flavus* EVs from corneal isolate and saprophyte.

Methods: *A. flavus* ATCC-200026 (Pea plant) and an isolate from a keratitis patient MTCC 13369 were used for EV isolation; the solid-state fermentation (SSF) technique was employed. EVs were isolated using ultracentrifugation. Nano-particle Tracking Analysis (NTA) was used to examine the particle concentration and size distribution of isolated EVs. Fungal EVs were stained with CM DiL and observed under confocal microscope. Mass spectrometry was used to identify the EVs proteome.

Results: The size of EVs was compared using NTA data, ATCC-26 EVs were found to be similar and MTCC 13369 EVs found to be in confocal microscopic image. Gene enrichment analysis of ATCC-26 EVs and MTCC-13369 EVs shows many structural proteins. Differentially expressed proteins between ATCC-26 and MTCC-13369 are displayed in a volcano plot.

Conclusion: State specific pathways or significant proteins unique to clinical sample and saprophyte.

PS-I-40

Regulation of vascular endothelial growth factor is significant in the pathology of ocular diseases

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Purpose: VEGF, a signalling molecule of the endothelial cells is intricately involved in the pathology of glaucoma, diabetic retinopathy (DR) and retinoblastoma. PI-3K pathway gets activated in ocular conditions of hyperglycemia, glutamate excitotoxicity and proangiogenic conditions which increases the expression of VEGF/VEGFR molecules. Dopamine (DA), Coenzyme Q10 and trolox and IGF-1 have proven to be effective therapeutic molecules to regulate the VEGF/VEGFR expressions and attenuating symptoms of DR, glaucoma and retinoblastoma respectively.

Method: DA alone at 10mg/kg body weight and DA + IGF-1 (2µgm/eye) was administered in animal models of DR maintained for a period of 12 as well as 16 weeks. Glaucoma was induced by causing glutamate excitotoxicity of the NMDA receptors by intravitreal administration of NMDA. Coenzyme Q10 and Trolox given at concentrations of 10mg/kg BW for a period of 7 days. Retinoblastoma model was setup by growing Y79 cells in vitro. The mRNA transcript levels of VEGFR1, VEGFR2, IGF-1R, Drd1,2 and 4, Grin2A and Grin2B are determined. Protein expressions of pVEGFR2, ERK, pERK, Akt and pAkt observed through western blotting and immunohistochemistry.

Result: VEGFR1, VEGFR2 expressions suppressed but Drd2 and IGF-1R enhanced in DR. VEGFR1, VEGFR2 and Grin2A expressions enhanced but diminished Grin2B expression in glutamate excitotoxicity model of glaucoma. Suppressed VEGFR1, VEGFR2 and IGF-1R expressions in Y79 model of retinoblastoma.

Conclusion: Regulation of VEGFR2 and VEGFR1 as downstream targets of Pi-3K/ERK/ Akt signalling pathways is crucial in the therapeutics of DR, glaucoma and retinoblastoma.

PS-I-41

Secreted frizzled-related proteins directed modulation of matrix metalloproteinases activity in keratoconus

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Purpose: Keratoconus is an ocular condition marked by progressive steepening and ectasia of corneal surface, leading to cone shaped deformation that compromises the structural integrity of the cornea, and significantly results in impaired vision. In this study we aim to investigate how secreted frizzled-related proteins (sFRPs) mediated modulations affects matrix metalloproteinase (MMPs) activity and to find intricate relationship of these molecules, particularly emphasizing on their ability to regulate key molecular processes underlying keratoconus progression.

Methodology: Following the acquisition of informed consent, participants underwent ophthalmic exams and corneal epithelium (CE) was collected from KC patients undergoing C3R surgery and control undergoing PRK. RNA was isolated, quantified, and evaluated via qPCR for gene expression. sFRPs, MMPs and fibrotic markers were studied, normalized using housekeeping genes, and fold changes recorded for further analysis. To assess the effect of sFRPs on different cells, Corneal stromal fibroblasts and limbal epithelial cells were treated with sFRPs and analyzed for MMPs and fibrosis.

Results: The level of sFRPs, MMPs and fibrotic markers were elevated in CE of KC patients. Our in-vitro experimentation demonstrated that treatment of sFRPs led to significant dysregulation in level of MMPs and fibrotic markers.

Conclusion: The elevated levels of sFRPs, MMPs, and fibrotic markers in the CE of patients, combined with our in-vitro findings showing significant dysregulation of these markers, highlights the crucial role of sFRPs in the pathogenesis of keratoconus. These findings support the hypothesis that sFRPs are involved in disrupting normal corneal homeostasis and contribute to disease progression.

PS-I-42

Integrative Metadata Analysis and Validation of Putative Markers for Adult Retinal Pigment Epithelial Stem Cells

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Purpose: The adult stem cells (SCs) in human Retinal Pigment Epithelium (RPE) were identified earlier to be present in the peripheral region based on functional characteristics. Since there are no RPESC specific marker, this study aims to identify putative SC markers by meta-data analysis and confirmation using human RPE.

Methods: Single cell RNA sequencing data of human RPE were retrieved (Voigt *et al.,* (2019); Orozco *et al.,* (2020); Xu *et al.,* (2021)) and the genes associated with stemness were identified through literature. The identified SC associated genes common in all three datasets were validated by quantitative real-time PCR (qRT-PCR). RNA was isolated from human RPE cells from the three regions: central, equatorial and peripheral RPE followed by cDNA conversion and qRT-PCR. Further, immunostaining of human RPE sections were carried out for the SC-specific markers.

Results: Twelve SC associated genes were identified to be common in all three datasets. Validation by qRT-PCR identified elevated expression of MET, BMP7, NEAT1, WWC1, ARHGAP18, SPOCK1, and RDH10 in the peripheral RPE. Immunostaining identified absence of MET and BMP7 expression in human RPE sections.

Conclusion: This study identified seven RPE-SC specific genes by literature search, and validated their differential expression in peripheral RPE at mRNA level but could not confirm the same at protein level. Further, proteomics studies are essential to identify a human RPE-SC specific marker.

PS-I-43

Phospholipases mediated inflammation and extracellular matrix remodelling drives keratoconus pathology

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Purpose: Keratoconus (KC) is a degenerative corneal disorder that leads to structural changes in the cornea and visual impairment. We identified differentially expressed genes (DEGs) and enriched pathways in the corneal epithelium (CE) of KC patients compared to controls using RNA sequencing. Phospholipases were the topmost upregulated genes. Further, validated these genes in different sets of patient samples and assess their impact on human corneal limbal epithelial cells (HCLE) and corneal stromal fibroblasts (CSF).

Method: Informed consent was obtained, CE tissue samples from four keratoconus patients undergoing corneal collagen cross-linking and individuals undergoing photorefractive keratoplasty were collected, followed by RNA isolation and RNA-sequencing. KC pathology associated DEGs and enriched pathways were selected, validated in different KC patient sets, and assessed their impact on HCLE and CSF for inflammation and extracellular matrix (ECM) remodelling.

Results: RNA sequencing analysis revealed 85 upregulated and 122 downregulated genes in the KC patients. We found out that level of phospholipase genes was upregulated in keratoconus patients as compared to control. Treating cells with phospholipase proteins lead to increased inflammation and dysregulated ECM synthesis.

Conclusion: Our findings suggest that phospholipases play a significant role in the cellular remodelling associated with keratoconus. The dysregulation of these enzymes contributes to inflammation and ECM disruption, which are critical factors in the development and progression of keratoconus. Further research into phospholipase-mediated pathways may provide new insights for targeted therapeutic strategies.

PS-I-44

Corneal regenerative capacity of bioengineered biopolymer-kuragel by ameliorating inflammation and enhancing corneal nerve repair

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Purpose: Neurotrophic keratitis (NK) is long-term manifestation of alkali-injury characterized by impairment of corneal innervation leading to reduced/absent corneal sensation. Weakening of corneal reflexes occur due to reduction in trophic neuromodulators that are essential for the corneal wound healing. It is also often associated with increase in pro-inflammatory markers such as IL-6 and extracellular matrix (ECM) modifications markers such as MMP-9. Here we propose the corneal nerve regeneration properties of our biodegradable hydrogel-Kuragel for regenerative treatment.

Methodology: Alkali burns were induced in New Zealand rabbits by treating the cornea with (i) filter paper soaked in 0.75 N NaOH for 10 s and (ii) trephination using a guarded trephine, followed by alkali burn with 0.75 N NaOH for 10 s. Cornea was rinsed immediately with 10 mL of normal saline to remove traces of NaOH. Kuragel was administered at site of excavation and animals monitored for 3 months. Corneal tissue was analysed for the biomarkers for inflammation, ECM remodelling and trophic neuromodulators.

Results: Alkali injury eye model of direct alkali burn and trephination followed by alkali burn caused an increase in pro-inflammatory and ECM milieu in the cornea. The bioengineered biopolymer-Kuragel was observed to ameliorate corneal wound healing in rabbits by targeting the IL1β, IFNᵞ along with neurotrophins such as epidermal growth factor (EGF), nerve growth factor (NGF) and neurotrophin-3 (NT3).

Conclusions: Neurotrophic keratitis is degenerative disease leading to corneal ulcer, corneal epithelial defects and debilitating corneal nerves. Our bioengineered biopolymer presents effective corneal wound healing and nerve regeneration in alkali injury model.

PS-I-45

Gene enrichment analysis and discovery of novel-splicing events in Behcet's disease: a sub-type of uveitis

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Purpose – Behçet's disease (BD) is a rare auto-inflammatory condition characterized by chronic uveitis and other clinical features. Alternative splicing events contribute to disease pathogenesis in number of auto-immune and inflammatory diseases. BD being an inflammatory disease, novel splice events might occur in BD patients and we aim to explore occurrence of possible novel splicing events in BD patients.

Methods – Total RNA extracted from the PBMCs of BD cases and healthy controls was sequenced. DESeq2 tools were used for downstream analysis. Gene enrichment analysis was conducted using Cluster Profiler. StringTie was used for the identification of novel transcripts. Novel splicing events, including exon skipping, were identified using rMATS package and visualized as sashimi plots. Genome-wide distribution of splice events were determined and viewed using karyoploteR tool.

Results – A total of 275 DEG's (229 upregulated; 46 downregulated) were identified. GO and pathway analysis showed significant enrichment molecular functions and pathways associated with inflammation. Selected loci in CAST gene showed significant involvement in exon skipping in BD. Formation of novel exon was discovered in many genes, particularly in PLB1 and BARD1 genes. Genome-wide distribution of the splice events showed that chr1 had significant number of splice events suggesting a potential regulatory role in the disease regulatory mechanisms.

Conclusions –Novel splice events in ophthalmic disease, particularly in BD has been explored for the first time in Indian cohorts. Genome-wide enrichment analysis of the splice variants underscores the importance of splicing in the regulation of disease pathology in Behçet's disease.

PS-I-46

A comparative study of eye lens protein α-crystallin in different ages of Rohu fish and recombinant human αb-crystallin

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Purpose: The objective of my study is to find out the changes of α-Crystallin, an eye lens protein in different ages of Rohu fish (1 year and 3 years) in comparison with Recombinant Human αB-Crystallin (as control).

Methods: α- Crystallin was isolated and purified from different aged Rohu fish eye lens, while Human αB-Crystallin was purified as recombinant form after over-expression in *E. coli*. To analyse the age-related changes, surface hydrophobicity in terms of bis-ANS binding, structural stability by urea denaturation study and chaperone activity by substrate lysozyme were measured. Dynamic Light Scattering was done to measure the oligomeric size of α-Crystallin.

Results: The results showed that in both time dependent and time independent kinetics of bis-ANS binding, preheated form of protein shows greater intensity of binding than native form in 3 years while 1 year showed a nominal value; but recombinant Crystallin showed highest affinity than fish. Urea unfolding assay showed that recombinant αB-Crystallin is most stable and Rohu 3 years is much stable than 1 year. The chaperone activity of human is 82% in 1:1 ratio while 3 years fish shows 74% in 1:6 dilution and in 1 year, it is 80% in 1:6 dilution. DLS study predicts recombinant protein diameter is 17-20 nm while in fish it is near about 4-7 nm for both ages.

Conclusions: During aging, the structural and functional properties change a little in different aged Rohu fish as it is primitive species, but a significant variation is shown in mammals (Recombinant Human αB-crystallin).

PS-I-47

Advancing DME management: the role of cytokine profiling in predicting anti-VEGF response

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Purpose: Diabetic Macular Edema (DME) is defined by fluid accumulation beneath the retina due to leakage in retinal blood vessels. Ranibizumab and Aflibercept are standard anti-VEGF therapy for DME, yet 30-40% of patients fail to improve visual acuity after years of intravitreal injections. DME-prognostic biomarkers are unknown. This study investigates DME patients' prognostic molecular signatures after anti-VEGF treatment.

Methods: Serum samples were collected from DME patients who had received a minimum of three anti-VEGF injections with six-month follow-up and grouped by central retinal thickness (CRT): responders (<300µm) and non-responders (>300µm). Bead-based flow cytometry analysis was used to investigate cytokines associated with vascular integrity, tissue healing, adhesion molecules, and inflammation. These cytokines were then correlated with the clinical outcomes of the patients.

Results: Angiopoietin-1 (Ang-1), a cytokine known for promoting vascular stability, was associated with significant improvements in CRT and visual acuity among responders. Additionally, elevated levels of TNF-α and VEGF were observed in responders, suggesting their contribution in vascular permeability repair with increase Ang-1/Tie-1 signalling. In contrast, higher levels of CD31 (PECAM-1) in non-responders indicate shedding and endothelial disruption; patients with high CD31 concentrations also showed poorer CRT improvement. These specific cytokine profiles could serve as prognostic markers for DME patients.

Conclusion: In conclusion, the study indicates that specific cytokine profiles, particularly higher levels of Ang-1, TNF-α, and VEGF correlate with better outcomes in DME patients. These profiles may serve as prognostic markers, enabling personalized treatment strategies. Conversely, elevated CD31 levels in non-responders highlight endothelial disruption, associated with poorer responses.

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PS-I-48

Comparative analysis of small EVs isolation techniques from tear fluid using different methods

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Purpose: Exosomes are widely used as biomarkers for various diseases and serve as a valuable source for liquid biopsy. Tear exosomes have the potential to be used as a non-invasive liquid biopsy source for conditions such as retinoblastoma, where tissue biopsies are not feasible. In this study, we aim to characterize the most effective method for isolating small extracellular vesicles (sEVs) from tear fluid, focusing on methods that offer high yield and purity while being time-efficient and cost-effective for clinical translation.

Methods: Tears and serum from volunteers were pooled separately, divided into aliquots and then subjected for sEVs isolation and comparison. We used three different methods to optimize sEVs isolation: ultracentrifugation (with three different protocols), PEG-based precipitation (with three different concentration), and a commercial kit-based isolation method. The isolated EVs were then characterized by nanoparticle tracking analysis (NTA) to access their concentration and purity.

Results: In the case of ultracentrifugation, none of the three protocols provided satisfactory yield or purity, indicating that further optimization is needed. In contrast, PEG8000-based precipitation, across all three concentrations (10%, 12% & 20% of PEG) along with commercial kit method, produced favourable results, with 80-90% of the sEVs consistently measuring less than 250 nm in diameter.

Conclusions: PEG8000-based precipitation and commercial kit protocols both showed good result in terms of yield, though some optimization is needed for purity. With further refinement, PEG8000-based precipitation could become a more cost-effective option for isolating tear exosomes compared to the commercial kit.

PS-I-49

Quantitative analysis method of rabbit corneal cells using in vivo confocal microscopy

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Purpose: To evaluate and quantify the corneal epithelium, keratocytes, sub-basal nerve plexus, and endothelium in healthy rabbit eyes using in vivo confocal microscopy (IVCM).

Methods: IVCM was performed on the central corneas of healthy New Zealand white rabbits. The corneas were scanned from the epithelium to the endothelium using a confocal microscope. Images were analysed to quantify cell densities in the epithelium, anterior and posterior stroma (keratocytes), and endothelium. Sub-basal nerve plexus parameters were also evaluated, including nerve fibre density, number of long nerve fibres, and total nerve count.

Results: The IVCM analysis revealed distinct cellular densities across the corneal layers. The epithelial layer showed a consistent density pattern. Keratocyte density was highest in the anterior stroma and decreased towards the posterior stroma. The endothelial cell layer exhibited a uniform distribution with a consistent cell density, crucial for maintaining corneal transparency. These findings establish a comprehensive baseline for normal rabbit corneal cell densities, serving as a reference for future research and clinical assessments.

Conclusion: This study demonstrates the effectiveness of IVCM in quantifying corneal cell densities in rabbits, providing essential baseline data for the corneal epithelium, keratocytes, and endothelium. These results are vital for future comparative studies involving corneal diseases and treatments, enhancing our understanding of corneal health and pathology in rabbits.

PS-I-50

Therapeutic targeting of 'aurora kinase A' in a chorioallantoic membrane xenograft model of RB

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Purpose: To assess the tumor forming potential of retinoblastoma (RB) cells on chorioallantoic membrane (CAM) and evaluate efficacy of Aurora Kinase A (AURKA) inhibition on the generated xenografts.

Methods: Rhode Island red eggs were incubated at 37.5°C and >55% humidity starting on embryonic development day 0 (EDD 0). On EDD 4, unfertilized eggs were separated, and fertilized eggs were fenestrated on EDD 6/7. RB cells, with or without DiI (Tetramethylindocarbocyanine Perchlorate) dye labelling, were implanted onto the CAM on EDD 9/10. The implanted cells were either pre-treated with an inhibitor (MK8745) or genetically silenced for AURKA. Tumors excised on EDD 14/15, were imaged, weighed, and their dimensions measured. Tumor volume was calculated as $4/3 \times \pi \times r^3$, {r = ½ × square root (diameter 1 × diameter 2)}. The DiI stained tumors were cryosectioned and visualized under a fluorescent microscope. FFPE sections were prepared from the remaining tumors and stained with Hematoxylin & Eosin. Tumor area and perimeter were analysed using ImageJ.

Results: Detection of DiI staining confirmed the presence of RB cells in xenografts, with histopathology showing hyperchromatic, viable cells with high nuclear-to-cytoplasmic ratios along with areas of necrosis and inflammation. Tumors treated with inhibitors displayed significant reductions in area, perimeter, weight, and volume compared to controls. These findings were consistent in xenografts harbouring RB cells having depleted AURKA.

Conclusions: AURKA inhibition led to reduced tumor sizes in RB xenografts. Therapeutic targeting of AURKA shows promise for further clinical development as a treatment strategy for human retinoblastoma.

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PS-I-51

Circulating markers as a diagnostic tool for POAG and PACG

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Purpose: This study was conducted to understand the variation in the circulating inflammatory and matrix-associated proteins between the primary open and angle-closure glaucoma.

Methods: Plasma samples were collected from patients of primary open angle glaucoma (POAG, n=14) and primary angle closure glaucoma (PACG, n=14). Total protein content was determined using Biuret reagent. Levels of IL-6, IL-10, MMP-9 and CK-13 were determined by ELISA. The difference between the groups was considered significant at $p<0.05$.

Results: Mean age of the cohorts were 64.8±4.7 (POAG) and 56.6±5.7 years (PACG). 86% of POAG group and 79% of PACG group were male participants. Median values of IL-6, IL-10 and MMP-9 concentration for POAG (0.004 0.002 and 12.95 pg/mg) and PACG (0.007, 0.005 and 15.72 pg/mg) were comparable. The IL-6 to IL-10 ratio was higher in PACG compared to POAG group. For CK-13, PACG group (0.043 pg/mg) had a higher median value as compared to POAG (0.055 pg/mg) ($p=0.09$).

Conclusions: For the first time, this study showed elevated levels of CK-13 levels in PACG group as compared to POAG. Future studies with larger cohort and onset of disease are required to validate these findings for any pharmacological intervention and/or biomarker establishment for PACG.

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PS-I-52

Bioengineered liquid cornea: instructing host tissue to prevents scarring in alkali injury wound model of mice

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Purpose: Loss of corneal transparency and poor refractive function are among the leading causes of blindness. Globally, around 200–300 million people are visually impaired, of which 5 million are affected with bilateral corneal blindness and 23 million by unilateral blindness. Corneal transplant is gold standard for management of corneal conditions. However, the balance between corneal donors and recipients is skewed towards recipient which leads to extensive wait time. To address this, artificial cornea is being employed, where an injured cornea will be removed and replaced with liquid cornea for treatment.

Material and Methods: C57BL/6 mice were used in the study. Corneal alkali wound injury of 2mm wide and 50um deep scar created using a graded trephine. Wounded animals were divided, first group received no treated (controls), second group, treated with bioengineered liquid cornea. Animals were imaged using the ophthalmological parameters; i) OCT, ii) Slit lamp and iii) Pentacam and iv) Galilei, over a period of 3 months. After 3 months, mice were sacrificed and eyes were enucleated and further processed for histopathology.

Results: Slit lamp revealed re-epithelization of the wound treated with bioengineered cornea, within 15 days. Central Corneal thickness of control mice ranged around 200-250um while bioengineered liquid cornea treated mice showed central corneal thickness (180-240um) almost similar to the control. In addition, the transparency of the regenerated cornea mimicked the native cornea as measured by opacity score.

Conclusions: The bioengineered liquid cornea is a regenerative treatment and this biopolymer acts as a sacrificial matrix for accelerating the growth of host tissue to cover the wound site.

PS-I-53

Comparison of low vs half dose ranibizumab in aggressive retinopathy of prematurity

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Purpose: To compare outcomes of low dose vs standard half-adult dose intravitreal ranibizumab (IVR) in eyes with aggressive retinopathy of prematurity (AROP).

Methods: This was a randomized, double-blinded investigator-initiated trial. We randomized 30 eyes of 15 eligible neonates with bilaterally symmetrical AROP to receive IVR 0.12 mg (intervention group) in one eye and 0.25 mg (control group) in the other eye. Additional interventions were reserved for eyes with progression or recurrence. Primary outcome was to assess disease regression at 12 weeks. Secondary outcomes were the area of retinal vascularization at 12 weeks, recurrence of disease, and need for secondary intervention.

Results: All eyes showed initial regression. Total area of retinal vasculature growth was 112.24±36.2 mm2 in intervention group and 115.74±39.69 mm2 in control group – both were statically significant. No significant difference was noted in final area of retinal vascularization between both groups. 7 eyes (58.3%) in each group had recurrence. 1 eye in the control group (6.6%) had retinal detachment.

Conclusion: With this preliminary investigation, we found that a lower dose of IVR may have similar outcomes compared to a half-adult dose of IVR for the treatment of AROP. This reduction of dose can reduce systemic side effects of anti-VEGFs in premature neonates as well as reduce the cost of treatment.

PS-I-54

Effect of long-term iron administration on mitochondrial dynamics and ferroptosis in aging rat retina

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Iron is essential for retinal function, but excess iron causes age-related macular degeneration (AMD). We investigated the effects of long-term iron administration on mitochondrial dynamics, mitophagy, and cell death in aging rat retinas. Male Wistar rats were grouped into 4-month control (4MC), 8-month control (8MC), 8-month experimental (8ME), 12-month control (12MC), and 12-month experimental (12ME) groups. Rats in the experimental groups were orally administered ferrous sulfate (500 mg/kg bw/week) from 4 months of age onwards until 12 months of age, control rats received water as a vehicle. They were sacrificed at 4, 8, and 12 months of age. Retinal iron levels, mitochondrial dynamics, signs of mitophagy and ferroptosis in retinas were assessed. The iron levels were significantly increased in 8ME and 12ME. H&E staining demonstrated thinning of the outer nuclear layer (ONL) and loss of the photoreceptor layer (PL). TEM confirmed the absence of ONL and PL, with a large capillary and vacuoles in the RPE. Confocal microscopy for smooth muscle actin revealed actin localization around blood vessels extending from the choriocapillaris into the inner retina, suggesting choroidal neovascularization in 12ME rats. Mitochondrial iron dysregulation was indicated by decreased mitochondrial ferritin and frataxin. Imbalanced mitochondrial dynamics were marked by increased Drp-1 and Mfn-2, with no correlation to Parkin and PINK1. Experimental rats exhibited autophagy initiation, evidenced by autophagosomes with disorganized mitochondria, increased LC3B, and decreased p62. Downregulation of glutathione and glutathione peroxidase-4 caused significant photoreceptor cell death in 12ME rats via ferroptosis. Immunohistochemistry revealed reactive gliosis in Müller cells, with GFAP upregulation in the 12ME group. This study concludes that iron overload disrupts mitochondrial dynamics, induces Parkin/PINK1-independent mitophagy, and triggers photoreceptor cell death via ferroptosis.

PS-I-55

Effects of Retinal Degeneration (RD) on inhibitory neurons and thalamic afferent terminals in primary visual cortex (V1)

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Purpose: Retinitis Pigmentosa is the most common form of RD that leads to gradual death of rods and cones. The loss of photoreceptors makes aberrant connections in the retina which further affects the downstream cortical signalling. The cortical functioning that depends on a fine balance of excitation and inhibition also gets affected. In this study we investigate whether changes in cortical function in RD are associated with alterations in the GABAergic neuron and its two important subtypes: somatostatin (SST) and parvalbumin (PV). We also measured vesicular glutamate transporter 2 (VGLUT2) expression, essential for transporting glutamate, in thalamocortical synapses.

Methods: We performed immunohistochemical staining, confocal microscopy, and neuron quantification using ImageJ software on wildtype (C57/BL6) and *rd1(C3H/HeJ)* brain sections to calculate GABA+, PV+, and SST+ neurons in all layers of V1. Western blot and immunolabeling was performed to estimate the expression of VGLUT2.

Results: We observed that the overall GABA+ population and PV+ was not affected, whereas SST+ neurons are significantly reduced in all layers of V1. There was also decreased immunolabeling and reduced expression of VGLUT2 in V1 of *rd1* mice.

Conclusions: The findings demonstrate the impact of impaired retinal input on GABAergic somatostatin neurons that modulate cortical processing by synapsing on to the distal dendrites of pyramidal neurons. In addition, the decreased excitatory thalamocortical expression causes hyperexcitability of the visual cortex disrupting the overall functioning. Our study provides insights into the changes that undergo in V1 and delineating their functional role will prove beneficial for a better experimental approach.

PS-I-56

TUDCA's impact on oxidative stress and microglia distribution in DR

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Purpose: Chronic hyperglycaemia causes neuro-inflammation, oxidative stress, and cell death in retina. It is one of the main causes of diabetic retinopathy. The activation of retinal microglia exacerbates this injury. Assessing TUDCA's impact on microglial activation is essential to comprehending its potential as a treatment for DR.

Methods: The rats were administered with 45 mg/kg BW of streptozotocin. Rats with 300 mg/dL or higher blood glucose were classified as diabetic. For four months following an onset of diabetes, 500 mg/kg BW of TUDCA was given every week. Immunohistochemistry was used to assess Iba-1, MHC-II, and CD-11b levels, while ELISA was used to assess VEGF, antioxidants, and pro-inflammatory cytokines.

Results: The Iba-1($p \leq 0.001$), MHC-II ($p \leq 0.001$), and CD-11b ($p \leq 0.01$) positive cells observed in the retina, the level of all three markers were significantly high in diabetic rat retina in comparison to control and treated. Level of VEGF-A $p \leq 0.001$(control+Diabetic) and $p \leq 0.01$(Diabetic+TUDCA treated) was significantly increased in DR as compared to control and treated. Glutathione ($p \leq 0.001$), SOD (DR+control $p \leq 0.001$ and DR+Qctn $p \leq 0.01$) and catalase ($p \leq 0.001$) levels were significantly lower in DR when compared to control and TUDCA treated.

Conclusions: Persistent hyperglycaemia in diabetic rats hyper-activates microglia, causing retinal damage. Diabetes-induced oxidative stress leads to decreased antioxidant levels. TUDCA therapy slows DR progression. Furthermore, other parameters of DR progression yet to be explored.

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PS-I-57

Elevated BCAT1 levels mediate autophagy inhibition and BCAA level dysregulation sustaining retinoblastoma growth

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Purpose: Retinoblastoma tumours (Rb) are caused by loss of the Rb protein due to mutations. BCAT1 (branched chain amino acid transferase 1) catalyses BCAA (branched chain amino acids) into branched chain keto acids and glutamate, the latter serves as an alternative energy source. BCAT1 levels were found altered in RB tissues. BCAA are potent regulators of mTOR complex, thus we hypothesised that their modulation may drive tumour specific metabolic and autophagic dysregulation. This study, therefore investigates mechanisms of BCAT1 mediated tumour growth.

Methods: BCAA and glutamate levels were measured in vitreous humour, collected at enucleation of Rb eyes. Immunohistochemical evaluation of BCAT1 and E2F2 levels was performed in tumour tissues. Gain or loss-of-function models for BCAT1 in WERI-RB1 cells were established by lentivirus packaging over-expression and Crispr-Cas9 constructs. Transduced cells were evaluated for cell proliferation, 3D tumour formation and chemosensitivity. Autophagy modulation was analysed by q-PCR and immunoblotting and compared with Trehalose and Chloroquine treated cells.

Results: The glutamate/BCAA levels and BCAT1 were significantly higher in vitreous humour of Rb eyes compared to paediatric controls ($p<0.05$). Ectopic BCAT1 expression in WERI-RB1 cells enhanced cell proliferation and 3D spheroid formation ($p<0.05$) BCAT1 ablation reduced proliferation ($p<0.05$). Immunoblot analysis of BCAT1 modulated WERI-RB1 cells revealed mTORC1 phosphorylation, increased LAMP1 and lipidation of LC3, indicative of autophagy activation upon BCAT1 ablation. Trehalose treated WERI-RB1 phenocopied the effects of BCAT1 ablation.

Conclusions: BCAT1 drives tumour growth by enhancing BCAA to glutamate conversion and inhibiting autophagy in Retinoblastoma. Therefore, BCAT1-BCAA pathway present a therapeutic avenue for managing tumour growth by BCAA pathway and autophagic intervention.

PS-I-58

Corneal wound healing using immature immune cells

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Purpose: Immature immune cells and their secretory components have been studied for regenerative potential. Such cells may be leveraged as therapeutic tools for tissue repair. We used a combination of dimethylsulfoxide (DMSO) and 1α,25-dihydroxyvitamin D_3 (D3) to induce myeloid leukemia (HL-60) cells towards an immature phenotype and examine their wound healing potential.

Method: HL-60 cells were induced to different immune cell phenotypes by culturing them with (i) 1.3 % DMSO; (ii) 100 nM D3, (iii) 100 nM RA (retinoic acid), (iv) 1.3 % DMSO + 100 nM D3, (v) 100 nM RA + 100 nM D3 for four days. Morphological analysis was carried out, followed by characterization of the immune cell markers of the immature phenotype by real time PCR. Conditioned media (CM) of these cells was tested for proliferation and percentage wound closure in human corneal epithelial cells (HCEC). HL-60 cells induced with different reagents were applied to alkali-injured goat corneas *ex vivo*. Healing was analyzed by slit lamp microscopy, histology and real time PCR.

Results: DMSO + D3-treated cells showed extensions, loss in uniformity of cells and increased expression of immature immune cell markers (CD11b, CD14, Arginase 1 and TGFβ1). Use of CM of these cells showed increased proliferation as well as increased percentage wound closure of HCEC cells. Goat corneas treated with DMSO+ D3-treated cells showed effective healing with formation of epithelium, and reduced fibrosis with decreased expression of fibronectin.

Conclusion: DMSO + D3-treated cells have characteristics of immature immune cells; such cells have the potential of healing.

PS-I-59

A pilot study to evaluate the health and nutritional values of Kachnar (*Bauhinia variegata*) among the tribal populations in the Hazaribagh, Jharkhand, India

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Purpose: Kachnar, also known as "*Bauhinia variegata*" is a species of flowering tree native to South Asia, particularly India and Pakistan. It belongs to the Fabaceae family and is known for its ornamental value and various traditional medicinal uses. Traditional medicine systems have used various parts of the Kachnar tree for their potential health benefits. It provides essential micro and macronutrients to tribal communities, especially in regions with limited food resources.

Method: A cross-sectional case-control pilot study was initiated to address estimate the plasma cytokine levels among the Kachnar-consuming and non-consuming groups (n=12 in each group). 5-mL of whole blood was collected in EDTA tubes after informed consent. Plasma was collected, and multiplex ELISA was performed using the Bio-Plex 200 automated immunoassay multiplex array system. Quality control measures were implemented during cytokine measurement, including calibration, validation, and control samples.MMP-9, TNF α, IL-8, IL-10 and IL-1 were multiplexed.

Results: Our study found reduced levels of inflammatory cytokines with an elevated anti-inflammatory IL-10 expression in Kachnar consumer groups compared to non-consumers.

Conclusion: Although our study failed to reach statistical significance due to the low sample size, still we could see a trend showing anti-inflammatory responses amongst Kachnar consumers. We are recruiting a large cohort to validate our present findings. It's important to note that the consumption of Kachnar and other wild plants is a practice deeply rooted in the cultural and dietary traditions of specific tribal communities. Additionally, the safety of plant consumption and potential toxicity should be considered in traditional practices.

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Indian Eye Research Group

30th Annual Meeting of the Indian Eye Research Group

Poster presentation: Day 2 (29th September 2024)

Abstract ID	Name of Presenter	Abstract ID	Name of Presenter
PS-II-1	Suchana S Shet Shirodker	**PS-II-27**	Ashi Gupta
PS-II-2	Manjuri Kurmi	**PS-II-28**	Muskan Garg
PS-II-3	Pritam Dutta	**PS-II-29**	Renu Venugopal
PS-II-4	Janak Buragohain	**PS-II-30**	Gazella Bruce Warjri
PS-II-5	Sayantika Chakarbarti	**PS-II-31**	Anwesha Bhattacharyya
PS-II-6	Zerin M Mollah	**PS-II-32**	Nikhil Kumar
PS-II-7	Rahul R	**PS-II-33**	Shalini Sanyal
PS-II-8	Rajvi Mehul Parikh	**PS-II-34**	Manisha Supriya
PS-II-9	Monika Chouhan	**PS-II-35**	Aastha Garg
PS-II-10	Keerthana T	**PS-II-36**	Seema Sen
PS-II-11	Nandhini C	**PS-II-37**	Prisha Warikoo
PS-II-12	Charul Jain	**PS-II-38**	Sahar Rafat
PS-II-13	Kritika Lohia	**PS-II-39**	Rathinavel Sethu Nagarajan
PS-II-14	Manasi Tripathi	**PS-II-40**	Kartik Goel
PS-II-15	Anannya Tuli	**PS-II-41**	Sheetal Chauhan
PS-II-16	Jay Sheth	**PS-II-42**	Sheetal Chauhan
PS-II-17	Mekhla Naik	**PS-II-43**	Rakshit Agarwal
PS-II-18	Sadab Khan	**PS-II-44**	Srijita Kundu
PS-II-19	Vinay Gupta	**PS-II-45**	Julfequar Hussain
PS-II-20	Ria Sachdeva	**PS-II-46**	Aarti Bhardwaj
PS-II-21	Nelaveni Rupa	**PS-II-47**	Sharma Shivam
PS-II-22	Jay Sheth	**PS-II-48**	Srilekha Sundaramurthy
PS-II-23	Garima Bansal	**PS-II-49**	Mrinal Singh
PS-II-24	Sai Sivani Koonapareddy	**PS-II-50**	Ashish Mishra
PS-II-25	Kalaiyarasi D	**PS-II-51**	Anshuman Verma
PS-II-26	Pragati Tiwari	**PS-II-52**	Poonam Kushan

Abstract ID	Name of Presenter	Abstract ID	Name of Presenter
PS-II-53	Rizza Abdul Nayeem	**PS-II-58**	Gorenka Sneha Pranahitha
PS-II-54	Janvi Patel	**PS-II-59**	Gaurab Kumar Jha
PS-II-55	Sundar Shiva Sankari	**PS-II-60**	Sripriya Sarangapani
PS-II-56	Manoj Yadav	**PS-II-61**	Afrin J
PS-II-57	Yuvashree Rajamanikkam		

PS-II-01

Comparison of flicker thresholds and microperimetry in patients with branchretinal vein occlusion

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Purpose: Visual acuity may not be sufficient to capture the loss of sensitivity in Branched Retinal Vein Occlusion (BRVO). This study aimed to compare flickerthresholds with retinal sensitivities obtained by microperimetry in patients with BRVO.

Methods: A prospective cross-sectional study was conducted on patients diagnosed with major BRVO. The inclusion criteria were as follows: a) only one eye affected b) both treatment naïve or previously treated, with a best corrected visual acuity of at least 20/125 in the affected eye. Flicker thresholds were psychophysically measuredwith a commercially available module called Flicker- *plus*. Retinal sensitivity was measured using a micro-perimeter NIDEK MP-3. The stimuli in both tests were presented at five locations, at the center (0°), and four eccentricities (12°) namely 45°, 135° (superior), -45°, and -135° (inferior) meridians.

Results: The mean±SD age of the BRVO patients (n=4) was 58.75±8.9 years and allwere males. In eyes with BRVO, 90% (18/20) of the tested points for flicker thresholds and retinal sensitivities were outside the normal age-matched values. Theaverage fold change (threshold in the diseased eye/normal average threshold) in parafoveal flicker thresholds (n=16 points) was 3.14 ((±1.67), compared to fold change observed in retinal sensitivities (n=11) 1.19 (±0.16) obtained by microperimetry.

Conclusions: The magnitude of relative flicker threshold deficits were larger in eyeswith BRVO as compared to corresponding microperimetry values. This could be because the flicker stimulus is metabolically more demanding on the retina than a static stimulus, thereby exacerbating the flicker thresholds.

PS-II-02

Comparative assessment of tear film dynamics in glaucoma: evaluating the impact of single-agent versus multi-agent pharmacological regimens using Spectral-Domain Optical Coherence Tomography (SD-OCT)

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Purpose: To assess the impact of single-drug versus multi-drug therapies on tear film height (TFH) and depth (TFD) in glaucoma patients and their correlation with ocular surface disease index (OSDI) scores.

Methods: TFH and TFD were measured using SD-OCT in three groups: controls without glaucoma, glaucoma patients on single-drug therapy, and those on multi-drug therapy. Glaucoma subjects had used topical medications for over one year, while controls had no history of glaucoma, dry eye, or other ocular surface conditions.

Results: The mean ± SD values for TFH and TFD were: controls (TFH: 227.44 ± 14.14 µm, TFD: 160.33 ± 77.07 µm), single-drug therapy (TFH: 112.67 ± 14.85 µm, TFD: 119.38 ± 26.16 µm), and multi-drug therapy (TFH: 64.42 ± 0.71 µm, TFD: 49.71 ± 4.24 µm). Significant differences were observed across groups for both TFH ($F = 326.82$, $p<0.001$) and TFD ($F = 134.32$, $p<0.001$). Post hoc analysis confirmed that the multi-drug therapy group had the most substantial reductions in TFH and TFD. OSDI scores were highest in the multi-drug therapy group ($p<0.001$) and correlated negatively with TFH ($r = -0.56$) and TFD ($r = -0.67$).

Conclusion: Multi-drug therapy in glaucoma patients is associated with significantly reduced tear film height and depth compared to single-drug therapy and controls. Higher OSDI scores, indicative of greater ocular surface discomfort, are correlated with lower TFH and TFD values. These findings highlight the impact of multi-drug therapy on tear film characteristics and ocular surface health in glaucoma patients.

PS-II-03

Comparative analysis of pupillary dynamics in individuals with single and repetitive concussions versus non-concussed controls: A quantitative pupillometric approach

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Purpose: This study aims to evaluate and compare pupillary dynamics among age-matched controls, single concussion, and repetitive concussion groups to identify distinguishing features.

Methods: A total of 50 individuals were assessed in each group: single concussion, repetitive concussion, and age-matched controls. Concussed subjects were recruited from a sports rehabilitation centre, each with a documented sports-related concussion with duration of 3 to 18 months. Pupillary dynamics were measured using iPhone-based pupillometry under standard room illumination.

Results: Age-matched controls showed superior pupillary function with Average Constriction Speed (ACS) of 1.79 ± 0.13 (95% CI: 1.74 to 1.84), Constriction Time (CT) of 1.95 ± 0.20 (95% CI: 1.87 to 2.03), Maximum Constriction Speed(MCS) of 3.81 ± 0.32 (95% CI: 3.69 to 3.93), Amplitude of 4.00 ± 0.26 (95% CI: 3.90 to 4.10), and Latency of 0.20 ± 0.05 (95% CI: 0.19 to 0.22), significantly differing from concussion groups ($p < 0.001$). Single concussion subjects had reduced ACS (1.11 ± 0.16, 95% CI: 1.05 to 1.17) and CT (0.90 ± 0.19, 95% CI: 0.83 to 0.97). Repetitive concussion subjects exhibited even lower ACS (0.84 ± 0.13, 95% CI: 0.79 to 0.89) and CT (1.57 ± 0.14, 95% CI: 1.52 to 1.62). ROC analysis revealed excellent discriminatory power with high AUC values for ACS (0.99), MCS (1.00), and CT (0.97).

Conclusion: Pupillary dynamics effectively differentiate between controls and concussion groups. Age-matched controls exhibit distinct pupillary responses, while progressive impairment in pupillary dynamics correlates with increased concussion severity.

PS-II-04

Influence of circadian rhythm on pupillary dynamics: A quantitative analysis using morningness-eveningness questionnaires

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Purpose: This study explores how different circadian rhythms, as determined by Morningness-Eveningness Questionnaires (MEQ), affect pupillary dynamics.

Methods: In this cross-sectional study, 200 participants aged 18-35 years were categorized into morning, intermediate, or evening types based on MEQ scores. Pupillary measurements were taken under controlled conditions (room illumination at 300 lux, temperature at 22°C, and noise levels below 30 dB). Inclusion criteria were normal vision, no ocular or systemic disease, and no medications affecting pupillary function. Exclusion criteria included neurological disorders, recent eye surgery, or sleep disorders.

Results: The one-way ANOVA revealed significant differences across the chronotypes in all measured pupillary parameters ($p < 0.001$ for all). Post hoc analysis demonstrated that morning types exhibited significantly faster latency (mean difference [MD] between morning and evening types: -0.04 seconds, 95% CI: -0.05 to -0.03, $p < 0.01$) and greater maximum constriction speed (MD between morning and evening types: 0.8 mm/s, 95% CI: 0.7 to 0.9, $p < 0.01$). Intermediate types generally displayed intermediate values, with significant differences observed between intermediate and evening types in latency (MD: -0.02 seconds, 95% CI: -0.03 to -0.01, $p = 0.03$) and other parameters. The findings indicate a clear gradation of pupillary response aligned with circadian preferences.

Conclusion: Circadian rhythm significantly influences pupillary dynamics, with morning types exhibiting more robust responses than evening types. These findings highlight the importance of considering circadian rhythms in ocular assessments and suggest potential for chronotype-based interventions.

PS-II-05

Construction and operation of an automated eye tracker for phoria assessment

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Purpose: Heterophoria has a global prevalence of nearly 4%, and if not detected early, it will eventually result in asthenopic symptoms. Studies show a significant inter-examiner variability in phoria evaluation, stressing the need for a standardized, objective test. Eye trackers have shown promise in improving accuracy and repeatability in measuring eye movements clinically, but high costs, portability and accessibility remain challenges preventing widespread adoption. Our study intends to overcome these issues by developing a cost-effective eye-tracker to measure phoria.

Methods: We included 15 pre-screened subjects in our study, all of whom had normal binocular vision parameters except for phoria. Our eye-tracking system featured two Raspberry Pi single-board computers, each connected to an infrared camera that was mounted on the subject's head using a head-gear similar to an indirect ophthalmoscope. An examiner conducted Alternating Cover Test while our system captured video of the subjects' eyes. Ocular positions were extracted by tracking the positions of the 1st Purkinje Image and Pupil using the cross-correlation technique. Re-fixation saccades following the cover-uncover were tagged using a velocity criterion and used to determine the direction and mean amplitude of the ocular deviation.

Results: The eye-tracking system successfully captured and analysed eye movements using a small, portable, and comfortable device, costing approximately 20,000 INR (~240 USD).

Conclusion: Our head-mounted eye-tracking system has demonstrated cost-effectiveness, patient comfort, and sufficient data quality, making it a viable option for clinical use. It also holds potential for use in community outreach, research, and training programs.

PS-II-06

The nexus between pupillary constriction characteristics and accommodative facility: A quantitative and correlational study

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Purpose: To analyse the interplay between pupillary constriction dynamics and variations in accommodative facility

Methods: This study included 400 eyes from 200 subjects, divided into high and low accommodative facility groups based on performance measured in cycles per minute (CPM) using accommodative flippers. Pupillary constriction dynamics were assessed using an iPhone-based pupillometer application, focusing on constriction speed and amplitude.

Results: The high accommodative facility group (mean ± SD: 14.2 ± 1.1 CPM) significantly outperformed the low facility group (mean ± SD: 9.4 ± 1.2 CPM, $p < 0.001$). Pupillary constriction speed was faster in the high facility group (mean ± SD: 3.8 ± 0.5 mm/s) compared to the low facility group (mean ± SD: 3.2 ± 0.6 mm/s, $p = 0.002$). Amplitude of constriction was greater in the high facility group (mean ± SD: 2.3 ± 0.3 mm) versus the low facility group (mean ± SD: 1.9 ± 0.4 mm, $p = 0.005$). Significant positive correlations were found between constriction speed and accommodative facility ($r = 0.58$, $p = 0.001$) and between constriction amplitude and accommodative facility ($r = 0.52$, $p = 0.003$). Multiple regression analysis indicated that constriction speed and amplitude collectively predicted accommodative facility, with an R^2 of 0.47 ($p < 0.001$), explaining 47% of the variance.

Conclusion: Increased pupillary constriction speed and amplitude are significantly associated with higher accommodative facility. These findings highlight the role of pupillary dynamics as predictors of accommodative performance and provide insights into the mechanisms underlying accommodative dysfunction.

PS-II-07

Use of 3D printing technology in developing assistive technology for visually impaired

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Purpose: To explore the use of 3D printers in developing assistive technology for the visually impaired.

Methods: A comprehensive review of present solutions and literature on using 3D printing technology for assistive technology for the visually impaired, including educational material, lower-cost braille material manufacturing, medical labels, etc. The study analyzes the potential benefits of 3D-printed assistive technologies for legally blind individuals.

Results: The study found that 3D printing technology benefits assistive technology for the visually impaired by providing lower-cost, quality products. The results displayed benefits in the area of rehabilitation, education, navigation, manufacturing and object identification. The usage of 3D printing shows promise in creating need specific solutions for the visually impaired.

Conclusions: 3D printing technology represents a valuable advancement in the development of assistive technologies for the visually impaired. By enabling the creation of time efficient production methods, affordable, customizable, and innovative solutions, this technology enhances accessibility, autonomy and quality of life. Continued exploration and refinement of 3D printing methods can improve the support available to visually impaired individuals.

PS-II-08

Application of digital accessibility principles to app design case studies of 5 award winning mobile application

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Background: The digital world is often inaccessible, with many apps not adhering to usability and regulatory requirements for people with disabilities. Despite growing awareness and legal mandates, practical implementation of accessibility principles in app design remains inconsistent, impacting user experience and app success. To address these issues, a review of five award-winning apps was conducted to illustrate how they effectively incorporated accessibility principles and guidelines.

Purpose: To explore and understand the digital accessibility principles and features that enhance the user experience for visually impaired individuals.

Methods: The accessibility features of the award-winning mobile application for example, Envision AI, Be My Eyes, etc. are examined, with particular attention to functionality, inclusive design principles, and Web Content Accessibility Guidelines (WCAG). The investigator included persons with visual impairment to offer a first-hand perspective. Similar unsuccessful applications were also analysed for comparison.

Results: The study shows the majority of the reviewed award-winning smartphone applications have effectively integrated key accessibility requirements, thus enhancing their usability for individuals with vision impairment. By utilizing features like text-to-speech and adjustable User Interface elements, these apps showed a strong incorporation of WCAG guidelines to offer an inclusive and accessible user experience.

Conclusion: The study provides a useful illustration of digital accessibility principles at work - thus providing key learnings for app designers and developers. It further emphasizes the fundamental soundness of the accessible app design concepts, and calls for a wider implementation of the same across the industry.

PS-II-09

Comparison of corneal densitometry data of humans and rabbits using different Scheimpflug devices

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Purpose: This study aims to compare and normalize corneal densitometry between rabbits and humans for use in pre-clinical trials.

Method: The study involved two-month-old New Zealand male rabbits weighing between 2-2.5 kg and healthy human participants aged 25-35 years. Imaging was performed using Pentacam, Galilei-G4, and RTVue devices. Densitometry values were obtained from Scheimpflug devices, while pachymetry values were gathered from all three devices. The interspecies differences in densitometry were analysed to facilitate their application in translational studies.

Results: The study found that corneal densitometry values are higher in rabbits compared to humans. This trend was confirmed by both Scheimpflug devices, although the values differed between the devices for each individual. To assess device accuracy, pachymetry values from Pentacam, Galilei-G4, and RTVue were compared, revealing inherent differences across all devices. While the data trends were consistent, there was a significant difference between the values from Pentacam and Galilei-G4, with Galilei-G4 showing higher values.

Conclusion: The normalization of densitometry data allows for direct translation from rabbits to humans. Based on the comparative data, Pentacam is deemed a more reliable device for densitometry, as it provides consistent data and is well-established in clinical practice.

PS-II-10

Characterization of pearl/regeneratory posterior capsular opacification

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Purpose - The aberrant fiber cell differentiation from human lens epithelial cells in response to the injury caused by cataract surgery is proposed to form pearl Posterior Capsular Opacification (PCO), However, the etiology of pearl PCO is not fully understood. This study aims to characterize the cellular components of human pearl PCO.

Methods - IOL implanted capsular bags from pseudophakic excised human donor globes were analysed under stereo zoom microscope to identify the size and number of pearls in the capsular bags. The pearls were subjected to (i) Haematoxylin and eosin staining and (ii) Immunostaining followed by confocal microscopic imaging to analyse the cytoarchitecture using F-actin; SOX2; E-cadherin; α-SMA; and β and γ crystallins.

Results – The number of pearls per donor was 153.6±46.2 (mean ± SD) and their size was 2.5±1.0 mm (mean ± SD). Haematoxylin and eosin and F-actin staining identified multilayered clusters of elongated cells, typical of pearl structures. SOX2 was expressed in 72.3 ± 22.6% cells within the pearls, and 29.9 ± 5.7% cells expressed the EMT marker α-SMA. While the expression of epithelial cell marker E-cadherin was low, the early fiber cell elongation marker β-crystallin showed intense expression in the pearls and the late fiber cell elongation marker γ-crystallin exhibited weak positivity, suggesting incomplete fiber differentiation.

Conclusion - Characterization of pearl PCO cells revealed (i) the presence of stem-like cells, (ii) cells undergoing EMT, and (iii) incomplete differentiation into lens fibers. Further research is essential to elucidate the molecular mechanism associated with pearl formation.

PS-II-11

The Sankara Nethralaya Tamil Nadu Essilor Myopia (STEM) study: Incidence and progression of myopia among school children in Tamil Nadu

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Purpose: To report the annual incidence and progression of myopia among school-going children in Tamil Nadu.

Methods: The STEM Study is a longitudinal, school-based cohort study conducted in children of 5-16 years from 11 schools in Tamil Nadu. All children underwent vision assessment and objective refraction with open-field autorefraction (Grand Seiko WAM-5500), binocular vision assessment, and ocular biometry measurement using non-contact biometry (IOL Master Version 500) for children with refractive error. Myopia and high myopia were defined as spherical equivalent (SE) refraction of ≤ -0.75D and ≤ -6.00D, respectively. The annual incidence and progression of myopia were reported from baseline data (2021-2022) matched with the first follow-up data (2023-2024).

Result: A total of 3778 children with a mean age of 11 years (range 5-16; SD: 2.38) participated in this study. The annual incidence of myopia was 14.6%. It was higher in the secondary grade children [Odds ratio (OR): 1.66; 95%CI: 1.31 – 2.10)] and among boys [(OR): 1.41; 95%CI: 1.13 – 1.76)]. The annual shift in SE myopia was -0.43 ± 0.67D. The mean annual progression of myopia was -0.98±0.63 D (SE) and was observed in 39.7% of children. The myopia progression was predominantly noted in the primary grade children (36.6%). The change in axial length of myopic children was 0.02 ± 0.79mm in the one-year follow-up period.

Conclusion: Among South Indian school children, the annual incidence was higher in the secondary grade children and progression was higher in the primary grade children.

PS-II-12

Cytokines interactions in HSV-1 keratitis

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Purpose: Herpes simplex virus 1 (HSV-1) is a prevalent cause of corneal blindness in developed countries. The most frequent clinical manifestation of HSV-1 infection is in corneal epithelial (CE) cells, resulting in epithelial keratitis. This study aims to investigate the levels of interleukin-6 (IL-6), secreted LY6/PLAUR domain-containing protein 1 (SLURP1) and nerve growth factor (NGF) in patients with HSV-1 epithelial keratitis, with the objective of examining the interactions between these molecules on the ocular surface and correlating their levels with disease severity.

Method: After obtaining informed consent, we collected tear samples and corneal epithelial scrapings from patients with HSV-1 epithelial keratitis during the active stage of the disease. We followed these patients through their antiviral treatment until clinical resolution, at which point we collected impression cytology of CE from the same individuals. Epithelial samples from individuals who underwent photorefractive keratectomy served as controls. Age-matched -control tears were also collected, followed by RNA isolation and cDNA conversion.IL-6 and SLURP1 gene expression were evaluated at each stage using quantitative PCR for CE samples and enzyme-linked immunosorbent assay for tears.

Results: Our results indicate that levels IL-6, SLURP1 and NGF were significantly elevated in both epithelial tissues and tears from patients with HSV1 epithelial-keratitis, contributing to the increased severity of the condition.

Conclusion: Our study reveals that elevated levels of IL-6, SLURP1 and NGF significantly contribute to the severity of HSV-1 epithelial keratitis. These findings show the crucial involvement of host-secreted factors in the immunopathogenic mechanisms underlying the disease

PS-II-13

Positional decoding of a continuously moving target in random 3D random motion in intermittent exotropia using fMRI

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Purpose: Individuals with Intermittent Exotropia (X(T)) often exhibit spontaneous and uncertain ocular motility behaviour. Here, we Identify visual system regions and underlying eye movement mechanisms by decoding frontoparallel (x & y) and depth (z) positions from fMRI response to a continuously moving 3D target.

Methods: We measured brain activity using fMRI in 6 X(T) & 7 healthy controls (HC) while they gazed at a target moving continuously in a 3D random walk. First, we binned target positions to match the time points (TP) in the fMRI signal. Next, we clustered these positions to 10 major locations in the stimuli space. Then, we decoded these positions from fMRI responses using one-vs-one Support Vector Classifiers. Positions were decoded for a range of 0 to 5 TP to account for delays in neural response at different levels of the visual system.

Results: In HC, x, y & z positions were decoded with above-chance accuracy in R-V3A, R-PEF, L-V4 & L-SMA for all lags. Particularly, z-positions were significantly decoded from R-FST/MT+ at a lag value of 3, affirming that depth-specific decoding is slower than that for frontoparallel positions. However, in X(T) we did not observe similar trends in aforementioned regions. Specifically, we noticed poor decoding performance in R-FST/MT+, L-V4, L-VIP, L-V3B, R-FEF in X(T) indicating their inability to maintain stable fixation in response to the depth component of 3D Brownian motion.

Conclusions: Our results show possibly compromised neural mechanisms for controlling eye movements to 3D motion in X(T) and understanding them may be useful for targeted interventions.

PS-II-14

Outcomes of Intralenticular Lens Aspiration with glued Scleral Fixated Intraocular Lens for ectopia lentis in young patients with Marfan's syndrome

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Purpose: To study the outcomes of ILLA with glued SFIOL in young patients with Marfan's Syndrome

Methods: Forty-two eyes of 23 patients with Marfan syndrome & ectopia lentis were enrolled in this study conducted at a tertiary care centre. Marfan syndrome was diagnosed as per modified Ghents criteria. All eyes underwent ILLA with glued SFIOL. Primary outcome measure was post-operative CDVA at 12 months. Secondary outcome measures were postoperative complications and residual refractive error.

Results: Mean age of patients was 12.52±6.6 years; 13 males & 10 females. Pre-operative mean CDVA 1.08±0.53 logMAR. On POD-1, mean CDVA was 0.68±0.49 logMAR. This difference was statistically significant (p-value<0.05). Post-op complications included vitreous hemorrhage (11.9%; n=5), hypotony (4.76%; n=2), corneal edema (4.76%, n=2), pupillary capture of IOL (4.76%; n=2), posterior dislocation of IOL (2.3%, n=1) and haptic extrusion through sclera (2.3%, n=1). At 6 months, 52.38% eyes had CDVA 20/20, 33.33% had ≥20/40, 14.06% had CDVA<20/40 with amblyopia. MRSE was -0.34±0.49 D; mean astigmatism was 1.20±0.84D

Conclusion: ILLA with glued SFIOL is a safe and effective modality for the management of ectopia lentis in young patients with Marfan's syndrome. This is the first study to assess the same. Management of amblyopia in post-operative period is essential to provide optimal visual outcomes.

PS-II-15

Association between plasma bisphenol levels and diabetic retinopathy

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Purpose: Emerging evidence suggests a link between endocrine-disrupting chemicals, such as bisphenols, and type 2 diabetes mellitus. Bisphenol exposure has also been associated with increased reactive oxygen species (ROS) production and activation of HIF/VEGF signalling. This study aimed to quantify bisphenol levels in the plasma of patients with various stages of diabetic retinopathy (DR) and investigate a potential association between bisphenol exposure and DR progression.

Methods: Plasma samples were collected from patients with mild (n=10), moderate (n=12), and severe non-proliferative DR (NPDR, n=11) and proliferative DR (PDR, n=12), along with controls (n=13). The levels of three common bisphenols (BPA, BPS, BPAF) were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Plasma levels of vascular endothelial growth factor (VEGF) was also assessed via ELISA.

Results: BPS was the most commonly detected bisphenol (*69.5%*), followed by BPA (*22.0%*), while BPAF was not detected. Total bisphenol levels were significantly higher in DR patients compared to controls (*p=0.0383*), with a rising trend from mild (*median=21.45 ng/mL*) to moderate (*median=27.40 ng/mL*) to severe NPDR (*median=35.2 ng/mL*) that plateaued with disease progression into PDR. Plasma VEGF levels were elevated in DR patients compared to controls (*p=0.0362*). No correlation was found between VEGF levels and total bisphenol content.

Conclusion: The study found elevated bisphenol levels in DR patients, indicating a potential link between bisphenol exposure and DR progression. However, future studies with large cohort are required to validate the findings.

PS-II-16

Quantitative assessment of minimum intensity-based changes in fluid on en-face OCT imaging following ranibizumab biosimilar treatment in neovascular AMD

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Purpose: This prospective study aimed to evaluate the efficacy of a new regulatory approved ranibizumab biosimilar (RzB), Oceva (Sun Pharmaceuticals, India), in treating neovascular age-related macular degeneration (nAMD) using Minimum-Intensity based changes observed on en-face optical coherence tomography (OCT) (en-face MI OCT).

Methods: Fifteen eyes with treatment-naïve nAMD underwent three loading doses of RzB. Best-corrected visual acuity (BCVA) and the proportions of eyes with subretinal fluid (SRF), intraretinal fluid (IRF), and subretinal hyperreflective material (SHRM) were assessed. En-face MI OCT-based analysis was conducted to quantify changes in fluid area and perimeter.

Results: At 12 weeks, there was a statistically significant improvement in BCVA from 0.94 {≈20/174} (± 0.59) logMAR to 0.84 {≈20/138} (± 0.61) logMAR (*P*=0.04). En-face MI OCT revealed a significant reduction in the median area of fluid from 0.9 mm² (IQR 0.62–4.56) to 0.32 mm² (IQR 0.1–0.64) (*P*=0.007), and in the median perimeter of fluid from 10.95 mm (IQR 7.26–25.67) to 6.02 mm (IQR 1.76–7.93) (*P*=0.0005). The proportion of eyes with SRF, IRF, and SHRM decreased from baseline (86.87%, 66.67%, and 60% respectively) to 12 weeks (60%, 46.67%, and 13.33%). No adverse events were reported.

Conclusions: Treatment with ranibizumab biosimilar Oceva showed promising outcomes in improving visual parameters and reducing fluid accumulation in patients with nAMD. Minimum Intensity-based analysis provided detailed insights into fluid dynamics, demonstrating its utility in evaluating treatment responses in nAMD. This study contributes to the initial assessment of Oceva in clinical practice, highlighting its potential efficacy in managing nAMD.

PS-II-17

Impact of screen time on paediatric dry eye disease in Indian children

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Purpose: This prospective cross-sectional study aimed to investigate the relationship between screen time and pediatric dry eye disease (PDED) in Indian children aged 4-16 years.

Methods: Forty-nine children were assessed for PDED using a combination of objective and subjective measures. Objective assessments included automated tear film breakup time (TBUT) and manual calculation of tear meniscus height (TMH) using the Visionix (VX-650) multimodal device. The validated Dry Eye Questionnaire (DEQ-5) was used for subjective assessment. The average daily screen time of participants was recorded, and its correlation with TBUT, TMH, and DEQ-5 scores was analyzed.

Results: The mean age of the participants was 10.61±2.87 years, with an average daily screen time of 3.29±1.94 hours. The mean values of TMH, TBUT, and DEQ-5 scores were 0.148±0.029 mm, 5.85±3.92 seconds, and 5.25±3.62, respectively. Analysis revealed a positive correlation between screen time and DEQ-5 scores (r=0.37), indicating an increase in dry eye symptoms with more screen usage. Screen time also showed a positive correlation with TMH (r=0.33), suggesting increased reflex tearing, and a negative correlation with TBUT (r=-0.16), indicating reduced tear film stability.

Conclusions: Extended screen time in children is associated with worsening symptoms of PDED, increased reflex tearing, and reduced tear film stability. These findings underscore the importance of advising children and their caregivers to limit screen use, take regular breaks, and undergo regular ocular health assessments to prevent or mitigate PDED. Healthcare providers should be proactive in educating families on strategies to protect children's eye health in the digital age.

PS-II-18

Exploring the bacterial and immune biomarkers profiles in Stevens-Johnson Syndrome patients before and after treatment

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Purpose: In the present study Quantitative Realtime PCR approach was used to correlate the abundance of predominant bacterial species in SJS with the inflammatory markers before and after treatment.

Methods: Tear samples were collected from Stevens-Johnson Syndrome (SJS) patients (n=15) along with healthy controls (HC, n=15) and SJS patients post treatment (SJS-PT, n=05). The presence or absence of specific organisms along with the inflammatory and anti-inflammatory markers were evaluated by Quantitative Realtime PCR (qRT-PCR). Student's t test was used to calculate significance. The p-value <0.05 was statistically significant.

Results: The relative abundance of the bacterial genera such as *Enterococcus, Corynebacterium* were found to be higher in the SJS group as compared to HC while an increase in genera such as *Cutibacterium* and *Prevotella* was seen in patients after treatment. Immune inflammatory markers such as IL-6, IL-1β and TNF-α showed significant increase in SJS patients and a decrease in patients post treatment was observed as compared to HC. SJS specific markers such as IL-36γ and Granulysin was seen to be increased and decreased respectively in SJS patients before and after treatment.

Conclusions: The higher relative abundance of the reported pro-inflammatory bacterial genera in SJS as compared to healthy controls, corelates with the increase in the fold change of the human inflammatory markers. The decrease of the expression of the inflammatory markers in SJS-PT along with the change in the relative abundance of bacterial genera can serve as a biomarker for SJS disease and its post treatment conditions.

PS-II-19

Efficacy of pharmacological vs optical intervention in preventing myopia progression among school-aged Indian children: first year results of parallel-group randomized clinical trial

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Purpose: To evaluate and compare the efficacy of pharmacological (atropine 0.01%) and optical [Spectacle with highly aspherical lenslets (HALs)] intervention in preventing myopia progression among school-aged Indian children.

Methods: 100 children (Age: 6-14years), with documented myopia progression ≥0.5D/year and myopia (-1 to -6D), were 1:1 randomized to either administer atropine 0.01% eye-drops in both eyes at bedtime (A01 group: 50 children) or wear HALs spectacles full-time(HALs group: 50 children). Ethical approval and informed consent were obtained.
The primary outcome measures were changes in spherical-equivalent refractive-error (SER) (measured through cycloplegic autorefraction), axial length (AL) (measured using IOL Master®700) and rate of myopia progression from baseline to 1-year follow-up. All analysis were performed based on intention-to-treat principle.

Results: There was no significant difference noted in age (A01:9.6±3.2years, HALs:9.2±2.8years; *P>0.05*), gender (A01:56%male, HALs:52%male; *P>0.05*), SER (A01: -4.32±1.5D, HALs: -4.14±1.7D;*P>0.05*) and rate of myopia progression (A01: -0.61±0.14D/year, HALs: -0.66±0.17D/year; *P>0.05*) at baseline between two groups.
The change in SER(ΔSER) from baseline to 1-year follow-up was -0.31 ±0.12D*(P:0.003)* in A01 group and -0.26±0.13D*(P:<0.001)* in HALs group. The difference of ΔSER between two groups was not significant *(P:0.11).* However, the change in rate of myopia progression was significant *(P:0.032)* between A01(0.3±0.11D/year) and HALs group(0.4±0.12D/year).
The change in AL(ΔAL) after 1-year was 0.29±0.12mm*(P:<0.001)* and 0.24±0.13mm *(P:<0.001)* in A01 and HALs group respectively; with no significant difference in ΔAL between groups *(P:0.17).*

Conclusions: Both pharmacological (atropine 0.01%) and optical (HALs) interventions are effective in preventing myopia progression among school-aged Indian children. However, efficacy of HALs spectacle is more than atropine 0.01% in reducing rate of myopia progression.

PS-II-20

Unravelling gene-driven phenotypic diversity in a North Indian primary congenital glaucoma cohort

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Purpose: To investigate genotype-phenotype correlations in Primary Congenital Glaucoma (PCG) patients in North India.

Methods: This retrospective study included patients clinically diagnosed with PCG. Whole exome sequencing was performed with 100% coverage of known PCG genes, including *CYP1B1, LTBP2, MYOC*, and *TEK*. Clinical parameters such as anterior segment findings, intraocular pressure (IOP), surgical outcomes, and associated comorbidities were assessed. Poor treatment outcomes were defined by persistent corneal edema, increasing corneal diameter or axial length, IOP >21 mm Hg, or the need for continued surgeries/antiglaucoma medications.

Results: Among 17 PCG patients (11 males, 6 females), 14 patients (82%) had mutations in known PCG-associated genes, with novel variants in 8 cases. Bilateral PCG was observed in 8/14 (57%) cases. Variants were found in *CYP1B1* (8/14= 57%), *TEK* (3/14= 21%), and other genes such as *LTBP2, FOXC1, VCAN, WDR36,* and *CPAMD8* (7.2% each). Missense mutations were most common (9/14= 64%), followed by deletions (3/14=21%) and nonsense mutations (2/14=15%). Five patients with mutations in *CYP1B1* gene presented at least one parameter of high disease severity; corneal scarring (2 cases), increased corneal diameter/ axial length (3 cases), multiple surgeries (4 cases) and persistent high IOP >21 mm Hg (3 cases). There was one case of arrested PCG with good vision. Patients with mutations in other genes were associated with comorbidities like lens subluxation and cataracts.

Conclusions: Genotype-phenotype correlations, especially regarding severity and outcomes, were observed within the PCG cohort. Further research with a larger cohort may help establish significant genetic prognostic markers for this population.

PS-II-21

Microbial composition shifts of the conjunctiva from neonates to adults

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Purpose: Ocular surface microbiome (OSM) is affected by the environmental factors and may potentially impact the bacterial colonisation and health in long term. Thus, the purpose of the present study is to understand the ocular surface microbiome composition after birth and in the healthy adults.

Methods: Conjunctival samples were collected from 36 healthy adults and 20 neonates using sterile Isohelix swabs. DNA was extracted and V3-V4 region of 16S rRNA was amplified by PCR. The amplicons were sequenced by Illumina platform. QIIME2 tool was used to process the data. R was used to assess the alpha and beta diversity. Statistical Significance between the groups was analyzed by Wilcoxon signed rank test.

Results: Alpha diversity indices such as Shannon and Simpson indicated significant differences between the groups. The observed genera significantly less in neonates compared to adult group. Neonate samples showed a higher abundance of genera *Staphylococcus* (37.62%)*, Pseudomonas* (12.6%) and *Stenotrophomonas* (6.82%). In contrast, adult samples were dominated by genera *Lactobacillus* (27.7%)*, Corynebacterium* (21.39%) and *Staphylococcus* (17.8%). The probiotic bacterial composition in neonates to adults is 7.5% to 34% of total abundance respectively. Further, Principal Coordinates Analysis showed distinct clusters for the 2 groups indicating clear differences in microbiome composition.

Conclusion: The study reveals that the ocular surface microbiome composition in neonates is different from adults and is colonized by opportunistic pathogens. While healthy eyes of adults had balanced composition of probiotic and opportunistic pathogens. Further studies are required to investigate the source of the microbiome at birth.

PS-II-22

The imaging correlation between outer retina-associated hyperreflectivity and outer retinal and choroidal microvascular changes in macular telangiectasia type 2

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Purpose: To elucidate outer-retinal choroidal microvascular abnormalities (ORCMA) observed in Macular Telangiectasia type 2 (MacTel 2), particularly in association with outer retina-associated hyperreflectivity (ORaH).

Methods: Retrospective analysis of 60 eyes with MacTel 2 having ORaH was conducted. OCT and OCTA images were meticulously reviewed to identify ORCMA patterns.

Results: All 60 eyes exhibited hyperreflective foci (HRF), with varying incidences of subsidence, ellipsoid zone (EZ) loss, pigment clumps, cystoids, and double-layer sign (DLS). Vascular invasion, retinochoroidal anastomosis (RCA), and macular neovascularization (MNV) were observed, with all cases demonstrating ORaH. OCTA revealed microvascular changes. Subsidence correlated significantly with invasion ($p=0.009$), while pigment clumps, cystoids, and DLS were not significant. RCA was associated with pigment clumps, cystoids, and DLS ($P<0.05$), whereas MNV correlated with subsidence and invasion ($P<0.05$).

Conclusions: The study delineates ORCMA in MacTel 2, identifying three patterns: vascular invasion, RCA, and MNV. Vascular invasion affects the outer retina, potentially leading to subsidence. RCA, a pre-proliferative stage, involves anastomosis between retinal and choroidal microvasculature, associated with pigment clumps, cystoids, and DLS. MNV, an advanced stage, exhibits extensions below the RPE and exudation, suggesting a continuum from invasion to neovascularization. Recognizing these stages aids in diagnosis, prognosis, and intervention development for MacTel 2.

PS-II-23

Application of augmented reality in vision rehabilitation: An expert perspective

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Purpose: To explore and evaluate the usage of advanced assistive technologies based on Augmented Reality in low vision management.

Methods: The research involved a comprehensive review of various advanced assistive technologies, electronic vision enhancement systems, smartphone applications, primarily functioned using augmented reality (AR) technologies. The study analysed the features, functionalities based on AR, and potential benefits of these technologies for individuals with varying degrees of visual impairment.

Results: The review revealed significant vision enhancement by AR based assistive technologies in low vision management. AR demonstrated improvement in magnification, contrast enhancement, color inversion, and expansion in field of view compared to traditional vision aids. Further, smartphone applications leveraging AR technology showed promise in vision enhancement for visually impaired users.

Conclusions: AR represents a significant evolution in low vision management. Devices based on AR offer enhanced visual capabilities, improved independence, and improved quality of life in individuals with visual impairments.

PS-II-24

Long-term impact of social, economic, and demographic variables on pediatric keratoplasty outcomes: Insights from a South Indian tertiary eye care centre

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Purpose: This study examines how the social and demographic factors affect pediatric keratoplasty outcomes in India, aiming to identify non-clinical factors that may influence outcomes to optimize surgical success and visual acuity in pediatric patients.

Methods: We conducted an observational, cross-sectional study of 255 patients aged 16 or younger who underwent keratoplasty at a single institution. All participants with their families completed a voluntary, IRB-approved survey either in-person or via telephone. Demographic data was self- reported, and clinical outcomes were gathered through chart review. The primary outcome measures were graft clarity and functional vision at the final follow-up.

Results: Among 255 participants, 165 (64.5%) had bilateral congenital corneal disease. Mean duration of follow-up was 74.4 ± 47.1 months, with 184 eyes (72.2%) achieving clear grafts in operated eyes. Multivariate analysis revealed significant associations between graft success and male gender ($p=0.047$), father's highest education level ($p=0.042$), a positive history of consanguinity ($p=0.048$), and small family units ($p=0.008$). Patients with a greater number of total visits ($p<0.001$) and those who received government support ($p=0.012$) were less likely to maintain graft success. Log MAR visual acuity improved from 1.43 ± 0.546 before keratoplasty to 1.08 ± 0.729 at the last follow-up visit ($p<0.001$).

Conclusions: Graft success in pediatric keratoplasty has been associated with demographic factors such as the father's level of education, consanguinity, and having fewer financial dependents. These findings underscore the critical role of health literacy and strong familial support in enhancing outcomes for pediatric keratoplasty patients.

PS-II-25

National programs in India contributing to the delivery of refractive services and their alignment with the WHO SPECS 2030 initiative

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Purpose: Around 54.5 million people in India have an uncorrected refractive error (URE), which is considered the major cause of preventable vision impairment. To address this huge burden and achieve the global target of effective refractive error coverage, the World Health Organization (WHO) initiated the WHO SPECS 2030, providing strategies to implement. This study reviews the national programs in India contributing to refractive service delivery and their alignment with WHO SPECS 2030.

Methods: A specific thematic review of the initiatives of national programs relating to the delivery of refractive services, identified from official government websites was conducted, analysing the thematic alignment of the initiatives with the five key strategies of the WHO SPECS initiative.

Results: The initiatives of 3 national programs, namely Rashtriya Bala Swasthya Karyakram (RBSK), National Program for Control of Blindness and Vision Impairment (NPCBVI), and Ayushman Bharat (AB) were identified to be contributing to the delivery of refractive services. NPCBVI's initiatives contribute to all the five SPECS strategies directly, except "strengthening of surveillance and research". AB and RBSK indirectly contribute to "improving access to refractive services" by integrating eye care with primary health care and screening for vision impairment as part of health screening, respectively. There are no initiatives that contribute directly to "strengthening surveillance and research".

Conclusion: Though national programs contribute to improving access to eye care, more specific initiatives to improve the delivery of refractive services should be implemented as per the country-level recommendations of the WHO, to address the huge burden of URE.

PS-II-26

Prospective cross-sectional study of the natural disease course of Congenital Hereditary Endothelial Dystrophy (CHED)

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Purpose: To evaluate and understand the various presenting clinical phenotypes and the natural course of CHED.

Methods: The study included 70 children with CHED reporting to a single referral tertiary care center. Demographic profiles, presenting vision, pachymetry, corneal features, and anterior segment OCT findings were recorded. Siblings, parents, and affected cousins were also assessed. Cases were graded based on the severity of corneal clarity. Changes in vision, intraocular pressure, corneal epithelium, stroma, and thickness were analysed to understand the clinical presentations and their evolution over time, including the need for surgical intervention.

Results: The age range of participants was from 3 months to 30 years, with 55.73% males and 44.26% females. About 59% of the patients were from southern India. The clinical phenotype varied from mild corneal haze to severely decompensated cornea with secondary corneal changes, such as hypertrophied epithelium, bullous changes, and pigmentation. The mean central corneal thickness at presentation was 1019µm, with some patients exhibiting severe loss of corneal endothelial cells.

Conclusion: Understanding the clinical presentations and corneal changes over time can aid in surgical decision-making and prognosis analysis. This knowledge is crucial for improving patient outcomes and developing targeted interventions.

PS-II-27

Aqueous angiography guided minimally invasive glaucoma surgery: A pilot randomized control trial

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Background: Glaucoma is a leading cause of irreversible blindness, with increasing prevalence. Minimally invasive glaucoma surgery (MIGS) offers a less invasive alternative to traditional methods, but the optimal approach for targeting aqueous outflow regions remains unclear.

Purpose: To compare the intraocular pressure (IOP)-lowering efficacy of Bent-Ab-Interno Needle Goniectomy (BANG) performed in high versus low aqueous outflow regions as identified by Aqueous Angiography (AA).

Method: A single centre randomized controlled trial involving 60 patients with Primary Open Angle Glaucoma and cataracts were divided into two groups, group 1 undergoing BANG in high aqueous outflow regions and group 2 in low aqueous outflow regions. Primary outcomes included IOP reduction and success rates, with secondary outcomes including changes in anti-glaucoma medications(AGM) and visual acuity. Overall success was defined as IOP $\leq$ 15 mmHg at 6 months follow up with AGM (qualified success) or without AGM (absolute success).

Results: AA image analysis on Image J software highlighted nasal as high flow outflow region and temporal as low flow outflow region. **The mean preoperative IOP were comparable in group 1(17.27**±3.43mmHg**) and 2(**17.60±5.42mmHg**).** The mean IOP at 6 months follow up in group 1 was (14.53 ± 4.06) and in group 2 was (13.82 ± 2.49) (inter-group p value= 0.692). Qualified success of 40% in group 1 and 86.6% in group 2 was noted (inter- group p value= 0.021). Significant reduction in AGM noted in both group (inter-group p value=0.668).

Conclusion: BANG performed in low aqueous outflow regions yields comparable IOP reductions to high-flow regions but with a higher success rate. Targeting low-flow regions may protect high-flow areas from scarring and enhance long-term outcomes. This approach could represent a valuable strategy in glaucoma management.

PS-II-28

Evaluation of Cognitive impairment in Primary Open Angle Glaucoma patients

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Methods: In this case-control study, individuals with POAG (cases, n=70) were compared with age- and sex-matched healthy individuals (controls, n=70) using detailed ophthalmological evaluation and cognitive assessment. Multitude of tests were employed to comprehensively assess various domains of cognitive function: Addenbrooke Cognitive Examination (ACE-III; Attention/orientation, memory, language, verbal fluency, and visuospatial skills), Post Graduate Institute Memory Scale (PGIMS; verbal and non-verbal memory), Wisconsin Card Sorting Test (WCST; Nonverbal executive functions), Go No-Go task (GNG; inhibitory control), and Trail Making Test (TMT; Attention and working memory).

Results: Intraocular pressure and cup disc ratio was significantly higher while mean deviation and retinal nerve fibre layer (RNFL) thickness was significantly lower in cases as compared to age and gender matched controls ($p<0.001$). Cases had significantly lower scores on ACE-III and PGIMS ($p<0.001$) and longer test completion time in TMT-A ($p=0.001$). The performance of cases was also significantly worse on majority of parameters of WCST and GNG task. Significant correlation was observed between serum cortisol level and WCST correct response ($p=0.04$), WCST error response ($p=0.002$) and total time taken in TMT-A ($p=0.03$)., Visual field mean deviation also exhibited a significant correlation with serum cortisol level ($p<0.001$) and total time taken on WCST ($p=0.03$) and TMT-A ($p=0.03$).

Conclusion: Individuals with POAG exhibited greater cognitive deficits and raised serum cortisol levels than age-matched healthy controls. Early recognition and management of cognitive impairment is pivotal for enhancing the quality of life and implementing comprehensive glaucoma care.

PS-II-29

Initial results of topical low-dose heparin adjuvant therapy for ocular SJS and associated molecular correlations

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Purpose: To elucidate the efficacy of topical low dose heparin as an adjuvant therapy in sub - chronic and chronic ocular SJS and its effect on Neutrophil extracellular traps (NETs)-associated gene expression.

Methods: Clinically diagnosed cases of SJS-associated ocular complications were randomly assigned to receive either topical low-dose heparin (LDH) with conservative treatment (CT) or CT alone for a month. Conjunctival imprints and tears were collected to identify NETs using ELISA and NETs-associated markers were evaluated by qPCR. Visual acuity, Schirmer's test, fluorescein staining, lissamine staining, OSDI score and ocular surface severity grading were assessed. NETs presence, clinical parameters, and gene expression patterns were correlated to identify potential biomarkers.

Results: NETs complex presence was confirmed providing insights into their potential contribution to SJS pathology. Upregulation of TLR9, MyD88, IL-8, TNFSF14 and C3a genes was observed on Day 0 of treatment. LDH treatment groups showed significant clinical improvement compared to CT group in both sub-chronic and chronic SJS eyes. This was concordant with reduction in gene expression in the respective groups post LDH treatment.

Conclusions: The study elucidated the beneficial role of low-dose heparin eye drops as adjuvant in managing SJS-associated ocular inflammation compared to standard care. Our results endeavours to shed light on the role of NETs in SJS ocular pathology and explore the therapeutic potential of low-dose heparin eye drops.

PS-II-30

Endocannabinoids and cortisol in plasma, aqueous and tear samples of primary angle closure glaucoma versus controls

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Purpose: To compare the levels of endocannabinoids (EC) in plasma, aqueous humor and tears, cortisol in plasma and aqueous, in primary angle closure glaucoma (PACG) and controls, while comparing the quality of life in both groups.

Methods: A total of 60 patients, ≥40years of age, with a diagnosis of PACG or cataract, 30 in each group were recruited. They were subjected to a detailed ophthalmic evaluation, a WHO Quality of Life (WHOQOL-BREF) questionnaire answering and collection of tear, aqueous and blood samples. The levels of endocannabinoids–anandamide (AEA), 2-arachidonoylglycerol (2AG) in plasma, aqueous humor and tears; cortisol in plasma and aqueous humor; and WHO-QOL score in each group were noted.

Results: Plasma AEA ($p=0.01$) and plasma 2-AG, ($p=0.002$) levels were significantly higher in the control group as compared to the PACG group. WHO-QOL score was better in controls ($p<0.001$). The EC were in undetectable levels in aqueous. Plasma and aqueous cortisol were significantly higher in PACG and both had the highest Area under the receiver operating characteristics (AUROC) curve value for differentiating PACG from controls. Tear 2AG and tear AEA had a moderately strong positive correlation with plasma 2-AG. Females had insignificantly higher levels of plasma and tear endocannabinoids.

Conclusions: Tear endocannabinoids were determined for the first time in PACG and normal with no difference between the two groups. Plasma and aqueous cortisol levels are a differentiating factor between normal and glaucoma patients with plasma endocannabinoids being remarkably higher in normals. Quality of life in glaucoma patients with high cortisol levels is poorer.

PS-II-31

Alterations in the Primary Visual Cortex (V1) due to deprived visual input in inherited Retinal Degeneration (RD)

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Purpose: Patients suffering from inherited RD undergo loss of light sensitive retinal photoreceptors such as rods and cones. The progression of photoreceptor loss results in imbalances in excitatory and inhibitory neurotransmission in V1. We investigated alterations in GABAergic neurons and its two important subtypes, parvalbumin (PV) and somatostatin (SST) that are important for visual perception. Additionally, we determined if degeneration affect excitatory thalamocortical transmission to V1.

Methods: We performed toluidine staining on the retina of C3H/HeJ (*rd1*), mouse to check the morphology and compared with C57BL6 mice (wt) at P40. Tissue sections from V1 region was used for immunofluorescence to detect the total neuronal population, GABA+ neurons, PV and SST. We analyzed the changes in excitatory neurotransmission using vesicular glutamate transporter 2 (VGLUt2) and quantified its levels using Western Blotting. Images were taken with confocal microscope and estimation of neuronal density in each layer of cortex was done with ImageJ.

Results: The general morphology of the retina showed absence of the photoreceptor layer in the rd1 retina while the bipolar and the retinal ganglion cells were intact. We observed reduced immunolabeling and marked decrease in the levels of VGLUt2 in degenerated mice. The total neuronal population (NeuN+) as well as GABA + neurons exhibited no changes. Quantitative analysis of PV+ neurons showed no differences in the overall density between the rd1 and wt mice whereas SST+ neurons showed significant reduction.

Conclusions: Our results demonstrate the implications of RD on GABA inhibitory neurons and glutamatergic thalamic afferents that lead to physiological alterations in V1.

PS-II-32

Hippo pathway dysregulation in uveal melanoma: prognostic implications of LATS and cell cycle kinases

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Purpose- Uveal melanoma (UM) is the potentially lethal intraocular tumor in adults, with alterations in *GNAQ/11* which regulates Hippo tumor suppressor pathway. Loss of LATS kinases could contribute to UM pathogenesis because of their significant role in inhibiting the central mediators (YAP/TAZ). Identification of druggable cancer-driving pathways could facilitate the development of effective treatments for UM patients. Here, we report the expression of LATS kinases, central mediators and CDK4/6 kinases in UM samples and correlate with patient outcomes.

Methods- Whole exome sequencing (WES) was conducted to evaluate the mutational status of GNAQ/11 in 56 prospective UM cases. Real-Time PCR was performed to measure the mRNA expression levels of LATS1, LATS2, YAP, TAZ, CDK4, CDK6.

Results- WES revealed GNAQ and GNA11 variations in 58% and 47% of cases, respectively. LATS kinases and central mediators (YAP/TAZ) were downregulated in more than 75% of cases. The cytoplasmic expression of phosphorylated LATS (pLATS1/2) was also reduced at protein level. CDK4 was upregulated in 67.9% of cases, whereas CDK6 was downregulated in 83.9% cases. Fifteen patients develop distant metastasis, of which seven died due to the disease. In the metastatic cases, the expression of these kinases was significantly reduced.

Conclusions- Our study highlights the significant alterations of several kinases and central mediators of Hippo pathway in UM. These changes are closely associated with the metastatic potential and poor prognosis of UM patients. Targeting the Hippo pathway and associated kinases could offer promising therapeutic strategies for management of UM.

PS-II-33

Analysing hypoxia-related and exosomes specific markers in high and low-risk retinoblastoma

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Purpose: Retinoblastoma (RB) is characterized by a complex tumour microenvironment involving interactions between tumour cells, immune cells, stromal cells, and the extracellular matrix. This study aimed to distinguish tumour heterogeneity between high and low-risk RB by analysing the immunohistochemical expression of hypoxia-related and exosome specific markers in enucleated eyes.

Methods: A retrospective observational study was conducted using clinically diagnosed, enucleated RB globes, which were categorized into two groups based on histopathological features. The low-risk group (n=5) exhibited no tumour invasion into the uvea, optic nerve, sclera, or extra-scleral tissue, while high-risk group (n=6) showed invasion into these structures. Histological sections were analysed for hypoxia-inducible factors (HIF-1, VEGF, TGF-beta), and exosome markers (CD9, CD81, TSG101). The protein expression patterns, intensity, and location were correlated with tumour differentiation, invasion, and TNM classification of malignant tumours.

Results: The high-risk group exhibited poor differentiation, with heterogeneous expression of hypoxia-inducible factors HIF-1 (83%) and VEGF (50%), and strong, homogeneous expression of exosome marker TSG101 (83%) in tumour and invasive areas. However, minimal expression was observed for TGF-beta (16%) and other exosome markers CD9 (16%), and CD81 (16%). In contrast, the low-risk group exhibited higher differentiation features with homogeneous expression of hypoxia-inducible factors HIF-1 (60%) and VEGF (80%), as well as exosome markers TSG101 (80%) and CD9 (60%). Notably, CD81 and TGF-beta were not expressed in the low-risk group.

Conclusions: Both high-risk and low-risk group showed elevated expression of HIF-1, VEGF, TSG101, with variable tetraspanin (CD9, CD81) levels, indicating a possible correlation that requires further investigations.

PS-II-34

Gene expression level of prolyl hydroxylase PHD1 AND HIF-1α in retinoblastoma patients

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Purpose: Retinoblastoma (Rb) is the most common intraocular malignancy of childhood. In normoxic condition, Hypoxia Inducible Factor (HIF-1α) and Prolyl Hydroxylase 1 (PHD1) together function as an oxygen sensor. The primary oxygen sensor in cells is PHD1, which is sufficient in stabilizing HIF-1α under normoxia. HIF-1α regulates hypoxia-mediated apoptosis, cell proliferation and tumor angiogenesis. Therefore, the purpose of this study was to explore the expression level of hypoxia (*HIF-1α*) in the regulation of oxygen sensing enzyme (*PHD1*) in Rb patients.

Methods: This prospective study includes a total of 30 Rb cases. mRNA expression level of *HIF-1α* and *PHD1* was investigated using quantitative Real-time PCR (qRT-PCR). Statistical analysis was performed to correlate their expression with clinicopathological parameters and patient outcome.

Results: There was a male preponderance (25/30) in this study. Massive choroidal invasion and retrolaminar optic-nerve invasion was observed in 18% and 12% cases, respectively. The mRNA expression of *HIF-1α* was found to be upregulated in 30% (10/30) cases, while *PHD1* was downregulated in 88% (26/30) cases. On statistical analysis, patients with massive choroidal invasion were statistically significant with HIF-1α expression (*p=0.001*), whereas optic nerve retrolaminar invasion statistically correlated with PHD1 expression (*p=0.015*).

Conclusions: This study highlights the dysregulation of the oxygen-sensing pathway in retinoblastoma. Elevated HIF-1α and decreased PHD1 expression were associated with specific clinical features, suggesting their potential role in disease progression. Targeting this pathway may hold promise for future therapeutic strategies in retinoblastoma.

PS-II-35

Gene expression alterations in Stevens-Johnson syndrome: The role of ECM and immune response in chronic conjunctival inflammation

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Purpose: Stevens-Johnson syndrome (SJS) is a severe type 4 hypersensitivity disorder, characterized by a vesicular reaction affecting the skin and mucosa, including the oral cavity, genitals, and ocular surface. The pathobiological underlying the onset of SJS and toxic epidermal necrolysis (TEN) are not fully understood. In the chronic stage of SJS, we hypothesize that there is increased expression of matrix metalloproteinases (MMPs), potentially released by infiltrating immune cells or activated tissue-resident cells in the conjunctiva. This elevated MMP expression may alter the extracellular matrix (ECM), leading to tissue remodelling that can influence immune cell migration, activation, and survival, contributing to both immune responses and clinical manifestations in the conjunctiva.

Methods: Conjunctival tissue samples were collected from five chronic SJS patients and five healthy individuals. RNA sequencing was performed to identify upregulated gene targets, which were further validated using real-time PCR (RT-PCR) on 10 conjunctival tissue samples from each group. Protein expression of validated genes was assessed via immunohistochemistry (IHC). The clinical severity and features of the tissue samples were documented using slit lamp imaging.

Results: Transcriptomic analysis identified several gene targets, including FN1 (fibronectin), ECM1, ECM2, MMP3, MMP28, TREM1, TNC (Tenascin C), CXCL-10, CXCL9, TGF-beta, and IL-1alpha. qRT-PCR validation showed significant upregulation of CXCL-10 and fibronectin in SJS patients, implicating their roles in ECM remodelling and SJS pathophysiology.

Conclusion: Enhanced expression of ECM-related genes contributes to immune cell dysregulation and chronic inflammation in the conjunctiva of SJS patients, underscoring the role of ECM remodelling in SJS progression.

PS-II-36

Expression of Acyl-CoA Cholesterol Acyltransferase- 1 (ACAT-1) in eyelid sebaceous gland carcinoma

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Purpose: Acyl-coenzyme A cholesterol acyltransferases (ACATs) are enzymes that convert cholesterol into less toxic cholesteryl esters, playing a critical role in cholesterol metabolism. Altered cholesterol metabolism has been identified as a key feature in various cancers. Eyelid sebaceous gland carcinoma (SGC) is an aggressive malignancy, and the role of ACAT1 in this cancer remains unclear.

Methods: ACAT-1 mRNA expression was performed by Real-time PCR in tissues obtained from 32 cases of eyelid SGC. Clinicopathological details were noted and the patients were followed up for 1–39 months (18.2 ± 10.65 months).

Results: The mean age of the 32 eyelid SGC patients was 58.6 ±12.252 years (range 27-80 years) with male preponderance and a male and female ratio of 1.3:1. Pagetoid spread was observed in 50%%, 25% had tumor size > 2 cm and 65% (21/32) were poorly differentiated. There were 4 cases each of recurrence (12.5%),.and metastasis, one patient (4%) died. mRNA overexpression of ACAT-1 was observed in 13 cases (40.6%). Patients with recurrence (2/4) and one patient who died had ACAT overexpression. Poor histologic differentiation correlated significantly with ACAT expression (p=0.04).

Conclusion: The study suggests that ACAT1 mRNA expression could serve as a marker for aggressive eyelid sebaceous gland carcinoma (SGC) patients. Insights into the role of ACAT-1 could lead to novel therapeutic strategies targeting cholesterol metabolism for refractory cases.

PS-II-37

Reactive oxygen species inducing pathways in the vernal keratoconjunctivitis pathology

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Purpose: Reactive oxygen species have been seen to induce oxidative stress, leading to an increase in overall inflammation and autophagy. Vernal keratoconjunctivitis is a disease characterized by repeated bouts of allergy, due to a mixture of environmental and potentially genetic factors. Previously NADPH Oxidase (NOX) and Angiotensinogen Converting Enzyme 2 (ACE2) were detected in VKC samples. Angiotensin II has been seen to modulate NOX production and thus acts as a key player in the formation of ROS production. Utilizing transcriptomics, this study further investigates the role of Angiotensinogen in VKC in the Indian population.

Methodology: RNA was extracted from Impression Cytologies and sent for transcriptomic analysis. The transcriptomic data was analysed using DAVID, and specific markers and pathways were narrowed down. Schirmers II test was conducted for tear fluid collection and Impression cytologies were collected of 15 patients, 5 unmedicated, 5 medicated, and 5 controls. RNA was isolated from the impression cytologies, which was then converted to cDNA and used to run qPCR to validate the transcriptomic data.

Results: Transcriptomics analysis showed the presence of pathways related to Autophagy, Ferroptosis and ER Stress. Markers were chosen to validate from this data including ATG7, Vimentin and ASCL4, all of which were upregulated. ELISA run on Angiotensin II as well as angiotensinogen yielded varied results.

Conclusion: The upregulation of autophagy-related markers and the presence of an inflammatory environment support the hypothesis that ROS is an underlying factor in VKC. Targeting these molecules could potentially provide lasting relief for affected individuals.

PS-II-38

Stearoyl-Coenzyme A Desaturase 1 (SCD1) expression in choroidal melanoma

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Purpose: Choroidal melanoma (CM) is the most common primary intraocular tumor in adults. The role of lipid metabolism in CM is not fully understood. SCD1 is an enzyme involved in lipid metabolism and is upregulated in various cancers. This study investigates Stearoyl-CoA desaturase (SCD1) expression in CM and assesses its clinical significance.

Methods: Clinicopathological data from 25 CM cases were evaluated, and SCD1 expression was evaluated by immunohistochemistry (IHC) and mRNA expression by real-time PCR. Patients were followed for 17-41 months (29.88±6.3).

Results: The mean age was 49.08 ±14.2 years, with a male predominance (68%). Tumors with larger diameters (>2cm) were present in 8% of cases. The cell types observed were epithelioid (32%), spindle (48%), and mixed (20%). Tumor cell infiltration to other ocular structures (such as conjunctiva, ciliary body, and sclera) was noted in 40% of patients (60% had high SCD1 mRNA expression) and 68% of cases in stage of T3/T4 (52.9 % of these showed high SCD1 mRNA expression. Six patients (24%) died, out of which 16.6% had high SCD1 expression, and one case (6.4%) had metastasis.
High SCD1 expression was observed in 28% of cases (IHC) and 60% (mRNA). A significant correlation was observed between SCD1 expression and tumor stage. The mRNA expression of SCD1 correlated with immunoexpression in 60% (15/25) cases.

Conclusion: The study concludes that CM patients showed the upregulation of SCD1 expression, suggesting its potential as a marker for identifying high-risk patients. SCD1 could be a molecular target for therapeutic intervention, providing new strategies for CM in drug-resistant cases.

PS-II-39

Patient-derived three-dimensional spheroid culture – a promising model for drug screening in retinoblastoma

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Purpose: Chemoresistance is emerging as a major challenge in retinoblastoma (RB) management and there is no suitable *in vitro* model replicating the clinical scenario. Hence we aim to establish RB patient-derived spheroid culture and evaluate its utility as an *in-vitro* drug screening model.

Methods: Several media compositions and methods were employed to optimize the establishment of spheroid culture. Sanger sequencing and Multiplex Ligation dependent Probe Amplification were done to confirm the *RB1* genotype of the spheroids. Drug responsiveness was determined by *in-vitro* cytotoxicity assay using CellTiter-Glo® Luminescence. The inherent features of spheroids were verified with the clinicopathological details.

Results: Spheroids were successfully cultured using DMEM-F12 media with defined growth factors in 9 tumor and 1 vitreous samples. *RB1* genotyping of spheroids showed the same mutational landscape as that of the tumor samples. Based on the *in-vitro* drug responsiveness to carboplatin, five cultures were segregated as chemo-resistant in which four of them were clinically known high-risk RB. Other five were categorized as chemo-sensitive, originated from tumors of low-risk RB. One of the spheroids were further expanded and used to evaluate the *in-vitro* efficacy of an FDA-approved drug to overcome resistance.

Conclusions: RB patient-derived spheroids with concordant clinicopathological features of patient tumors were established. These spheroid culture has been demonstrated as a tool for drug screening in improved RB management.

PS-II-40

Exploring the molecular landscape of ocular surface squamous neoplasia

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Purpose: Ocular Surface Squamous Neoplasia (OSSN) is a malignancy ranging from dysplasia to invasive squamous cell carcinoma. This study aims to investigate the molecular pathways involved in OSSN pathophysiology to enhance understanding of the disease.

Methods: Transcriptomic profiling was conducted on OSSN tumor samples compared to healthy conjunctival tissues. Differentially expressed genes (DEGs) were identified, and the top hundred significant DEGs were analysed for their role in OSSN. Ten key genes were validated using quantitative reverse transcription PCR (qRT-PCR) on OSSN patient samples.

Results: Significant DEGs were identified, including KLK6, CXCL11, CXCL9, IL36RN, KRT6A, KRT16, KRT10, CDSN, KLK13, MMP12, and RAET1L, which are involved in critical processes such as epithelial differentiation, cell cycle regulation, and immune surveillance. Tumor suppressor genes like VSIG2, IGFBP3, and KRT4 were downregulated, while NID1, IGLC3, and CDA were upregulated aggressive OSSN cases.

Conclusion: This study highlights key molecular pathways and genes significantly associated with OSSN pathophysiology. These findings provide important insights into the mechanisms driving OSSN, potentially informing future diagnostic and therapeutic strategies.

Clinical Implications: The identified genes are crucial in understanding OSSN pathogenesis, offering potential insights for improving diagnostic and therapeutic approaches. However, the primary focus remains on the role these genes play in the disease's development and progression.

PS-II-41

Prognostic and therapeutic implications of immune microenvironment in ocular lymphoma

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Purpose: To explore the expression of Immune checkpoint regulators (PD-1, PDL1, and CTLA-4) and components of the immunosuppressive microenvironment (tumor-associated macrophages and T regulatory cells) in ocular lymphoma patients and correlate with therapeutic outcome.

Methods: Seventeen ocular lymphoma cases and 15 controls were studied. PD-1, PD-L1, and CTLA4 gene expression profiles and immunoexpression of tumor-associated macrophages and T regulatory cells were analysed. Survival analysis was performed to assess the clinical significance of immune checkpoint, tumor associated macrophages, and T regulatory cells in ocular lymphoma patients.

Results: PD1 mRNA overexpression was observed in 76%, PDL1 in 64% and CTLA4 in 59% cases. Of the 3 immune-checkpoint regulators analysed overexpression of CTLA4 gene was significantly associated with worst disease free survival (P=0.008) in ocular lymphoma patients. All the cases showed positivity for CD68 immunoexpression and 53% (9/17) cases showed immunoexpression of iNOS. However, all the cases were negative for CD163 expression. CD4 immunoexpression was seen in all the cases and FOXP3 positivity was seen in 12% (2/17) cases. Of all the TAMs and Tregs, markers analyzed we observed that only iNOS expression was significantly associated with worst disease free survival (P=0.03) of ocular lymphoma patients.

Conclusion: CTLA4 gene overexpression and iNOS immunoexpression could prove to be useful biomarkers for identifying high-risk ocular lymphoma patients. Overexpression of CTLA4 and iNOS in patients with poor prognosis suggests the need for further investigation of anti-CTLA-4 immunotherapy combined with iNOS as potential partners in the treatment of refractory/relapsed ocular lymphoma cases.

PS-II-42

Therapeutic potential of receptor tyrosine kinase gene expression profiling in ocular squamous cell carcinoma

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Purpose: In the present study, the expression pattern of receptor tyrosine kinases and its association with cyclins immunoexpression and clinical outcome in ocular squamous cell carcinoma was explored.

Materials and Methods: Sixty-one histopathologically confirmed ocular squamous cell carcinoma cases and 15 normal conjunctival tissues were included in this study. We generated comprehensive qPCR gene expression profiles of 19 RTKs (VEGFR1, VEGFR2, VEGFR3, EGFR1, EGFR2, EGFR3, EGFR4, AXL, MET, ROS, RET, PDGFRα, PDGFRβ, IGF1R, IGF2R, FGFR1, FGFR2, FGFR3, FGFR4) in all the cases and controls. Immunoexpression profiling was done to check the status of cyclin D, E and A in all the cases. Patients were followed up for 36 to 90 months (mean 54 ±23 months).

Results: Overexpression of different receptor tyrosine kinases ranges between 20-90%. Overexpression of EGFR4. FGFR1, FGFR2. MET, AXL. IGFR1 mRNA was seen in 20-40%; EGFR1, EGFR2, FGFR4, RET, ROS, PDGFR1, PDGFR2, VEGFR1, VEGFR3 in 40-60%; EGFR3, FGFR3 and IGFR2 in 60-80% and VEGFR2 in 90% ocular SCC cases. Immunohistochemical expression of cyclin D1 was seen in 49%, Cyclin B1 in 51%, and Cyclin E1 in 29% of ocular SCC cases. Of the 19 markers analyzed overexpression of EGFR4, AXL, FGFR4, VEGFR1, VEGFR3, and IGFR1 genes were significantly associated with the worst disease-free survival of patients with ocular SCC and cyclin D1 expression. Spearman's rank correlation coefficient and semi-supervised gene cluster analysis revealed significant co-expression of EGFR4, AXL, FGFR4, VEGFR1, VEGFR3, and IGFR1 genes.

Conclusions: Overexpression EGFR4, AXL, FGFR4, VEGFR1, VEGFR3, and IGFR1 genes may prove to be a useful biomarker for identifying high-risk patients with ocular SCC. The association of these RTKs with cyclin D1 indicates their role in pathogenesis of ocular SCC. A significant and direct correlation was observed between RTKs gene expressions. Therefore, the effect of multiple receptor tyrosine kinase inhibitors should be analyzed to explore the therapeutic potential of these receptors in ocular SCC patients.

PS-II-43

Faith of a grafted AMG - from the operating room to a cell culture lab

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Purpose: To Analyse the Histology and Immunohistochemistry of an in vivo amniotic membrane grafted for epithelialization of the cornea.

Methods: A case of Pseudophakic Bullous Keratopathy operated for lamellar keratoplasty (DSEK) along with Amniotic Membrane Grafting was sequentially followed up for epithelial healing. On 3rd week post-op, the grafted AMG was loosely adherent over the central cornea which was removed easily with forceps that resulted in clearing of the visual axis and improvement in patient's subjective correction. There was complete epithelial healing that was ensured by fluorescein staining. This loose AMG was collected in a micro centrifuge tube containing 100ul of DMEM and sent to the laboratory for histological and immunohistochemical studies for the expression of CK-12, E-Cadherin and ZO-1.

Results: H and E revealed multilayered growth of epithelial cells over the amniotic membrane like positive control (NSFS corneal tissue) and in contrast to a single-layered epithelium of negative control (cryopreserved AM). The qRT-PCR revealed a 46-fold rise in CK-12, 1.8 times rise in ZO-1 and 109 times rise in E-Cadherin with the test AMG compared to the negative control.

Conclusions: This suggests that the amniotic membrane has well-differentiated corneal epithelium (CK-12 expression), increased tight junctions (ZO-1) and that AMG promotes cell adhesion and migration (E-Cadherin). This indicated that well-differentiated corneal epithelium covers the amniotic membrane over both the stromal as well as epithelial sides. Also, the increased expression of ZO-1 and E-cadherin suggest that amniotic membrane promotes migration of well-differentiated corneal epithelium in on-lay method of amniotic grafting.

PS-II-44

Determination of different oxidative stress markers in formalin fixed paraffin embedded (FFPE) tissues of mucormycosis patients

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Purpose: Mucormycosis is a life threatening, angioinvasive, fungal infection, caused mainly by *Rhizopus spp*. and *Mucor spp.* At our hospital, LV Prasad Eye Institute, several cases of mucormycosis have been reported that led to orbital exenteration. We aim to determine the presence of oxidative stress markers in the formalin fixed paraffin embedded (FFPE) surgically removed tissue section after orbital exenteration from patients with mucormycosis and COVID associated mucormycosis to understand molecular mechanism of disease pathogenesis and host immune response in ocular mucormycosis.

Methods: FFPE tissue sections (5µm) were obtained from ophthalmic pathology department, LVPEI. We checked the presence of some oxidative stress markers by the standard method of immunohistochemistry. Immunostaining was performed with antibodies specific for oxidative stress. Also, as most of the patients had a prior history of COVID 19 infection, we checked the presence of SARS COV 2 nucleocapsid protein in the exenterated FFPE tissue sections by the standard method of immunohistochemistry.

Results: We have stained 10 infected and 7 control tissue and increased expression of all the oxidative stress markers were observed in infected patient tissue sections compared to control. We saw decreased expression of the enzyme antioxidants in the infected tissues compared to the control. Presence of SARS COV 2 nucleocapsid protein was also observed in the infected tissues.

Conclusion: This indicates oxidative stress may play an important role in the disease pathogenesis of mucormycosis. This will further help us to understand the molecular mechanism that governs the pathogenesis of this disease.

PS-II-45

Investigation of association of CFH Y402H allelic variants with clinical phenotypes of age-related macular degeneration

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Purpose: Complement Factor H (CFH) is a key regulatory protein in the immune system that plays an important role in the innate immune response and inflammation. The (Y402H) Rs1061170 polymorphism in the CFH gene has been identified as a major genetic risk factor for increased susceptibility to AMD. This Y402H variant alters the protein's ability to regulate complement activation, leading to increased inflammation and tissue damage in the retina. Here we correlate the genotype of CFH with clinical phenotypes of AMD patients.

Methods: A total of 106 AMD patients and 54 control patients were recruited for this study. Their CFH gene was analyzed for (Y402H) Rs1061170 allelic variants and categorized into wild type (TT), Heterozygous risk (CT), and Homozygous risk (CC). Further, the patient's clinical outcomes were correlated with the CFH gene allelic variants.

Results: Genetic analysis showed that the AMD group consisted of 21%, 52%, and 27% in the wild type (TT), Heterozygous risk type (CT), and Homozygous risk type (CC) allelic variants respectively whereas the control group consisted of 46%, 45%, and 9% in the (TT), (CT), and (CC) respectively. In addition, large drusen were seen in 16% (n=14), 54% (n=45), and 30% (n=25) of the (TT), (CT), and (CC) groups respectively. Moreover, disciform scars were seen in 2% (n=2), 14% (n=15), and 8% (n=9) of the (TT), (CT), and (CC) groups respectively.

Conclusion: AMD patients with the CFH risk alleles (CT and CC) showed a strong correlation ($p = 0.05$) with drusen size and disciform body phenotypes.

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PS-II-46

A rare rhodopsin gene mutation in an Indian family with autosomal dominant retinitis pigmentosa spanning over five generations

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Purpose: *RHO* gene is the most frequently implicated for autosomal dominant retinitis pigmentosa (ADRP). This study was conducted to screen *RHO* gene in five generations of an Indian ADRP family.

Methods: Genomic DNA was extracted from peripheral blood of 29 available family members from the pedigree. The coding regions of *RHO* gene were examined using Sanger sequencing. Identified missense mutations were predicted for pathogenicity with six different online algorithms. Structural changes of the protein were analyzed using Garnier–Osguthorpe–Robson, PyMol, ChimeraX, and Molecular Dynamic simulations.

Results: All the affected family members have been experiencing night blindness and reduced visual acuity since childhood, along with the classical triad of retinitis pigmentosa (RP). A total of five sequence variants were detected in *RHO*, consisting of one pathogenic missense mutation (p.C187S). The p.(C187S) was identified as a heterozygous mutation in the proband and other sixteen affected family members but was absent in the remaining twelve unaffected members.

Conclusion: This is the first worldwide report of missense mutation p.(C187S) being associated with ADRP. This mutation disrupts the disulphide bond between C110 and C187 within the rhodopsin protein which leads to the misfolding and altered structure of protein, impairing its function in visual signal transduction cascade. The resulting dysfunction contributes to the development of RP due to rod cells degeneration. These findings add more information to the catalogue of pathogenic *RHO* gene mutations. This may aid in better clinical management, and genetic counselling in familial cases of RP.

PS-II-47

The intersection of mitochondrial genetics and keratoconus: new perspectives on corneal morphology and disease progression

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Purpose: Keratoconus (KC) is thinning of corneal stroma & irregular astigmatism, resulting in diminution of vision. Emerging evidence suggests a dysregulation of oxidative balance and mitochondrial function in KC corneas. Therefore, our study conducted the whole mitochondrial DNA (mtDNA) sequencing to study the mtDNA variations along with ultrastructural analysis of mitochondrial morphology and oxidative stress in KC patients.

Method: Whole mtDNA sequencing was performed on 20 KC blood and corneal tissues. Corneal tissue was also processed for transmission electron microscopy (TEM) and mRNA expression levels of oxidative stress genes.

Results: In our study, variations were found in both non-coding and coding region. Total of 389 variations at different position were found in coding region in which 237 variations reported as pathogenic. Most common variations were T to C and C to T followed by A to G in D-loop & coding region. TEM showed degraded mitochondria with dissolved cristae. qRT-PCR showed the downregulation of anti-oxidative genes. These variations were significant with disease severity and diminution of vision.

Conclusion: This is the first study showing high frequency of mtDNA variations in Indian population of KC corneas which might be due to abnormal morphology of mitochondria. Our findings highlight the potential utility of mtDNA variations and structural alterations as prognostic marker for KC patients. Mitochondrial dysfunction may be considered as a genetic risk factor contributing indirectly through the oxidative stress mechanism to the development and/or progression of KC.

PS-II-48

Identifying autosomal recessive Leber hereditary optic neuropathy, a new neuro-opthalmo-genetic paradigm in Indian cohort

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Purpose – Recent reports from India suggest that about 60-70% of individuals with Leber hereditary optic neuropathy (LHON) test negative for the common primary mitochondrial mutations and thus lack a definitive genetic diagnosis. This study aimed to explore alternative mechanisms and inheritance patterns by conducting whole mitochondrial and nuclear gene sequencing on samples that were negative for these primary mutations.

Methods – Thirty-five individuals suspected of having Leber hereditary optic neuropathy (LHON) were recruited from the Neuro-Ophthalmology Clinic. Clinical exome sequencing (CES) and mitochondrial genome sequencing were performed using NGS on the HiSeqX Ten platform, with a 2×150bp paired-end setup. The sequencing reads were aligned to both the human mitochondrial genome and the reference human genome (hg19). Variants were filtered using the VARIMAT tool (v.2.3.9), and haplogroup analysis

Results – CES revealed that approximately 20% of individuals (n=7) had homozygous mutations primarily affecting Complex I nuclear genes, while another 20% (n=7) had heterozygous mutations in other nuclear-encoded mitochondrial genes. Additionally, 17% of individuals (n=6) had mutations in the *OPA1* gene, with the remaining 43% showing no identifiable mutations.

Conclusions – Comprehensive genetic evaluations are crucial, and this study underscores the importance of screening both nuclear and mitochondrial genes to determine the diagnosis, inheritance patterns, and potential future therapies.

PS-II-49

Analysis of genetic variants in patients with xeroderma pigmentosum presenting at a tertiary eye care centre

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Purpose – The purpose of this study is to analyse clinical and genetic features of 19 XP patients from Indian cohort who presented at a tertiary eye care centre. By correlating the identified genetic variations with clinical presentations, we seek to enhance diagnostic accuracy and prime therapeutic management and strategies.

Methods - Whole exome sequencing (WES) was performed on Illumina NovaSeq, targeting ~99% of coding regions. Variant annotation, filtration, and interpretation followed ACMG guidelines using VarSeq and Varsome. Variants were filtered based on frequency and clinical significance, considering inheritance patterns and clinical data. NGS data was then confirmed by Sanger Sequencing.

Results - A total of 38 eyes of 19 patients were included in the study. The mean age of patients at first visit to clinic was 15.97 ± 9.75. The median age of the patients at presentation was 13 years (IQR 8.5 – 21 years). There was a significant male predominance with male to female ratio of 13:6. 11/19 patients were born out of consanguineous marriage with 2nd and 3rd degree consanguinity being more frequent. All 19 patients (total 38 eyes) had complaints of photophobia and hypopigmented naevi was seen in 100% patients. The ocular abnormalities included congestion, eyelid pigmentation and varying degrees of corneal haze/ scarring in all eyes, poor vision in 38/38 eyes, lid margin abnormalities in 21/38 eyes, conjunctival hyperemia in 25/38 eyes, conjunctival pigmentation in 13/38 eyes, pterygium in 05/38 eyes, corneal scarring in 16/38 eyes, corneal vascularization in 11/38 eyes, corneal edema in 03/38 eyes, ocular surface neoplasia in 11/38 eyes. Whole-exome sequencing succeeded by sanger sequencing identified - 14 homozygous variants IN XPC gene. These were predominantly frameshift (n=6) and stop-gain (n=5) variants and (n=1) deletion mutation, (n=1) substitution and (n=1) intronic variation, (n=6) variants introduced premature stop codons due to frameshift disruptions while (n=4) variants resulted in a premature termination of the protein. 10 out of 14 variants were novel, absent from population databases like gnomAD, 1000 Genomes and clinical variant repositories like ClinVar and HGMD while 4 variants were already reported.

Conclusions - We found that XPC was the most common complementation type in our cohort of patients. We suggest patients with severe XP ocular manifestation can be screened for mutations in the XPC gene

PS-II-50

The involvement of the mitochondrial genome in primary congenital glaucoma

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Purpose: This study aims to understand the involvement of the mitochondrial genome in primary congenital glaucoma (PCG) pathogenesis.

Methods: Deep sequencing of the entire mitochondrial genome (mtDNA) was undertaken in PCG cases (n=305) and ethnically matched normal controls (n=356). The complete mitochondrial genome was amplified using two sets primers of 8.3kb and 8.6kb. DNA library was prepared and sequenced on an Ion torrent S5 platform using Ion Ampliseq chemistry. Sequencing was performed at an average depth of 2000X to account for heteroplasmy. The data was aligned to the revised Cambridge reference sequence (rCRS) and the further analysis was done using GATK and VarSeq. The variants were annotated using MSeqDR tool.

Results: We observed 23 rare pathogenic variants across PCG cases (32/305; 10.49%). These variants were either absent or present in very low frequency in controls. There were 135 common variants in the coding and tRNA region across PCG cases and controls. Out of these, 12/135 (34.28%) variants in nine genes were found to be associated with the disease ($p \leq 0.05$). Among these, 3/12 (25%) were conferred to risk and 9/12 (75%) were protective to the disease. The PCG cases harboring pathogenic variation were found to cluster on nine major haplogroups. The M (34.38%), U (25%) and R (18.75%) haplogroups were prevalent among the cases.

Conclusions: Our study highlighted the involvement of pathogenic and potentially disease-associated mtDNA variants in PCG. These findings suggested the involvement of mtDNA in disease pathogenesis and susceptibilities to potential haplogroups that needs further investigations.

PS-II-51

Analysis of genetic variants in Indian patients with Xeroderma Pigmentosum presenting at a tertiary eye care centre

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Purpose: This study analyzed the clinical features and genotypes of 19 Indian patients with Xeroderma Pigmentosum (XP) presenting with ocular symptoms at a tertiary eye care center. The goal was to enhance diagnostic accuracy and prime therapeutic strategies by correlating genetic variations with clinical presentations.

Methods: Whole-exome sequencing (WES) on the Illumina NovaSeq platform targeted ~99% of coding regions. Variants were annotated, filtered, and interpreted using ACMG guidelines via VarSeq and Varsome, with confirmation by Sanger sequencing.

Results: The study included 38 eyes from 19 patients, with a median age of 13 years. There was a male predominance (13:6 ratio), and 11 patients were from consanguineous marriages. All patients exhibited photophobia and hypopigmented naevi, along with other ocular abnormalities such as lid margin abnormalities in 30/38 eyes, conjunctival hyperemia in 23/38 eyes, conjunctival pigmentation in 14/38 eyes, pterygium in 6/38 eyes, corneal scarring in 14/38 eyes, corneal vascularization in 11/38 eyes, corneal edema in 03/38 eyes, and ocular surface neoplasia in 9/38 eyes. WES identified 19 homozygous variants in the XPC gene, including 13 novel variants. Most were frameshift or stop-gain mutations, leading to premature stop codons.

Conclusions: XPC was the most common complementation group in this cohort, contrasting with previous Indian studies where XPA predominated. Patients with severe XP ocular manifestations should be screened for XPC mutations, as XPC frameshift variants were associated with a more severe phenotype and poorer ocular outcomes.

PS-II-52

Impact of ARMS2 and HTRA1 homozygous risk alleles on the severity and clinical phenotypes of Age-related Macular Degeneration

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Purpose: Age-related macular degeneration (AMD), a leading cause of vision loss, is significantly influenced by genetic variants in ARMS2 and HTRA1. ARMS2 rs10490924 affects mRNA stability and mitochondrial function, while HTRA1 rs11200638 risk allele "A" increases transcriptional activity, driving AMD progression. This study investigates how SNPs in ARMS2 and HTRA1 impact AMD severity by examining their association with clinical features. In doing so, we want to improve our understanding of AMD pathophysiology and genetic risk assessment.

Methods: A total of 160 participants were studied, including 106 AMD patients and 54 controls. Genotyping for ARMS2 rs10490924 and HTRA1 rs11200638 SNPs was performed, and clinical phenotypes between risk and non-risk genotype groups of AMD were assessed.

Results: Among the AMD patients, the GG (normal) allele for ARMS2 was observed in 19.8% and for HTRA1 in 21%, compared to 46.3% and 42% in controls, respectively. The TT risk allele for ARMS2 was present in 34.9% of AMD cases and 7.4% of controls. Patients with the TT genotype had larger drusen (25.9%), pigmentary changes (21%), PED (Pigment Epithelial Detachment) (12%), and disciform scars (13%) compared to those with the GG genotype. For HTRA1, 34% of AMD patients had AA risk alleles, with 11% developing disciform scars compared to 3% of those with GG genotypes.

Conclusion: Clinical phenotypes, including drusen size, pigmentary changes, PED, and disciform scars, were significantly associated with the ARMS2 homozygous risk allele and disciform scars were linked to HTRA1 homozygous risk allele, when compared to homozygous normal alleles.

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PS-II-53

GWAS study to identify genomic risk factors for DR in the South Indian population: A SIGNATR study

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Purpose: Diabetic Retinopathy (DR) is a microvascular sight-threatening complication. This abstract focuses on the genomic risk factors for DR and its subtypes specifically in the South Indian population identified by a genome-wide association study.

Methodology: 2538 patients with South Indian ancestry with T2DM and with/without DR were recruited for the study. The patients were from a retrospective and a prospective cohort from Sankara Nethralaya. Imaging data and clinical data were collected for the same cohorts. The imaging data included fundus photography and optical coherence tomography. The clinical data included HbA1c, lipid profile, and microalbuminuria. GWAS was carried out using Infinium Expanded Multi-Ethnic Genotyping Array. Covariate adjustments were made for significant principal components such as HbA1c levels, duration of diabetes, and DR status. A polygenic risk score was performed for T2DM, overlapping DR and PDR.

Results and Conclusion: A novel locus on chromosome 10 was observed to be significantly associated with Proliferative Diabetic Retinopathy (PDR). However, a significant genome-wide association was not observed for most traits. Polygenic risk scores also identified associations with T2DM, with risk for PDR. This is the first GWAS for DR in a South Indian cohort. Further studies with larger cohorts will identify additional genetic variants for this population.

PS-II-54

Genomic exploration of antifungal drug resistance in *Fusarium solani*

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Purpose: Fungal keratitis is predominantly caused by the *Fusarium* species, which is becoming less susceptible to antifungals, making treatment challenging. The objective of our work was to understand the genetic determinants that contribute to drug resistance in *Fusarium solani*, which can shed light on the underlying mechanisms responsible for increased drug resistance to antifungal agents.

Methods: Antifungal Drug Susceptibility Testing of *F. solani* (n=20) against 6 antifungal Drugs employing broth Micro Dilution Method was done and MIC was determined. Whole-genome sequencing of clinical isolates (n=4) was done using Illumina Hiseq. The bioinformatics analysis was performed using various tools and software, including FASTQC, SPAdes, AUGUSTUS, and NCBI Blast. The molecular docking of CYP51A was done by Autodock vina tool with voriconazole.

Results: The Antifungal Drug Susceptibility with average MIC value were found as Fluconazole (512 µg/ml), Itraconazole (32 µg/ml), Natamycin (16 µg/ml), Amphotericin B (8 µg/ml), Voriconazole (4 µg/ml), and Posaconazole (4 µg/ml). Genomic study identified many types of genes associated with resistance to antifungal drugs, such as CYP51, ERG, Efflux pumps, myosin, Multidrug resistance protein, and Beta tubulin, in the isolates. Molecular docking showed the inability of drug voriconazole to bind with heme group of CYP51A gene.

Conclusions: The presence of mutations in the sequence of CYP51A protein in all isolates is likely to be the cause of resistance to azole drugs. This study, which utilizes phenotypic and genotypic characterization, aims to address the issue of antifungal drug resistance.

PS-II-55

Clinical profile of neuro-ophthalmological conditions at a tertiary eye care centre: A six-year retrospective study

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Purpose: Neuro-ophthalmology integrates the aspects of neurology and ophthalmology. Studies specific to neuro-ophthalmology disorders profiling are scarce in South India. Therefore, this study aims to delineate the clinical and demographic characteristics of patients presenting with neuro-ophthalmological conditions at a tertiary eye care centre.

Methods: A retrospective hospital-based study was conducted at the neuro-ophthalmology clinic of Aravind Eye Hospital (AEH), a tertiary eye care centre in Madurai, Tamil Nadu, India, spanning six years from 2018 to 2023. The data were retrieved from the electronic medical records, including patient demographics, visit details, clinical presentations, management, treatment and outcomes. Data analysis was performed using GraphPad Prism.

Results: A total of 5,129,848 outpatients visited AEH, Madurai of which 53,769 (1%) were referred to the Neuro-Ophthalmology Clinic. Among them, 19,834 (37%) patients had conclusive diagnosis for neuro-ophthalmological disorders, in which 10,029 males, 9,803 females, and 2 transgender individuals, resulting in a male-to-female ratio of 1.02:1. The mean age of the disease presentation was recorded as 47.35 ± 16.93 years (ranging from 2-103 years) with the highest incidence occurring in the 41-60 age group (39.73%). Furthermore, the study identified the most frequently diagnosed conditions among patients as Optic Neuropathy (26.69%), Papilledema (15.72%), Optic Neuritis (6.98%), Non-Arteritic Anterior Ischemic Optic Neuropathy (NAAION) (6.6%), Ocular Myasthenia Gravis (6.07%), and Toxic Optic Neuropathy (5.4%).

Conclusions: This study highlights the diverse spectrum of neuro-ophthalmic disorders encountered in a tertiary eye care centre. Neuro-ophthalmology in India requires significant advancement as a specialized field to more effectively meet the healthcare needs of the country's population.

PS-II-56

Identification and structural analysis of novel pathogenic variants in the *MYOC* and *CYP1B1* genes in Indian JOAG patients

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Purpose: Juvenile onset open-angle glaucoma (JOAG) manifests in individuals under the age of 40, resulting in elevated intraocular pressure and significant optic nerve damage. To broaden the spectrum of mutations associated with JOAG and to determine their specific structural implications, we examined *MYOC* and *CYP1B1* gene in a cohort of 111 unrelated North Indian patients diagnosed with JOAG.

Methods: PCR-DNA sequencing screened the coding exons and intron-exon junctions of the *MYOC* and *CYP1B1* genes in 111 unrelated JOAG patients and 100 controls. Identified sequence variations were searched in the ClinVar database, HGMD, and dbSNP. Six different online available algorithms including REVEL, SIFT, Mutation Taster, SNAP2, IMutant2.0, and MutPred2 were used for the pathogenicity prediction of missense variations. The Structural consequences of detected possible pathogenic variations were predicted by using PyMol, Chimera and MD simulation of these changes.

Result: Potentially-pathogenic variations were observed in thirty patients (27.02%) within the *MYOC* and *CYP1B1* genes, encompassing both novel and previously documented variants. Analysis reveals a higher prevalence of *CYP1B1* gene variants (22.5%) relative to *MYOC* gene variants (4.5%), suggesting that *CYP1B1* is the predominant gene implicated in JOAG among Indian patients. Structural predictions of novel potentially-pathogenic mutations indicate altered stability and flexibility.

Conclusion: Our findings enhance the mutation spectra and frequencies of *MYOC* and *CYP1B1*gene in JOAG among the North Indian population. Structural predictions of novel pathogenic mutations could enhance the understanding of JOAG pathogenesis and support subsequent functional analysis.

PS-II-57

Differential gene expression analysis in Uveitis sub-type VKH (Vogt-Koyanagi-Harada) disease: a case-control study in Indian cohort

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Purpose – Vogt-Koyanagi-Harada (VKH) disease is a multisystem, autoimmune disease characterized by bilateral granulomatous panuveitis affecting young adults. Around 55.9% of posterior uveitis is due to VKH disease. To understand gene regulatory mechanisms in VKH disease, we have explored the differential gene expression in VKH cases and compared it to healthy controls in this study.

Methods – PBMC was isolated from the blood samples and total RNA was extracted from them for VKH patients and controls using TRIzol reagent. Illumina HiSeqX system was used for deep sequencing of total RNA. DESeq2 package was used to identify the Differentially Expressing Genes (DEG) and also to do the downstream analysis of the RNA-Seq data. The DEG data was further visualized as Heat-map, Volcano Plot, PCA plot, Category Network plot using Bioconductor packages in R. Gene Set Enrichment Analysis was done using “Clusterprofiler”. Selected candidate genes were experimentally validated by RT-qPCR.

Results – In total, 214 gene candidates (142 up-regulated and 72 down-regulated) Gene Ontology and pathway analysis of these genes revealed significant enrichment of autoimmune and inflammatory related GO and pathways (eg: Chemokine, Rap1, MAPK signalling pathway). RT-qPCR validation confirmed expression pattern of RNA seq data.

Conclusions –This is the first study for the transcriptome analysis of uveitis in Indian cohort and resulted in the identification of candidate genes and pathways associated with VKH. This study identified immune-associated genes and pathways. Few of the genes have the potential to be novel biomarkers for VKH.

PS-II-58

Prevalence, demographics, and clinical characteristics of cornea plana

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Purpose: To determine Cornea plana's prevalence, demographics, and clinical characteristics through a single-centric study in India.

Methods: This is a retrospective study of patients diagnosed with CP from 1994 to 2024 using electronic medical records.

Results: A total of 163 eyes from 87 patients were studied. Most were from southern India (66.6%), followed by eastern India (4.59%), and other regions including the north, west, central, northeast, southwest, northwest, and international locations. The median age was 13.06 years (IQR, 3.61-25.11), with a Mean Spherical Equivalent of 2.92±6.53. Males constituted 52.87% of the cohort. Parental consanguinity: 31.03%. Clinical features included high hyperopia, indistinct scleral-corneal borders, and early arcus lipoides. Among the total cohort, 20.24% were intervened where 6.7% underwent cataract surgery and 1.84% PK.

Conclusion: A high incidence of Cornea plana was reported in southern India. Understanding cornea plana's demographics, progression, and clinical characteristics is vital to further improve management strategies.

PS-II-59

Analysis of gene expression patterns of epigenetic factors in retinoblastoma

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Purpose: In retinoblastoma, epigenetic deregulation of tumor-promoting pathways, alongside RB1 gene loss or MYCN amplification, may contribute to tumor heterogeneity. However, a thorough analysis of epifactor genes in retinoblastoma is lacking. This study aims to determine whether the epifactor gene expression can effectively categorize patients into distinct subgroups.

Methods: Gene expression datasets from previously published microarray analyses of RB tumors were downloaded from the NCBI-GEO database. These datasets were used to construct a cohort, followed by unsupervised clustering using R. Consistently differentially expressed genes within the cohort were identified using edgeR. Additionally, a gene set of epigenetic factors and regulators was sourced from EpiFactors, a curated database detailing epigenetic regulators, their complexes, targets, and products. The expression of these epigenetic factors was then analyzed within the RB cohort.

Results: After compiling all the datasets, we established a comprehensive cohort consisting of 250 tumor tissue and 7 normal retina samples. As expected, principal component analysis revealed a clear separation between the normal retina samples and retinoblastoma tissues. Overall, epigenetic factors were differentially regulated in retinoblastoma patients compared to normal tissues. Profiling of 815 epigenetic factors uncovered further heterogeneity among the retinoblastoma patients. Notably, DNA and histone methyltransferases were among the most highly regulated genes within a group of patients.

Conclusions: Epigenetic gene expression analysis of the retinoblastoma gene expression dataset revealed the presence of epigenetic heterogeneity among retinoblastoma patients. This heterogeneity can be further explored to understand its impact on clinical outcomes and drug responses.

PS-II-60

Utility of genetic testing and genetic counselling in a tertiary eye hospital

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Purpose: Technological advances, availability of gene-based clinical trials, and interventional therapeutic strategies have allowed an increased opportunity for genomic testing in ophthalmic diseases. The inheritance patterns and risk of recurrences derived based on the genetic test results defines the role of genetic counsellors to communicate with the patients and families to further, help them in making informed choices and planning. This report summarizes the outcome of genetic testing and genetic counselling in a tertiary ophthalmic setup in India.

Methods: The patients underwent a standard pretest genetic counselling procedure after detailed clinical evaluation and referral from ophthalmologists. Genetic tests performed were either for a specific candidate/panel genes using the NGS platform followed by detailed post-genetic discussions.

Results and Conclusions: The data compiled for 10 years showed that genetic testing and counselling were performed for around 40 different ocular diseases. Retinal and pediatric diseases were the most frequent referral and mutations suggestive of syndromic forms of inherited retinal degenerations, and myopia were identified in a subset of these patients. The outcome of the genetic tests in these families has enabled carrier testing and predictive testing in the families.

PS-II-61

Characterizing Bardet-Biedl syndrome patients for DNA methylation alterations associated with the comorbidities

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Purpose: Bardet-Biedl syndrome is an autosomal recessive disorder with metabolic dysfunction. Disease-specific methylation signatures are being explored for many monogenic diseases. We performed genome-wide methylation analysis for BBS patients to identify unique disease-specific methylation patterns.

Methods: Array-based (850K) DNA methylation profiling was performed in the peripheral blood samples of BBS patients and compared with controls (N=10 cases and no disease controls). Raw intensity data files (.idat) were processed in ChAMP R package using the intensity data files along with the sample annotation sheet containing patient phenotype and array data. Quality control, normalization, probe correction was performed on the datasets, and batch effects were eliminated. Pairwise comparisons between cases and controls were performed to identify differentially methylated positions (DMPs) and regions (DMRs) with a minimum of 5 probes and p-value < 0.05. Visualization plots were generated followed by gene set enrichment analysis (GSEA) using Enrichr database.

Results: A total of 269 DMPs and 296 DMRs corresponding to 43 genes were identified with a statistical significance of $p < 0.05$. Among these, 158 DMRs were in the CpG dense promoter region of 23 genes, and these differentially methylated genes were associated with calcium signalling, LDH activity, peroxisome biogenesis, dilated cardiomyopathy as well as Wnt and Hedgehog signalling pathways.

Conclusion: In this study, we have explored and identified disease-specific DNA methylation signatures in Bardet Biedl syndrome that could also explain the probable mechanisms involved in the associated comorbidities. However, these pilot results need further validation.

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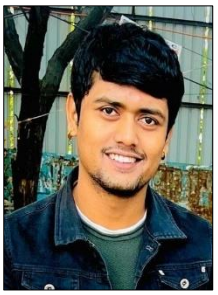

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