Indian Eye Research Group

IERG

19th Annual Meeting

July 30-31, 2011

25 YEARS LVPEI
So that all may see

CSIR - INDIA
Centre for Cellular and Molecular Biology

LV Prasad Eye Institute

LV Prasad Eye Institute Celebrates 25 Years of Excellence in Eye Care

Supported in parts by an unrestricted educational grant from

GE Healthcare

Pearala Agencies
Hyderabad

Program Schedule

Day 1 30/07/2011 Saturday	Time	Subject	Venue	Invited Speaker I	Invited Speaker II	Chair	Co-chair
Registration	8.15-9.00 am	Art Gallery, Level VI					
Breakfast	8.15-9.00 am	Terrace, Level VII					
Inauguration	9.00-9.10 am	Inauguration of the Meeting	Patodia Auditorium			D Balasubramanian	G Chandrasekhar
Session I	9.10-10.00 am	Bireswar Chakrabarti Oration Lecture	Patodia Auditorium	UP Andley		D Balasubramanian	
Tea Break	10.00-10.30 am	Terrace, Level VII					
Session II	10.30-12.30 pm	Biology of Eye Diseases	Patodia Auditorium	K Dharmalingam		S Krishnakumar	G Kumaramanickavel
Session III	12.30-1.15 pm	Poster Session I	Art Gallery, Level VI				
Lunch Break	1.15-2.00 pm	Terrace, Level VII					
Session IV	2.00-4.05 pm	Retina, Oncology and Oculoplasty	Patodia Auditorium	N Angayarkanni		T Velpandian	P Sundaresan
Tea Break	4.05-4.30 pm	Terrace, Level VII					
Session V	4.30-6.15 pm	Cornea and Lens	Patodia Auditorium	N Lini		GB Reddy	A Vasavada
Special event	6.30-7.30 pm	Special talk on NGS	Patodia Auditorium	T Jose		M Denton	S Chakrabarti
Dinner	8.00-10.00 pm	Taj Banjara Hotel					
Day 2 31/07/2011 Sunday	**Time**	**Subject**	**Venue**	**Invited Speaker I**	**Invited Speaker II**	**Chair**	**Co-chair**
Registration	8.00-8.30 am	Art Gallery, Level VI					
Session VI	8.30-10.00am	Community Eye Health, Low Vision and Rehabilitation	Patodia Auditorium	J Keeffe	V Nangia	V Muthukarruppan	R Khanna
Tea Break	10.00-10.30 am	Terrace, Level VII					
Session VII	10.30-11.30am	Prof D Balasubramanian Oration Lecture	Patodia Auditorium	R Augusteyn		G N Rao	
Session VIII	11.30-1.00 pm	Visual Neurosciences and Optometry Research	Patodia Auditorium	SP Arun	NK Challa	TP Das	S Bharadwaj
Session IX	1.00-1.45pm	Poster Session II	Art Gallery, Level VI				
Lunch Break	1.45-2.30 pm	Terrace, Level VII					
Session X	2.30-4.00pm	Glaucoma and Uveitis	Patodia Auditorium	V Umashankar	A Mukhopadhyay	Kunal Ray	G Swarup
Valedictory	4.00-4.30 pm	Prize Distribution and Vote of Thanks	Patodia Auditorium	D Balasubramanian and Inderjeet Kaur			
Tea Break	4.30- 5.00 pm	Terrace, Level VII					

Day 1 30/07/2011 Saturday

Registration	*8.15-9.00 am*	*Art Gallery, Level VI*	
Breakfast	*8.15-9.00 am*	*Terrace, Level VII*	
Inauguration	9-9.10 am	D Balasubramanian G Chandrasekhar	**Inauguration of the meeting**
Session I	9.10-10.00 am	Usha P Andley	**Bireswar Chakrabarti Oration Lecture:** Protein Aggregation in Cataract
Tea Break	*10.00-10.30 am*	*Terrace, Level VII*	
Session II	10.30 am -12.30 pm **Biology of Eye Diseases**		
	10.30-11.00 am	K Dharmalingam	Proteomics Approaches to Understand Ophthalmic Diseases
	11.00-11.15 am	G B Reddy	Micronutrient Status in Diabetic Retinopathy
	11.15-11.25 am	S Krishnakumar	Reversal of Stathmin Mediated Microtubule Destabilization Senstizes Retinoblastoma Cells to Low Dose of Anti Microtubule Agents: A Novel Synergistic Therapeutic Intervention
	11.25-11.35 am	V Gopakumar	An Approach of Novel Tissue Engineering for Bioengineering Corneal Epithelial Cells from Non-Ocular Epithelial Cells
	11.35-11.45 am	ID Rastogi	Evolution of Higher Stability in Lens γ-Crystallins at the Cost of Calcium Binding
	11.45-11.55 am	Anshul Arora	Cell Junction Proteins in Cataractous Lens Epithelial Cells
	11.55-12.05 pm	Srilatha Jasty	Neural Potential and Gene Expression Profile of Stem/Progenitor Cells Derived from Human Ciliary Pigment Epithelium
	12.05-12.15 pm	T Velpandian	Functional Assessment of the Involvement of Organic Cation Transporter (OCT) in the Tear Kinetics
Session III	12.15-1.00 pm	**Poster Session – I (Art Gallery, Level VI)**	
Lunch Break	*1.00-2.00 pm*	*Terrace, Level VII*	
Session IV	2.00-4.05 pm	**Retina, Oncology and Oculoplasty**	
	2.00-2.15 pm	N Angayarkanni	Paraoxonase (PON) in Diabetic Retinopathy
	2.15-2.25 pm	AR Patel	Expression Analysis of Antiangiogenic Factors in Lens Epithelial Cells of Persistent Fetal Vasculature Patients
	2.25-2.35 pm	S Senthilkumari	Levels of Macular Xanthophylls in Indian Donor Eyes
	2.35-2.45 pm	S Rathi	Quantitative Analysis of Vitreous Humor Reveals Distinct Protein Profiles in Patients with Retinopathy of Prematurity
	2.45-2.55 pm	A Tiwari	Design and Engineering of a Therapeutic Agent for Proliferative Vitreoretinopathy

	2.55-3.05 pm	R Danda	Tissue Specific Cytotoxicity Mediated Suicidal Gene Therapy
	3.05-3.15 pm	S Jalali	Complications of Intravitreal Bevacizumab in Retinopathy of Prematurity. The Indian Twin Cities ROP Screening (ITCROPS) data base report number 6
	3.15-3.25 pm	T V Dave	Template Technique: A Novel Method for Eyelid Debulking in Patients with Orbitopalpebral Plexiform Neurofibroma
	3.25-3.35 pm	P Jayaraj	Expression of E-Cadherin In Sebaceous Cell Carcinoma of the Eye Lid an Immunohistochemical Study
	3.35-3.45 pm	R Modi	An Unusual Case of Pedunculated Upper Lid Mass
	3.45-3.55 pm	P Mahendradas	Fundus Autofluorescence Imaging in Eyes with Serpiginous Choroiditis- Our Experience
	3.55-4.05 pm	N Murugan	A Single Tube Multiplex Polymerase Chain Reaction for Detection of *TEM, SHV, OXA* Gene Mediated Extended-Spectrum β-Lactamases Among Gram-Negative Bacilli Recovered from Ocular Specimens.
Tea Break	*4.05-4.30 pm*	*Terrace, Level VII*	
Session V	4.30-6.15pm	**Cornea and Lens**	
	4.30-4.45 pm	N Lini	Host Response In Aspergillus flavus Corneal Ulcers And Secretome Analysis of Clinical Isolates of a Flavus
	4.45-4.55 pm	A Mohamed	Ultrastructural Features of Nuclear Cataracts with Different Morphologies
	4.55-5.05 pm	RSG Karthikeyan	Expression of TLRs, Dectin-1, And Pro-Inflammatory Cytokines in Human Corneas Infected with Filamentous Aspergillus and Fusarium Sp
	5.05-5.15 pm	S Basu	Clinical Outcomes of Repeat Autologous Cultivated Limbal Epithelial Transplantation for Ocular Surface Burns
	5.15-5.25 pm	M Mathew	Microbiological Profile and Role of AC Wash in the Management of Microbial Keratitis
	5.25-5.35 pm	D Das	Corneal Collagen Cross-linking Procedure Leads to Apoptosis in Limbal Epithelial Cells.
	5.35-5.45 pm	S Hazra	Therapeutic Modulation of Matrix Metalloproteinase Prevents Posterior Capsular Opacification
	5.45-5.55 pm	GP Chidambaranathan	Ex-vivo Expansion of Limbal Epithelium With Known Stem Cell Content under GMP Compliance

	5.55-6.05 pm	VPR Vendra	Why Do Some Mutations in Human Gamma D Crystallins Lead to Congenital Nuclear Cataracts while Others Lead to Peripheral Cataracts? A Protein Structural Rationale
	6.05-6.15 pm	MLS Chalasani	A Cataract Causing Mutant of Connexin-50 Shows Defect in Transport to the Plasma Membrane and is Retained in the Endoplasmic Reticulum
Special event (Sponsored by Sandor Proteomics)	6.30-7.30 pm	T Jose	**Special talk:** "Next Generation DNA Sequencing: A step towards Personalized Healthcare"
Dinner	*8-10.30 pm*	*Taj Banjara Hotel, Road No.1, Banjara Hills, Hyderabad*	

Day 2 31/07/2011 Sunday

Registration	*8.00-8.30 am*	*Art Gallery, Level VI*	
Session VI	8.30-10.00 am	**Community Eye Health, Low Vision and Rehabilitation**	
	8.30-8.45 am	J Keefe	Population-based Surveys: Which Method Should I Use?
	8.45-9.00 am	V Nangia	Central India Eye and Medical Study (CIEMS) - A Clinical Translational Study
	9.00-9.10 am	S Sheeladevi	Knowledge, Attitude and Practices on Diabetes and Diabetic Retinopathy in Rural Andhra Pradesh in India
	9.10-9.20 am	P K Rani	Rural Vision Health Guardians role in Eye Health, Diabetes and Hypertension Education
	9.20-9.30 am	TP Das	The e-kit. One Pack Solution for First- line Bacterial Endophthalmitis Treatment
	9.30-9.40 am	R Sumalini	Development and Validation of the Children Visual Impairment Questionnaire (CVIQ)
	9.40-9.50 am	S Patil	Why Does Pediatric Cataract Surgery in India have a Poor Follow-up?
	9.50-10.00 am	A Mallipatna	The feasibility of Using a Digital Camera to Elicit Red Reflexes for the Detection of Vision-Threatening Eye Disease in Pre- School Children
Tea Break	*10.00-10.30 am*	*Terrace, Level VII*	
Session VII	10.30-11.30am	R Augusteyn	**Prof D Balasubramanian Oration Lecture:** Growth of The Human Eye Lens
Session VIII	11.30 am-1.00 pm	**Visual Neuroscience and Optometry Research**	
	11.30-11.45 am	SP Arun	What Do Neurons in Inferior Temporal Cortex Tell Us About Object Recognition
	11.45-12.00 pm	NK Challa	Nature Of L- and M-Cone Input to Cone-Opponent and Non-Opponent Mechanisms as a Function of Retinal Eccentricity
	12.00-12.10 pm	UK Addepalli	Effect of Spectrum Bias on the Diagnostic

			Accuracy of Spectral Domain Optical Coherence Tomograph in Glaucoma
	12.10-12.20 pm	N Srividya	Visual & Refractive Outcomes of Intraocular Lens Implantation in Congenital Cataracts less than 6 Months of Age
	12.20-12.30 pm	M Kumar	Clinically Significant Macular Edema can be a Pitfalls in Axial Length Measurement before Cataract Surgery
	12.30-12.40 pm	S Sarkar	The Relation between Pharmacological Dilation, Pupil Diameter and Accommodative Response Magnitude
	12.40-12.50 pm	A Narasaiah	Inter And Intra Subject Variability of Luminanace - Slope Calibration In Eccentric Photorefraction
	12.50-1.00 pm	T Kumbar	Correlation of Back Optic Zone Radius Measurement of Rigid Contact Lenses with Radiuscope and Keratometer
Session IX	**1.00-1.45 pm**	**Poster Session – II (Art Gallery, Level VI)**	
Lunch Break	*1.45-2.30 pm*	*Terrace, Level VII*	
Session X	**2.30-4.00 pm**	**Glaucoma and Uveitis**	
	2.30-2.45 pm	V Umashankar	Genotype To Phenotype Annotation of SNP's – An Insilico Approach
	2.45-3.00 pm	A Mukhopadhyay	Genetic Association and Gene-Gene Interaction of *HAS2, HABP1* and *HYAL3* in POAG Implicate Role of Hyaluronan in Glaucomatous Neurodegeneration
	3.00-3.10 pm	AK Mandal	Surgical Outcome of Primary Trabeculotomy-Trabeculectomy in Glaucoma with Sturge-Weber Syndrome
	3.10-3.20 pm	NS Choudhari	Repairing Goldmann Applanation Tonometer is Easy
	3.20-3.30 pm	J Mollah	Efficacy of Split Hours Part Time Patching Vs Continuous Wear Part Time Patching for Treatment of Unilateral Amblyopia in Children
	3.30-3.40 pm	K Sirohi	A Glaucoma-Associated Optineurin Variant, M98k Induces Retinal Ganglion Cell Death by Reducing Transferrin Receptor Level Through Lysosomal Degradation
	3.40-3.50 pm	M Umesh	Rate of Visual Field Progression in Medically and Surgically Treated Glaucoma Patients
	3.50-4.00 pm	VK Gothwal	Rasch Analysis of the Glaucoma Quality of Life (GQL-15) Questionnaire
Valedictory	4.00-4.30 pm	**Prize distribution and Vote of thanks**	
Tea Break	*4.30- 5.00 pm*	*Terrace, Level VII*	

Poster Session I July 30, 2011 12.15-1.00pm		
S. No	***Names***	***Title***
1	R Sankaranarayanan	Aberrant Expression and Regulation of Calpain 3 (*CAPN3*) a nd Transglutaminase 2 (*TGM2*) in Human White Mature Cataract
2	A Pal	Quantification and Characterization of Melanin Pigment Isolated from Pathogenic Fungi Causing Keratitis
3	D A Ganatra	Trichostatin-A Prevents Transforming Growth Factor Mediated Changes in Lens Epithelial Cells: *In Vitro*
4	F Kayastha	Response of Lens Epithelial Cells to Injury
5	MJ Bharathi	Utility of Routine Conventional Light Microscopic Examinations in the Diagnosis of Microsporidial Keratitis
6	K Rangachari	Biophysical Characterization of Human Myocilin And Purification of C-Terminal Domain
7	J Jeyalaxmi	Glaucoma Database (Bioinformation. 2011 Feb 7;5(9):398-9)
8	S Chauhan	p16INK4A as a Predictor of HPV in Ocular Surface Squamous Neoplasia
9	Y Bhadange	Clinical and Genetic Evaluation in Patients with Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES)
10	K Kaviarasan	Elevated Levels of Serum Oxidized LDL-antibody in Diabetic Retinopathy
11	MB Selvi	Increased Thiolation and Homocysteinylation of Proteins are Responsible for Protein Damage in Eales Disease
12	S Bhattacharjee	Proteomic Analysis of Arsenic Toxicity on Lens Proteins of LABEO ROHITA
13	A Verma	Screening of RPE65 Gene in Southern Indian Cohort of LCA Patients
14	S Tiwari	Evaluation of Human Lacrimal Gland Cultures for Secretory Function and Putative Stem Cells
15	J Samuel	NIS Expression in Retinoblastoma
16	J Sudhakar	Presence of Hypoxic Regions in Human Retinoblastoma: A Possible Role in Hypoxia Regulated Multi Drug Resistance
17	U Mangalangi	Characterization of Human Ciliary Pigmented Epithelial (CPE) Cells
18	S Gaddipati	Outcome of Cultured Oral Mucosal Epithelial Transplantations (*COMET*) in the treatment of Bilateral Severe Limbal Stem Cell Deficiency
19	S Maddileti	Plasma Polymer Coated Therapeutic Contact Lenses for Expansion and Delivery of Limbal Epithelial Cells
20	CN Sharma	Genetic Screening of Genes Involved in Leber's Congenital Amaurosis (LCA) in Indian Patients
21	S Katta	The del443ins54 (ARMS2) Variation in an Indian Cohort with Age-Related Macular Degeneration
22	C Ramachandran	Comparison of Limbal Epithelial Cell Growth on a Synthetic Polymer Scaffold and Human Amniotic Membrane (HAM)

23	KN Rao	Variations at the 7q31 Locus Harbouring *CAV1* and *CAV2* are not Associated with Primary Glaucomas in an Indian Population
24	PK De	A Heterodimeric Secretoglobin of Lacrimal Gland Origin is Present Male-Specifically in Body-Fur of Syrian Hamsters
25	RM Nair	Evaluation of Cancer Stem Cells in Human Y79 retinoblastoma Cell Line
26	PK Balne	DNA Sequence Based Identification of Nonsporulating Molds Isolated from Ocular Infections
27	GR Musada	Molecular Genetic Analysis of Norrie Disease Pseudoglioma (*NDP*) Gene and Tetraspanin 12 (*TSPAN 12*) Gene in Indian Familial Exudative Vitreo Retinopathy (FEVR) Patients
28	N Sahini	Identification of Disease Genes in Indian Population with *ARRP* by Homozygosity Screening
29	S Siddiqui	Quantitative Analysis of Plasma proteins in Patients with Age Related Macular Degeneration
30	MK Janani	A Study on An Epidemic of Acute Keratoconjunctivitis in Chennai: Isolation of a Novel Human Adenovirus Identified Based on Phylogenetic Analysis
31	L Dhanurekha	*In Vitro* Antifungal Susceptibility Testing by Disk Diffusion Method Against Amphotericin B and Voriconazole on Ocular Isolates of Non Sporulating Moulds
32	M V Jeyalatha	Study to Evaluate the Effect of UV Rays on Riboflavin Treated Cadaveric Corneal Limbal Stem Cells
33	M RajyaLakshmi	Structural Features and Stability of a $\beta\gamma$ -Crystallin Domain of a Non-Lens Vertebrate Protein *CRY* $\beta\gamma$ 3
34	L Yeramala	Stability Tuning Knob in $B\gamma$-Crystallins and its Evolution in Vertebrate Homologues
35	N Lini	Analysis of the Serum Proteomic Patterns for the Early Detection Diabetic Retinopathy
36	S Singh	Quantification of the Expression of Sex Steroid Receptors in Lacrimal Gland and Cornea in the Experimental Models of Dry Eye
37	Vijayakumar	Evaluation of Ethambutol Induced Retinal Toxicity by ERG Changes: Role of Trimetazidine
38	P Gupta	Antiangiogenic, Antioxidant and Anticataract Potential of Marine Invertebrate Species
39	K Mahender	Screening of Antiangiogenic and Antiproliferative Potentials of Marine Cynobacterial Strains
40	B Arora	Controlled Experimental Study on Ocular Distribution of Systemic Barbiturate: Forensic Relevance
41	A Sirohiwal	Ocular Kinetics of Extraporaneous Natamycin Formulation in Ex-Vivo Studies after Intrastromal Administration
42	H Anuradha	Evaluation of Retinal Toxicity of Systemically Administered Drugs: Effect of Quinidine on Rat Using Electroretinogram
43	S Patnaik	Evaluation of Intraocular Penetration of a Polyherbal Formulation and its Antiangiogenic Potential

44	T Prasad	Use of Two Molecular Markers in Combination can Identify Precisely Adult Human Corneal Epithelial Stem Cells
45	S Kar	Comparison of Methods for the Detection of Methicillin Resistance and Antibiotic Susceptibility Profile of Staphylococcus Aureus Isolates from Ocular Infections
46	M Balasubramanian	Serotyping of Toxoplasma Gondii by ELISA in Ocular Toxoplasmosis Patients
47	J Lakshmipriya	Phenotype and Genotype of Effector Molecules of Pseudomonas aeruginosa Type III Secretion System (*T3SS*) from Human Corneal Ulcer
48	N Velusamy	Identification of Methicillin Resistant Staphylococcus aureus by Conventional and Molecular Methods and its Antimicrobial Susceptibility Pattern
49	R Shukla	Molecular Genetic Analysis of Leber's Congenital Amaurosis (LCA) in Indian Patients
50	M Mondal	Molecular Genetic and Functional Studies on Oculocutaneous Albinism
51	VR Anandula	Homozygosity Mapping in Consanguineous South Indian Families with Ophthalmic Genetic Disorders.
52	V Vauhini	Characterization and Differentiation of Induced Pluripotent Stem Cells (iPSCs) Towards Retinal Lineages
53	A Varghese	Levels of Tumor Necrosis Factor-Alpha in Aqueous Humor of Patients with Primary Open Angle Glaucoma
54	D Gajjar	A Prospective Study on Etiology and Antibiotic Resistance Pattern of Infections of the Eye
55	GP Manderwad	Prevalence of Multidrug Resistant Pseudomonas Aeruginosa in Ocular Infections
56	A Kannan	Comparison of Commercial Kits - IgM Microlisa and Leptocheck with MAT in the Serodiagnosis of Leptospiral Uveitis
57	A Anthony	Oxidative Stress Markers in Patients with Primary Open Angle Glaucoma
58	BP Ramachandran	Seropositivity Rate among Cornea Donors at Eye Donation Centres Attached to Ramayamma International Eye Bank

Poster Session II July 31,2011 1.00-1.45pm		
S.No	***Names***	***Title***
1	P Chandra	Economic Burden of Diabetes in Urban Indians
2	S Patil	Causes of Blindness and Visual Impairment in Sindhudurg, Coastal Maharashtra
3	P Gogate	Long Term Outcome of Pediatric Cataract Surgery in Western India
4	M Srinivas	Uncorrected Refractive Errors, Presbyopia and Spectacle Coverage In South India
5	T Nagachandrika	Is Rod Function Normal in Subjects with Rod Monochromatism
6	PS Reddy	Revised NEI-VFQ or IND-VFQ in Visually Impaired: A Conundrum
7	P Veerendranath	Changes in Astigmatism Components with Accommodation
8	N Srividya	Assessment of Retinal Nerve Fiber Layer and Ganglion Cell Complex Thickness in Amblyopic Eyes
9	WD Prakash	Assessment of Inter- Observer Variability in the Clinical Measurements of Ptosis
10	M S Preeji	Corneal Erosions With Silicone Hydrogel Lens Wear: Clinical Features and Outcome
11	S Basu	Clinical Outcomes of Allogenic Cultivated Limbal Epithelial Transplantation for Bilateral Limbal Stem Cell Deficiency
12	BV Preethi	Computerized Graphical Representation of the Zone of Clear And Single Binocular Vision
13	DK Bagga	The Quality of Life Associated with Glaucoma
14	A Sati	Bilateral Granular Dystrophy: A Clinicopathogenetic Correlation after Alcohol Assisted Debridement with Phototherapeutic Keratectomy
15	S Tirumala	Effect of Room Lighting on the Visual Performance of School-Age Children
16	A Singh	Clear Corneal Tunnel Infection after Uneventful Phaco Emulsification
17	P Harikumar	Analysis of Visual Parameters in Subjects with and without Diabetic Retinopathy
18	S Pahuja	Clinicopathological Analysis of Repeat Descemet Stripping Endothelial Keratoplasty or Penetrating Keratoplasty for Failed Descemet Stripping Endothelial Keratoplasty
19	M Fatima	RGP Contact Lens Fitting in Keratoconus using Fitscan Technology
20	P Kumar	Role of Corneal Topography in RGP Contact Lens Fitting in Keratoconus
21	A Narasaiah	Inter and Intra Subject Variability of Luminanace - Slope Calibration in Eccentric Photorefraction

22	R Rao	Reasons for Loss to Follow-Up & Visual Outcomes after Cataract Surgery at Secondary Eye Centre in Adilabad District of Andhra Pradesh
23	MV Devi	Axial Length Measurement using B-Scan Vs A-Scan Ultrasonography
24	G Sushma	Axial Length Measurement using B-Scan Vs A-Scan Ultrasonography
25	VP Dave	Retrospective Analysis of Choroidal Neovascular Membranes Secondary to Central Serous Chorio Retinopathy
26	TV Dave	Outcomes of Cataract Surgery Following Treatment for Retinoblastoma (RB)
27	MN Begum	Profile of Patients Presenting For 1-Week Postoperative Visit Following Uncomplicated Phacoemulsification for Senile Cataract
28	M Joseph	Part Time Occlusion therapy for Anisometropic Amblyopia Patients in 4 to 15 Year Old Children
29	M Joseph	A Case Series of Posterior Microphthalmos with Pappilomacular Folds
30	A Fatima	Measurement of Retinal Nerve Fibre Layer (RNFL) Thickness by Spectral Domain Optical Coherence Tomography. (OCT)
31	S Chaurasia	Outcomes of Endothelial Keratoplasty (EK) without Stripping Descemet's Membrane (DM)
32	M Ramappa	Bilateral Congenital Hyper Plastic Pupillary Membrane: A Clinical and Histopathological Correlation after Successful Surgical Excision
33	S Dumpati	Boston Scleral Contact Lens in Coexisting Steven Johnson Syndrome and Keratoconus
34	V Raghuram	Impact of Lens Induced Anisometropia on Accommodative Responses to Step and Ramp Stimuli In Adults
35	R Kekunaya	Comparison of Accuracy of Intraocular Lens (IOL) Power Calculation Formulae in Children < 2 Years
36	V Sachdeva	Hyperhomocystenemia in isolated sixth cranial nerve palsy: culprit or bystander
37	A Gupta	Topical Brimonidine and Phenylephrine in Achieving Homeostasis during Strabismus Surgery
38	R Narayanan	Natural History of Macular Telangiectasia
39	D Kommanapalli	Ganglion Cell Complex (GCC) in Normal & Macular Dystrophies - A Spectral Domain Optical Coherence Tomography (SD OCT) Study
40	P Chandra	Scleral Thickness: A Normative Data Using Ultarasound
41	A Karsolia	Wavefront Aberrations in Normal Indian Aging Population
42	S Chary	Collagen Cross Linkage – One Year Review
43	S Pahuja	Deep Anterior Lamellar Keratoplasty in Children
44	J Ashar	Documentation of Keratoconus Progression in Patients with Vernal Keratoconjunctivits using Orbscan

45	A Mathur	Outcomes of Penetrating Keratoplasty in Mooren's Ulcer
46	V Amarnath	Intrasession and Intersession Repeatability of the Orbscan System on Corneal Topography Assessment in the Normal Human Eye
47	K Sargod	Spatial Profile of Macular Pigment Density and Correlation with Central Foveal Thickness in Normal Asian Indian Eyes
48	P Kalaiselvan	Management of Post-Lasik Keratectasia with Contact Lenses
49	A Mallipatna	Assessing the ability of High School Children as Key Informants in the Detection of Childhood Blindness
50	S Mandal	Comparison of Macular Hole Measurement between Spectral Domain Cirrus SD OCT and Digital Photography
51	S Mandal	Corneal Endothelial Cell density and Morphology of Indian Population in Different Age Groups.
52	M Kumar	Macular Folds after Topiramate Induced Angle Closure
53	M Kumar	Comparison of Central Corneal Thickness Measurement in Keratoconus with Specular Microscopy, Spectral Domain OCT and Orbscan-II Vs Ultrasound Pachymetry
54	B Sahoo	Retinal Nerve Fiber Layer Thickness in Normal Indian Children Measured with Spectral Domain Optical Coherence Tomography
55	S Pawar	Preferential Hyperacuity Perimeter in AMD
56	C Malhotra	"EPI" on versus "EPI" off Corneal Collagen Crosslinking- an Intraoperative Study
57	MK Sinha	Delayed- onset Endogenous Salmonella Typhi Endophthalmitis following Typhoid: A Case Report and Review of literature
58	V Nangia	Optic Disc Size and its Correlates in High Myopia in Central India

Dr Bireswer Chakrabarti Oration Lecture

Protein Aggregation in Cataract

Usha P Andley
Department of Ophthalmology and Visual Sciences, and of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, Missouri, USA.

The basis of lens transparency is the normal interactions between constituents of the cellular cytoplasm. To understand how these interactions are perturbed by mutation or stress, model organisms have been utilized successfully to define the protein aggregation states that lead to lens opacities. Multiple ocular effects have been identified in mouse models for hereditary cataracts. These models are relevant for understanding the in vivo functions of lens proteins. Stress-induced changes in proteins of the lens epithelium and fibers can be expected to be important for development of opacities in age-related cataracts. Global identification of proteins that change with stress suggests several candidates important in development of human cataract.

Prof D Balasubramanian Oration Lecture

Growth of the Human Eye Lens

Robert C Augusteyn
Vision CRC, Ivanhoe Vic 3079 Australia.

Purpose: A thorough understanding of lens growth and properties is essential for studies on age related impairment of vision such as cataract and presbyopia. This study aims to identify factors contributing to the growth and properties of the lens.
Methods: Data were collected on the changes in lens wet and dry weights and/or dimensions, as a function of age, for over 140 species. Logistic and allometric analyses were used to examine what factors contributed to these changes. Crystallin, refractive index and stiffness distributions within the lens were obtained for some species.
Results: Lens growth is monophasic and continues throughout life. Weight depends only on age and is independent of gender, environment and nutritional state. In all species, except primates, growth is asymptotic and the final lens size is dependent on globe volume. Compaction generates continuously increasing refractive index gradients. By contrast, human lens growth is biphasic, asymptotic prenatally and linear postnatally, Lens diameter increases with the axial length of the eye whereas postnatal thickness decreases in childhood and then increases for the rest of life. Compaction generates a plateau of constant refractive index in the nucleus and a cortical gradient.
Conclusions: Lens growth in humans is very different from that in other species and produces an organ comprised of two distinct tissues, the nucleus and cortex, with different functions and properties. Care should be taken in using animal models for studying human lens pathologies.

Proteomics Approaches to Understand Ophthalmic Diseases

K Dharmalingam
School of Biotechnology, Madurai Kamaraj University, Madurai-625021, India.

Three different ophthalmic disease states are being examined using proteomic approaches to understand the disease mechanism and also to look for appropriate biomarkers for the differentiation of stages of these diseases. Diabetic Retinopathy (DR), Mycotic keratitis and Primary Open-Angle Glaucoma (POAG) are the major diseases we are focusing at present. Our approach of completely defining the proteome in the various biological compartments (e.g. cornea, tear, aqueous humor, vitreous humor, serum, plasma etc.,), affected in these diseases will provide a comprehensive understanding of the complexity of disease condition and the stage specific alterations in the patients. The top down approach involves whole-protein level quantitative proteome analysis using DIGE and mass spectrometry. Differentially regulated proteins identified using proteomic approaches are validated using immunochemical methods. This analysis has given valuable information about the disease process and also led to the identification of stage specific changes.

Paraoxonase (PON) in Diabetic Retinopathy

Angayarkanni Narayanasamy
Biochemistry and Cell Biology Department, Vision Research Foundation, Sankara Nethralaya Chennai 600 006, India.

Paraoxonase (PON, EC: 3.1.1.2) is a calcium dependent 45kD glycoprotein synthesized in liver microsomes and localized in the high density lipoprotein. It has three types of enzyme activities depending upon the substrates, namely the arylesters, organophosphates and thiolactones. It exists in three isomeric forms namely PON 1, PON 2 and PON 3. It is considered as an antioxidant enzyme as it prevents the formation of OxiLDL, owing to its arylesterase activity. Therefore extensive studies on its protective role in atherosclerosis are reported apart from various other diseases. However, very few studies have looked into its implication in ocular diseases. We made a first report of lowered serum PON1-ARE (arylesterase) activity as a risk factor for CRVO, which was found to significantly correlate with oxidative stress. A similar finding was also observed in ARMD cases. Recently we had reported on a significant decrease in the PON-AREase activity in the vitreous of PDR cases along with an increase in the PON-HCTLase (thiolactonase) activity. This study revealed for the first time that the homocysteine thiolactone HCTL level is increased in the vitreous of PDR cases. The increase in PON-HCTLase is probably a protective effect to eliminate homocysteine thiolactone that contributes to endothelial cell dysfunction. *In vitro* studies in primary bovine endothelial cells showed a dose dependent increase in the PON-HCTLase activity with increasing concentration of both Homocysteine (Hcys) and HCTL associated with decreased PON-AREase activity. *In silico* analysis based on the PON 2 structure proposed by us, revealed that Hcys plays a modulatory role in regulating both the activities.

Host Response in *Aspergillus flavus* Corneal Ulcers and Secretome Analysis of Clinical Isolates of *A.flavus*

Lalitha Prajna[1], Lini Nirmala[1], N V Prajna[1], K Dharmalingam[2]
[1]Dr G Venkataswamy Eye Research Institute, Aravind Medical Research Foundation, Madurai; [2]Madurai Kamaraj University, Madurai, India.

Fungal corneal ulcer is an enormous public health problem in India affecting young-adults in their most economically productive period. The progression of this disease is a combination of both host immune mediators and the virulence factors of invading pathogens. In order to understand the disease pathogenesis, the knowledge about both host as well as pathogen is essential. For this purpose, we studied the expression of the innate immune response in corneal tissue from patients infected with *Aspergillus flavus* and found up regulation of specific PRRs as well as inflammatory cytokines.

The invasiveness and morphogenesis of the fungal agents maybe implicated in the pathogenesis. Fungi differ in virulence, even among strains of the same species. In order to understand disease progression, the role of the pathogen was examined. Insects are being widely used as surrogate host to study the virulence of medically important fungi. Our studies in *Galleria mellonella* infection model suggest that, it could potentially be applied to study the virulence of *A. flavu*s, and clinical strains showed more virulence than the environmental strains. SEM analysis of *A. flavus* indicated that there is no morphological or structural difference between the spores of clinical isolates and environmental strains. Examination of the secreted proteome of the pathogen under infection condition is a complementary approach, to understand disease. Secreted proteins were collected from *A.flavus* grown on solid state fermentation and analyzed using 2D SDS-PAGE and nano LC MS/MS. Results indicated that even though there are no morphological or structural changes observed between the clinical and the environmental isolates, the clinical isolates are more virulent and their secretion profile was distinct. Our studies imply that both host as well as pathogen specific factors are playing an inevitable role in the early pathogenesis of fungal keratitis.

Population-based Surveys: Which Method Should I Use?

Jill Keeffe
Centre for Eye Research Australia, Melbourne, Australia.

Population-based surveys are required to plan effective eye care services. They provide information on the prevalence, causes and risk factors for eye diseases and vision impairment and barriers to use of eye care services. In addition to the very large surveys such as APEDS and the Melbourne VIP, new methods have been developed for the “rapid assessment” of prevalence, causes and barriers. The rapid assessment methods used in the field of eye health include RAAB, RARE and RAVI. This presentation will discuss the advantages and disadvantages of each type of survey in terms of the teams needed to conduct the studies, eye examinations, data and specimens able to be obtained, skills needed to analyse results, the area of a country able to be covered, time and cost. Methods for surveys of vision loss in children such as in schools for the blind or using comprehensive sources of data collection will be outlined and discussed.

Central India Eye and Medical Study (CIEMS) - A Clinical Translational Study

Vinay Nangia, Jost Jonas, A Matin, K Bhojwani, M Kulkarni, A Sinha, M Yadav, R Gupta, A Khare, S Ughade, P Joshi, S Agarwal, K Bhate, P Nangia, R Jonas, S Lambat, S Giradkar
Suraj Eye Institute, Nagpur, India.

Purpose: The Central India Eye and Medical Study (CIEMS) is a population based study. Normative Data for ocular paramters are important as they serve to define what abnormal. In addition clinical data are important since they serve to provide data that influence public health. It was the purpose to study a population in rural Central India for which normative ocular, and public health data were not available.
Methods: Study included 8 randomly chosen villages at a distance of 40 kms. The inclusion criteria were age 30+ years. There were 4711 study participants (response rate 80.1%). The mean age was 49.5+-13.4 years. All participants underwent a questionnaire, including regarding the socioeconomic background, alcohol and tobacco consumption, exercise and diet intake. An ophthalmic evaluation, including refraction, slit lamp biomicroscopy, applanation tonometry, biometry, indirect ophthalmoscopy, fundus photography of the optic disc and macula was done. Medical examination included blood pressure, ECG and x ray. Biochemistry tests including haemoglobin, ESR, urine examination, blood sugar, glycosylated haemoglobin, kidney function tests and lipid profile were done.
Results: The mean IOP was 13.6±3.4 mmhg. The mean systolic blood pressure was. A strong correlation was seen in univariate and multivariate analysis with systolic blood pressure ($P<0.001$) and diastolic blood pressure ($P=0.003$). The Central Corneal Thickness was found to be 514±33 um. The CCT showed a significant correlation with IOP ($P<0.001$). The mean axial length was 22.6±0.91mm and the anterior chamber depth was 3.22±0.34mm.
1049 (22.3%) subjects were found to be visually impaired (WHO Standards) and 35 (0.7%) were blind. Using refraction visual impairment could be reduced in 729 (67%) of subjects. PRVA - Visual impairment and blindness following cataract surgery was seen in 60% of patients less than 70 yrs and in 69% in >70 years. It was seen in 88% of patients with aphakia and in 66% patients with pseudophakia.
Conclusions: The CIEMS enabled the determination of the normative values for IOP, CCT, Axial length, Anterior Chamber depth and lens thickness, in addition to determining the mean values of blood pressure In the community. From the public health perspective. It highlighted the importance of uncorrected refractive error as a significant cause of visual impairment and its impact on improving visual acuity in the community. The CIEMS may have significant translational clinical impact on public health and clinical management.

What do Neurons in Inferior Temporal Cortex Tell Us About Object Recognition?

S P Arun
Indian Institute of Science, Bangalore, India.

It was thought for a long time that there was nothing more to seeing than the sensing of light in the eye. But we now know that visual information from the eyes is processed along a series of visual areas that occupy nearly 40% of our brains. This transformation is important to understand because specific damage to these areas results in very specific disorders of high-level vision that are difficult to treat. This transformation is even more remarkable because despite tremendous advances in computing, we still cannot make computers match human vision. So how does the brain accomplish vision? Can we understand this at the level of single neurons? Can we use this knowledge to treat disorders of high-level vision? In my talk I will describe some of my work on understanding how objects are represented by neurons in the monkey visual cortex, and how we can relate this to human vision.

Nature of L- and M-Cone Input to Cone-Opponent and Non-Opponent Mechanisms as a Function of Retinal Eccentricity

N K Challa[1], D McKeefry[1], N R A Parry[2], J. Kremers[3], I J Murray[4], A Thanasis[4]
[1]Department of Optometry and Vision science, Bradford University, UK; [2]Visual Sciences Centre, Manchester Royal Eye Hospital, Oxford Rd, Manchester, UK; [3]Department of Ophthalmology, University Hospital Erlangen, Erlangen, Germany; [4]Faculty of Life Sciences, University of Manchester, Manchester, UK.

Chromatic processing at the retinal level was examined using the electroretinograms (ERGs) and psychophysical procedure (2AFC), for which cone isolating stimuli were used to assess the nature of L and M cone inputs to cone-opponent mechanisms. In order to investigate this we recorded L and M cone isolating ERGs and also cone detection thresholds using psychophysical procedure. ERGs and detection thresholds were obtained using a sequence of stimuli with different spatial configurations comprising; i) circular stimuli of different sizes or ii) annular stimuli with a 70° outer diameter but with different sized central ablations. L- and M-cone isolating ERGs were obtained from five colour normal subjects using a DTL fibre electrode. Fourier analysis of the ERGs was performed and measured the amplitude of the first harmonic of the response. Cone detection thresholds on the other hand were measured in three subjects using Two Alternate Temporal Force Choice (2ATFC) procedure.

From the ERG experiments, It has been shown that the low (12Hz) and high (30Hz) temporal frequency flickering stimuli can isolate the chromatic and luminance processing mechanisms in the retina. For low temporal frequency ERGs, the L:M ratio was close to unity and L/M phase difference was close to 180°. For high temporal frequency ERGs, the L:M ratio was more than unity and L/M phase difference was close to 90°. Psychophysical results have also shown good correlation to the ERG findings. In addition to this, the variation in L:M ratio across the retinal eccentricity was also examined. These results suggest, for the chromatic processing, L:M ratio is close to unity independent of retinal eccentricity and individuals. For the luminance processing, L:M ratio is more than unity and depends upon the region of the retina being stimulated. These findings indicate the maintenance of cone selective input for the chromatic processing across the human retina. Important to note from the above findings is that the filtering process exists at the cortex level for both chromatic and achromatic signals that originate at the retina.

Genotype to Phenotype Annotation of SNP's – An *In Silico* Approach

V Umashankar
Centre for Bioinformatics, Sankara Nethralaya Chennai – 600006, India.

Currently, studies on genetic polymorphisms in human pertaining to Ocular diseases are expanding rapidly. However, Studies explaining pathogenic phenotypic effects arising due to the reported polymorphisms are very limited due to cost and time expensive experimental methods. To overcome this constraint, Bioinformatics approaches can be effectively implemented to discriminate the pathogenic variants from the non-pathogenic ones. Presently, many optimized sequence and structure based *Insilico* methods for SNP analysis available which aids annotation of pathogenicity. Many of the Novel SNPs relevant to ocular disease conditions were reported and the respective phenotypic analysis was done in a similar fashion at CENTRE FOR BIOINFORMATICS, SANKARA NETHRALAYA. Hence, my talk will emphasize on the methods implemented for SNP analysis with relevant case studies.

Genetic Association and Gene-Gene Interaction of *HAS2*, *HABP1* and *HYAL3* in POAG Implicate Role of Hyaluronan in Glaucomatous Neurodegeneration

Kaustuv Basu[1], Indian Genome Variation Consortium[2], Abhijit Sen[3], Kunal Ray[4], Ilora Ghosh[1], Kasturi Datta[1], Arijit Mukhopadhyay[5]

[1]Biochemistry Laboratory, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi. [2]Nodal Laboratory, CSIR-Institute of Genomics and Integrative Biology, Delhi,[3]Dristi Pradip, Jodhpur Park, Kolkata, [4]Molecular and Human Genetics Division, CSIR-Indian Institute of Chemical Biology, Kolkata, [5]Genomics & Molecular Medicine, CSIR-Institute of Genomics and Integrative Biology, Delhi, India.

Purpose: In Primary Open Angle Glaucoma (POAG), extensive remodelling of extracellular matrix (ECM) occurs in response to elevated intraocular pressure (IOP). Hyaluronan (HA) as an important component of ECM, plays a significant role in maintaining aqueous humor outflow in trabecular meshwork in anterior part of the eye. However, no study has yet been reported on the possible role of the polymorphisms in HA metabolising genes in glaucoma.

Methods: We examined potential association of the single nucleotide polymorphisms (SNPs) of the HA synthesizing gene – hyaluronan synthase 2 (*HAS2*), hyaluronan binding protein 1 (*HABP1*) and hyaluronidase 3 (*HYAL3*) – involved in HA catabolism in the POAG patients and the status of these SNPs in 24 populations across India.

Results: We observed an allelic association (rs6651224; p = 0.03; OR: 0.49) at the second intron (C>G) of *HAS2* with POAG. rs1057308 revealed a genotypic association (p = 0.03) at the 5' UTR of *HAS2* with only HTG. The associated GG genotype indicated increased risk for the disease. TCT haplotype (rs1805429, rs2472614, rs8072363) in *HABP1* was found to be significantly high ($p<0.05$) for both HTG and NHTG patients compared to controls. The frequency of TCT among 24 normal Indian populations varied between 0.02 to 0.32. TTAG & TTGA (rs2285044, rs3774753, rs13100173, rs1076872) haplotypes in *HYAL3* were found to be significantly higher frequency ($p<0.05$) in both HTG and NHTG. Gene-gene interaction revealed *HABP1* predominantly interacts with *HAS2* in HTG while in case of NHTG it associates both with *HYAL3* and *HAS2*.

Conclusion: The study demonstrates for the first time the potential for involvement of three important genes in hyaluronan metabolism with POAG pathogenesis.

Support: Grant (numbers SIP-0006, SIP-0007, MLP-0016 and 60(0078)/07/EMR-II) from Council for Scientific and Industrial Research (CSIR), Govt. of India.

ABSTRACT OP-1

Micronutrient Status in Diabetic Retinopathy

G B Reddy[1], A Satyanarayana[1], N Balakrishna[1], M Sivaprasad[1], K Viswanath[2], Radha Ayyagari[3]
[1]National Institute of Nutrition, Hyderabad; [2]Pushpagiri Vitreo Retina Institute, Hyderabad, India; [3]Ophthalmology, University of California, San Diego, USA.

Purpose: Multiple factors are likely to be involved in predisposing diabetic subjects to diabetic retinopathy (DR), as evidenced by the fact that many but not all diabetic patients develop DR. Though, studies have focused on the genetic susceptibility of DR, information on nutritional status of DR are meager. In this study we have evaluated the micronutrients status in DR with an ultimate goal to understand the role of genotype-nutrient interaction in DR.
Methods: A hospital based cross-sectional case-control study was conducted with 300 type-2 diabetic subjects with retinopathy (DR) (n=100) and without retinopathy (DNR) (n=200) along with 100 normal control (CN) subjects based on strict inclusion and exclusion criteria. Diabetic subjects on nutritional supplements and history of nephropathy and other complications were excluded. Based on ophthalmic examination including FFA, patients were classified as DNR or DR. In addition to regular clinical profile, the blood levels of all the vitamins and trace elements were analyzed by HPLC, atomic absorption, spectrophotometric methods.
Results: Among trace elements, an inadequacy in blood levels of manganese, cobalt and zinc was found in DR patients compared to CN and DNR. Excepting vitamins folic acid B6, B12 and D, blood levels of all the vitamins were not different between the groups. While, levels of vitamin B6, folic acid and vitamin D were significantly lower in diabetic groups as compared to controls, there was no difference between DNR and DR groups. This study revealed a significantly lower level of plasma vitamin-B12 in DR patients compared to CN and DNR groups. While plasma homocysteine levels were found to be higher in diabetes patients compared to control subjects, homocysteine was further higher in DR group.
Conclusions: These results suggest an association between vitamin-B12 deficiency and hyperhomocysteinaemia in DR and further indicate that vitamin-B12 deficiency could be an independent risk factor for DR.

ABSTRACT OP -2

Reversal of Stathmin Mediated Microtubule Destabilisation Sensitizes Retinoblastoma Cells to Low Dose of Anti Microtubule Agents: A Novel Synergistic Therapeutic Intervention

S Krishnakumar[1], M Mitra[1], M Kandalam[1], C S Sundaram[2]
[1]L and T Department of Ocular Pathology, Vision Research Foundation, Sankara Nethralaya and [2]Advanced Proteomics laboratory, Centre for Cellular and Molecular Biology, Hyderabad, India.

Purpose: The purpose of the present study was to explore the possibility of stathmin as an effective therapeutic target and to evaluate the synergistic combination of stathmin RNAi and anti-microtubule agents, paclitaxel and vincristine to retinoblastoma Y79 cells.
Methods: RNAi mediated specific inhibition of stathmin expression in Y79 cells was demonstrated by real time quantitative reverse transcriptase PCR (RT-Q-PCR) and studied its effect on cell proliferation by MTT assay, cell invasion using matrigel, microtubule polymerization by immunohistochemistry, apoptosis, cell cycle analysis by flow cytometry analysis and the changes in FOXM1 protein expression by western blotting. The effect of combination treatment of stathmin siRNA and paclitaxel/vincristine was studied by assessing the cell viability and apoptosis.
Results: siRNA mediated transient stathmin down regulation resulted in marked inhibition of retinoblastoma cell proliferation and cell invasion in vitro. Stathmin inhibition promoted Y79 cells to G2/M phase and ultimately there was an increased apoptotic events as evidenced by higher caspase-3 activation and cleaved PARP expression. Cells transfected with stathmin siRNA showed long and bundled microtubule polymers and sensitized the Y79 cells significantly to paclitaxel and vincristine.
Conclusions: Stathmin may be a pivotal determinant for retinoblastoma tumorigenesis and chemosensitivity. Strategies to inhibit stathmin will help to enhance the cytotoxic effect of paclitaxel while reducing toxicity (or side effects) to normal cells caused by high doses.

Support: DBT Proteomics Task force sanction (BT/PR7152/MED/14/961/2006) to SK and CSS

ABSTRACT OP-3

An Approach of Novel Tissue Engineering for Bioengineering Corneal Epithelial Cells from Non-Ocular Epithelial Cells

Vidya Gopakumar, K Sasirekha, S Saranya, S Charanya, S Krishnakumar
Department of Ocular Pathology, Sankara Nethralaya, 18, College Road, Chennai 600 006, Tamil Nadu, India.

Purpose: Transplantation of autologous corneal cells to treat ocular surface disease, namely Limbal stem cells deficiency (LSCD) is an alternative to allograft transplantation and does not require immunosuppression, but it is practically not possible in many cases where bilateral disease produces total corneal stem cell deficiency in both eyes. Therefore, sources of autologous tissue that can functionally replace the corneal epithelium have been considered as an alternative to allogenous limbal transplants
Methods: Experiments were performed using skin tissue (n=6) obtained from patients who underwent oculoplastic surgery at Sankara Nethralaya. Confluent cultures of epidermal keratinocytes and transdifferentiated corneal epithelium were characterized by their specific markers using reverse transcriptase polymerase chain reaction (RT-PCR). The morphological characteristics of these cultivated cells were analyzed by haematoxylin & eosin staining and phase contrast microscopy.
Results: Keratinocyte cells were isolated and cultured from skin epidermis and expressed keratinocyte specific markers. Epidermal keratinocytes were subjected to transdifferentiation in corneal specific microenvironment and the cultured cells had the morphological features of corneal epithelia and expressed corneal specific markers.
Conclusions: Our study summarizes that human skin is a viable alternative for the generation of corneal tissue. Skin has emerged as a promising source for the growth of corneal epithelium and its capacity to transdifferentiate opens up new avenues in transplantation research. The future direction would be about facing the issues of fibroblast cells occurring during the passaging of keratinocytes. Thus we propose in our study that the corneal epithelium reconstructed from skin keratinocyte stem cells, may be an alternative for constructing autogenously bioengineered corneas in the future.

ABSTRACT OP-4

Evolution of Higher Stability in Lens γ-Crystallins at the Cost of Calcium Binding

Ishan D Rastogi, Amita Mishra, Shashi Kumar Suman, Lahari Yermala, Yogendra Sharma
Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Habsiguda, Hyderabad, India.

Purpose: The eye lens is made of different crystallins forming a transparent glassy structure. Over the course of evolution, it is speculated that the more primitive microbial βγ-crystallins recruited as present vertebrate lens βγ-crystallins. The major difference between the microbial homologues and lens βγ -crystallins is in their ability to bind Ca^{2+}. Lens βγ-crystallins are the thermodynamically stable proteins, but do not possess the canonical Ca^{2+}-binding motif. To investigate the reasons for the loss of Ca^{2+}-binding ability over the course of evolution from microbial homologues to vertebrate βγ-crystallins following study has been done. Many mutations in lens b- and g-crystallins have been reported in patients with hereditary cataract. Recently, a mutation (R77S) has been identified in lens g-crystallin which is implicated in cataract, which caught our attention since this is the conserved Arg which is located at the 5th position of the N/D-N/D-X-X-S/T-S motif. This residue in most microbial homologues is Ser and ligates Ca^{2+} via side chain coordination.
Methods: Non-functional Ca^{2+}-binding site of bovine lens γ-crystallin was converted to functional (canonical) by mutating appropriate amino acid residue(s) in the N/D-N/D-X_1-X_2-S/T-S motif in the N-terminal domain. Residues selected were N34S and R77S, (both in the 1st and 2nd Greek key motifs of gB-crystallin). The mutants were over-expressed, purified and the changes in domain stability by a particular mutation were evaluated. In addition, we also investigated the role of Ca^{2+} on influencing the stability using equilibrium unfolding.
Results: Mutations of Arg to Ser (R77S) and Asn to Ser (N34S) in the Ca^{2+}-binding motif appears to us that it partially converts a vertebrate g-crystallin towards a microbial type, thus we investigated the implications of such mutations on the protein stability, vis-a-vis the effect of Ca^{2+}-binding. While mutations in the non-functional motif in g-crystallin favouring Ca^{2+}-binding, we observed significant decrease in domain stability. Incidentally, Ca^{2+}-binding further destabilizes a domain. This is contrary to that seen in any Ca^{2+}-binding protein where Ca^{2+} never decreases the stability of a protein, thus appears to be the only known case where Ca^{2+}-binding destabilizes a protein domain. However no such destabilising effect was observed for the similar mutations in full length βγ -crystallin signifying the role of C-terminal domain in conferring extra stability to the protein.
Conclusions: We suggest that such alterations in domain properties directly lead to cataract. Additionally, our data suggest that while ancestors used to bind Ca^{2+}, most of the binding sites in lens βγ -crystallins were disabled to suit to their role in a lens. Our data also predict that any mutation in the Ca^{2+}-binding motif would be deleterious to the health of an eye lens obviously due to Ca^{2+}-binding. R77S mutation in βγ -crystallin (N

terminal domain mutant) significantly destabilized leading to loss of structure. This is possibly one of the reasons as to why R77S mutation is responsible for congenital cataract. However mitigation of such destabilizing effect in case of full length R77S mutant of bovine γ-crystallin implies the role of C-terminal domain in providing extra stability. Moreover from evolutionary point of view, the vertebrate γ-crystallin had their calcium binding ability compromised for the extra stability to better suit their role in eye lens as is shown from the destabilization in presence of calcium.

ABSTRACT OP-5

Cell Junction Proteins in Cataractous Lens Epithelial Cells

Anshul Arora, Siddharth Mehta, SR Kaid Johar, Devarshi Gajjar, Abhay Vasavada
Iladevi Cataract and IOL Research Centre, Gurukul Road, Ahmedabad-380052, Gujarat, India.

Purpose: To detect the expression of cell junction proteins Connexin 43 (Cx43), Zonula Occludens (ZO-1), α-catenin and β-catenin in Lens Epithelial Cells (LECs) of patients with different types of cataract.
Methods: Specimens of anterior lens capsule with attached monolayer of LECs were obtained from patients (n=31) undergoing cataract surgery at Iladevi Cataract and IOL Research Centre, Ahmedabad. Specimens were divided in three groups: nuclear cataract, cortical cataract and posterior subcapsular cataract (PSC). 16 specimens were used for Immunofluorescence localization of Cx43, ZO-1, α-catenin and β-catenin. 15 specimens were used to quantify mRNA levels of these genes using Real Time PCR. Clear lenses (n=6) obtained from donor eyes were used as control. Statistical analysis was done using students t-test. Values of $P<0.05$ were considered significant.
Results: Immunofluorescence results showed that α-catenin and β-catenin were localized at the cell boundaries in all the types of cataracts. Punctate localization of Cx43 at cell boundaries was observed in control, nuclear cataract and PSC. Cytoplasmic pools of Cx43 with no localization on cell boundaries were observed in cortical cataract. Real time PCR results showed significant upregulation of Cx43, α-catenin and β-catenin in cortical cataracts. Also, α-catenin and ZO-1 were significantly down regulated in nuclear cataract.
Conclusions: The altered expression of different cell junction proteins may play a crucial role in formation of different types of cataract.

ABSTRACT OP-6

Neural Potential and Gene Expression Profile of Stem/Progenitor Cells Derived from Human Ciliary Pigment Epithelium

Srilatha Jasty, Gunisha Pasricha, Nivedita Chatterjee, Subramanian Krishnakumar
Department of Ocular Pathology, Sankara Nethralaya, 18, College Road, Chennai 600 006, Tamil Nadu, India.

Purpose: The objective of our study was to isolate and characterize the cellular properties of neurospheres and differentiated progeny derived from ciliary pigment epithelial (CE) cells of human cadaveric eyes. In this study we also investigated the gene expression profiles of the stem/progenitor cells and the differentiated progeny derived from CE cells, as the changes underlying differentiation of the stem/progenitor derived from the CE cells from human cadaveric eye are essentially unknown.
Methods: CE cells from human cadaver eyes were cultured in the presence of mitogens to generate neurospheres (NS) and the growth characteristics were evaluated. The Neurospheres (NS) were plated under differentiation conditions to assay the differentiation potential of their progeny. The Neurospheres and differentiation potential was analyzed by RT-PCR, immunocytochemistry and calcium imaging. We also applied whole genome cDNA microarray to measure the dynamic gene expression changes in the process of differentiation.
Results: The CE cells could generate NS in the presence of mitogens as demonstrated by our sphere suspension assay, RT-PCR and immunocytochemistry studies, the NS were capable of producing different retinal cell types, which was confirmed by RT-PCR and immunocytochemistry. The gene expression profiling reveals the dynamic changes occurring during the course of CE neurospheres differentiating into neural progeny, obtained by cluster analysis of the differentially expressed genes. Our results provide new insights into the cellular and genetic pattern for the differentiation of CE cells derived Stem/progenitor cells (SCs) into neural progeny. This gives strong hints to the unknown changes that occur during differentiation of NS into neural progeny.
Conclusions: We have demonstrated expansion and maintenance of SCs from CE of cadaveric eyes. These cells maintain their self-renewal properties and the ability to differentiate along neural cell lineages and hence could be a practical source of donor cells for ex-vivo Stem cell therapy for degenerative disorders.

Support: Department of Biotechnology (BT/PR7956/MED/14/1196/2006)

ABSTRACT OP-7

Functional Assessment of the Involvement of Organic Cation Transporter (OCT) in the Tear Kinetics

T Velpandian, J Nirmal, T Vasantha, R V Azad, A Bhatnagar, S Ghose
Department of Ocular Pharmacology & Pharmacy, Dr. RPC, AIIMS, New Delhi-110029, India.

Purpose: The present study has been attempted to investigate the functional importance of OCT in precorneal area *invivo*. The effect of OCT blockade on the tear residence of OCT substrates has been evaluated using pharmacological tools.
Methods: Tetraethylammonium (TEA), metformin were used as substrates and quinidine, atropine as OCT blockers. Albino rabbits of either sex weighing 1.5-2.0 kg were used for the study. Tear residence of OCT substrates after topical and intravenous administration were assessed in the presence and absence of blocker. Tear samples were collected using calibrated Shrimer's strips were subjected for simultaneously analyzes using LC-MS/MS. *Invivo* rabbit Gamma Scintigraphy was also used to image the fate of ^{99}Tc labeled tetraethylammonium with or without OCT transporter blockade.
Results: OCT blockers atropine and quinidine (topical pretreatment) 30 min prior to substrate treatment significantly decreased the precorneal clearance of OCT substrates thereby increased its tear residence levels after topical administration. Interestingly, intravenous administration of TEA reached the tear after single intravenous administration and reached its Tmax at 60 min. Whereas, the topical blocker pretreatment significantly reduced the TEA reaching the tear after intravenous administration at all the studied time points (upto 2h).
Conclusions: OCT are functionally involved in corneal disposition of topically applied drugs. This study further reveals that intravenously administered xenobiotics which are OCT substrates, might be capable of reaching precorneal area through tear secretion. It shows that OCT in tear glands might be positioned from basolateral to apical side to enable the transport of biochemical mediators which are of relevance for drug targeting.

Support: AIIMS intramural grant and CSIR SRF to NJ.

ABSTRACT OP-8

Expression Analysis of Antiangiogenic Factors in Lens Epithelial Cells of Persistent Fetal Vasculature Patients

Alpesh R Patel, Devarshi Gajjar, Sajni Shah, Abhay Vasavada
Iladevi cataract and IOL Research Centre, Gurukul Road, Ahmedabad–380052, Gujarat.

Purpose: Persistent fetal vasculature (PFV) is a pathological condition occurred due to failure in the regression of hyaloids vasculature. The sequel of cellular and molecular processes involved in the normal involution of the hyaloid vessels remains unclear. Hence, present study was undertaken to evaluate the role of antiangiogenic factors in etiology of PFV.
Methods: The study population included children from 1 month to 9 years. A preoperative bimicroscopic examination was carried out under anesthesia in younger children and B-scan ultrasound were performed to detect any malformation of the posterior segment. Anterior capsules from pediatric cataract patients with (n=9) and without PFV (n=15) were collected from operation theatre. Samples were immediately subjected to RNA extraction and c DNA construction. The mRNA levels of tumstatin, canstatin, arresten, MMP-2 and MMP-9 were analyzed using quantitative real-time PCR.
Results: Results showed that out of 16 PFV cases 4 patients showed remnants of the anterior hyaloid vasculature, 11 patients showed posterior segments without pathology and 1 patient has band to optic disc. Expression analysis revealed that mRNA level of MMP-2, tumstatin and canstatin was 4, 2.5 and 2.3 times higher respectively in PFV cases than control subjects. Whereas, there was no significant (p=0.82) change observed in the MMP-9 mRNA levels. Arresten showed 1.6 times decreased (p=0.01) levels of mRNA in PFV cases.
Conclusions: So, far from our data we speculate that increased level of canstatin and tumstatin could be to cope up with increased angiogenesis in the pathological condition of PFV. Decreased levels of arresten could be because of the developmental shift in collagen IV subunits.

ABSTRACT OP-9

Levels of Macular Xanthophylls in Indian Donor Eyes

Srinivasan Senthilkumari, Ravichandran Ranjith Kumar
Department of Ocular Pharmacology, Aravind Medical Research Foundation (AMRF), Dr G Venkataswamy Eye Research Institute, Anna Nagar, Madurai-20, Tamilnadu, India.

Purpose: Our previous studies demonstrated the levels of macular carotenoids in both North and South Indian donor eyes along with the levels of carotenoids in commonly consumed fruits and vegetables (*J Nutr Sci Vitaminol, 2010; 56: 411-420*). The present study was undertaken to correlate the levels of serum carotenoids with the levels of maculae in South Indian donor eyes.
Methods: Both young (below 50 yrs; N=11) and old (above 50 yrs; N=15) human donor eyes with serum were obtained after the removal of cornea for transplantation with the approval of the Standing Institutional Human Ethics Committee. Macular region of each donor was dissected out using 8mm trephine. Both samples were subjected for the quantification of lutein (L) and zeaxanthin (Z) by HPLC method as reported earlier.
Results: The young and old maculae showed detectable amount of lutein and zeaxanthin. The mean (+/- SE) macular lutein and zeaxanthin concentration of old donor eyes was found to be 24.42 +/- 5.84 and 31.46 +/ - 12.1ng / mg. The young donor eyes showed 32.4 +/- 16.13 ng / mg of lutein and 14.29 +/- 3.83 ng / mg of zeaxanthin in their maculae. The mean serum levels of lutein in young and old donors ranged from 13-119nM and 4-156 nM and their corresponding zeaxanthin levels were found to be 0.07 – 96 nM and 21-255nM respectively.
Conclusions: The young maculae showed less concentration of zeaxanthin as compared to old donors which correlates well with their decreased serum levels. So, the macular health of an individual purely depends upon the dietary intake of colored fruits and vegetables.

ABSTRACT OP-10

Quantitative Analysis of Vitreous Humor Reveals Distinct Protein Profiles in Patients with Retinopathy of Prematurity

Sonika Rathi[1], Musada Ghaneshwer[1], Subhadra Jalali[2, 3], Ramesha Kekunaya[3], Padmaja K Rani[2], Subhabrata Chakrabarti[1], Inderjeet Kaur[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Smt. Kannuri Santhamma Centre for Vitreo Retinal Diseases, [3]Jasti V Ramanamma Children's Eye Care Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Retinopathy of Prematurity (ROP) is a vaso-proliferative eye disease in premature babies (GA ≤32 weeks) weighing≤1,500 g and is characterized by the interruption of normal retinal vascular development leading to retinal detachment. The present study aimed to evaluate the level of inflammatory and angiogenic factors in ROP.
Methods: Vitreous humor (50-100µl) were collected from stage IV and V of ROP babies (n=30) and cataract controls (n=30) during vitrectomy with prior written informed consent and stored at -80°C. The concentrations of 28 proteins and other factors involved in neuro-degeneration, extracellular matrix modeling, angiogenesis and inflammatory pathways in pre-diluted vitreous (1:5) samples were evaluated using multiplex bead immunoassays based on powerful Luminex xMAP technology. Differences between levels of these proteins were analyzed using appropriate statistical tests.
Results: Of the 28 analytes evaluated, 21 were detectable in the vitreous humor samples. Patients with ROP exhibited significant elevations in MMP9 (p=0.035), CFH (p=0.002), C3 (p=0.006), C4 (=0.0009), Prealbumin (p=0.012), SAP (p=0.026), APO A1 (p=0.002) and APOC3 (p=0.006) compared to the controls. No significant differences were observed in vitreous humor concentrations of 14 other proteins between patients and controls (p>0.05).
Conclusions: Significant alterations in 21 cytokines were associated with ROP, suggesting that abnormal immune environments and ECM components may contribute to the development of ROP in premature babies.

ABSTRACT OP-11

Design and Engineering of a Therapeutic Agent for Proliferative Vitreoretinopathy

Maryada Sharma[1], Anil Tiwari[1], Shweta Sharma[1], Amod Gupta[2], Manni Luthra-Guptasarma[1]
[1]Department of Immunopathology, [2]Department of Ophthalmology, Postgraduate Institute of Medical Education and Research, Chandigarh–160012, India.

Purpose: Proliferative vitreoretinopathy (PVR) is stimulated by serum, following break in the blood-retinal barrier. Fibronectin, an important component of serum, together with retinal pigment epithelial (RPE) cells plays a crucial role in PVR, in which RPE cells attach to the retinal surface and migrate into the vitreous, with subsequent formation of cellular membranes, proliferation of cells, deposition of extracellular matrix (ECM) on both sides of the retina, leading to contraction of retinal membranes and tractional retinal detachment. As yet, no pharmacological adjuvant has been found suitable for use in the treatment of PVR. The present study was designed to develop an antibody cognate to the N-terminal region of fibronectin, to inhibit matrix formation, and prevent RPE-ECM interactions.
Methods: Phage display-based antibody library screening technology was used to generate an scFv antibody against the 30 kDa N-terminal region of fibronectin (scFv Fn52). The scFv was further engineered to incorporate an integrin-binding “RGDS” site (scFv FnRGDS). The two scFv antibodies were assessed for RPE cell migration (transwell assay), viability (MTT assay), collagen gel contraction, and fibronectin and actin polymerization (immunostaining).
Results: Both antibodies showed reduced cell viability, migration, collagen gel contraction and actin and fibronectin polymerization; scFv FnRGDS was more potent than scFv Fn52.
Conclusions: scFv FnRGDS could be a promising therapeutic which needs to be tested further for efficacy in a model of PVR.

ABSTRACT OP-12

Tissue Specific Cytotoxicity Mediated Suicidal Gene Therapy

K Gopinath, Ravikanth Danda, S Krishnakumar
Department of Ocular Pathology, Vision Research Foundation, Sankara Nethralaya, 18, College Road, Chennai: 600 006, India.

Purpose: We report here the suitability of the epithelial glycoprotein-2 (EGP-2) promoter for suicide gene therapy in Epithelial cell adhesion molecule (Ep-CAM) expressing cancer cells.
Methods: In this study, we used an epithelial glycoprotein-2 (EGP-2) promoter to a suicide gene, Herpes simplex virus Thymidine kinase (HSV-*TK)*. This construct was delivered into Retinoblastoma cells Y79, breast carcinoma cells MDA-MB-453, MCF-7 and cervical cancer cell line HeLa, using Lipofecatmine transfection reagent. The cells were then treated with the prodrug Ganciclovir (GCV).The therapeutic effect was evaluated in vitro.
Results: Our study showed that the EGP-2 promoter driven, Lipofecatmine -delivered suicide gene was only expressed in Ep-CAM -positive Y79, MDA-MB-453, and MCF-7 cancer cells , not in Ep-CAM –negative HeLa cells, and resulted in significant cytotoxicity after administration of the prodrug GCV in vitro.
Conclusions: In this study, we demonstrate that the EGP-2 promoter mediates efficient and selective killing of tumor cells in vitro and maintains activity and selectivity in vitro. We show that the EGP-2 promoter is a promising tool in cancer gene therapy to tackle a broad range of tumor types.

Support: Indian Council of Medical Research (ICMR), Grant No. BMS-35/06/2010.

ABSTRACT OP-13

Complications of Intravitreal Bevacizumab in Retinopathy of Prematurity. The Indian Twin Cities ROP Screening (ITCROPS) Database Report Number 6

Subhadra Jalali,[1] Divya Balakrishnan, [1] Zarifa Zeynalova, [1,3] Padmaja K Rani, [1]Tapas R Padhi[2]

[1]Srimati Kannuri Santhamma Centre for Vitreo Retinal Diseases, Kallam Anji Reddy campus, LV Prasad Eye Institute, Hyderabad Eye Research Foundation, Hyderabad, [2]Department of Retina Vitreous, LV Prasad Eye Institute, Bhubaneswar, Orissa, India; [3]National Eye Centre, Baku, Azerbaijan.

Purpose: To report the ocular and systemic complications and clinical outcomes of intravitreal Bevacizumab (IVB) in vascularly active Retinopathy of prematurity (ROP).
Methods: From the prospectively collected Indian Twin Cities ROP Screening (ITCROPS) data base, efficacy and safety data was analyzed of all babies who had received IVB with or without subsequent surgery.
Results: 26 eyes of 14 babies received single dose 0.75-0.60 mg of IVB after informed consent, followed by surgery if needed. Indications included severe stage 3plus after failed laser in 7 eyes, stage 4A plus in 8 eyes , stage 4B/ 5 with plus in 9 eyes and as primary treatment for advanced APROP in two eyes. Drug was injected intravitreally in 25 eyes and intracamerally in one eye. New vessels regressed promptly in all eyes. Vision could be salvaged in 17 of 26 eyes at risk of blindness. Complications included macular hole leading to rhegmatogenous retinal detachment in one eye; bilateral vascular attenuation and perivascular exudation in one baby, severe retinal edema and exudative detachments in both eyes.of one baby with APROP, and progression of detachment bilaterally from stage 4A plus to an inoperable stage 5 in one baby who did not return for timely surgery. One baby who received intracameral injection developed hepatic dysfunction after 10 days. This manifested as raised liver enzymes to levels more than five times above normal lasting for 3 weeks. One eye of this baby also developed RPE/choroidal rupture. One baby with APROP developed
Conclusions: Uniocular or binocular IVB at half adult dose, may be considered an effective and possibly safe adjunct to laser/surgery of acute stage vascularly active ROP. We report one systemic and five ocular adverse events that may be related to the procedure or the drug.

ABSTRACT OP-14

Template Technique; A Novel Method For Eyelid Debulking In Patients With Orbitopalpebral Plexiform Neurofibroma

Tarjani V Dave, Anirban Bhaduri, Sima Das, Savari Desai, Mohd J Ali, Milind Naik, Santosh G Honavar
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To report a novel technique of initial eyelid debulking, simultaneously addressing horizontal and vertical laxity and improving outcome in orbitopalpebral plexiform neurofibroma.
Methods: Seventeen patients underwent debulking with a template of the contralateral normal eyelid being used to determine the amount of tissue excision.
Results: The mean reduction in horizontal and vertical palpebral fissure was 8.27 mm and 9.1 mm respectively at 1 year. Nine patients with severe ptosis subsequently underwent ptosis surgery with adequate correction.
Conclusions: The template technique of eyelid debulking in orbitopalpebral plexiform neurofibroma, followed by ptosis correction provides gratifying outcome.

ABSTRACT OP-15

Expression of E-Cadherin in Sebaceous Cell Carcinoma of the Eye Lid: An Immunohistochemical Study

P Jayaraj[1], S Sen[1], S Kashyap[1], N Pushker[3], MS Bajaj[3], A Sharma[2], S Ghose[2]
[1]Department of Ocular Pathology, [3]Microbiology and [2]Ophthalmoplasty services, Dr. Rajendra Prasad Centre for Ophthalmic sciences, All India Institute of Medical sciences, New Delhi 110029, India.

Purpose: E-cadherin is an important component of normal epithelial tissues where it is responsible for homotypic cell-to-cell adhesion. Reduced expression of E-cadherin complex is frequently observed in various types of human cancers and has been correlated with invasion, metastasis, and recurrence. The aim of the the present study was to investigate the association of E-cadherin with the clinicopathological features of eyelid SbCC.
Methods: Paraffin section of 66 histopathologically proven SbCC cases (2007-2010) were subjected to immunohistochemistry using mouse monoclonal antibody (HECD-1) to E-cadherin. E-cadherin expression was correlated with clinicopathologic features including age, tumor differentiation, size, site, pagetoid spread and tumor stage. Patients were followed up for a period of (6-24 months).
Results: Of the 66 cases of eyelid SbCC analyzed (29 males and 37 females, mean age 58.5 years), loss of membranous E-cadherin expression was observed in 83% (55/66) cases. Loss of E-cadherin expression correlated significantly with orbital invasion (*P* value 0.0276) and lymph node metastasis 100% (6/6). Five of the seven patients (71%) with tumor recurrence were also negative for E-cadherin expression. Correlation with histological features revealed loss of E-Cadherin in all Well differentiated SbCC 100% (16/16) and in 78% (39/50) Poor differentiated SbCC. However, a statistical significant correlation was not achieved.
Conclusions: Loss of E-cadherin expression is a frequent event in SbCC of the eyelid and correlates positively with orbital invasion, lymph node metastasis and tumor recurrence. These results suggest that loss of E-cadherin protein expression may contribute to the aggressive behavior associated with SbCC. Thus E-cadherin may be a used as poor prognostic indicator of eyelid SbCC and such patients may warrant aggressive treatment.

ABSTRACT OP-16

An Unusual Case of Pedunculated Upper Lid Mass

Rohit Modi , C K Barua , P K Goswami, Himanto Hazarika
Regional Institute of Ophthalmology, Guwahati, India.

Purpose: To report an unusual case of mass arising from the tarsal conjunctiva of right upper lid.
Methods: Along with detailed history, comprehensive examination was done. It was found there is no history of trauma, pain. On examination it was 4cmx3cmx3cm, pedunculated polypoidal mass arising from lid margin. It had an irregular red to yellowish in colour with granular surface. It was firm in consistency and non tender. Transillumination was negative. On Xray of right orbit, no erosion of bones was seen. Provisional diagnosis of basal cell carcinoma was made. Lid mass was excised and subjected to histopathological examination.
Results: Histopathological examination revealed multiple endothelial lined vascular channels (proliferating capillary buds) along with an infiltrate of mononuclear cells, characteristic of pyogenic granuloma .A diagnosis of pyogenic granula was made.
Conclusions: Though a relatively a rare cause, differential diagnosis of pyogenic granula should be considered for a lid.

ABSTRACT OP-17

Fundus Autofluorescence Imaging in Eyes with Serpiginous Choroiditis - Our Experience

Padmamalini Mahendradas, Kavitha Avadhani, Rajesh Babu, Rohit Shetty, Bhujang K Shetty
Narayana Netralaya, Bangalore, India.

Purpose: To study the fundus autofluorescence (FAF) images of eyes with serpiginous choroiditis**.**
Methods: A prospective observational case series of nineteen patients with serpiginous choroiditis was done in a teritiary eye care centre at South India.
Results: Fifteen patients were male and five patients were female. Spectralis revealed three different patterns of Autofluorescence in serpiginous choroiditis. They are (1) Hyperautofluorescence was seen in 5 eyes at the time of presentation and at final follow up none of the patients had hyperautofluorescence pattern, (2) Sixteen eyes had hypoautofluorescence with hyperautofluorescence pattern at the time of presentation. At final follow up 17 eyes had the same pattern and were on treatment and (3) Hypoautofluorescence pattern was seen in 9 eyes at the time of presentation, At final follow up 13 eyes had hypoautofluorescence pattern.
Conclusions: Fundus autofluorescence can be used as an additional investigational tool in the diagnosis and management of serpignous choroiditis. Fundus autofluorescence in eyes with serpiginous choroiditis reflect the changes in the outer retinal layers corresponding with the activity of the disease.

ABSTRACT OP-18

A Single Tube Multiplex Polymerase Chain Reaction for Detection of TEM, SHV, OXA Gene Mediated Extended-Spectrum β-Lactamases among Gram-Negative Bacilli Recovered from Ocular Specimens

M Jayahar Bharathi, Nandagopal Murugan, R Ramakrishnan
Department of Microbiology and Molecular Biology, Aravind Eye Hospital and PG Institute of Ophthalmology, Tirunelveli, Tamil Nadu, India.

Purpose: To develop and apply a novel multiplex PCR for rapid detection of TEM, SHV, OXA genes of Extended-Spectrum β-Lactamases mediated resistance among gram-negative bacilli recovered from ocular specimens.
Methods: As per CLSI guidelines, total of 67gram-negative bacilli (GNB), which were recovered from ocular specimens between February 2011 and May 2011, were subjected to phenotypic evaluation for ESBL production and molecular confirmation by applying multiplex PCR targeting TEM, SHV, OXA genes in a single reaction tube using group specific primer for TEM, SHV and OXA genes.
Results: Of 67, 6(9%) isolates showed positive results in phenotypic method, while in molecular method, mPCR detected β-Lactamases mediating genes in 50(75%) isolates. The most frequently detected gene is TEM (78%(39 of 50); in 19 isolates TEM alone, in 20 isolates along with OXA (18) and SHV (2)) followed by OXA gene (46% (23 of 50); in 2 isolates OXA alone, in 21 isolates along with TEM (18) and SHV (3) and SHV gene (22%(11 of 50); in 6 isolates SHV alone and in 5 isolates along with OXA (3) and TEM (2). The prevalence of β-Lactamases mediating genes are found to be higher among GNB from contact lens related infections (100%; 9 of 9), lacrimal system infections (100%; 4 of 4), conjunctivitis (100%; 2 of 2) and donor corneal rim (100%; 6 of 6), and 20%(2 of 10) among corneal pathogens. While among GNB from pre-operative conjunctival cultures, it accounted as 74% (25 of 34). The most commonly encountered gene is TEM.
Conclusions: β-Lactamases mediating genes are prevalent more among GNB of ocular infections (75%) and also among GNB of normal conjunctiva of healthy individuals (74%).

ABSTRACT OP-19

Ultrastructural Features of Nuclear Cataracts with Different Morphologies

Ashik Mohamed[1], *Kurt O. Gilliland*[2], *Sangeetha Metlapally*[2], *Dorairajan Balasubramanian*[1], *Sönke Johnsen*[4], *W. Craig Fowler*[3], *M. Joseph Costello*[2]
[1]Hyderabad Eye Research Foundation, L V Prasad Eye Institute, Hyderabad, India; [2]Department of Cell and Developmental Biology, [3]Ophthalmology, [2]University of North Carolina, Chapel Hill, NC; [4]Department of Biology, Duke University, Durham, NC, USA.

Purpose: To identify the characteristic ultrastructural damage in a variety of human nuclear cataracts those have very different appearances after extracapsular extraction. Common age-related nuclear cataracts are compared with less common hypermature, black and white cataracts.
Methods: Fresh human lens nuclei, from the US and India, were Vibratome sectioned, immersion fixed and en bloc stained prior to dehydration and embedding in epoxy. In 70 nm sections of different nuclear regions, fiber cells were imaged with a CCD camera on a Tecnai 12 transmission electron microscope.
Results: Cell damage in age-related nuclear cataracts was mainly minor damage to membranes with narrow extracellular space (ECS) and some deposits within the ECS, slight increase in cytoplasmic texturing, and presence of 1-4 µm multilamellar bodies. Hypermature cataracts exhibit enlarged ECS and more damage around membranes as well as in situ fiber cell disintegration. Black cataracts have essentially the same ultrastructure as age-related cataracts. Gross examination of white cataracts reveals that nuclei have little coloration and are opaque due to extensive white scattering. These nuclei have highly textured cytoplasm and numerous globular cell disruptions.
Conclusions: Nuclear cataract types have distinguishing ultrastructural features. The increased cell breakdown and enlarged ECS in hypermature cataracts are consistent with cortical cell disintegration and extensive white scattering of the nucleus. The similarity of black to age-related cataracts suggests that the gradual build-up of lens pigments towards opacification can occur with only minor alterations to cell structure. Conversely, limited pigment build-up in white cataracts is coupled with extensive cell disruption.

ABSTRACT OP- 20

Elevated Expression of TLRs, Dectin-1, and Pro-Inflammatory Cytokines in Human Corneas Infected with Filamentous *Aspergillus* and *Fusarium Sp*

Rajapandian S G Karthikeyan,[1] Sixto Leal[2], N V Prajna[1], E Pearlman[2], David Geiser[4], K Dharmalingam[3], Lalitha Prajna[1]
[1]Microbiology, G. Venkatasamy Eye Research Institute, Aravind Medical Research Foundation, Madurai, India; [2]Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH, USA; [3]School of Biotechnology, Madurai Kamaraj University, Madurai Kamaraj University, Madurai, India; [4]Department of Plant Biology, Pennsylvania State University, University Park, PA, USA.

Purpose: The purpose of this study is to examine immune response in human corneas infected with *Aspergillus* and *Fusarium sp*.
Methods: Total RNA from *Fusarium* or *Aspergillus* species infected human corneal ulcer materials were extracted by the TRIzol method, and quantitative PCR was performed for expression of TLR (Toll Like Receptor) 2, TLR4, Dectin-1, Dectin-2, IL-1α, IL-1β, IL-4, IL-8, IL-17, TNF-α, and IFN-γ. Donor corneas obtained from Aravind Rotary Eye Bank were used as controls for relative quantification. Corneal tissues after transplant surgery were examined for infiltrating cells by immunohistochemistry as well as by qPCR for expression of IL-17, IL-4 and IFN-γ.
Results: For the current study, 81 patients corneal ulcer materials, and 23 patients post transplanted corneal tissues were analyzed by quantitative PCR, and histopathology respectively. We found, increase in TLR2, TLR4, Dectin-1, IL-17, IFN-γ and IL-1β in infected groups. The immunohistochemistry analysis showed the presence of neutrophils, macrophages and $CD4^+$ T-lymphocytes.
Conclusions: Elevated expression of TLR2, TLR4, TLR9, Dectin-1 and pro-inflammatory and chemotactic cytokines in the infected tissue implicates, these mediators plays a crucial role in fungal recognition, and recruitment of neutrophils to the infected cornea. Increase in the IL-17 and IFNγ indicates the possible involvement of the Th1 and Th17 mediated T cell response. These studies will lead to an increased understanding of the human host response in fungal keratitis, and may identify novel targets for immunotherapy.

ABSTRACT OP-21

Clinical Outcomes of Repeat Autologous Cultivated Limbal Epithelial Transplantation for Ocular Surface Burns

Sayan Basu[1,2]*; Md Hasnat Ali*[3]*; Virender S Sangwan*[1,2]
[1]Cornea and Anterior Segment Services, [2]Sudhakar and Sreekanth Ravi Stem Cell Biology Laboratory, [3]Department of Clinical Epidemiology and Biostatistics, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Road No.2, Banjara Hills, Hyderabad, India.

Purpose: To evaluate the outcomes of repeat autologous cultivated limbal epithelial transplantation (CLET) after failed primary CLET for limbal stem cell deficiency (LSCD).
Methods: This study was a retrospective chart review of 68 eyes of 68 patients who underwent repeat autologous CLET for unilateral LSCD due to ocular surface burns between 2001 and 2009. A limbal biopsy was obtained from the healthy contralateral eye. The limbal epithelial cells were expanded ex-vivo on human amniotic membrane using a xeno-free and feeder-free culture system. The resulting cultured monolayer sheet was transplanted on the patient's affected eye after surgical preparation. All patients underwent a comprehensive ophthalmic examination of both eyes at every visit. Primary outcome measure was success of repeat CLET, clinically defined as stable ocular surface with absence of conjunctivalization or peripheral corneal neovascularization at 1 year post-operatively.
Results: Most patients were young (mean age: 17.3±12.3 years) males (72.1%) with history of alkali burns (83.8%) and median follow-up of 19 (range 12 to 90) months. Kaplan Meier survival probability of repeat autologous CLET was 46.3±0.07% at 1 year (median survival: 10 months). Sub-group analysis showed that success of CLET was significantly better (80.4%, P=0.0004) in eyes without symblepharon (hazard ratio: 4.6) and simultaneous keratoplasty (hazard ratio: 2.6). In 82% of successful cases vision improved to 20/40 or better post-operatively (P<0.0001). All donor eyes had normal ocular surface post-operatively.
Conclusions: Autologous CLET is safely repeatable and is effective in treating eyes with failed primary CLET and LSCD due to ocular surface burns.

ABSTRACT OP-22

Microbiological Profile and Role of AC Wash in the Management of Microbial Keratitis

Sujata Das[1], Magie Mathew[2], Savitri Sharma[1], Srikant K Sahu[1]
[1]L V Prasad Eye Institute, Bhubaneswar, Orissa, [2]L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To analyse the demographics, risk factors, pathogenic organisms, complications and clinical outcome in patients with infectious keratitis who underwent anterior chamber wash for diagnostic (Group 1) or therapeutic (Group 2) purpose
Methods: Retrospective review of all the medical records of patients of infectious keratitis who underwent anterior chamber wash from July 2007 to July 2010 was done
Results: 25 eyes of 25 patients were included in the study. Mean age was 44.92 +/- 19.64 (3 -75) years. Risk factors were seen in 21 eyes ; injury in 17 eyes, surgery in 2 eyes, instillation of plant extract in one eye, poor ocular surface in one eye
Out of the 20 eyes in Group 1, microorganisms were isolated in 11(55%), (3 smears, 10 cultures). Cultures revealed fungus in 4, bacterial in 4, mixed infection in 2 and only positive smears in one eye. Of the 20 eyes resolution of infection was achieved in 14, 2 eyes required tissue adhesive and BCL, 2 eyes required PPV+PPL+IOAB, 2 eyes required therapeutic penetrating Keratoplasty(TPK)
All the 5 eyes in Group 2 were microbiologically inconclusive. Resolution was seen in 3 eyes, one eye required TPK and the other eye was lost to follow-up.
Complications included corneal perforation in 2 eyes, increased IOP in 2 eyes and anterior lens capsule rupture in one eye
Conclusions: AC wash may be considered in cases of microbial keratitis predominantly involving the deep stroma and the anterior chamber. Despite the complications, it serves both diagnostic and therapeutic purposes.

ABSTRACT OP-23

Corneal Collagen Cross-Linking Procedure Leads to Apoptosis in Limbal Epithelial Cells

Debashish Das, Himanshu Matalia, D Kamesh, S Murali, A A Vincent, Rohit Shetty
Stem Cell Laboratory, Narayana Nethralaya, Bangalore, India.

Purpose: To study the apoptotic effect of riboflavin and ultraviolet-A (UV-A) irradiation on *ex vivo* cultivated limbal epithelial cells (LECs).
Methods: *Ex vivo* cultured LECs on denuded amniotic membrane were exposed to UV-A light used in corneal collagen cross-linking (C3R) in the presence and absence of photo-sensitizer riboflavin. These cells were then used for extraction of RNA, cDNA conversion or used for staining. Quantitative PCR and immunoflourscence staining were done for evaluating the apoptotic state of the cells in different treated and untreated conditions. The statistical analysis was finally done using Student-T test.
Results: It was noted that anti-apoptotic genes such as Bcl-2 was down-regulated whereas Bax, a pro-apoptotic gene was up-regulated. Significant up-regulation was detected in the levels of both caspase 3 and caspase 9 in cell exposed to UV-A in comparison to untreated cells.
Conclusions: The results indicate that exposure of LECs to the UV-A dosage used in C3R procedure enhances the apoptotic genes as well as caspase activity. In the presence of riboflavin the damage caused by UV-A is marginalized but not totally abrogated.

ABSTRACT OP-24

Therapeutic Modulation of Matrix Mettaloprotinase Prevents Posterior Capsular Opacification

Sarbani Hazra[1], Himangshu Palui[1], Rajdip Guha[2], Akelesh Mishra[1], Jeram Joonkey[1], Geeta Vemuganthi[3], Samar Basak[4], Tapan K Mandal[1], Aditya Konar[2]
[1]West Bengal University of Animal & Fishery Sciences. Kolkata-700037, India; [2]Indian Institute of Chemical Biology, Kolkata-700032, India; [3]University of Hyderabad.India; [4]Disha Eye Hospital, Barrackpore, India.

Purpose: MMP inhibition has been established as a potent pathway for the prevention of posterior capsular opacification. We have evaluated the use of EDTA as MMP inhibitor for prevention of posterior capsular opacification in a rabbit model.
Methods: One eye of White New Zealand rabbits underwent phacoemulsification surgery for lens removal and was treated intracamerally into the capsular bag with EDTA, the control group received normal saline. The kinetics of the drug was evaluated by HPLC of the aqueous humor at different time points. Gelatine zymography of aqueous humor was performed to evaluate the modulation of MMP2 by EDTA.The PCO formation was assessed by slit lamp biomicroscopy and ophthalmoscopy. After one month the eyes were enucleated and PCO was evaluated by Miyake Apple view and histology and immunolocalization of MMP2. The degree of posterior capsular opacification was graded with a score of 0- 3.Toxicity of the drug on was studied by histopathology and specular microscopy and electroretinography (ERG).
Results: EDTA remained available in aqueous up to 72hrs. Suppression of MMP2 activity and subsequent prevention of posterior capsular opacification was observed in EDTA treated group compared to the control group. Histology, specular microscopy and ERG did not reveal any toxicity of EDTA on intraocular structures.
Conclusions: EDTA successfully prevented posterior capsular opacification without causing any toxicity to the intraocular structures.

Support: Council of Scientific and Industrial Research, India.

ABSTRACT OP-25

Ex-vivo Expansion of Limbal Epithelium with Known Stem Cell Content Under GMP Compliance

Gowri P Chidambaranathan, T Lalitha, Saumi Mathews, Minu Jennifer, V R Muthukkaruppan

Department of Immunology & Cell Biology, Aravind Medical Research Foundation, Dr. G. Venkataswamy Eye Research Institute, Madurai-625020, India.

Purpose: To validate the simple method of explant culture for *ex-vivo* expansion of limbal epithelial stem cells (LESCs) in compliance with Good Manufacturing Practice (GMP).

Methods: The limbal tissues from cadaver eyes were cultured as 2 mm explants along with stroma in SHEM with autologous serum and forskolin instead of FBS and cholera toxin, or in StemPro® MSC SFM (Invitrogen). The native and cultured epithelial cells were isolated, immunostained for p63 or Cx-43 with PI counterstaining and the SC content was quantified on the basis of our two parameter analysis (high p63 expression in small cells with large nucleus/cytoplasmic (N/C) ratio) using confocal microscopy. Functionally the cultured cells were analysed for their label retaining property and colony forming efficiency (CFE).

Results: We demonstrate that the explant culture provides the original limbal niche for SC expansion based on: (1) the migration of SCs in the outgrowth, (2) a 10-fold increase in the number of SCs, (3) the presence of small Cx-43 negative cells with high p63 expression near the explant, (4) the presence of label retaining cells on the explants even after three weeks of culture and (5) the explants having the ability to generate another sheet of epithelium. In compliance with GMP, we establish that (i) SC content in StemPro® MSC SFM (approved for human application) is similar to SHEM with autologous serum and (ii) there is an increase in SC content by forskolin on the basis of CFE.

Conclusions: The simple method of explant culture along with stromal cells provides the required limbal niche for ex-vivo expansion of LESCs. In compliance with GMP, validation of cultured epithelium by quantifying the SC content is essential to assess the clinical outcome after transplantation in limbal stem cell deficient patients.

ABSTRACT OP-26

Why Do Some Mutations in Human Gamma D Crystallins Lead to Congenital Nuclear Cataracts While Others Lead to Peripheral Cataracts? A Protein Structural Rationale

Venkata P R Vendra[1], Dorairajan Balasubramanian[1], Garima Agarwal[2] Narayanaswamy Srinivasan[2]
[1]C-TRACER, Prof Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad 500034; [2]Molecular Biophysics Unit, Indian Institute of Sciences, Bangalore 560012, India.

Purpose: As many as 16 mutations are known in human γD crystallin, mutations in the N-terminal domain (Ntd) are associated with milder forms of cataract while those in the C-terminal domain (Ctd) are associated with nuclear cataract. We address this dichotomous issue by comparing the structural properties of three Ctd mutants with wild-type and two Ntd mutants.
Methods: Wild-type and mutant proteins were over-expressed and purified. Structural characterization was done by circular dichroism and fluorescence. Wild-type and mutant clones were transfected in to human lens epithelial cells to study the formation of light scattering particles. Molecular modeling was also done, using standard methods.
Results: Ctd mutants are far less soluble than wild-type, and Ntd mutants; Secondary structure of the Ctd mutants was quite similar to the full length protein; R140X and G165fs molecules showed red shifts in intrinsic fluorescence compared to wild-type; A remarkable increase in the surface exposure of several nonpolar residues was seen in the Ctd compared to Ntd mutants and wild-type; Wild-type and Ntd mutants bound Ca^{2+} ions with affinity, Ctd mutants showed little or no binding of Ca^{2+} ions; Transfection of Ctd mutants into HLE cells led to scattering particles while the wild-type and Ntd mutants did not.
Conclusions: Loss of C-terminal residues in the mutants causes the loss of a Greek key motifs and exposure of normally buried apolar side chains to the solvent, leading to self-aggregation and scattering both *in vitro* and *in situ*, thus offering an insight to the mechanism of opacification.

ABSTRACT OP-27

A Cataract Causing Mutant of Connexin-50 Shows Defect in Transport to the Plasma Membrane and is Retained in the Endoplasmic Reticulum

Madhavi L S Chalasani[1], Madhavi Muppirala[1], Surya Prakash G Ponnam[2], Chitra Kannabiran[2], Ghanshyam Swarup[1]
[1]Centre for Cellular and Molecular Biology, Hyderabad, India; [2]Kallam Anji Reddy Molecular Genetics Laboratory, L.V. Prasad Eye Institute, Hyderabad, India.

Purpose: Congenital cataracts account for one-tenth of childhood blindness in Southern India. In a previous study, we identified a frameshift mutant of connexin50 (c.670insA, p.Thr203AsnfsX47) in a family with autosomal recessive cataract. The mutant protein is smaller and contains 46 aberrant amino acids at the C-terminus. The objective of this study is to determine the cellular behaviour of the frameshift mutant of connexin50 in order to understand the pathological consequences of the mutation.
Methods: Wild type-CX50 (WT-CX50-myc-his) and Fs-CX50-myc-his (frameshift mutant) and GFP constructs were transiently transfected in HeLa cells, stained with markers of various cell organelles and examined by confocal microscopy to understand the localization properties and transport to plasma membrane. The protein levels were analysed by Western blotting.
Results: In transiently transfected HeLa cells, WT-CX50 localised to appositional membranes and cytoplasmic foci whereas the frameshift mutant showed localization to the endoplasmic reticulum (ER) and to some extent in the ERGIC (ER-Golgi intermediate compartment). The frameshift mutant did not reach the plasma membrane. Moreover, expression of FS-CX50 showed disintegration of the ERGIC and breakdown of Golgi. Expression of FS-CX50 resulted in reduced level of ERGIC-53 protein. Transport of VSVG-GFP to plasma membrane was inhibited by expression of frameshift mutant but not by wild type connexin50.
Conclusions: Our results show that the frameshift mutant of connexin50 (c.670insA, p.Thr203AsnfsX47) is defective in its transport to the plasma membrane and is retained in the ER which is likely to cause the disease.

ABSTRACT OP-28

Knowledge, Attitude and Practices on Diabetes and Diabetic Retinopathy in Rural Andhra Pradesh in India

Satyanarayana Sristi[1, 2], *S Sheeladevi*[2], *Padmaja K Rani*[2,3]
[1]University of New South Wales, Australia, [2]International Centre for Advancement of Rural Eye Care, L V Prasad Eye Institute, Hyderabad, India, [3]Smt. Kanuri Santhamma Retina Vitreous Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To ascertain the knowledge, attitudes and practices (KAP) of the general population and persons with diabetes on diabetes and diabetic retinopathy in rural southern India.
Methods: A total of 380 people with diabetes (Group 1) and 380 people from the general population (Group II) aged ≥40 years were enrolled from randomly selected rural villages of Phirangipuram mandal in Guntur district in Andhra Pradesh in southern India. A KAP questionnaire was used and answers were scored on a continuous scale — minimum 60% in each section was taken as the correct knowledge, a positive attitude and good practices.
Results: The mean score of knowledge (P value <0.001), attitude (P value <0.001) and practices (P value <0.001) was significantly higher among persons with diabetes. In group I, 28.2%, 36.1% and 40.3% had the correct knowledge, a positive attitude and good practices, respectively, whereas the scores of the group II were comparatively low. Among the people with self-reported diabetes 58.2% (221) never had a dilated examination and there was no statistically significant difference between the gender and dilated examination. However, 92.5% (147) had dilated eye examination only once although 46.3% are with diagnosed diabetes for more than 5 years. Education of the subjects is the main influencing factor for correct knowledge (P value <0.001) and a positive attitude (P value <0.001) in both the groups.
Conclusions: Health education programs needs to be organised and targeted for both communities and practitioners to increase the awareness resulting in positive practices.

ABSTRACT OP-29

Rural Vision Health Guardians Role in Eye Health, Diabetes and Hypertension Education

Suresh Kuruganti, Padmaja K Rani, Sheela Sethudevi, Jachin Williams, Anil K Reddy
International Centre for Advancement of Rural Eye Care, L V Prasad Eye Institute, Hyderabad, India, Smt. Kanuri Santhamma Retina Vitreous Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Sight to Resight project was envisaged to address some of the identified barriers namely (I) Lack of awareness (2)Apathy in health care seeking behavior in accessing Eye care and primary health care services in remote rural communities duly utilizing the services of volunteers(vision health Guardians)
Methods: The project is launched in september 2010 in twenty villages surrounding four vision centres attached to Mudhole secondary centre(Bhainsa and Kallur),and Thudukurthy secondary centre{Nagar kurnool & Achampet). Nineteen volunteers from the mostly women with high school education were shortlisted and, were imparted a brief training in identifying eye problems affecting vision, and high risk population for Hypertension and Diabetes mellitus.
Results: About 35,570 persons were screened for visual acuity of which 9131 were identified and referred to vision centres, 2431 persons visited the vision centre after examination, 986 were prescribed glasses and 521 persons were referred to Secondary level centre. At secondary centre cataract surgery was advised for127 persons of whom 64 underwent surgery and 4 persons were referred to tertiary centre. 9110 households were screened for diabetes risk, 555 persons with high risk for diabetes were identified besides 333 patients with known diabetes.7650 persons were screened for hypertension and 684 persons were found to have hypertension and were referred to physician besides 714 known hypertension subjects.
Conclusions: The rural community accepted the vision health guardians as one of their own, and volunteers are able to serve as eyehealth, diabetes and hypertension educators.

ABSTRACT OP-30

The E-Kit. One Pack Solution for First- Line Bacterial Endophthalmitis Treatment

Taraprasad Das [1], *Ravindran D Ravilla* [2], *Sriram D Ravilla* [3]
[1]L V Prasad Eye Institute, Bhubaneswar, [2]Aravind Eye Care System, Madurai, [3] Aurolab, Madurai, India.

Purpose: To provide essential first-intervention treatment material for bacterial endophthalmitis in a ready-to-use kit.
Methods: We have developed the E-kit that contains most essential devices and antibiotics for initial treatment of bacterial endophthalmitis. The three essential contents of the E-kit are eye speculum, 23 g butterfly needle (for vitreous biopsy), and antibiotics. The easy-to-prepare includes vancomycin 100 mg and ceftazidime 250 mg in vials. One can is required to add 10 ml of water for injection, provided in the kit and 0.1 ml of the prepared solution contains 1 mg vancomycin in 0.1 ml and 2.5 mg of ceftazidime in 0.1 ml recommended for intravitreal injection.
Results: The visual analogue scale (VAS) on the utility and ease of use of the E-kit was measured with 15 scrub nurses. The VAS was highest for the antibiotics and least for the eye speculum.
Conclusions: The E-kit benefit includes accurate and easy preparation of antibiotics, safe vitreous biopsy and intravitreal injection. It also reduces the ordering time and inventory cost.

ABSTRACT OP-31

Development and Validation of the Children Visual Impairment Questionnaire (CVIQ)

Rebecca Sumalini, Vijaya K Gothwal, Seelam Bharani, Shailaja P Reddy, Deepak K Bagga
Meera and L B Deshpande Centre for Sight Enhancement, Vision Rehabilitation Centres, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To develop a reliable and valid questionnaire to assess visual difficulties in day-day activities in school-going children with visual impairment (VI) in India.
Methods: A list of 67 themes was generated from focus group discussions (FGDs) with children with VI and their parents. Items were also extracted from the existing questionnaires for children. The 32-item questionnaire was piloted in 25 children with VI following FGDs with low vision specialists. Six items were deleted as 80% of the participants reported 'no difficulty'. A final 27-item (including a last global rating item) Children Visual Impairment Questionnaire (CVIQ) was administered to 150 participants (mean age, 12.3 years). Response to each item was rated on a 3-category scale. Rasch analysis was used to assess the psychometric properties of the 26 items.
Results: Rating scale was used by participants as was intended to. Four items related to mobility were deleted as these did not measure the same underlying construct as rest of the items. The most difficult item was copying small letters from board (-2.04 logits) and easiest was watching movie at theatre (1.59 logits). The final 22-item unidimensional CVIQ was able to discriminate among four strata of participant ability and the items were reasonably matched well to the participants' visual abilities (targeting, -1.02 logits).
Conclusions: The Rasch-developed CVIQ is a psychometrically sound questionnaire to measure visual disability in school-going children in India. It provides interval-level measures of visual disability, so is advantageous as an outcome measure for low vision rehabilitation services in children.

ABSTRACT OP-32

Why Does Pediatric Cataract Surgery in India Have a Poor Follow-Up?

Shailbala Patil, Parikshit Gogate, Rahin Tamboli, Rekha Mane, Anil Kulkarni, G V Rao
Gogate's Eye Clinic, Pune, India.

Purpose: To study the cause of poor follow-up after successful pediatric cataract surgery and understand its implications.
Methods: 257 children with 378 pediatric cataracts were examined 3-5 years after surgery and demographic data, visual acuity and causes of poor follow-up were noted.
Results: Only 20 (7.8%) of the children had attended a regular annual follow-up. 38 (14.8%) of children did not visit the hospital as their parents did not feel the need for it; 53 (20.6%) parents said no one told them that they had to visit regularly, 63 (24.5%) reported that their child was seeing well; 53 (20.6%) reported that hospital was too far; 65 (25.3%) that they could not afford to travel and 69 (26.9%) that they did not find the time (parents could give more than one cause, so total>100%). There were no gender of child ($p=0.91$) or socioeconomic class (Kuppusamy classification) ($p=0.51$) difference in follow-up by parents. Outcome was better in children who attended a regular follow-up ($p<0.01$), there was less PCO ($p<0.01$) and they were more likely to wear correct spectacles.
Conclusions: Ophthalmologists must actively counsel parents for follow-up in order to have the optimal benefit of the intervention.

ABSTRACT OP-33

The Feasibility of Using a Digital Camera to Elicit Red Reflexes for the Detection of Vision-Threatening Eye Disease in Pre-School Children

Ashwin Mallipatna, Alefia Merchant,Kaushik Hegde, Vasudha K.,Jyoti Matalia, Ravindra Battu, Nasrin Najm-Tehrani, Ken Nischal, Mathew Kurian,Pradeep Banandur, Helen Dimaras, Robert Downie, Cynthia VandenHoven, Lindsay Hampejsková, Brenda Gallie
Narayana Netralaya, Bangalore, India.

Purpose: The prevalence of visual impairment and blindness in children is high in India. There is no organized eye-screening program for the identification of such children. A red-reflex exam with a direct ophthalmoscope (DO) is currently recommended for the early detection of eye diseases that threaten vision, but may not be suitable screening tool in a context where medical resources are limited. In previous research, we determined the parameters needed to obtain a clinically-relevant red reflex in photographs using a consumer digital camera with a flash. This study compares the ability of a digital camera to detect vision-threatening eye conditions in comparison to the standard DO.
Methods: Children under the age of five were screened for red-reflex abnormalities in a walk-in pediatric ophthalmology clinic in Bangalore, India. A red reflex examination was performed by a pediatric ophthalmologist with a DO. Red reflex photographs of the children were obtained with a compact digital camera using standardized parameters. The photographs were analyzed for the presence of abnormalities by a pediatric ophthalmologist, independently of the DO examination. A white reflex or a total loss of reflex was defined as a vision-threatening abnormality (Level 1 abnormality). More subtle abnormalities (Level 2 abnormality) were ignored for the purpose of this analysis. Clinical diagnoses obtained from medical records were classified into vision and non-vision threatening eye disease based on the clinical expertise of a pediatric ophthalmologist. Exam sensitivities and specificities for the detection of potentially vision-threatening disease were calculated.
Results: 186 children were screened for red-reflex abnormalities. 169 were included in analysis. 101 had a vision-threatening eye condition. 17 Level 1 abnormalities were detected with the DO; 57 were detected with the digital camera. The sensitivity and the specificity of the DO was 0.17 and 1.00, respectively. The sensitivity and specificity of the digital camera was 0.57 and 0.78, respectively.
Conclusions: Level 1 abnormalities detected with a compact digital camera demonstrate substantially greater sensitivity for vision-threatening eye disease when compared to those detected with a DO, although they do so with lesser specificity. Compact digital cameras show potential for use as a screening tool, especially if used in conjunction with the DO. While photographs could be used to rule in the possibility of disease, the DO could be used to rule out false positives. A camera could be a practical screening toolfor a community-based programs because it is more widely available and creates a permanent record of the examination for remote evaluation.

ABSTRACT OP-34

Effect of Spectrum Bias on the Diagnostic Accuracy of Spectral Domain Optical Coherence Tomograph in Glaucoma

Uday K Addepalli,[1,3] Harsha BL Rao,[1,2] Tukaram Kumbar,[1] Neha Bharathi,[1] Sirisha Senthil,[1] Nikhil S Choudhari,[1] Chandra S Garudadri[1]
[1]VST Glaucoma Center, [2]Center for Clinical Epidemiology and Biostatistics, L V Prasad Eye Institute, Banjara Hills, Hyderabad, India, [3]Vision CRC, UNSW, Sydney, Australia.

Purpose: To evaluate the influence of the control group on the diagnostic accuracy of spectral domain optical coherence tomograph (SDOCT) in early glaucoma.
Methods: All participants underwent optic nerve head (ONH), retinal nerve fiber layer (RNFL) and ganglion cell complex (GCC) imaging with the commercially available SDOCT. Diagnostic accuracies of SDOCT parameters obtained in analysis 2 were compared with that obtained in analysis 1.
Results: AUCs of all ONH parameters were significantly lesser ($p<0.001$ for all comparisons) in analysis 2 compared to analysis 1. AUCs of all RNFL parameters in analysis 2 were comparable ($p>0.05$) to that in analysis 1. Though the AUCs of GCC thickness parameters were comparable in the two analysis, AUCs of GCC focal loss volume (FLV) and global loss volume (GLV) were significantly lesser ($p<0.001$) in analysis 2 (0.749 and 0.706 respectively) compared to analysis 1 (0.921 and 0.886 respectively).
Conclusions: The diagnostic accuracy of SDOCT in glaucoma is influenced by the control group. When a clinically relevant control group, consisting of normal subjects with suspicious looking optic nerves was used instead of a control group consisting of normal subjects with no suspicious findings of glaucoma, the diagnostic accuracies of all ONH parameters and the best GCC parameters significantly reduced while that of the RNFL parameters remained unchanged.

ABSTRACT OP-35

Visual and Refractive Outcomes of Intraocular Lens Implantation in Congenital Cataracts Less than 6 Months of Age

N Srividya[1], *Amit Gupta*[1], *Ramesh Kekunnaya*[1]
[1]Department of Pediatric Ophthalmology, Strabismus and Neuro-ophthalmology, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India.

Purpose: To analyze the visual outcomes and changes in refractive status in children with primary intraocular lens (IOL) implantation before 6 months of age.
Methods: 36 eyes of 18 children underwent lens aspiration with primary IOL implantation before 6 months of age. The mean follow up was 16.5 ± 6.59 months (ranged 10 to 36 months). Visual acuity, refraction, intraocular pressure, anterior and posterior segment evaluation was regularly evaluated.
Results: Of the 36 eyes, 8 (33%) had stable visual acuity and 28(77%) had a significant improvement in acuity. Refraction showed a myopic shift in the follow-ups (1st month refraction was +6.5Ds ± 1.5, 3rd month +5.5Ds ± 1.68, 6th month +5Ds ± 1.32, 12th month +4Ds ± 1.2 , 24th month +3 Ds ± 1, 36 th month +0.85Ds ± 1.2). A total of 8 number of complications were seen following the surgery.
Conclusions: Primary IOL implantation in less than 6months of age group is a safe procedure with a good visual outcome. Regular follow up is of utmost importance for a successful visual rehabilitation.

ABSTRACT OP-36

Clinically Significant Macular Edema can be a Pitfall in Axial Length Measurement Before Cataract Surgery

Mukesh Kumar, Bhaktiprada Sahoo, Santanu Jana, Taraprasad Das
L V Prasad Eye Institue, Bhubaneswar, Orissa, India.

Purpose: To compare axial length (AL) measurements obtained with ultrasound (US) AND IOL Master eyes with diabetic clinically significant macular edema and study its significance.
Methods: In 39 eyes of 39 diabetic patients with clinically significant macular edema, we measured axial length using both instruments and the retinal thickness of the macula using optical coherence tomography. Wilcoxon Signed ranks test was used to measured the difference between the axial length measured by 2 methods, the relationship between macula thickness and axial length difference was analyzed by Spearman's correlation coefficient .
Results: The axial length was 22.91 ±0. 84 mm (mean ± standard deviation) by US and 23.37 ± 0.89 mm (mean± standard deviation) by IOL Master. There was a significant difference between axial length measurements ($p<0.001$). In addition, the thickness of the macula and the difference in axial length measurement were positively correlated (correlation coefficient=8.52, p-0.002).
Conclusions: Axial length measurement using applatation A-scan US and IOL Master in eyes with CSME differ significantly both statistically and clinically. This study may suggest that IOL Master is ideal for measuring axial length in eyes with clinically cystoid macular edema and improving post cataract surgical refractive outcome.

ABSTRACT OP-37

The Relation Between Pharmacological Dilation, Pupil Diameter and Accommodative Response Magnitude

Samrat Sarkar, Shrikant R Bharadwaj
Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Phenylephrine Hydrochloride (PHCl), a commonly used mydriatic agent, has been shown to reduce accommodative performance. This experiment determined the magnitude of this reduction and explored if this reduction was due to the pharmacology effect of PHCl or due to the optical effect of pupil dilation.
Methods: Accommodative responses of ten emmetropic adults (21 to 30 years) were measured using a dynamic (60Hz) photorefractor before dilation and after dilating both eyes with 2.5% or 5% or 10% PHCl. The experiment commenced one-hour after instillation of three drops of PHCl in each eye. The right eye viewed a high-contrast visual target that switched between two LCD screens placed at 67 and 33 cm (1.5D stimulus) before the subject with their natural pupils or through 1mm or 4mm or 6mm or 8mm artificial pupils. The left eye was occluded using an IR transmitting filter and consensual accommodative response was recorded.
Results: Two-factor ANOVA showed a statistically significant main effect of pupil size on accommodative amplitude ($p<0.01$). Accommodative responses with natural pupils and with 8mm, 6mm and 4mm pupils were not significantly different from each other ($p>0.43$ for all) but they were all significantly larger than those obtained with 1mm pupils ($p<0.03$). The main effect of PHCl concentration and interaction between the two factors were not statistically significant ($p>0.44$).
Conclusions: PHCl does not appear to have a significant impact on the accommodative amplitude of adults. The reduction is accommodative response with 1mm pupil is somewhat expected due to an increase in optical depth-of-focus.

ABSTRACT OP-38

Inter and Intra Subject Variability of Luminanace - Slope Calibration in Eccentric Photorefraction

Asa Narasaiah[1], Geeta Sravani[1], T Rowan Candy[2], Shrikant R Bharadwaj[1,3]
[1]Bausch & Lomb School of Optometry, [3]Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India; [2]Indiana University School of Optometry, Bloomington, Indiana, USA.

Purpose: Refractive power measured using eccentric photorefraction depends on the calibration of luminance slope formed across the pupil into dioptric units. This study determined two aspects of this defocus calibration factor: Its inter and intra-subject variability and the impact of experimental set-up on the calibration factor.
Methods: 57 emmetropic adults (20.3 to 31.3yrs) fixated on the photorefraction unit at 75cm with their left eye. Anisometropia was induced by placing trial lenses (+8D to -8D in 1D step) before the IR filter occluded right eye and the defocus calibration factor was calculated by plotting luminance slope for each lens against the induced anisometropia. This paradigm was repeated (n=24) twice within 5 minutes and twice within two weeks to determine intra-subject variability and it was repeated (n=15) with an IR reflecting hot-mirror and an IR transmitting cold-mirror to determine variability due to experimental set-up.
Results: Mean (+/-95% CI) calibration factor obtained within the +/-5D linear range was 0.69+/-0.28Ls/D. Mean calibration factors in sessions one (0.64+/-0.22Ls/D) and two (0.66+/-0.25Ls/D) were not statistically significantly different from each other (p=0.38).Mean calibration factor with hot mirror (0.56+/- 0.13Ls/D) and with cold-mirror (0.51 +/- 0.19Ls/D)were not significantly different from each other (p=xx) but both were significantly different from direct viewing of the camera(0.621 +/- 0.17Ls/D)($p<0.02$).
Conclusions: Large variability in the defocus calibration factor across subjects and across experimental set-up suggests that individual calibration factors should be used to convert luminance slope in to diopetrs in each subject and with each experiment.

ABSTRACT OP-39

Correlation of Back Optic Zone Radius Measurement of Rigid Contact Lenses with Radiuscope and Keratometer

Tukaram Kumbar[1], T Shyam Sunder[3], Jawalkar Swati[2], Addepalli Uday Kumar[1,5], Asma Fathima[2], Harsha L Rao[4], Varsha Rathi[3]
[1]VST Glaucoma Centre, Dr Kallam Anji Reddy campus, L V Prasad Eye Institute, Hyderabad, [2]Bausch & Lomb School of Optometry, [3]Bausch & Lomb Contact Lens Centre, [4]Center for Clinical Epidemiology and Biostatistics, L V Prasad Eye Institute, Hyderabad, India; [5]Vision CRC, UNSW, Sydney, Australia.

Purpose: To investigate the agreement of Radiuscope and Keratometer for Back Optic Zone Radius (BOZR) measurements of Rigid Contact lenses.
Methods: 100 spherical PMMA contact lenses (Flexilens Pvt Ltd, Mumbai and Classic Pvt Ltd, Banglore) were selected randomly. One investigator measured the BOZR with Radiuscope. The second investigator measured the same lens for BOZR with Keratometer. As the keratometers is designed to measure a corneal surface, the BOZR readings are less than actual radii. A correction factor of 0.025 was used to have corrected keratometers readings.
Results: The two investigators were masked for the readings. The mean differences between uncorrected and corrected Radiuscope-keratometry measurements were 0.003 and -0.024 respectively. The p value was statistically significant for both methods measurements. The 95% limit of agreement for uncorrected and corrected Radiuscope-keratometry measurements is -0.11 to 0.11 and -0.14 to 0.09 respectively.
Conclusions: With unavailability of Radiuscope in majority of contact lens practice, Keratometer can be used to measure the BOZR of contact lenses.

ABSTRACT OP-40

Surgical Outcome of Primary Trabeculotomy-Trabeculectomy in Glaucoma with Sturge-Weber Syndrome

Anil K Mandal, Pradeep K Reddy, Sannapaneni Krishnaiah
LV Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To evaluate the safety and efficacy of Primary Combined Trabeculotomy-trabeculectomy for glaucoma in Sturge –Weber syndrome.
Methods: Retrospective analysis of 21 eyes of 21 patients who underwent Combined Trabeculotomy-trabeculectomy over a 12 year period by a single surgeon between 1994 and 2006.
Results: Mean age of the patients was 33.7 ± 64.4 months (0.1-252, range). IOP was reduced from 25.19 ± 6.76 to 18.42 ±11.42 ($p<0.001$) with a mean follow up 0f 63.27±57.2 months (3 -168 months, range). Corneal edema was noted in 57.1% of patients before surgery and it persisted in 14.3% of patients following surgery. Final visual acuity > 20/200 was seen in 38.1% of the patients. Hyphaema was noted in 3 patients, cataract was noted in 1 patient, shallow AC in 1 patient, choroidal detachment in 2 patients. Success was 68% at the end of 8 years.
Conclusions: Combined Trabeculotomy-Trabeculectomy is safe and effective with good IOP control and moderate visual outcome.

ABSTRACT OP-41

Repairing Goldmann Applanation Tonometer is Easy!

Nikhil S Choudhari, Harsha B L Rao, Sirisha Senthil, G Chandra Sekhar
V S T Glaucoma Centre, Dr. Kallam Anji Reddy campus, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To investigate the feasibility and utility of calibration of the Goldmann applanation tonometer (GAT). Calibration error of GAT, the Gold standard for intraocular pressure measurement, is much more common than expected. The manufacturers recommend returning faulty tonometers for calibration. The logistics and time delay do not permit such arrangement in most ophthalmic practices.
Methods: We ourselves learned the techniques of calibrating GAT by trial and error. This prospective study included 22 faulty Haag-Streit GATs (calibration error more than ± 2 mm Hg at any level of check). We repaired the instruments by lubrication followed by calibration (adjustment of counter-weight or weight), when lubrication did not suffice.
Results: We could repair all faulty instruments. Twenty one (95.4%) faulty GATs required lubrication alone. One (4.5%) faulty GAT required adjustment of the counter-weight.
Conclusions: Lubrication of GAT, if not repair, can be carried out by ophthalmologists or technicians and may drastically cut down the downtime of the instrument.

ABSTRACT OP-42

Efficacy of Split Hours Part Time Patching vs Continuous Wear Part Time Patching for Treatment of Unilateral Amblyopia in Children

Joseph Mollah,[1] S Anand,[2] Virender Sachdeva,[2] Ramesh Kekunnaya,[1] Amit Gupta,[1] Vaibhev Mittal, [2]Rekha Gunturu[2]
[1]Department of Pediatric Ophthalmology, Strabismus and Neuro-ophthalmology, LV Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad; [2]Department of Pediatric Ophthalmology, Strabimsus and Neuro-ophthalmology, L V Prasad Eye Institute, GMR Varalakshmi Campus, Vishakapatnam, India.

Purpose: To compare efficacy of 'Continuous Wear part time patching' and 'Split part time patching' in treatment of unilateral amblyopia.
Methods: Children between 4-11 years with with unilateral strabismic, anisometropic or stimulus deprivation amblyopia were treated with either continuous wear (Gp A) or split hours part time patching (Gp. B). All children were also given treatment of the underlying condition such as glasses or surgery for ptosis, strabismus, etc. Children were followed up for the improvement in visual acuity and the compliance at each follow-up visit.
Main outcome Measures: Improvement in BCVA, rate of Improvement in BCVA.
Results: 21 and 52 children were recruited in Gp A and Gp B respectively. Both groups were matched for baseline characteristics, VA, causes for amblyopia and hours of patching. Mean follow up duration was 14.5 and 15.6 months respectively.
At 3 months follow-up, 42.9% and 28.8% patients had improvement in BCVA>2 lines in Gp A and B respectively (p=0.21),while at 6 months 42.9% and 67.3% patients had improvement > 2 lines (p=0.13) in Gp A and Gp.B. Rate of improvement in BCVA was slower in the split hours patching group but at 6 months follow-up both groups showed similar improvement. There was no difference in the compliance to the patching therapy in 2 groups (p>0.05).
Conclusions: Split hours part time patching may be at least as effective as continuous wear part time patching.

ABSTRACT OP-43

A Glaucoma-Associated Optineurin Variant, M98K Induces Retinal Ganglion Cell Death by Reducing Transferrin Receptor Level through Lysosomal Degradation

Kapil Sirohi[1], Madhavi L S Chalasani[1], Dorairajan Balasubramanian[2], Vegesna Radha[1], Ghanshyam Swarup[1]
[1]Centre for Cellular and Molecular Biology, Hyderabad, India; [2]L V Prasad Eye Institute, Hyderabad, India.

Purpose: To understand how the glaucoma associated M98K variant of optineurin alters cellular function and causes pathology.
Methods: Optineurin and its mutants were overexpressed and effects on cell survival, vesicle dynamics and turnover of transferrin receptor (TfR) examined. A cell line model for retinal ganglion cells (RGC-5) was used and compared with other cell types.
Results: Overexpression of M98K induced death of RGC-5 cells, but not of other neuronal and non-neuronal cell lines. This cell death was inhibited by coexpression of dominant negative mutants of caspase-1 and caspase-9, but not by anti-apoptotic protein Bcl2. Cell death induced by overexpression of M98K mutant was dependent on a functional ubiquitin-binding domain (UBD) of optineurin. Compared to wild type optineurin (WT), the M98K mutant showed stronger colocalization and better interaction with TfR. It impaired the trafficking of TfR as shown by reduced uptake of transferrin and larger size of M98K-containing vesicles compared to wild-type optineurin. Lysosomal degradation is involved in maintaining cellular TfR levels. Overexpression of M98K but not WT or E50K reduced cellular TfR levels specifically in RGC-5 cells dependant on its UBD. The degradation of TfR by M98K is mediated by lysosomal pathway and not by proteasomal pathway. Coexpression of TfR or supplementation of media with an iron donor or inhibition of TfR degradation reduced M98K induced cell death.
Conclusions: M98K variant of optineurin reduces cellular TfR levels through lysosomal degradation and causes death selectively in retinal ganglion cells providing a molecular mechanism for its association with etiopathogenesis of glaucoma.

ABSTRACT OP-44

Rate of Visual Field Progression in Medically and Surgically Treated Glaucoma Patients

Madhulata Umesh, A Uday Kumar, Harsha B L Rao, S Sirisha, A K Mandal, G Chandrasekhar
VST Glaucoma Services, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To compare the rates of visual field (VF) progression in medically and surgically treated glaucoma patients.
Methods: Data of consecutive primary glaucoma patients treated either medically (272 eyes of 192 patients) or surgically with trabeculectomy (62 eyes of 48 patients), and with ≥5 Visual field examinations during the follow-up were analyzed. Rates of progression of a new global index, visual field index (VFI) in medically vs surgically treated eyes were compared.
Results: Median mean deviation of surgical group at baseline (-15.9 dB) was worse than ($p=0.01$) medical group (-9.4 dB). Median intraocular pressure over the follow-up was lesser ($p<0.001$) in the surgical group (14.1 mm Hg) than medical group (15.9 mm Hg). Median rate of progression of VFI in surgical group (-0.43% per year) was similar ($p=0.63$) to that in medical group (-0.44% per year).
Conclusions: Rates of progression of VF damage were similar in medically and surgically treated glaucoma patients.

ABSTRACT OP-45

Rasch Analysis of the Glaucoma Quality of Life (GQL-15) Questionnaire

Vijaya K Gothwal,[1] Shailaja P Reddy,[1] Seelam Bharani,[1] Deepak K Bagga,[1] Rebecca Sumalini,[1] Garudadri Chandrasekar,[2] Harsha B L Rao,[2] Sirisha Senthil,[2] Vanitha Pathak-Ray,[2] Anil K Mandal[2]
[1]Meera and L B Deshpande Centre for Sight Enhancement, Vision Rehabilitation Centres, [2]VST Centre for Glaucoma Care, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Previous psychometric evaluation of the Glaucoma Quality of Life questionnaire (GQL) focused on classic assessments of reliability and validity. Our aim was to investigate the psychometric properties of the GQL and its four subscales in a glaucoma population using Rasch analysis.

Methods: 220 patients with glaucoma (mean age, 59.6 years) recruited from the glaucoma clinic of a tertiary eye care centre were administered the 15-item GQL. Rasch analysis was used to investigate the following properties of the GQL and its subscales: measurement of a single construct (unidimensionality), discrimination among strata of patient ability (person separation) and targeting of item difficulty to person ability.

Results: The GQL discriminated four strata of patients. However, some items did not contribute towards measurement of a single construct indicating a secondary dimension. This comprised 2 peripheral vision and 1 dark adaptation/glare item. Elimination of these 3 items resulted in GQL being a unidimensional measure. However, further item deletion was required as 2 items did not measure the same construct. The resultant 10-item measure was unidimensional and the items were reasonably well to the patient ability (-0.94 logits). All the subscales were dysfunctional. There was a significant relationship between visual disability assessed using the GQL and high-contrast visual acuity ($r=0.32$, $p<0.0001$), contrast sensitivity ($r=-0.35$, $p<0.0001$) and mean deviation of better eye ($r=-0.44$, $p<0.0001$).

Conclusions: The reduced 10-item GQL has good psychometric properties and is unidimensional. The GQL is essentially a measure of visual disability and can be used as an outcome measure in glaucoma interventions.

Aberrant Expression and Regulation of Calpain 3 (*CAPN3*) and Transglutaminase 2 (*TGM2*) in Human White Mature Cataract

Rajkumar Sankaranarayanan, Mamidipudi R. Praveen, Harsha Tripathi, Alpesh Patel, Devarshi Gajjar, Anshul Arora, Anuradha Pal, Forum Kayastha, Darshini Ganatra, Abhay R Vasavada
Iladevi Cataract and IOL Research Centre, Ahmedabad-380052, Gujarat, India.

Purpose: To analyze the expression and regulation of calcium dependant protease calpain 3 *(CAPN3)* and transglutaminase 2*(TGM2)* in white mature cataracts.
Methods: Lens and LECs were collected from cataract patients undergoing extra capsular cataract extraction and phacoemulsification surgical procedure respectively. Total RNA from LECs was isolated using TRIZOL method. Level of expression of calpain *(CAPN3),* and transglutaminase *(TGM2*) were analyzed by RT-PCR. Total and free calcium concentration was analyzed by colorimetric and fluorimetric techniques. Level of calpain activity was analyzed by spectrophotometric and zymographic methods. Profile of soluble and insoluble proteins was analyzed by Glycine-SDS PAGE. Immunoblotting and Immunofluorescence cytochemistry was carried out to analyze vimentin and cross-linking of vimentin with beta-crystallin.
Results: RT-PCR analysis revealed aberrant expression of CAPN3, and TGM2 in WMC. Protein profiling revealed excessive degradation of soluble proteins in WMC. Immunoblotting analysis showed degradation of vimentin in all type of cataracts. Immunofluroresence analysis revealed vimentin cross-linked with beta crystallin in LECs of WMC. There was significant increase in total and free intracellular calcium concentration in LECs of WMC ($p<0.01$). In lens of WMC the total calcium concentration was significantly higher. Calpain activity was found to be higher in both cortical and nuclear region of other types of cataract than WMC.
Conclusions: The increased level of total and intracellular free calcium levels, increased calpain activity, vimentin degradation and vimentin-beta crystallin cross link in LECs of WMC provides sufficient clue for the pathogenesis. However, their definite role in pathogenesis of WMC could be further evaluated with huge sample size.

ABSTRACT BS-2

Quantification and Characterization of Melanin Pigment Isolated from Pathogenic Fungi Causing Keratitis

Anuradha Pal, Devarshi Gajjar, Nutan Badgujar, Bharat Godadra, Abhay Vasavada
Iladevi Cataract and IOL Research Centre, Gurukul Road, Ahmedabad-380052, Gujarat, India.

Purpose: Melanins are implicated in the pathogenesis of several human diseases, including some microbial infections. The present study was designed to understand the properties and characterization of melanin from fungal isolates causing keratitis.
Methods: Ten fungal species were used in the present study. All the fungi in the present study were isolated from the infected cornea, from the eyes having keratitis. Studies of extracellular and intracellular melanin were carried out by method of Gadd (1982). The nature of melanin pigment and quantification was done by spectral properties. Characterization of melanin was also done by studying the effect of two inhibitors Kojic acid and tricyclazole on growth and melanin production of fungus in the solid media at different concentration of inhibitors.
Results: Melanin pigment extracted from the *Aspergillus* spp (n=3) and *Exserohilum* spp (n=1) species answered positively to all chemical tests that are characteristics property of melanin pigment. UV spectra of most of the species resemble the spectra of standard melanin. The quantity of melanin was found maximum in *Aspergillus* spp (211.5µg/ml). In *Curvularia* spp extracellular melanin was absent. In 8 species [Aspergillus (n=3), *Curvularia* (n=3), *Exserohilum* (n=1) and *Cladosporium* (n=1)] melanin production was inhibited by tricyclazole indicating the DHN pathway. *Aspergillus niger* (n=1) was inhibited by kojic acid indicating DOPA pathway. In *P. phoenicicola* melanin synthesis was inhibited by both the pathways.
Conclusions: All fungal isolates showed the presence of melanin either intra or extra cellular. Characterization studies showed that the type of melanin in high number of isolates was DHN.

ABSTRACT BS-3

Trichostatin-A Prevents Transforming Growth Factor Mediated Changes in Lens Epithelial Cells: *In Vitro*

Darshini A Ganatra, Mudra Kansara, Devarshi Gajjar, Kaid Johar, Abhay Vasavada
Iladevi Cataract and IOL Research Center, Gurukul road, Ahmedabad- 380052, Gujarat, India.

Purpose: To determine the effect of Trichostatin–A (TSA); a Histone deacetylase (HDACs) inhibitor on Transforming growth factor- β (TGFβ) induced epithelial mesenchymal transition (EMT) of lens epithelial cells (LECs).
Methods: LECs from clear lenses of cadaver eyes (n = 4) were allowed to grow in a 12 well plate containing EMEM-10% FBS. On 80% confluency, cells were serum starved for 24 hours and then treated with 1μM TSA or 10ng/ml of TGFβ or TGFβ and TSA (T+T group) or left untreated as control for 24 hours. Cells were then collected for histological analysis, western blotting, immunofluorescence localization of αSMA, E-cadherin and β-catenin, and for analyzing DNA damage by comet assay. Two tailed student's t-test for independent samples was used for comparison of data sets and $p<0.05$ was considered significant.
Results: Immunofluorescence revealed loss of cell membrane proteins; E-cadherin and β-catenin on TGFβ treatment with very few cells showing positive staining at cell membranes. But treatment of TSA helped in preserving these proteins at cell membrane. TGFβ treated cells developed lamellipodia-like protrusions and αSMA was localized more at cell membrane but showed weakest staining on TSA treatment. Co-treatment of TSA and TGFβ led to heterogenous distribution of αSMA with no lamellipodia-like protrusions. Western blotting confirmed increased αSMA level in TGFβ group and cleavage of β-catenin. TSA lead to increased DNA damage ($p<0.001$) in LECs compared to control as well as TGFβ.
Conclusions: TSA helps in inhibiting TGFβ induced EMT by maintaining cell membrane proteins, E-cadherin and β-catenin, decreasing αSMA, and increasing DNA damage.

ABSTRACT BS-4

Response of Lens Epithelial Cells to Injury

Forum Kayastha, Rinal Chaudhary, Devarshi Gajjar, Abhay Vasavada
Iladevi Cataract and IOL Research Centre, Gurukul Road, Ahmedabad-380052, Gujarat, India.

Purpose: To study the changes in the expression of proteins: vimentin, filamentous actin (f-actin), proliferating cell nuclear antigen (PCNA), alpha smooth muscle actin (α-SMA), β- crystallin and matrix metalloproteinase-2 (MMP2) by immunofluorescent staining in human lens epithelial cells (hLECs) after cataract surgery (*in situ*) and in cultured hLECs (*in vitro*) after injury.
Methods: hLECs after cataract sugery (n=18) were collected from Iladevi Cataract and IOL Research Centre, Ahmedabad. hLECs (n=4) obtained from cadaver eyes were cultured and wounded. Cells were harvested and media was collected at 0, 1, 2, 4 hours respectively. Both *in situ* and *in vitro* groups were subjected to immunofluorescent localization of above mentioned markers. Extracellular MMP 2 was detected by gelatin zymography in *in vitro* group.
Results: Results showed increased expression of vimentin and f-actin at the leading edge of the cells at the site of injury with time in *in vitro* and *in situ* groups. Expression of α- SMA increased with time and it showed distributed pattern in cytoplasm in *in vitro* group, while it was localized at leading edge of cells in *in situ* group. Expression of MMP 2 increased with time in intracellular and extracellular conditions. PCNA was localized after 2 hours in *in vitro* group and was not detected *in situ*. No significant change was observed in the expression of β-crystallin within 4 hours of injury.
Conclusions: In immediate response to injury, hLECs start expressing vimentin, f-actin, PCNA, α- SMA to promote migration, proliferation and EMT. Increased expression of MMP 2 promotes cell motility and wound contraction.

ABSTRACT BS-5

Utility of Routine Conventional Light Microscopic Examinations in the Diagnosis of Microsporidial Keratitis

M Jayahar Bharathi[1], *R Ramakrishnan*[2], *V Anitha*[2]
[1]Dept. of Microbiology and Molecular Biology, [2]Cornea and External Eye Diseases, Aravind Eye Hospital & Postgraduate Institute of Ophthalmology, Tirunelveli, Tamil Nadu-627001, India.

Purpose: To describe the utility of conventional light microscopic examinations in the diagnosis of microsporidial keratitis.
Methods: Patients case records of four cases of microsporidial keratitis diagnosed between July 2010 and February 2011 were reviewed retrospectively for microbiological evaluation. A diagnosis of infectious keratitis was kept in a mind, corneal scrapings were performed using standard protocol and the material obtained was subjected to direct microscopic examinations (such as 10% KOH wet mounting, and Gram's, Giemsa's and 1% acid fast stainings) and culture (on sheep's blood agar, chocolate agar, Sabouraud's dextrose agar and non-nutrient agar).
Results: All inoculated culture media showed no growth after seven days of the incubation. While, the 10% KOH wet mounting of corneal scrape showed numerous, large, oval to round refractile bodies, unlike inflammatory cells which appear irregular, often clustered in groups of Microsporidial spores. The Gram-stained smear showed numerous; large, oval to round, bluish purple in color though some remained unstained spores of Microsporidia often clustered in groups. The Giemsa-stained smear revealed large, oval to round, bright purple color Microsporidial spores and are further confirmed by 1% acid fast stain, that demonstrated microsporidia as large, oval to round, bright red color spores against a bluish background, often clustered in groups.
Conclusions: Simple light microscopic techniques, such as 10% KOH wet mounting, Gram-staining and 1% acid-fast staining are remains the standard test for rapid identification of microsporidia and are recommended in all clinics without exception for establishing timely treatment.

ABSTRACT BS-6

Biophysical Characterization of Human Myocilin and Purification of C-Terminal Domain

K. Rangachari[1], P J Eswari Pandaranayaka[1], J. Jeyalaxmi[1], Prasanthi Namburi[2], P Sundaresan[2], S R Krishnadas[3], S Krishnaswamy[1]
[1]Dept. of Genetic Engineering, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu, India; [2]Department of Genetics, Aravind Medical Research Foundation, Madurai, Tamilnadu, India; [3]Glaucoma clinic, Aravind Eye Hospital, Madurai, Tamilnadu, India.

Purpose: To study, using biophysical methods the human myocilin and the C-terminal region purification.
Methods: Myocilin (55 kDa mol wt, 504 aa, Swissprot Q99972) is localized both intracellularly and extracellularly at multiple sites and may exert diverse biological functions. Based on a model of myocilin built by us, four regions have been identified. These regions are N-term, coiled coil, hinge and C-term. The C-term region contains the olfactomedin domain. This region has been cloned in *E.coli* for over expression and purified in soluble form.
Results: In order to study the structural features of the olfactomedin domain containing region we have over expressed it in *E.coli* by using pET-20b+ vector, purified, optimized refolding conditions. Biophysical studies have been done on full length human myocilin which has also been purified.
Conclusions: The C-term region of Myocilin has been purified and characterized biophysically and needs to be further studied. The characterization of the full length myocilin using spectroscopic and light scattering studies suggests the presence of beta sheet region and the tendency to aggregate.

ABSTRACT BS-7

Glaucoma Database

J Jeyalaxmi[1] , K Rangachari[1], P J Eswari Pandaranayaka[1], Prasanthi Namburi[2], P Sundaresan[2], S R Krishnadas[3] , S Krishnaswamy[1]
[1]Dept. of Genetic Engineering, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamilnadu, India; [2]Department of Genetics, Aravind Medical Research Foundation, Madurai, Tamilnadu, India; [3]Glaucoma clinic, Aravind Eye Hospital, Madurai, Tamilnadu, India.

Purpose: To develop a user-friendly medium of data retrieval on genes related to Glaucoma and sequence analysis.
Methods: The database is constructed using SQL as a back end and html is used to display the web page. Perl language is used to fetch the data from sql to html.
Results: *MYOC*, *OPTN*, *CYP1B1* and *WDR36* are the important candidate genes. Nearly 4% of the glaucoma patients have mutation in any one of these genes. Mutation in any of these genes causes disease either directly or indirectly and the severity of the disease varies according to position of the genes. We have compiled all the related mutations and SNPs in the above genes and developed a database.
Conclusion: This database was developed to help access statistical and clinical information on particular mutation that cause glaucoma.

Bioinformation. 2011; 5(9): 398–399. Published online 2011 February 7

ABSTRACT BS-8

P16^{INK4A} as a Predictor of HPV in Ocular Surface Squamous Neoplasia

S Chauhan[1], S Sen[1], A Sharma[2], S Kashyap[1], N Pushker[3], R Tandon[3], S Ghose[3]
[1]Department of Ocular Pathology, [2]Microbiology and [3]Ophthalmoplasty services Dr. Rajendra Prasad Centre for Ophthalmic sciences, All India Institute of Medical sciences, New Delhi 110029, India.

Purpose: Ocular Surface Squamous Neoplasia (OSSN) encompasses a spectrum of conjunctival, limbal or corneal neoplasias which include dysplasias and invasive squamous cell carcinoma (SCC). p16^{INK4A} is a cyclin dependent kinase inhibitor involved in cell cycle regulation and is overexpressed in HPV infected cervical, head and neck cancers. In the present study we have correlated the expression of p16^{INK4a} with the presence of HPV in OSSN.
Methods: Twenty cases of OSSN (6 exenterations and 14 biopsies) diagnosed between 2008-2010 were included in the study. Immunohistochemistry for p16^{INK4a} was performed on formalin fixed paraffin embedded sections using mouse monoclonal antibody (clone-6H12) .HPV L1 capsid region was detected by multiplex PCR using PGMYO9/11 consensus primers. The clinical and histopathology features were also recorded. The results of p16^{INK4a} were correlated with presence of HPV.
Results: The mean age of the patients was 57 years (SD ±12.6) with male preponderance (2.5:1). Histopathology revealed 5 cases to be dysplastic lesions and 15 SCC. HPV was detected in 3/15 (20%) cases of SCC (2 poorly differentiated and one well differentiated). p16^{INK4a} expression was seen in 4/15 (27%) cases of SCC. Of the 3 cases positive for HPV, 2 cases showed p16^{INK4a} expression (66%). All the dysplastic lesions were found to be negative for p16^{INK4a} expression and HPV.
Conclusions: Our preliminary results suggest that p16^{INK4a} expression in OSSN may prove to be useful in suspecting HPV infection. Further studies on a larger cohort need to be done to determine the utility of p16^{INK4a} expression as a biomarker for HPV in OSSN.

ABSTRACT BS-9

Clinical and Genetic Evaluation in Patients with Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES)

Bhavna Chawla[1], Yogesh Bhadange[1], Rima Dada[2], Manoj Kumar[2], Neelam Pushker[1], Mandeep S Bajaj[1], Mahesh Chandra[1], Supriyo Ghose[1]
[1]Dr Rajendra Prasad Centre for Ophthalmic sciences, AIIMS, [2]Department of Anatomy, AIIMS, New Delhi, India.

Purpose: To study the clinical and genetic features in patients with BPES in Indian population.
Methods: Patients with BPES who presented between March 2009 to April 2011 were evaluated. All patients underwent detailed clinical workup and ocular investigations. Genetic study included cytogenetic and molecular analysis.
Results: Twenty cases and twenty age matched control enrolled. Mean age at presentation was 12 y (Range 4-32 y). Strabismus was noted in 40%, refractive error in 95% (oblique astigmatism in 55%), amblyopia in 60% cases. No significant difference was observed between cases and controls in Axial length, Keratometry, AC depth, Lens thickness, Pachymetry, Colour vision, Contrast sensitivity and Visual evoked response ($p<0.05$). Pedigree analysis shows disease inheritance in four cases. On karyotyping 25% harbored chromosomal abnormalities. These included 46XY;del(3qter), 46XY;del(3q26.3), 46XX;del(3q24-25) and 46XY;del(3q26q-ter). One novel mutation was detected on molecular analysis. Cytosine to Thymidine change was found at codon position 1635 of exonic region.
Conclusions: BPES is associated with a high incidence of refractive error, strabismus and amblyopia. This study suggests that it is possible that there are other loci other than *FOXL2,* which may regulate development of eyelids in Indian population.

ABSTRACT BS-10

Elevated Levels of Serum Oxidized LDL-antibody in Diabetic Retinopathy

K Kaviarasan[1], M Jithu Mohanlal[1], S Barathi[1], Mohammad Arif Mulla[2], Vishal R Raval[2], U N Das[3], N Angayarkanni[1]
[1]Biochemistry and Cell Biology Department, Vision Research Foundation, Chennai-600006, [2]Shri Bhagwan Mahavir Vitreo Retinal Services, Medical Research Foundation, Chennai-600 006, [3]School of Biotechnology, Jawaharlal Nehru Technological University, Kakinada-533 003, India.

Purpose: Enhanced oxidative stress contributes to the pathogenesis of diabetic retinopathy. The aim of this study is to investigate oxidative stress, lipid profile and oxidized LDL antibody (oxLDL-Ab) levels in patients diabetics, patients (Type 2-DM) with non proliferative (NPDR) and proliferative diabetic retinopathy (PDR) compared to the healthy controls
Methods: N^{ε}-hexanoyllysine (HEL), a marker of oxidative stress and oxLDL-Ab were measured by ELISA in serum of patients with Type 2DM (n=8) NPDR (n =9) and PDR (n =8) and normal control subjects(n =9). The clinical details and routine biochemical investigations were documented for all the cases and controls that were age and sex matched.
Results: A significant increase in the serum levels of ox LDL-Ab, was seen in the PDR cases (30.1±19.78 U/mL) compared to the control (15.14±6.42 U/mL; p=0.025). The serum concentration of HEL in PDR group (25.72 ± 6.9 nmol/mL) was significantly higher than controls (18.82±6.77 nmol/mL; p=0.046). In DM and NPDR a progressive raise in the levels of these parameters were seen but was not statistically significant. Though a signficant increase was seen in VLDL levels and triglyceride levels in the PDR group, a significant positive correlation was seen only between serum total cholesterol and serum oxLDL levels in the PDR cases (p=0.0003; r= 0.81).
Conclusions: Higher levels of HEL and oxLDL-Ab were seen in PDR compared to control. These results suggest that augmented oxidative modification of LDL in diabetes may contribute to the disease progression. OxLDL-Ab can serve as a prognostic marker for diabetic retinopathy.

ABSTRACT BS-11

Increased Thiolation and Homocysteinylation of Proteins are Responsible for Protein Damage in Eales Disease

M Bharathselvi, N Angayarkanni, S Ramakrishna, K N Sulochana
Department of Biochemistry and Cell Biology, Vision Research Foundation, Sankara Nethralaya, Chennai – 600006, India.

Purpose: Eales disease is an idiopathic condition of eyes mainly affecting young adult males and characterized by inflammation, vascular occlusion, neovascularization and recurrent vitreous haemorrhages. Since homocysteine is implicated in vascular occlusion. our objective was to measure the extent of thiolation and homocysteinylation of plasma proteins in patients with Eales Disease.
Methods: Blood samples were collected from patients and healthy volunteers of the same age group of 20 – 40 years with approval from Institutional Ethic Board and consent from the participants in this study (Test, n=20 and control, n=15).Thiolated, homocysteinylated protein were estimated by RP-HPLC after OPA derivitization.Thiolactone was estimated by ion-exchange HPLC. Total antioxidant capacity (TAC) and Thio barbituric acid reactive substances (TBARS) were measured by Spectrophotometer.
Results: Our results show 7 fold increases in thiolactone, 2 fold increased homocysteinylated and thiolated proteins in patients of Eales disease when compared to control subjects. Oxidative stress assessed in terms of TBARS showed elevation with a lowered TAC in Eales group compared to control subjects.
Conclusions: Homocysteine-thiolactone is a reactive metabolite causes protein homocysteinylation which alters the protein function is already observed by Jakubowski *et al*. This is the first report on elevated thiolactone, thiolated and homocysteinylated protein in ocular disease with retinal vascular occlusion, neovascularization and inflammation. Thus, increased Homocysteine alter the protein function by homocysteinylation and thiolation of proteins.

ABSTRACT BS-12

Proteomic Analysis of Arsenic Toxicity on Lens Proteins of *Labeo rohita*

Soma Bhattacharjee, S Samanta, A P Sharma and B P Mohanty
Biochemistry Laboratory, Central Inland Fisheries Research Institute, Barrackpore, Kolkata, India.

Purpose: Arsenic is a ubiquitous environmental contaminant and a potent human carcinogen. It has been reported that the arsenic inhibits the growth and induces apoptosis of human lens epithelial cells. In the present study we have used proteomic approach to identify specific lens proteins which are affected by arsenic toxicity.
Methods: Juveniles of Indian major carp (IMC) *Labeo rohita,* were exposed to arsenic (sodium meta-arsenite, $NaAsO_2$) at different concentrations (0, 5, 10, 15, 20, 25 ppm), for 10 days. The soluble lens protein extracts, from both control and arsenic exposed fish lens were separated by 2-D gel electrophoresis. αA-crystallins were identified by 1- and 2-D immunoblot analysis. Peptide mass fingerprinting (PMF) of specific spots of interest, from 2-D gels, were carried out by MALDI-TOF-Mass spectrometry followed by database interrogation.
Results: Exposure to arsenic (>20ppm conc.) led to development of cataract. Proteomic analysis of lens proteins revealed that the intensity of several proteins including βA2, βA4, βA2b, βB2, small heat shock protein and skeletal α-actin significantly decreased in arsenic exposed fish lens indicating generalized damage to the lens proteins and decrease in the expression level. Fifteen discrete αA-crystallins spots seen on 2-D Immunoblot in normal lens, decreased at different levels of exposure. αA-crystallins appeared severely degraded at high concentration (>20ppm) of arsenic.
Conclusions: Arsenic exposure altered the protein expression profile of the lens; αA-crystallins which are molecular chaperons were severely affected and at ≥ 20ppm these protein were completely damaged. This is the first report showing arsenic exposure increases the risk of cataract, which is of predictive value for the endemic population of arsenic-contaminated areas, who have got life-long exposure to arsenic through food chain, albeit at lower doses.

ABSTRACT BS-13

Screening of *RPE65* Gene in Southern Indian Cohort of LCA Patients

Anshuman Verma[1], Vijayalakshmi Perumalsamy[2], Sundaresan Periasamy[1]
[1]Department of Genetics, Aravind Medical Research Foundation, Madurai, India; [2]Department of Pediatric Ophthalmology, Aravind Eye Hospital, Madurai, India.

Purpose: Leber congenital amaurosis (LCA) is a most severe form of visual impairment found in infants and children. At present around 15 genes have been reported for disease causing mutations. The *RPE65*, one of these genes accounts for around 6% of LCA cases globally. The genetic spectrum of *RPE65* mutation is essentially required in support of emerging gene therapy treatment. Therefore, in order to know the mutational spectrum of *RPE65* in southern Indian cohort we screened 15 clinically well diagnosed LCA cases. We also tried to find out the frequency of the identified mutation.
Methods: Direct PCR and sequencing were used to screen 14 exons along with the immediately flanking intronic sequences of *RPE65* gene in 15 LCA cases. In addition 100 ethnically matched healthy control samples were sequenced to validate the pathogenic nature of identified mutation. The frequency of identified mutation was checked by sequencing the exon harboring the mutation in replicative group of 25 LCA and 25 juvenile Retinitis Pigmentosa (RP) patients.
Results: A novel homozygous thymine insertional mutation at c.361 (c.361insT) in exon5 was identified in one patient. This insertion was not detected in 100 control samples. Parental DNA analysis demonstrated the mutation in heterozygous condition. This change predicts a frame shift at position 120th amino acid onwards leading to truncated protein formation of *RPE65.* The replicative group of 25 LCA and 25 juvenile RP patients did not show the presence of c.361insT mutation indicating it as a rare variant.
Conclusions: To the best of our knowledge the c.361insT mutation has not been described before. This novel c.361insT mutation seems to be rare event. Our results contribute in LCA mutation spectrum.

ABSTRACT BS-14

Evaluation of Human Lacrimal Gland Cultures for Secretory Function and Putative Stem Cells

Shubha Tiwari[1], Mohammad Javed Ali[2], Murali Mohan Sagar Balla[1], Milind N Naik[2], Santosh G Honavar[2], Vijay Anand P Reddy[2], Geeta K Vemuganti[1,3,4]
[1]Sudhakar and Sreekant Ravi Stem Cell Biology Laboratory, [2]Ophthalmic Plastic Surgery, Orbit & Ocular Oncology, [3]Ophthalmic Pathology Services, L V Prasad Eye Institute, Hyderabad. India; [4] School of Medical Sciences, University of Hyderabad, Hyderabad, India.

Purpose: Dry eye syndrome is a chronic disabling disease caused by functional disruptions in the lacrimal gland. The current treatment involves palliative ocular surface lubrication and rehydration. Cell therapy involving replacement of the gland is a promising alternative for long-term relief to patients. This study aimed to establish functionally competent human lacrimal gland cultures *in–vitro* and explore the presence of stem cells in the native gland and established cultures.
Methods: Fresh human lacrimal gland from patients undergoing exenteration was harvested after IRB approval. The freshly isolated cells were evaluated for expression of stem cell markers ABCG2, high ALDH1 levels and c-kit. Cultures were established on Matrigel, collagen and HAM and these investigated for the presence of stem cell markers and differentiating markers of epithelial (E-cadherin, EpCAM), mesenchymal (Vimentin, CD90) and myoepithelial (α-SMA, S-100) origin. The conditioned media was tested for secretory proteins (scIgA, lactoferrin, lysozyme) by ELISA.
Results: Native human lacrimal gland on flow cytometric analysis showed the expression of ABCG2, high ALDH1 and c-kit. Lacrimal gland cultures formed a monolayer, in order of preference on Matrigel, collagen and HAM within 15-20 days, containing a heterogeneous population of stem-like and differentiated cells. The epithelial cells formed 'spherules' with duct-like connections. The levels of secretory proteins in the conditioned media were significantly higher than the negative controls ($p < 0.05$ for all comparisons) indicating ongoing differentiation.
Conclusions: The study reports the novel finding of establishing functionally competent human lacrimal gland cultures *in-vitro*. It also provides preliminary data on the presence of potential stem and duct-like cells in the fresh and *in-vitro* cultured human lacrimal gland, which warrant further studies. These significant findings could pave way for cell therapy in future.

ABSTRACT BS-15

NIS Expression in Retinoblastoma

Jaisy Samuel[1,2], *S Krishnakumar*[2], *Archana Mukherjee*[3]
[1]Deakin University, [2]Department of Ocular Pathology, Vision Research Foundation, Sankara Nethralaya, 18, College Road, Chennai 600 006, Tamil Nadu, India; [3]Radiopharmaceuticals division, RLG building, Bhabha Atomic Research Centre, Mumbai 400085, Maharashtra, India.

Purpose: In many cancers, radiotherapy is sensitive because of Sodium Iodide Symporter. This study was conducted to identify the presence of Sodium Iodide Symporter (NIS) in retinoblastoma. It was first determined whether NIS is expressed in retinoblastoma and then whether NIS reactivity correlates with tumor aggressiveness.
Methods: NIS presence was evaluated by immunohistochemistry in 20 retinoblastoma tumors using the anti-NIS mouse monoclonal antibody. NIS reactivity was correlated with invasion and differentiation of the tumors. Flow cytometry, Immunofluorescence and Western Blot were also performed to confirm the NIS expression in the RB tumors and Y79 cells
Results: Among the 20 tumors, Immunohistochemistry and Flow cytometry revealed that NIS reactivity was significantly higher in the invasive than the noninvasive tumors. Blotting in RB samples showed a 25kD band apart from a faint 50kD band. Immunofluorescence revealed strong membrane positivity in Y79 cells.
Conclusions: In conclusion we found that NIS is expressed in RB but it is not clear to what extent NIS is active in these tissues. The detailed study of NIS molecule at RNA and protein level, its functionality and the fate of transported iodine are required before NIS may be targeted for therapeutic use.

Support: Board of Research in Nuclear Sciences, Bhabha Atomic Research Centre, Grant No. BARC/2009/35/17/BRNS

ABSTRACT BS-16

Presence of Hypoxic Regions in Human Retinoblastoma: A Possible Role in Hypoxia Regulated Multi Drug Resistance

Job Sudhakar[1], *Nalini Venkatesan*[1], *K Vikas*[2], *J Biswas*[1], *S Krishnamumar*[1]
[1]Department of Ocular Pathology, Vision Research Foundation, [2]Department of Vitro Retinal services, Medical research foundation, Sankara Nethralaya, 18 College Road, Chennai 600 006, Tamil Nadu, India.

Purpose: To Investigate the Presence of hypoxia in human retinoblastoma tumors to rule out its role in hypoxia induced drug resistance and correlate with clinicopathological features
Methods: We have evaluated the expression of HIF-1a and BNIP3 by immunohistochemistry in 44 archival retinoblastoma (RB) specimens. The tumors were divided into 2 groups: Group A tumors (n =22 tumors) with no invasion and Group B tumors (n =22 tumors) with invasion of the choroid, optic nerve, and/or orbit. The expression of HIF-1a and BNIP3 were correlated with invasion/differentiation of the tumors. The presence of Hypoxia is validated using Carbonic anhydrase IX; a hypoxia marker, in 35 tumors of similar cohort.
Results: The overall expression of HIF-1a was observed in 37/44 tumors(84%). Among the 22 low risk tumors HIF-1a was expressed in 86% (19/22) tumors and among 22 high risk tumors, HIF-1a was observed in 81% (18/22) tumors. Similarly BNIP3 was 93% (41/44) in RB tumors. Among the 22 low risk tumors BNIP3 was observed in 95 % (21/22) tumors and among 22 high risk tumors BNIP3 was observed in 90% (20/22) tumors. We observe no significant correlation of HIF - 1a and BNIP3 expression with invasion and differentiation ($p>0.005$). Unaffected retina, and retina from healthy donor showed negative expression for HIF-1a.
Conclusions: Hypoxia is associated with resistance to radiation therapy and chemotherapy, and is also associated with poor outcome regardless of treatment modality in many cancers, indicating that it might be an important role in drug resistance. There are reports of hypoxia in murine retinoblastoma tumors contributing to therapy resistance. This is the first report of hypoxia in a large cohort of primary RB tumors. We observed an overall expression of HIF-1a expression in 84% of tumors; we did not find any correlation between low risk and high risk tumors. The absence of HIF-1a expression in unaffected retina makes HIF-1a a potential target. Further investigations are in progress to understand the molecular mechanisms of hypoxia related drug resistance in retinoblastoma.

Support: Indian Council of Medical Research (ICMR), Grant No: 5/4/6/2/2008/NCD-II.

ABSTRACT BS-17

Characterization of Human Ciliary Pigmented Epithelial (CPE) Cells

Mangalangi U, Savitri Maddileti, Indumathi Mariappan
Sudhakar and Sreekanth Ravi Stem Cell Biology Laboratory, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To culture and characterize the Ciliary Pigmented Epithelial (CPE) cells and to check for their potential to dedifferentiate into retinal cell types *in vitro.*
Methods: Human cadaveric donor eyeballs were collected. The CPE cells isolated from one eyeball was subjected to culture, whereas CPE cells from the other eyeball was used for RNA isolation. RNA was also isolated from the Retinal Pigmented Epithelial (RPE) cells and Retinal cells. Reverse transcription PCR (RT-PCR) was done to check for the relative gene expression patterns of some of the key regulatory factors and signaling molecules like the Chx10, MITF, Nestin, Pax6, LEF1 and BMP7.
Results: The pigmented cells of the ciliary epithelium adhered and underwent cell proliferation in culture. Growing cultures of CPE was established both by adherent and suspension culture methods. The pigmented cells gradually lost their pigmentation upon expansion and showed capacity to form neurospheres in suspension cultures. Adherent cultures showed some differentiating neural-like cells. These cells have to be further validated for marker gene expression. RT-PCR results revealed that BMP7 and MITF expression is high in RPE cells. Retina showed higher levels of Chx10 and Nestin expression as expected. Interestingly, CPE cells also expressed MITF and BMP7 similar to the levels in RPE cells and also retained some moderate levels of Chx10 expression, suggesting that CPE cells share a common genetic program.
Conclusions: CPE cells could be induced to proliferate in culture using suitable growth factors and they appear to share a common gene expression signature with the retinal and RPE cells.

ABSTRACT BS-18

Outcome of Cultured Oral Mucosal Epithelial Transplantations (COMET) in the Treatment of Bilateral Severe Limbal Stem Cell Deficiency

Subhash Gaddipati[1], Muralidhar Ramappa[2*], Indumathi Mariappan[1], Geeta K Vemuganti[1,3], Virender S Sangwan[2]*

[1]Sudhakar and Sreekanth Ravi Stem Cell Biology Laboratory, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India; [2]Cornea and Anterior Segment Services, Hyderabad Eye Institute, Hyderabad, India; [3]Hyderabad Central University, Hyderabad, India.

Purpose*:* To assess the outcome of cultured oral mucosal epithelial cell transplantation (COMET) in the treatment of patients with bilateral limbal stem cell deficiency (LSCD).
Methods: After IRB approval and informed consent, autologous oral mucosa was harvested and cultured on denuded human amniotic membrane (hAM) using xenobiotic-free culture medium. After surgical debridement of ocular surface of affected eye, the patients were then transplanted with cultured oral mucosal epithelial cells on hAM. Patients were followed up and evaluated for ocular surface integrity, symptomatic relief and visual recovery.
Results: The mean age of 16 patients (18eyes) who received COMET for severe bilateral limbal stem cell deficiency was 28.5±11.25 years (18-48years). Majority (80%) of them had suffered acid injuries. The mean duration from the time of injury to COMET treatment was about 17.5 months. One case underwent penetrating keratoplasty for further visual rehabilitation at 11 months following COMET; the corneal button provided histology proof of integration and stratification of the transplanted oral epithelium on corneal surface. The average time required for re-epithelization after COMET was 7±4 days. The clinical prognosis was encouraging in terms of symptomatic relief in 62% of the cases, marginal improvement of vision in about 45% of the treated eyes. The postoperative complications were stromal melting with corneal perforation (1), preexisting secondary glaucoma (5). Some degree of superficial and peripheral corneal neovascularization was also observed in all the eyes.
Conclusions: COMET is safe in severe bilateral LSCD and offers symptomatic relief with marginal visual recovery, and obviates the need for immunosupression. Peripheral vascularization seen in all cases warrants further studies to understand the pro and anti-angiogenic signaling between native avascular cornea and vasculared oral epithelium.

*Contributed equally to the work.

Plasma Polymer Coated Therapeutic Contact Lenses for Expansion and Delivery of Limbal Epithelial Cells

Savitri Maddileti[1], Indumathi Mariappan[1], Suet Peng Low[2], Karl David Brown[3], Mark Daniell[3], David Steele[2], Dorairajan Balasubramanian[1], Virender S Sangwan[1]
[1]Sudhakar and Sreekanth Ravi Stem Cell Biology Laboratory, Hyderabad Eye Research Foundation, LV Prasad Eye Institute, Hyderabad, India; [2]Mawson Institute, University of South Australia, Adelaide, Australia; [3]Centre for Eye Research in Australia, Melbourne, Australia.

Purpose: To produce surface modified therapeutic contact lenses for the culture and delivery of corneal epithelial cells for the treatment of limbal stem cell deficiency.
Methods: Silicone hydrogel contact lenses coated with acrylic acid (AA) and 1-7 Octadiene (OCT) monomers in ratios of 100% AA, 75:25 AA:OCT, 50:50 AA:OCT, 25:75 AA:OCT and 100% OCT were used. Applicability of these lenses for culturing human corneal epithelial cells was tested by explant and suspension culture methods using immortalized HCE cells and primary human limbal epithelial cells. Cell culture parameters like cell adhesion, migration and proliferative properties were assesed. Efficiency of cell transfer from the contact lens surface onto the extracellular matrix (ECM) coated surfaces and a corneal wound bed model was also tested.
Results: HCE cells adhered and proliferated well on all surfaces containing the hydrophilic acid groups, while the hydrophobic and uncoated surfaces did not allow cell adhesion and the cells formed non adherent and floating cell clumps. Total cell viability checked by MTT and Alamar Blue assay showed that the number of adhered and viable cells decreased with the increasing concentrations of octadiene. Similar trend was observed with the suspension cultures of primary human limbal epithelial cells, which also stained positive for the corneal epithelial marker, CK12. Explant culture showed an increase in impedence for cell migration at the growth zone with the increase in octadiene concentration. Transfer experiments on ECM coated surfaces and on corneal organ culture model revealed that the acidic surfaces promoted better cell transfer.
Conclusions: Plasma polymers providing acid functional groups like the acrylic acid supported better cell adhesion, migration, proliferation and transfer of corneal epithelial cells. This suggests that these plasma polymer coated contact lenses can be used as synthetic carriers for the culture expansion of limbal epithelial cells and for their transfer on to the corneal wound bed.

ABSTRACT BS-20

Genetic Screening of Genes Involved in Leber's Congenital Amaurosis (LCA) in Indian Patients

C Neeraja Sharma[1], *Subhadra Jalali*[2], *Chitra Kannabiran*[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Smt Kannuri-Santhamma Centre for Vitreo-Retinal Diseases, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Leber Cogenital amaurosis (LCA), the most severe form of inherited disorder is one of the main causes of blindness in children due to the retinal degeneration within one year of age. Research exploring the molecular mechanisms underlying LCA pathogenesis led to the identification of fourteen genes involved in early onset of retinal degeneration. We aimed to screen for the mutations in one of the fourteen candidate genes, *TULP1* (Tubby-like protein 1) in LCA patients, because mutations in the *TULP1* gene are known to be associated with LCA and autosomal recessive RP.
Methods: Probands with LCA were recruited with informed consent (n = 130). Patients were clinically evaluated and diagnosis was made according to pre-defined criteria. Additionally, 75 normal subjects were enrolled who served as controls. Genomic DNA was isolated according to standard protocol. The *TULP1* gene was screened in probands using PCR and direct sequencing.
Results: Preliminary screening of coding regions of *TULP1* led to the identification of 5 reported variations in patients. The reported variations include two intronic homozygous changes-IVS 5+26 C/T, IVS 8-62 G/A and 3 missense heterozygous variations-Ile259Thr (ATA/ACA), Lys261Asn (AAG/AAC), Lys269Gln (AAA/CAA) in exons 5 and 8 respectively.
Conclusions: This will be the first study on *TULP1* gene screening in LCA patients in Indian population. Screening of the *TULP1* gene i this patient cohort is in progress.

ABSTRACT BS-21

The del443ins54 (*ARMS2*) Variation in an Indian Cohort with Age-Related Macular Degeneration

Saritha Katta,[1] Inderjeet Kaur[1],Rajeev R Pappuru,[2] Raja Narayanan,[2] Annie Mathai,[2] Ajit B Majji[2], Subhabrata Chakrabarti[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Kannuri Santhamma Centre for Retina and Vitreous, LV Prasad Eye Institute, Hyderabad, India.

Purpose: The *ARMS2/HTRA1* (10q26) has been implicated as a major susceptibility locus for AMD. Single nucleotide polymorphism (SNP) in the coding region of *ARMS2* (A69S; rs10490924) and promoter of *HTRA1* have exhibited strong associations with AMD worldwide. Subsequently, an insertion-deletion (indel) polymorphism (del443ins54; EU427539) in the 3'UTR that affects mRNA stability was shown to be associated with an increased risk of AMD. We aimed to determine if the del443ins54 variant conferred additional risk of AMD in an Indian cohort, wherein, the *ARMS2* (rs10490924) and *HTRA1* (rs11200638) SNPs were previously implicated with the disease.
Methods: The del443ins54 was screened in clinically well characterized cases of AMD (n=227) and normal controls (n=206) by a combination of agarose gel electrophoresis and resequencing techniques. Estimates of Hardy Weinberg equilibrium, allele, genotype and haplotype frequencies, linkage disequilibrium (LD) and their odds ratios were calculated using the PLINK and Haploview softwares.
Results: There were no deviations from Hardy Weinberg equilibrium for these SNPs in the controls. The del443ins54 variant was strongly associated ($p=1.78x10^{-13}$) with AMD (OR=2.80, 95%CI, 2.12-3.70) compared to the *HTRA1* variant. But the major risk detrminant was the A69S SNP ($p=1.84x10^{-15}$; OR=3.06, 95%CI, 2.31-4.04), which was in tight LD with the indel variant (D'=0.87, 95%CI, 0.80-0.90). Two and three locus haplotype analysis could not dissociate the indel for any additional risk contribution in AMD.
Conclusions: Our data confirms the involvement of the del443ins54 variant with AMD in an Indian cohort. However, it does not confer any additional disease risk compared to the A69S variant.

ABSTRACT BS-22

Comparison of Limbal Epithelial Cell Growth on a Synthetic Polymer Scaffold and Human Amniotic Membrane (hAM)

Charanya Ramachandran[1], Pallavi Deshpande[2], Farshid Sefat[2], Indumathi Mariappan[1], Dorairajan Balasubramanian[1], Anthony J Ryan[3], Geeta Vemuganti[4], Sheila MacNeil[2], Virender Sangwan[1]

[1]Sudhakar and Sreekant Ravi Stem Cell Lab, L V Prasad Eye Institute, Kallam Anji Reddy Campus, L V Prasad Marg, Hyderabad, India; [2]Tissue Engineering Group, Department of Engineering Materials, Kroto Research Institute, University of Sheffield, Sheffield, United Kingdom; [3]Department of Chemistry, Univeristy of Sheffield, Sheffield, United Kingdom; [4] School of Medical Sciences, University of Hyderabad, Hyderabad, India.

Purpose: To evaluate the suitability of a synthetic biodegradable PLGA (co-polymer of poly-lactic and -glycolic acid)-based polymer for culturing limbal epithelial cells as an alternative to the use of hAM.

Methods: After obtaining IRB approval and informed consent, suspension and explant cultures of human and rabbit limbal epithelial cells were established on hAM and PLGA scaffolds. The influence of different extracellular matrix proteins and fibrin on cell outgrowth from the explants on PLGA was also determined. Cultured cells were characterized by immunolabeling using markers to identify differentiated (Cytokeratin 3/12) and stem cell populations (p63, ABCG2). A corneal organ culture model was employed to study the transfer of cultured cells from scaffolds onto the denuded corneas.

Results: Limbal cultures could be established by suspension and explant cultures on hAM and PLGA in 14 days. Explant outgrowth on hAM was much better than on PLGA but the addition of fibrin and other extracellular matrix proteins improves outgrowth on the synthetic scaffold. Immunostaining revealed the presence of both stem cells and differentiated cells similar to that reported in hAM. Preliminary results show cells cultured on PLGA can be successfully transferred onto the denuded ex vivo cornea in 2 weeks.

Conclusions: Preliminary data suggests that PLGA provides sufficient support for the proliferation and migration of limbal epithelial cells with the maintenance of stem cell population. Similar to hAM, PLGA scaffold along with the cultured cells could be transplanted on the human cornea in an organ culture system, suggesting its suitability for future clinical application.

ABSTRACT BS-23

Variations at the 7q31 Locus Harbouring *CAV1* and *CAV2* are not Associated with Primary Glaucomas in an Indian Population

Kollu N Rao,[1] Inderjeet Kaur,[1] Sirisha Senthil,[2] Anil K Mandal,[2] Harsha BL Rao,[2] Garudadri Chandrasekhar,[2] Muralidhar Ramappa,[3] Sunita Chaurasia,[3] Virender S Sangwan,[3] Subhabrata Chakrabarti[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]VST Centre for Glaucoma Care, [3]Department of Cornea and Anterior Segment, LV Prasad Eye Institute, Hyderabad, India.

Purpose: Recently, a genome-wide association study implicated variations on 7q31 near *CAV1* and *CAV2* genes in primary open angle glaucoma (POAG) in large and diverse cohorts of Caucasian and Asian origin. We tried to replicate this association in an Indian cohort comprising cases of POAG and primary angle closure glaucoma (PACG).
Methods: Seven single nucleotide polymorphisms (SNPs) harbouring regions in and around *CAV1* and *CAV2* (rs4236601, rs4730742, rs8940, rs1052990, rs10227696, rs10258482 and rs926198) were screened in clinically well characterized cohorts of POAG (n=196), PACG (n=111) and ethnically matched normal controls (n=270). Screening was accomplished by a combination of resequencing and PCR-based restriction digestion. The data were analyzed using appropriate statistical softwares. The magnitude of effect of the major SNP rs4236601 on POAG susceptibility was ascertained by a meta analysis of 12 cohorts with 4429 cases and 38,015 controls.
Results: All the 7 SNPs were in Hardy Weinberg equilibrium among the normal controls ($p>0.05$). There were no significant differences in the allele or genotype frequencies of these SNPs between the normal controls and cases of POAG and PACG and none of them withstood Bonferroni correction ($p<0.007$). Meta analysis indicated a moderate evidence for association between rs4236601 and POAG (Pooled OR=1.22, 95%CI, 1.11-1.34).
Conclusions: The present study did not indicate any involvement of the 7q31 SNPs in POAG and PACG in an Indian population. The results indicated that the rs4236601 SNP was very rare in Asian populations and its effect was largely due to its high frequency among individuals of European descent.

ABSTRACT BS-24

A Heterodimeric Secretoglobin of Lacrimal Gland Origin is Present Male-Specifically in Body-Fur of Syrian Hamsters

Mahabub P Mohammad, Wenson D Rajan, Ramesh B Kasetti, Ved P Dubey, Prabir K De
Centre for Cellular & Molecular Biology, Hyderabad, India.

Purpose: Lacrimal gland (LG) and tears contain pheromones, which can be volatile or non-volatile. In mice, some sex-specifically expressed proteins in LG and tears are contact-pheromones (Nature, 466, 118-22, 2010) and these are additionally present in body-fur but whether these fur proteins are of LG origin, is uninvestigated. Upon encounter, hamsters nuzzle each other's facial region and fur, which might also contain protein-pheromones. LG extracts of male hamsters contain a ~30-35 kDa smear-like banding major protein (GP-30) and a similar protein is detectable in their body-fur extracts. This study investigates, i) presence of GP-30 in tears of male hamsters and its presence in LG, tears and body-fur of females, ii) investigates whether GP-30 in fur is of LG origin, and also iii) identifies the protein by purifying and characterizing it.
Methods: Tears, LG and fur from male, female and LG-ablated males were assessed for presence of GP-30 in stained-SDS-PAGE gels or Western blots. Purified GP-30 from male LG was subjected to N-terminal sequencing, peptide mass fingerprinting, glycan and CD analysis.
Results: GP-30 is abundantly present in LG and tears of hamsters in both sexes. Although, GP-30 is present in fur of males, it is undetectable in females. GP-30 is obliterated from fur of LG-ablated males. Purified GP-30 separates into small (non-glycosylated) and large (N-glycosylated) subunits in reducing SDS-PAGE; the latter binds the lectins, MAA and DSA but not GNA, SNA or PNA and was markedly shortened by N-glycosidase-F treatment. CD analysis showed that GP-30 is a α-helical protein. Proteomic analysis revealed that GP-30 was identical to a previously cloned heterodimeric protein of secretoglobin family of unknown function, expressed in hamster parotid gland (J Biol Chem. 277, 233-42, 2002).
Conclusions: GP-30 is present in LG and tears in both sexes of hamster. GP-30 is a highly N-glycosylated, disulfide-linked heterodimeric secretoglobin. Although GP-30 in male fur is sourced from LG, it is unclear why the protein is undetectable in female fur. The male-specific presence of lacrimal GP-30 in fur suggests a sex-related function for this protein.

ABSTRACT BS-25

Evaluation of Cancer Stem Cells in Human Y79 Retinoblastoma Cell Line

Rohini Nair,[1,2] Murali M S Balla,[2] Chitra Kannabiran,[3] Santosh G Honavar,[4] M J Ali,[4] Imran Khan,[5] Ravi K R Kalathur,[5] P Kondaiah,[5] Geeta K.Vemuganti[1,2]
[1]School of Medical Sciences, University of Hyderabad, Hyderabad, [2]Ophthalmic Pathology Laboratory, L.V.Prasad Eye Institute, Hyderabad, [3]KallamAnji Reddy Molecular Genetics Laboratory, L.V.Prasad Eye Institute, [4]Ocular Oncology Services, L.V.Prasad Eye Institute, [5]Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, India.

Purpose: Evaluation of Cancer Stem Cells (CSCs) in primary tumours is not only of academic interest but of potential therapeutic application. However, with the limitation of clinical samples and animal models, some of the studies need to be done in cell lines. This study attempts to evaluate the CSC properties in RetinoblastomaY79 cell line.
Methods: Phenotypic characterization of Y79 cells was done by FACS using putative CSC markers (CD133, CD90 and CD44). The tumor cells were sorted using CD133 and analyzed for differential gene expression (using RT-PCR, qPCR and Microarray), cell cycle statusand clone forming ability.
Results: Y79 cells expressed stem cell genes (Oct-4, Nanog, ABCB1 and Bmi-1) and differentiated retinal cell surface markers (CD133 and CD90). $CD133^-$cells showed high expression of PROX1, Oct4, Bmi-1, Pax6 and Nanog. Gene expression profile showed deregulation of Purine metabolism pathway (p=0.009), TGF-beta pathway (p=0.009), p53 signalling (p=0.017), Jak-Stat pathway (p=0.047), cytokine-cytokine receptor interaction pathway (p=0.034) and oxidative phosphorylation pathway (p=0.012) in $CD133^-$ cells. Several embryonic stem cell genes (HoxB2, HoxA9, Sall1 and Lefty) and neural stem cell genes (ABCB1, ABCB5, Mushashi 2 and Bmi-1)were upregulated in these cells. Majority of $CD133^-$ cells (83.3±4.1%) were in G_0-G_1 phase as against $CD133^+$ cells, which were mostly (81.1±4.1%) in S/G_2/M phase. Clone forming ability of $CD133^-$ cells was 23±4.3% whereas $CD133^+$ cells showed 9.3±0.5%. Expansion of $CD133^-$ clone showed gradual increase of CD133 expression -12.4±0.55, 14.4±1.1%, 34.5±0.1 to 38.8±1.3% at respective passages.
Conclusions: The study confirms the presence of CSCs in Y79 cell line using the potential marker CD133. This also supports our previous data on Retinoblastoma primary tumor cells that $CD133^-$cells appear to have CSC properties, i.e. primitive gene expression, drug resistance genes, quiescent in nature, better clone forming ability, and differentiation.

ABSTRACT BS-26

DNA Sequence Based Identification of Nonsporulating Molds Isolated from Ocular Infections

Praveen Kumar Balne[1], *Savitri Sharma* [2], *Ashok Kumar Reddy*[5], *Suma Nalamada*[1], *Chitra Kannabiran*[3], *Prashant Garg*[4]
[1]Jhaveri Microbiology Centre, L.V. Prasad Eye Institute, Hyderabad, India; [2]Ocular Microbiology Services, L V Prasad Eye Institute, Bhubaneswar, India; [3]Kallam Anji Reddy Molecular Genetics Laboratory, [4]Cornea and Anterior Segment services, L V Prasad Eye Institute, Hyderabad, India; [5]GHR Micro Diagnostics, 145 Dwarakapuri colony, Punjagutta, Hyderabad, India.

Purpose: Nearly 16-20% of both hyaline and dematiaceous fungi, isolated in ocular microbiology laboratories, remain unidentified owing to absence of specific sporulation in the medium used routinely for their isolation. The purpose of this study was to determine the species of nonsporulating fungi isolated from ocular infections in our laboratory.
Methods: 131 nonsporulating molds isolated from patients with ocular infections from January 2009 to March 2011were included in the study. DNA was extracted from the fungal cultures after 2 - 4 weeks of incubation. PCR and DNA sequencing were done by using the panfungal primers targeted the internal transcribed spacer (ITS) region of the rRNA gene.
Results: Out of 131 fungal isolates 110 isolates were identified to species level and 21 were identified to genus level. All identified fungal isolates fell in 35 genera, of which 17 isolates belonged to the genus *Lasiodiplodia* and 12 belonged to its teleomorph *Botryosphaeria*; *Fusarium* 12 and its teleomorphs *Nectria* 4 and *Gibberella* 3; *Bipolaris* 6, *Curvularia* 3 and its teleomorph *Cochliobolus* 19; *Colletotrichum* 5 and its teleomorph *Glomerella* 1; *Aspergillus* 6, *Macrophomina* 5, *Pythium* 4, *Edenia* 4, *Pleosporaceae* 3, *Penicillium* 3, *Phialemonium* 2, *Cladorrhinum* 2, *Scytalidium* 2, *Corynespora* 2, *Thielavia* 2, *Rhytidhysteron* 1, *Neocosmospora* 1, *Engyodontium* 1, *Septogloeum* 1, *Myrothecium* 1, *Tetraplosphaeria* 1, *Dothideomycete* 1, *Neodeightonia* 1, *Graphium* 1, *Neoscytalidium* 1, *Periconia* 1, *Pezizomycotina* 1, *Cladosporium* 1 and *Chaetomium* 1.
Conclusions: In absence of specific spores, PCR-based DNA sequencing technique targeting ITS region can be used for species level identification of nonsporulating fungi.

ABSTRACT BS-27

Molecular Genetic Analysis of Norrie Disease Pseudoglioma (*NDP)* Gene and Tetraspanin 12 (*TSPAN12*) Gene in Indian Familial Exudative Vitreo Retinopathy (FEVR) Patients

Ganeswara Rao Musada[1], Subhadra Jalali[2], Padmaja K Rani[2], C Anupama Reddy [3], G Pramod Reddy [3], Subhabrata Chakrabarti[1], Inderjeet Kaur[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, Prof Brien Holden Eye Research Centre, Hyderabad Eye Research Foundation, [2]Smt Kanuri Santhamma Retina Vitreous Centre, L V Prasad Eye Institute, Kallam Anji Reddy Campus, [3]Fernandez Hospital, Hyderabad, India.

Purpose: Familial exudative vitreo-retinopathy (FEVR) is a genetically heterogenous disorder characterized by impaired development of retinal vasculature. Norrin β-catenin signalling is the recognized pathway responsible for the disease phenotype. The present study explored the relative role of Norrie disease pseudoglioma (*NDP*) and Tetraspanin 12 (*TSPAN*12) genes, which encode for ligand, norrin and a ligand-frizzled4 receptor complex facilitator protein, TSPAN12 respectively, in Indian FEVR spatients.
Methods: A total of 110 unrelated clinically well characterized FEVR cases (61 familial and 49 sporadic) with different stages of FEVR, their available family members and 100 normal controls were enrolled in the study with a prior informed written consent. Genomic DNA was isolated from peripheral leukocytes. The entire coding region and 5' and 3' untranslated regions of *NDP* and *TSPAN*12 genes were analyzed by polymerase chain reaction based direct sequencing. The variations identified were further assessed for segregation patterns and genotype-phenotype correlation.
Results: Taken together 16 different mutations were identified in *NDP* and *TSPAN12* genes in 21 different families, of which 13 were novel. The novel changes included 6 missense, 3 frame shift, 3 intronic and one UTR mutation. The six novel missense mutations were predicted to be pathogenic as they replaced highly conserved amino acids in the functional domains of the proteins with a SIFT score < 0.005. Variable expressivity of mutations was observed in some of the families.
Conclusions: *NDP* and *TSPAN12* gene mutations were identified in 12% and 7% of the FEVR cases respectively indicating their potential role in FEVR pathogenesis in the Indian patients.

ABSTRACT BS-28

Identification of Disease Genes in Indian Population with ARRP by Homozygosity Screening

Nishika Sahini[1], Hardeep Pal Singh[1] Gayathri Mohan[1], Subhadra Jalali[2], Chitra Kannabiran[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Smt Kannuri-Santhamma Centre for Vitreo-Retinal Diseases, Hyderabad Eye Research Foundation, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Retinitis Pigmentosa (RP) belongs to a clinically and genetically heterogenous group of retinal dystrophies. Genes involved with RP show autosomal dominant, autosomal recessive (ARRP) or X-linked inheritance pattern. Till date 40 genes are known to cause recessive RP. To identify genes involved in ARRP, SNP genotyping on a 6K genotyping array followed by homozygosity mapping was done. The present study aims to screen candidate genes in these homozygous regions to detect the pathogenic changes.
Methods: Blood samples from families with ARRP cases were collected after IRB approval and informed consent. Diagnosis of RP was made after complete ophthalmic evaluation of all family members SNP genotypes were evaluated for homozygous regions shared among all affected individuals. Known RP genes mapping to these intervals were screened by PCR-based amplification of coding regions and direct sequencing. The detected variations in sequence were further genotyped in family members and 75 controls by PCR-RFLP/sequencing.
Results: Candidate genes- *MFRP*, *TULP1*, *NR2E3*, *GNAT1*, and *PROM1* were screened for mutations in 5 families based on corresponding regions of homozygosity. We identified a novel homozygous complex rearrangement of 2 bp deletion + 25 bp insertion in exon2 p.Arg47GlnfsX65 in *NR2E3*, a large deletion from exon8 to exon25 in *PROM1*, a single base pair missense substitution Asn349Lys in *TULP1* and a single base deletion c.493delC (Tyr164TyrfsX26) in *MFRP* genes were observed which co-segregated with the disease. Three novel intronic variants IVS5+49 T/G, IVS7-16delG and IVS7-14 T/C in *GNAT1*; reported variations in *NR2E3* (2) and *PROM1* (6) were also observed.
Conclusions: There is a need to further learn about the other genes and loci involved in causing ARRP. The present study shows that homozygosity mapping is a useful way of identifying disease genes in families with consanguinity.

ABSTRACT BS-29

Quantitative Analysis of Plasma proteins in Patients with Age Related Macular Degeneration

Saika Siddiqui[1], Inderjeet Kaur[1], Saritha Katta[1], Rajiv K Reddy[2], Raja Narayan[2], Annie Mathai[2], Subhabrata Chakrabarti[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Smt. Kannuri Santhamma Centre for Vitreo Retinal Diseases, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Several genes involved in complement cascade, extra cellular matrix modeling, oxidative stress and cholesterol metabolism pathways have been implicated in age-related macular degeneration (AMD) The present study aimed to evaluate the expression of these genes in plasma samples in order to understand their potential role in AMD pathogenesis.
Methods: Plasma (3ml) were collected from patients with different stages of AMD (n=60) and age adjusted cataract controls (n=60) having no systemic history of diabetes, hypertension and any long illness with prior written informed consent, aliquoted and stored at -80°C. The concentrations of 13 proteins involved in neuro-degeneration, extracellular matrix modelling, angiogenesis and inflammatory pathways; a2-Macroglobulin, Apolipoprotein A1, Apolipoprotein C3, Apolipoprotein E, Complement Component 3 (C3), Complement Factor H (CFH), Prealbumin and Tissue Inhibitor of Metalloproteases (TIMPs) were evaluated in pre-diluted plasma samples (1:1000) using multiplex bead immunoassays based on powerful Luminex xMAP technology. Differences between levels of these proteins were analyzed using appropriate statistical tests.
Results: All the 13 analytes were detectable in the plasma samples. Patients with AMD exhibited significant elevations in C3 ($p=0.0001$), while CFH was relatively higher in the controls ($p=0.023$). Among the ECM-related proteins, TIMP1 exhibited a higher concentration in AMD ($p=0.023$). The other proteins did not exhibit significant differences in the plasma of patients and controls ($p>0.05$).
Conclusions: Significant alterations in C3 and TIMP1 in AMD patients suggest that abnormal immune activity and ECM components may contribute to the disease pathogenesis. The present data on C3 also corresponds with the genomic association of *C3* with AMD.

ABSTRACT BS-30

A Study on an Epidemic of Acute Keratoconjunctivitis in Chennai: Isolation of a Novel Human Adenovirus Identified Based on Phylogenetic Analysis

M K Janani, J Malathi, H N Madhavan
L and T Microbiology Research Center, Sankara Nethralaya, Old no 18, New no 24 , College Road, Chennai -600006, India.

Purpose: To identify the causative agent of epidemic conjunctivitis outbreak in Chennai in 2010.
Methods: Seventeen patients (30 specimens) with conjunctivitis were subjected to virological investigations. Culture and PCR for detection of Adenovirus and Enterovirus was carried out. PCR Positive products were further subjected for DNA sequencing. The nucleotide sequences of the hexons of isolates were analyzed by comparison with all 51 human Adenovirus strains. Phylogenetic tree was constructed using DAMBE software.
Results: Among 30 conjunctival swabs collected from 17 patients, 7 were positive for adenovirus by PCR on the direct specimen, none of them were positive for Enterovirus. Eleven of 30 conjunctival swabs showed cytopathic effect in HEp-2 cell line and were confirmed as HAdV by PCR. The DNA sequence data of the 11 isolates had equal percentage of homology with HAdV 6 and 2 on blast analysis. On Phylogenetic analysis with GeneBank data of 51 Adenovirus strains, 11 isolates from patients during the epidemic outbreak of conjunctivitis during year 2010 formed a separate clade indicating a new novel strain
Conclusions: Based on phylogenetic analysis it is concluded that the recent conjunctivitis epidemic that occurred in Chennai was caused by a novel adenovirus strain.

ABSTRACT BS-31

In Vitro Antifungal Susceptibility Testing by Disk Diffusion Method against Amphotericin B and Voriconazole on Ocular Isolates of Non Sporulating Moulds

L Dhanurekha, K Lily Therese, S Prasanna, H N Madhavan
L and T Microbiology Research Center, Sankara Nethralaya, Old no 18, New no 24, College Road, Chennai -600 006, India.

Purpose: To apply PCR based DNA sequencing targeting ITS region for the identification of ocular Non-sporulating moulds (NSM) isolates to species level and to apply *in vitro* antifungal susceptibility testing by disk diffusion method against Amphotericin B and Voriconazole

Methods: 20 non-sporulating moulds (NSM) isolates 15 corneal scraping, 3 corneal buttons, 1 donor corneal rim (DCR), and1 necrotic tissue collected from cornea and infected suture were included in this study. Identification of NSM was done by PCR based DNA sequencing targeting ITS region and *in vitro* antifungal susceptibility testing was performed using Disk Diffusion Method using Mueller Hinton Agar supplemented with 2% Glucose for Amphotericin B (10 µg/disc)and Voriconazole(1µg/disc).

Results: The 20 NSM ocular isolates were identified by PCR based DNA sequencing as 17 belonging to newer emerging pathogens [*Botryosphaeria species* (5)*, Lasiodiplodia* species (5), *Glomerulla singulata* (2), *Macrophomina phaseolina*(2)*, Pythium*(1), *Amylomyces rouxii*(1) *Phomopsis species*(1) and 3 was established pathogens (*Bipolaris*(1) *Paecilomyces sp*(2)]. Of 20 isolates, 16 isolates were sensitive to both Amphotericin B and Voriconazole, 2 ocular isolates from corneal buttons (*Pythium insidiosum*, *Amylomyces rouxii*) were resistant to both Amphotericin B and Voriconazole and 2 isolates of *Glomerella cingulata* from 2 corneal scrapings were resistant to Voriconazole.

Conclusions: *In Vitro* Anti fungal susceptibility testing by disk diffusion method is a rapid, reliable and cost effective method which can be adapted in clinical microbiology laboratory.

ABSTRACT BS-32

Study to Evaluate the Effect of UV Rays on Riboflavin Treated Cadaveric Corneal Limbal Stem Cells

M Vimalin Jeyalatha[1], *J Malathi* [1], *H N Madhavan* [1], *Prema Padmanaban*[2]
[1]L and T Microbiology Research Centre, Vision Research Foundation, Chennai, [2]Cornea Service, Sankara Nethralaya, Chennai, India.

Purpose: Keratoconus is a disorder of the cornea characterized by progressive thinning of the cornea resulting in irregular cornea i.e. cornea bulges forward in a conical shape. The widely used treatment modality involves the application of riboflavin phosphate eye drops to the cornea followed by the exposure of UV radiation (365nm) for duration of 30 minutes. As it is a known fact that UV light causes damage of the cells, it was proposed to study the effect of UV rays on the corneal limbal stem cells.
Methods: Ten donor eyes were collected. Biopsies were taken before treatment with riboflavin, after exposure of UV rays and from the site protected from UV rays and by metal ring. Viable cells were enumerated by trypan blue exclusion method. Biopsies were cut into bits and were placed over the Denuded amniotic membrane. DMEM/F12 mix was added to plates and was incubated in CO_2 at 37^0 C.
Results: The ten donor eyes collected for the study were subjected for biopsy. The average number of the viable cells counted before exposure was 2405 (25%), after exposure 2320 (23%) and 2765 (28%) when covered with metal ring. None of the biopsies exposed to UV rays showed outgrowth of cells.
Conclusions: Trypan blue exclusion method showed a 2% reduction in the number of viable cell count when exposed to UV rays. Preliminary study showed that the UV exposed on cadaveric corneal stem cells treated with riboflavin has harmful effect on the cells. Further specific marker studies are needed to strengthen

ABSTRACT BS-33

Structural Features and Stability of a βγ -Crystallin Domain of a Non-Lens Vertebrate Protein Crybg3

M. Rajyalakshmi, V Rajanikanth, Shanti Swaroop Srivastava, Rajan Sankaranarayanan, Yogendra Sharma
Centre for Cellular and Molecular Biology (CCMB), Council of Scientific and Industrial Research (CSIR), Uppal Road, Hyderabad-500 007, India.

Purpose: To study the structural features and stability of vertebrate non-lenticular βγ-crystallins and compare with homologues of lens βγ-crystallins. This gives insights into the modifications that occurred during their recruitment into non-lens tissues.
Methods: With known signature sequences of βγ-crystallin superfamily, BLAST search was performed. We selected a protein in human genome possessing βγ-crystallin domain annotated as hypothetical protein. Its homologue is present in *Mus musculus* genome. Total RNA was extracted and the gene corresponding to putative second βγ-crystallin domain was cloned, overexpressed, purified and crystallized. The crystal structure of this recombinent protein was solved and its stability was determined by performing various biophysical experiments and compared with other homologous crystallin domains.
Results: The second βγ -crystallin domain (90 residues) was more typical to a lens crystallin. This domain has a conventional sequence of AB type arrangement of Greek key motif typical of vertebrate crystallins and is also closely related to the βγ-crystallin domains of AIM1 in sequence. The canonical Ca^{2+}-binding sites are absent in this domain. Though this domain structurally resembles the eye lens γ-crystallin, it has a relatively moderate thermal (49°C, ΔH 1.29 x 10^4 ± 216 kJ mol^{-1}) and equilibrium stability of $C_{1/2,\ [GdmCl]}$ of 1.14 M. This domain upon unfolding retains significant tertiary structure with a considerable loss of secondary structure. During equilibrium unfolding (at sub-molar concentrations of GdmCl), the protein is precipitated indicating the aggregation of partially unfolded species, a phenomenon exhibited by its cataract-related mutants of γ-crystallin. This partially unfolded species binds Bis-ANS more strongly than native protein indicating the exposure of hydrophobic core, thus suggesting that this is an intermediate state. The far-UV CD, gel filtration, 2D [^{15}N, ^{1}H]-HET-ex-SOFAST-HMQC data reveals that this intermediate has a near native-like conformation.
Conclusions: This study provides further insight on the sequence-structure-stability relationship of various βγ -crystallin domains, and would be used to explore the mutations seen in lens βγ -crystallins. The fact that βγ -crystallin domains are also the part of diverse non-lens proteins, redefines the recruitment and evolution of lens crystallins.

ABSTRACT BS-34

Stability Tuning Knob in βγ-Crystallins and its Evolution in Vertebrate Homologues

Lahari Yeramala, Shashi Kumar Suman, Amita Mishra, Daddali Ravindra, Yogendra Sharma
Centre for Cellular and Molecular Biology (CCMB), Uppal Road, Habsiguda, Hyderabad, India.

Purpose: βγ-Crystallin domain is a highly diverse domain and has evolved to acquire high domain stability in lens homologues. The evolution of stability and its control in βγ-crystallins that possess either a canonical (mostly from microorganisms) or a non-canonical (found in vertebrate homologues) Ca^{2+}-binding motif is not known. Our goal was to understand how the stability across these members was attained and controlled.
Methods: βγ -Crystallin domains from various taxa were selected and cloned. Calculated mutations were performed in these βγ-crystallin domains within the Ca^{2+}-binding motif i.e. N/D-N/D-X_1-X_2-S/T-S. The various proteins were over-expressed and purified. Equilibrium unfolding of more than 22 wild type domains and their mutants in the apo and holo forms were performed.
Results: We observed that there exists a stability gradient across members of the superfamily (from low stable domain to high stable domain). We observed that a Ser at the 5^{th} position in the N/D-N/D-X_1-X_2-S/T-S motif is related with a higher stability as well as Ca^{2+}-binding over Thr at the same position. In the reverse case, mutation of Ser to Thr decreased the domain stability. Polar residue at the 3^{rd} position of the motif decreased the stability whereas a hydrophobic residue at the same position increased the stability of the domain. When Phe was mutated to Asp at the first position, there was an increase in the stability of the domain. Conversion of a functional motif of a microbial crystallins to a non-functional motif i.e., a vertebrate–type, by the conversion of the 5^{th} Ser/Thr to Arg/Lys augmented the domain stability. However, there was a loss of Ca^{2+}-binding as well as Ca2+-induced gain in stability. Conversely, when a non-functional motif was modified to a functional one, by mutating Arg to Ser (at the 5^{th} position) resulted in the decrease of stability.
Conclusions: Occurrence of a polar, a hydrophobic or a serine residue at the 1^{st}, 3^{rd} or 5^{th} position of the motif respectively resulted in a domain with a higher stability. Partial conversion of the microbial-type domain to the vertebrate-type domain disabled the Ca^{2+}-binding but augmented the stability of the domain. Our results demonstrate that the N/D-N/D-X-X-S/T-S motif fine-tunes the stability of a domain thus acting as a stability tuning knob and also governs the Ca^{2+}-dependent gain or loss in domain stability.

ABSTRACT BS-35

Analysis of the Serum Proteomic Patterns for the Early Detection of Diabetic Retinopathy

N Lini[1], M Valar Nila[1], T P Vignesh[2], V R Muthukkaruppan[1], K Dharmalingam[3]
[1]Dr.G. Venkataswamy Eye Research Institute, Aravind Medical Research Foundation, Madurai, India, [2]Retina Clinic, Aravind Eye Hospital, Madurai, India, [3]National Facility for clinical Proteomics, School of Biotechnology, Madurai Kamaraj University, Madurai, India.

Purpose: Diabetic retinopathy (DR), an ocular complication of patients suffering from Diabetes mellitus (DM), is a retinal vascular disorder, in which the retinal microvasculature gets affected. The aim of the study was to establish and optimize a two-dimensional polyacrylamide gel electrophoresis (2-DE) technique for serum proteomics to improve the resolution and reproducibility and to explore the same for possible biomarkers.
Methods: Patients and healthy controls, above 45 years of age of either extending Aravind Eye Hospital, Madurai were enrolled. Serum samples from each group of patients were examined, using Two Dimensional Polyacrylamide Gel Electrophoresis (2D PAGE). Proteins showing altered expression levels were then identified using nano LC MS/MS. Image analysis and statistical analysis of 2D gels was performed using Image Master Platinum 7.0 (IMP 7.0) Software (GE Healthcare).
Results: Among the 56 identified proteins, proteins including Apolipoprotein A-IV, alpha-1-antitrypsin, Haptoglobin precursor, Haptoglobin α2 chains were up regulated in PDR. The relative quantity of these proteins is less in NPDR.
Conclusions: This study provides baseline information about the serum protein profile of Diabetic Retinopathy patients of south Indian origin and this will enable us to identify a marker for the early diagnosis of retinopathy in diabetic patients. Most of the up regulated proteins are acute phase proteins involved in inflammation and may be associated with the disease progression, thereby giving fundamental information for further research. Based on our results, we propose that the analysis of serum proteins will allow us to uncover a biomarker for early diagnosis and disease progression.

ABSTRACT BS-36

Quantification of the Expression of Sex Steroid Receptors in Lacrimal Gland and Cornea in the Experimental Models of Dry Eye

S Smrita, T Velpandian, N Sharma, J S Titiyal, N R Biswas, S Ghose
Department of Ocular Pharmacology & Pharmacy, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India.

Purpose: To Quantification of the expression of sex steroid receptors in lacrimal gland and cornea in the experimental models of dry eye
Methods: Either sex of Wister rats weighing 180-250g were used. Group-A (female rats, n=5) & Group-B (male rats, n=5) served as control. Group-C rats (n=5) were ovariectomized and kept for 4 cycles. Group-D (female, n=5) and Group-E (male, n=5) were fed finasteride (1.16mg/Kg, p.o.) for 10 days. At the end of experiment, all rats were sacrificed. Lacrimal gland and cornea were enucleated. Total mRNA was isolated from above tissues and optimized for cDNA synthesis. All samples were run in triplicate and mean Ct value was taken for the quantification.
Results: All values are exhibited as compared to control. Ovariectomized rats exhibited significant down regulation of the expression of ER-α, β and androgen receptors (72.77%, 97.80% & 95.81% decrease in cornea and 97.69%, 98.47% & 99.84% decrease in lacrimal gland , $p<0.05$ for both tissues). Finasteride treated groups showed significant ($P<0.05$) down regulation in the expression of all steroid receptors in the lacrimal gland of both female and male rats.
Conclusions: Present study indicates the down regulation of sex steroid hormone receptor (ER-α & β and androgen) expression involved in the progression of sex steroid deficient experimental models of dry eye.

Support: Department of Biotechnology, Government of India.

ABSTRACT BS-37

Evaluation of Ethambutol Induced Retinal Toxicity by ERG Changes: Role of Trimetazidine

A R Vijayakumar, T Velpandian, N R Biswas, V Menon, R Saxena, S Ghose
Department of Ocular Pharmacology & Pharmacy, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India.

Purpose: To study effect of Trimetazidine against ethambutol induced retinal toxicity on rats using Electroretinogram (ERG).
Methods: White albino rats of either sex, weighing 150-225 gm were used for the study. Ethambutol (at two dose levels viz. 200mg/Kg and 400 mg/Kg, p.o.) was administered to two separate groups of rats pre-treated with Trimetazidine (3mg/Kg, i.p.). ERG recordings were taken in dark-adapted condition on day 0, day 1, day 7 and day 14 of drug treatment. ERG recordings were compared against normal, vehicle treated, and ethambutol alone treated animals in order to evaluate effectiveness of Trimetazidine against ethambutol induced retinal toxicity in rats.
Results: Ethambutol induced ERG changes (a and b wave amplitude and latency) were recorded with a help of custom made corneal electrodes in rats with both white and green light excitation. Ethambutol at the dose of 400mg decreased the amplitude and latency of both a & b waves in white light condition. In green light stimulation increased the latency of both wave forms. Trimetazidine at the studied doses significantly reduced the ERG changes induced by ethambutol in green light condition.
Conclusions: Trimetazidine showed some benefit in the ERG changes induced by ethambutol toxicity induced in rats. Further studies are in progress.

ABSTRACT BS-38

Antiangiogenic, Antioxidant and Anticataract Potential of Marine Invertebrate Species

P Gupta[1], T Velpandian[1], M Arumugam[2], R V Azad[1], R Saxena[1], N R Biswas[1], S Ghose[1]
[1]Department of Ocular Pharmacology & Pharmacy, Dr R P Centre, AIIMS, New Delhi-110029, India; [2]CAS in Marine Biology, Annamalai University, Tamil Nadu, India.

Purpose: To evaluate the antiangiogenic, antioxidant and anticataract potential of the methanolic extracts of twenty four marine invertebrate species (MIV_{1-24}).
Methods: The antiangiogenic activity of all the extracts was evaluated using Chorio-Allantoic Membrane (CAM) assay. Briefly for the CAM assay, fresh fertilized *white Leghorn* chick eggs were incubated at 37 ± 2°C in a humidified incubator. 2-3ml albumin was withdrawn on day 3 and 200µg extract was loaded on the CAM on day 7 followed by photography on day 12 with subsequent quantification of angiogenesis. *In vivo* anti-angiogenic activity was carried out using the corneal neovascularization assay in rats. The antioxidant activity was evaluated using the diphenylpicryl-hydrazyl (DPPH) radical scavenging assay and anticataract activity was evaluated using hydrocortisone induced cataract in developing chick embryo's.
Results: The methanolic extracts of MIV-3, 4, 17 and 18 showed significant ($p \leq 0.001$) antiangiogenic activity in both the tested assays whereby they profoundly inhibited the growth of new blood vessels in comparison to the controls. Among all the extracts, MIV-4, 7, 14, 15, 18, 21 and 24 showed noticeable antioxidant activity in comparison to the standard (sodium ascorbate) and among these species MIV-21 showed noticeable delay in the progression of cataract.
Conclusions: The methanolic extract of MIV-4 showed significant antiangiogenic activity as it profoundly inhibited the VEGF induced proliferation of new blood vessels. Further studies are in progress to isolate the components responsible for the observed effect.

Support: CSIR for providing SRF for this Research work.

ABSTRACT BS-39

Screening of Antiangiogenic and Antiproliferative Potentials of Marine Cynobacterial Strains

K Mahender [1], T Velpandian[2], F Tasneem[3], P Malliga[4]
[1]Integrated Institute of Technology, Dwarka, New Delhi-110077; [2]Department of Ocular Pharmacology & Pharmacy, [2]Dr. RPC, AIIMS, New Delhi; [3]Department of Bio-Sciences , Jamia Millia Islamia, New Delhi-110025; [4]Department of Microbiology, Bharathidasan University, Tirchy, Tamilnadu, India.

Purpose: To study the antiangiogenic and antiproliferative potential of different Marine Cynobacterial (MCB) strains.
Methods: Chick Chorio Allontoic Membrane (CAM) Assay: The different strains (MCB_1-MCB_{15}) were subjected for CAM assay at different concentrations using fertilized hen eggs. VEGF (50 ng) served as positive control and albumin as vehicle control. On 12^{th} day images were captured using imaging software and were analyzed. Corneal Neovascularization (CN): Albino rats weighing 100-250 g/bw were divided into two groups (n=8). Right cornea of all the rats was cauterized using 75% silver nitrate and 25% potassium nitrate at approximately 2.5mm from the corneoscleral limbus for 5 sec under anaesthesia.10 µl of MCB_5 instilled (3times/day) upto 5 days and control rats were treated with normal saline. Cornea was subjected for analysis using imaging software. Antiproliferative Assay: MTT assay was performed in HeLa Cells using 96-well flat bottomed culture plates in complete culture medium. After 24hrs incubation with test compounds cells were subjected for assay. Percent of inhibition was calculated from the mean values of experiments and the data were expressed as percentage of control.
Results: MCB_5 showed significant ($P<0.001$) antiangiogenic activity in the CAM assay. In the CN assay, the MCB_5 inhibited the growth of new blood vessels and it was found to significant ($P<0.001$) as compare to sham treated group. MCB_5 extract was showed significant antiproliferative effect compared with control group.
Conclusions: The extract of MCB_5 showed significant antiangiogenic and antiproliferative activity, it may be due to the inhibition of VEGF induced proliferation of new blood vessels.

ABSTRACT BS-40

Controlled Experimental Study on Ocular Distribution of Systemic Barbiturate: Forensic Relevance

Beauty Arora[1], T Velpandian[1], S Lalwani[1], T D Dogra[1], R Saxena[1], V K Goyal[2], S Ghose[1]
[1]Department of Ocular Pharmacology & Pharmacy, Dr. R. P. Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India; [2]Forensic Science Laboratory (FSL), Government of NCT of Delhi, New Delhi-85, India.

Purpose: To evaluate vitreous as a suitable forensic marker in an experimental study involving pentobarbitone overdosage using rabbits.
Methods: A controlled study was conducted in New Zealand albino rabbits (n=8), randomizing it into two groups: perimortem (n=4) and postmortem (n=4). In both the groups, pentobarbitone (60 mg/kg) was intravenously administered through marginal ear vein. Immediately after death of rabbits, whole blood, plasma and eyeball were collected in perimortem group and after 17 hr of death in postmortem group. Eyeball was dissected and vitreous, aqueous & ocular tissues were collected. All the samples were analyzed by LC-MS/MS.
Results: In vitreous and aqueous humor, increase in the levels of pentobarbitone was observed at postmortem as compared to perimortem. Correspondingly, in plasma and whole blood, decrease in the levels of pentobarbitone was observed at postmortem. All the ocular tissues, i.e. conjunctiva, iris, sclera, retina-choroid, cornea and lens also showed an increase in the pentobarbitone concentration at 17 hrs. Interestingly, this study found a ratio of 1:30 between postmortem vitreous and perimortem whole blood.
Conclusions: Decrease of pentobarbitone in postmortem plasma and blood concentration and its increase in vitreous humor suggest that redistribution of pentobarbitone has taken place which is getting diffused from blood to vitreous. Redistribution is further confirmed, as postmortem pentobarbitone ocular tissue levels were also found to be higher. This study demonstrates the importance of vitreous in pentobarbitone estimation in forensic investigations when the blood sample is lacking or is putrefied. Further studies are in progress regarding the mechanisms involved.

Financial support: The Directorate of Forensic Science, Ministry of Home Affairs, Government of India.

ABSTRACT BS-41

Ocular Kinetics of Extraporaneous Natamycin Formulation in *Ex-Vivo* Studies after Intrastromal Administration

A Sirohiwal, R Sharma, T Velpandian, A K Ravi, N Sharma, S Ghose
Department of Ocular Pharmacology & Pharmacy, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India.

Purpose: To study the ocular kinetics of Intrastromal injection of extraporaneously prepared natamycin formulation.
Methods: Goat eyes were procured from the slaughter house immediately after sacrifice. The eyes were transferred to the laboratory after storing in the ice packed box. The eyes were washed thoroughly with normal saline and extra ocular tissue was removed using standard technique. The eyes (n=4) were mounted in ex-vivo chamber maintained at 37ºC and anterior segment of the eyes were cannulated using custom made probe. Balanced salt solution was passed through the probe at the flow rate of 5µl /min and was allowed to stabilize for the period of 30 min. After stabilization, 20 µl of natamycin was injected intrastomally at the concentration of 20µg. The effluents were collected in the preweighed micro centrifuge tubes at different time interval of 0.5, 1, 2, 4, 6, 8 & 12 hr and were stored in -80°C till LC-MS/MS analysis.
Results: Maximum concentration (93ng/ml) of natamycin was observed at 60 min and it was maintained at a steady state level upto 12hr. The continuous monitoring of eyes showed there is no dislocation of the probe and effluents collected and weighed showed that the standard deviation of the effluent mass was within the allowable limit.
Conclusions: Present study indicates that natamycin reached maximum level in aqueous humor at 1hr and levels were maintained upto 12hr, which may be beneficial in fungal endophthalmitis.

ABSTRACT BS-42

Evaluation of Retinal Toxicity of Systemically Administered Drugs: Effect of Quinidine on Rat Using Electroretinogram

H Anuradha[1], A K Ravi[1], T Velpandian[1], R V Azad[1], J R Bellare[2]
[1]Department of Ocular Pharmacology & Pharmacy, Dr. RPC, AIIMS, New Delhi-110029, India; [2]Department of Chemical Engineering, IIT Bombay, Mumbai-76, India.

Purpose: Quinidine is a widely employed anti-arrhythmic agent known to precipitate visual disturbances. However, the cause of this visual side effect is not well understood. This study has been undertaken to evaluate retinal toxicity by observing changes in rat electroretinogram following intravitreal injection of quinidine.
Methods: Wister rats (either sex) weighing 250-300 gm have been used for this study. Rats were divided into three groups, viz. control, vehicle control and drug (Quinidine 1.6 µg /µl) treated group. Animals were dark adapted at least 30 min prior to ERG study. Scotopic ERGs were recorded after pupillary dilatation for a period of four hours under anesthesia (specially standardized) with the help of custom made & standardized gold electrode.
Results: ERG records against intravitreal quinidine were observed at 5, 15, 30, 60, 120 and 240 min time points. Changes in various components of ERG such as a-, b-, early receptor potentials, oscillatory potentials, PhNR and i-wave have been observed.
Conclusions: The present study indicates that quinidine (open state sodium channel blocker) is interfering with the photoreceptor activity and in addition might have an influence with signal conduction in the first inter-plexiform layer. Further studies are in progress correlating intraocular penetration with retinal toxicity.

ABSTRACT BS-43

Evaluation of Intraocular Penetration of a Polyherbal Formulation and its Antiangiogenic Potential

S K Patnaik, T Velpandian, P Gupta, A K Ravi, N R Biswas, S Bandhopadhya, S Ghose
Department of Ocular Pharmacology & Pharmacy, Dr R P Centre for Ophthalmic Sciences, All India Institute of Medical Sciences (AIIMS), New Delhi-110029, India.

Purpose: Herbal formulations have been implicated in the traditional Indian system of medicine for the treatment of various ocular morbidities. Therefore, the objective of the present study was to evaluate the intraocular penetration of Itone eye drops using LC-MS/MS and to evaluate its antiangiogenic potential.
Methods: Intraocular penetration of polyherbal formulation was carried out using New Zealand albino rabbits of either sex weighing 2–2.5 kg body weight. 50µl of Itone formulation was instilled into lower fornix and 50µl aqueous humor was aspirated at different time points (15, 30, 60 and 120min) via paracentesis. The samples were stored at -80°C until analysis. The antiangiogenic activity of Itone was evaluated using Chorio-Allantoic Membrane (CAM) assay and corneal neovascularization assay in rats. Briefly for the CAM assay, fresh fertilized chick eggs were incubated at 37°C in a humidified incubator. 3ml albumin was withdrawn on day 3 and 50µl of Itone (50ng) was spreaded over pre-coated VEGF coverslip that were loaded on the CAM on day 7 followed by photography on day 12 with subsequent quantification of angiogenesis. *In vivo* antiangiogenic activity was evaluated using corneal neovascularization assay in rats.
Results: Intraocular Penetration of Itone showed a maximum concentration of borneol at 30 min and curcumin at 15 min. Itone showed significant antiangiogenic activity in both the antiangiogenic assays and significantly inhibited the proliferation of new blood vessels.
Conclusions: The polyherbal eye drop showed significant intraocular penetration and antiangiogenic activity.

Support: Research grant from Deys Medical Stores, Kolkata, India.

ABSTRACT BS-44

Use of Two Molecular Markers in Combination can Identify Precisely Adult Human Corneal Epithelial Stem Cells

Tilak Prasad , C Gowri Priya, V R Muthukkaruppan
Department of Immunology and Cell Biology, Aravind Medical Research Foundation, Dr. G. Venkataswamy Eye Research Institute, Madurai, India.

Purpose: Identification of corneal epithelial stem cells (SCs) has been a major challenge due to lack of specific markers. Earlier we have established a specific method to identify and quantify SCs based on two parameter analysis (high p63 expression combined with high N/C ratio) (Arpitha et al., 2005; 2008 a,b). The present study is extended to demonstrate a simpler method to identify and quantify SCs using two molecular markers.
Methods: Cytospin smears of native limbal epithelial cells (from cadaver limbal rings) at a density of 2.5 x 10^4 cells/slide were prepared and immunostained for ABCG2 and/or Connexin 43 (Cx-43), with PI as counter stain. Images of 100 cells per slide were acquired and analyzed for (i) N/C ratio and ABCG2 (ii) N/C ratio and Cx-43 (iii) Cx-43 and ABCG2, using fluorescence microscope equipped with ISIS V 5.4.2 software.
Results: Analysis of limbal epithelial cells revealed 39.75 ± 7.5% (mean ± SD) to be high positive for ABCG2 and 12.6 ± 7.3% cells to be negative for Cx-43. These cells were characterized for N/C ratio along with either one of these markers 7.0 ± 2.9% cells were found to be ABCG2 high and N/C ratio high (>0.7) and this distinct population was found to be absent in cornea. A similar result was obtained by combining N/C ratio with Cx-43 negative cells (4.01 ± 2.5%). By combining the two molecular markers –ABCG2 high and Cx-43 negative, it was possible to identify stem cells.
Conclusions: A single marker is not sufficient to identify adult epithelial SCs. This simplified method of using two markers in combination to quantify SC content in a given population using fluorescence microscope will aid in the validation of culture conditions for transplantation in the field of regenerative medicine.

ABSTRACT BS-45

Comparison of Methods for the Detection of Methicillin Resistance and Antibiotic Susceptibility Profile of *Staphylococcus Aureus* Isolates from Ocular Infections

Sarita Kar[1], Sasmita Panda[2], Savitri Sharma[1], Durg V Singh[2], Sujata Das[1], Srikant K Sahu[1]
[1]L V Prasad Eye Institute, Bhubaneswar, [2]Institute of Life Sciences, Bhubaneswar, India.

Purpose: To compare phenotypic methods for determination of antibiotic resistance among *S. aureus* isolates from ocular infections and detection of *mecA* gene by PCR.
Methods: Forty-five out of 105 staphylococcal ocular isolates were identified as *S. aureus* by ATB™ reader (bioM'erieux). Both Etest® (AB bioM'erieux) and broth microdilution method (BD) were performed for all the isolates to determine MIC of oxacillin, chloramphenicol, ciprofloxacin, gatifloxacin, moxifloxacin, ofloxacin and vancomycin. MIC of cefazolin was determined by broth microdilution method. Methicillin resistance was further determined by disc diffusion technique using cefoxitin, oxacillin and methicillin discs (HiMedia) as per CLSI guidelines and confirmed by detection of *mecA* gene by PCR.
Results: Four of the 45 (8.9%) *S. aureus* isolates were methicillin resistant (MRSA) by BD and by the amplification of the *mecA* gene. Among the MRSA, resistance to ciprofloxacin and ofloxacin was 100% while it was 75% (n=3) to gatifloxacin by BD. Only 14.6% (n=6) of methicillin sensitive isolates (MSSA) were sensitive to gatifloxacin, ofloxacin and ciprofloxacin. Susceptibility of MRSA and MSSA to vancomycin was 100% while that of cefazolin was 75% (n=3) and 100% (n=41) respectively. Chloramphenicol and moxifloxacin showed variable results by the two methods. Detection of *mecA* gene followed by cefoxitin disc diffusion method showed highest sensitivity and specificity.
Conclusions: Prevalence of MRSA was low in this study. While vancomycin and cefazolin were effective against the *S. aureus* isolates, resistance to fluoroquinolones was high. Cefoxitin disc diffusion assay seems to be a simple yet reliable method for detection of MRSA.

ABSTRACT BS-46

Serotyping of Toxoplasma Gondii by ELISA in Ocular Toxoplasmosis Patients

B Mahalakshmi[1], K L Therese[1], N Sundar[2], M E Grigg[2], J Biswas[3], H N Madhavan[1]
[1]L and T Microbiology Research Centre, Vision Research Foundation, Chennai-6; India; [2]Laboratory of Parasitic Diseases, NIH, Maryland, USA; [3]Department of Uvea, Sankara Nethralaya, Chennai -6, India.

Purpose: To apply a serotyping ELISA to type *Toxoplasma gondii (T. gondii)* strains infecting patients with ocular toxoplasmosis.
Methods: ELISA for serotyping of *T. gondii,* (standardized by Dr Grigg ME, at the Laboratory of Parasitic Diseases, NIH, USA) was applied on 96 ocular toxoplasmosis (OT) patients' sera (male-71 and female 25 with mean age of 35years and 27years respectively) were included in the study. OT sera were first tested by ELISA against lysate of *T. gondii* (standard RH strain). Only serum samples testing positive for the *T. gondii* lysate were subjected to ELISA using polymorphic peptides of SAG1, GRA6-220 (Type I / III), GRA6-214 (type II) and GRA7 -225 (type II). Serotypes (Type I / III, Type II, and atypical: combination of all three types) was based on the positive results with one or more of the four antigens established by Kong et al., 2003.
Results: Among the 96 sera tested, anti-*T. gondii* antibodies were positive by ELISA with *T. gondii* lysate in 75(78.1%). Of the 75 positive sera, type I / III was detected in 16 (21.3%), type II in 20(26.7%), atypical in 17 (22.7%) and 22(29.35) were positive to only SAG1 peptide and could not be typed further.
Conclusions: To the best of our knowledge, this study is the first attempt in India to apply ELISA for Serotyping of *T. gondii*. Genotyping data is required from Indian isolates of *T. gondii* from which new polymorphic peptides can be derived to identify the *T. gondii* strain types circulating in Indian population.

ABSTRACT BS-47

Phenotype and Genotype of Effector Molecules of *Pseudomonas aeruginosa* Type III Secretion System (T3SS) from Human Corneal Ulcer

Lakshmi Priya Jeganathan[1], SivaGanesa Karthikeyan Rajapandian[1], Arne Rietch[2], E Pearlman[3], Lalitha Prajna[1]

[1]Department of Microbiology, Dr.G.Venkataswamy Eye Research Institute, Aravind Medical Research Foundation, Madurai, Tamilnadu, India; [2]Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH;[3]Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH USA.

Purpose: *Pseudomonas aeruginosa* is one of the common bacterial pathogen associated with human corneal infection. The Type III Secretion System (T3SS) of *Pseudomonas aeruginosa* is one of the important virulence determinants. The purpose of this study is to characterize the effector molecules of the Type III Secretary System of *Pseudomonas aeruginosa* from human corneal infections.

Methods: 50 *Pseudomonas aeruginosa* isolates from patients with corneal ulcer were used for this study. The colony PCR was performed targeting the type III secretory effector genes ExoU, ExoS, and ExoT using specific primers. For the western blot, the organisms were grown in modified LB Broth with high mineral salts and low calcium. The culture supernatant proteins were precipitated by TCA and resolved in 12% poly acrylamide gel. The proteins are transferred to NC membrane and the expression of specific effector proteins are confirmed by using specific antibodies. The antibiotic resistant pattern of the clinical isolates was determined by disc diffusion method according to the NCCLS guidelines. The clinical parameters of the infected corneal ulcer patients were correlated with the presence of T3SS effector molecule to determine the disease severity.

Results: Among the 50 isolates, 16 (32%) isolates are expressing ExoST phenotype, 7 (14%) are expressing ExoUT, and 6 (12%) are expressing ExoS, 1 (2%) is expressing ExoU, 10 (20%) are expressing ExoS phenotypes. Only one isolate expressing all the 3 effector molecules and 9 (18%) of them are not expressing any of the effector molecules. There was no statistically significance was observed between the presence of the effector molecules with that of clinical outcome of the disease. Multi drug resistant pattern was not observed in any of the isolates.

Conclusions: Majority of the clinical isolate causing keratitis are having Type 3 secretory mechanism. Better understanding of the virulence factor of these pathogens may lead to development of new therapeutic target for treating this disease.

ABSTRACT BS-48

Identification of Methicillin Resistant *Staphylococcus aureus* by Conventional and Molecular Methods and its Antimicrobial Susceptibility Pattern

Nithya Velusamy, Lalitha Prajna
Department of Ocular Microbiology, Aravind Medical Research Foundation, Aravind Eye Hospital, Anna Nagar, Madurai-625020, Tamilnadu, India.

Purpose: To compare the results of conventional and molecular methods in detection of Methicillin resistance (MR) and to determine the in vitro susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) ocular isolates to various antibiotics.
Methods: *Staphylococcus aureus* strains isolated from various ocular infections were subjected to the antibiotic sensitive test by standard disc diffusion method. The organism which is found to be resistant to oxacillin and cefoxitin were selected and screened for the presence of the *mecA* gene using specific PCR primers. The isolates were also subjected to susceptibility test to various other recommended antibiotics. The sensitivity and resistant pattern was determined as per the CLSI guidelines.
Results: 40 strains were tested for the presence of methicillin resistance. PCR analysis revealed that all these strains were positive for *mecA* gene. 35 (87.5%) of the 40 isolates were resistant to both cefoxitin and oxacillin and remaining 5 of them showed resistant to any one of these antibiotic. All the MRSA strains were found to be susceptible to linezolid, rifampin, synercid, clindamysin, and azithromycin.
Conclusions: The *mecA* gene acting as the conservative gene was carried by all MRSA strains. Detection of MR by *mecA* gene was easier and less time consuming compared to conventional methods. PCR assay was superior in identifying intermediate and heterogeneous MR in shorter duration of time. MRSA strains showed higher percentage of sensitive to linezolid, rifampin, synercid, clindamysin, and azithromycin, and these drugs can be considered as an alternative for treating ocular MRSA infections.

ABSTRACT BS-49

Molecular Genetic Analysis of Leber's Congenital Amaurosis (LCA) in Indian Patients

Rachna Shukla[1], *Subhadra Jalali*[2], *Chitra Kannabiran*[1]
[1]Kallam Anji Reddy Molecular Genetics Laboratory, [2]Smt Kannuri-Santhamma Retina Vitreous Service, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Leber's congenital amaurosis (LCA) is a congenital; severe retinal dystrophy involving both rods and cones. Till date, 14 genes have been reported for LCA, of which *Crumbs homolog 1* (*CRB1*) accounts for ~10% of LCA cases in other populations studied. The CRB1 protein plays a key role in the regulation and maintenance of cell polarity or plasticity in the retina, in intracellular and extracellular protein-protein interactions, and possibly in signal transduction. Mutations in the *CRB1* gene are known to be associated with autosomal recessive LCA. The purpose of present study was to screen the *CRB1* gene for pathogenic alterations in LCA.
Methods: Probands with LCA were recruited with informed consent (n = 130). Patients were clinically evaluated and diagnosis was made according to pre-defined criteria. Additionally, 75 normal subjects were enrolled who served as controls. Genomic DNA was isolated according to standard protocol. The *CRB1* gene was screened in probands using PCR and direct sequencing.
Results: Preliminary screening of coding regions of CRB1 in 30 probands led to the identification of 4 reported and 2 novel variations. The reported variations include IVS 2-83 A/T, IVS3-60 T/G, IVS3-36 T/C, and IVS4-53 T/G. Two homozygous novel variations (Gln1124X, Tyr1269X) were identified in exon 9 and exon 10 respectively. Both of these variations were observed in one patient each and were predicted to be pathogenic.
Conclusions: This is the first study to report *CRB1* gene variations in an Indian cohort of LCA patients. So far, *CRB1* mutations reported in other populations were not observed in our study.

ABSTRACT BS-50

Molecular Genetic and Functional Studies on Oculocutaneous Albinism

Maitreyee Mondal[1], *Mainak Sengupta*[1], *Swapan Samanta*[2], *Asim Sil*[3], *Kunal Ray*[1]
[1]CSIR-Indian Institute of Chemical Biology, Kolkata; [2]Calcutta National Medical College, Kolkata, [3]Netra Niramay Niketan, Purba Medinipur, West Bengal, India.

Purpose: Oculocutaneous albinism (OCA), which results in congenital hypopigmentation of ocular and cutaneous tissues, is reported as one of the major causes of childhood blindness in India. In continuation of our effort on the molecular genetic study on OCA we are interested to reveal the wider spectrum of tyrosinase (TYR) mutations causing OCA1A (complete lack of melanin) and OCA1B (minimal melanin with progression of age) and the molecular basis of phenotypic variability.
Methods: Thirteen patients and their first degree relatives from 11 pedigrees from eastern India were enrolled in the study. PCR-sequencing based approach was taken for screening 7 genes associated with pigmentation. To decipher the molecular basis of OCA1 pathogenesis, 23 missense mutations were introduced in normal *TYR* cDNA clone by site-directed mutagenesis, expressed in HEK293 cells, their expression levels examined by western blot analysis, dual enzymatic activities measured and retention in ER monitored by Endoglycosidase H digestion.
Results: We identified 4 different mutations in *TYR* and 1 in *OCA2*. All the OCA1A related mutations lacking TYR activity are retained in ER irrespective of their position in the protein. Interestingly, only 3 out of 14 reported OCA1B mutations were found to retain DOPA oxidase and Tyrosine hydroxylase activities and the rest were devoid of any enzymatic activity and were retained in ER.
Conclusions: Overall defects in TYR are the major cause of OCA among Indians while OCA2 defects are less prevalent. The observed lack of TYR activity in "OCA1B mutations" demonstrates need for assessment of the biological activity of the implicated variants for correlation with phenotype of the patients.

Supported by Council of Scientific & Industrial Research (CSIR). MM and MS are supported by CSIR Fellowships.

ABSTRACT BS-51

Homozygosity Mapping in Consanguineous South Indian Families with Ophthalmic Genetic Disorders

Venkata Ramana Anandula[1], V Ramprasad[2], R Shetty[1], G Kumaramanickavel[1]
[1]Narayana Nethralaya, Bangalore, India; [2]Spinco-Biotech, Chennai, India.

Purpose: To map the genes for autosomal recessive keratoconus and foveal hypoplasia families.
Methods: A consanguineous keratoconus and foveal hypoplasia families were subjected for gene mapping using Affymetrix SNP 6.0 genechip by the homozygosity mapping technique.
Results: The probable regions of linkage were chromosomes 12 and 14 and the SNPs were rs1544671 and rs3811259 respectively in keratoconus family and LOD score of 2.3 was obtained for the marker rs254347 in chromosome 16 and the disease was segregating with the haplotypes in the foveal hypoplasia family.
Conclusions: The probable regions of keratoconus gene to be in chromosome 14 and the foveal hypoplasia gene in chromosome 16 is high.

ABSTRACT BS-52

Characterization and Differentiation of Induced Pluripotent Stem Cells (iPSCs) Towards Retinal Lineages

Vasundhara Vauhini[1], Subbarao Mekala[1,2], Savitri Maddileti[1], Subhash Gaddipatti[1], Indumathi Mariappan[1]
[1]Sudhakar and Sreekanth Ravi Stem Cell Biology Laboratory, L V Prasad Eye Institute, Hyderabad, India; [2]Asian Institute of Gastroenterology, Hyderabad, India.

Purpose: To derive and characterize induced pluripotent stem cell (iPSC) lines and establish a protocol for differentiating them into retinal cell types.
Methods: Recombinant retroviruses carrying the genes for the mouse Oct4, Sox2, Klf4 and cMyc were used for deriving miPSCs from mouse embryonic fibroblasts. The reprogrammed colonies showing ES-like morphology were picked, expanded and molecular characterization was done to confirm successful reprogramming. Differentiation was initiated by forming embryoid bodies and subsequently cultured in differentiation medium supplemented with RPE conditioned medium.
Results: Using retroviral methods, miPSCs were derived at an efficiency of 0.01%. 4 clones were expanded beyond 20 passages and one of the clones (4F3) was completely characterized. The endogenous copies of all the four transgenes were upregulated in this clone and also expressed the pluripotent stem cell markers like the Oct4, Nanog, SSEA1 and the alkaline phosphatase enzyme. RAPD fingerprinting confirmed its genotype to be identical to the parental MEFs. The hypomethylated status of the nanog gene promoter confirmed the successfully reprogrammed status. Expression of different germ layer specific marker genes in the differentiated cells in EBs was confirmed by RT-PCR. On extra cellular matrix coated dishes, patches of weekly pigmented, RPE like cells were observed after 6-8 weeks. These cells were found to be positive for ZO-1 and also had phagocytic activity. Further characterization of the differentiated cells using retinal markers is in progress.
Conclusions: The mouse iPSC lines generated by us behaved like ES cells in terms of their stemness and pluripotency and were capable of differentiating into RPE-like cells *in vitro*.

ABSTRACT BS-53

Levels of Tumor Necrosis Factor-Alpha in Aqueous Humor of Patients with Primary Open Angle Glaucoma

Anju Varghese[1], Parul Saxena[1], Arpna Srivastava[1], Sandeep Goswami[1], Ramanjit Sihota[2], Vinay Gupta[2] and Jasbir Kaur[1]
[1]Department of Ocular Biochemistry, [2]Glaucoma Research Facility and Clinical Services, Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Purpose: Glaucoma, a common cause of blindness that affects millions of people, is characterized by optic nerve atrophy from progressive apoptotic neuronal degeneration secondary to elevated intraocular pressure over a long period of time. Cytokine release and oxidative stress contribute to glaucomatous degeneration. Tumor necrosis factor-alpha (TNF-α), a potent immunomodulatory cytokine, is upregulated in a patient with post-traumatic brain injury. The pathogenesis of glaucoma is a result of a neuroinflammatory process. Pro-inflammatory cytokines such as TNF-α may contribute to this process. In the present study, we have evaluated the levels of TNF-α in aqueous humor of patients with primary open angle glaucoma.
Methods: Twenty patients with primary open angle glaucoma were included in the study. Twenty age-matched cataract patients were taken as controls. Aqueous humor samples were collected at the beginning of surgery through paracentesis. Concentration of TNF-α in aqueous humor was measured by enzyme-linked immunosorbent assay.
Results: The mean TNF-α level in aqueous humor of patients with primary open angle glaucoma (2.81±1.36 pg/ml) was significantly higher than control subjects (1.54±0.49 pg/ml) ($p<0.001$).
Conclusions: TNF-α levels are elevated in aqueous humor of patients with primary open angle glaucoma as compared to control group. TNF-α may be used as a reliable biomarker in the progression of primary open angle glaucoma.

ABSTRACT BS-54

A Prospective Study on Etiology and Antibiotic Resistance Pattern of Infections of the Eye

Devarshi Gajjar, Anuradha Pal, Bharat Ghodadra, Abhay Vasavada
Iladevi Cataract and IOL Research Centre, Gurukul Road, Ahmedabad 380052, Gujarat, India.

Purpose: To find out the prevalence of bacterial and fungal ocular infections at Iladevi Cataract and IOL Research Centre (ICIRC). To test the antibiotic and antifungal sensitivity pattern of these bacteria and fungi against the most commonly used ocular antibiotics.
Methods: Consecutive patients with eye infections at the out patient department of ICIRC from the period of July 2009 to February 2011 were recruited in the study. All clinical isolates (fungal and bacterial) were prospectively collected, maintained and identified by routine microbiological tests.
Results: Total of 306 cases of ocular infections were analyzed, of which 54.54% were keratitis, 36.2% were conjunctivitis, 6.5% were endophthalmitis, 0.65% were stye and 1.63 % were of infected buckle. The predominant bacterial species isolated were Coagulase negative staphylococci, S epidermidis and Pseudomonas spp. *Fusarium* spp., *Aspergillus* spp. Followed by Curvullaria spp. were the most common fungi causing keratitis. Endophthalmitis was mainly caused by *Aspergillus* spp. and *Pseudomonas* spp. Coagulase negative Staphylococci were isolated from high number of patients (34%) with conjunctivitis. High number (84.2 and 81.6%) of coagulase negative *Staphylococcus* spp. were sensitive to tobramycin and chloramphenicol and high number of *Staphylococcus* isolates (39.5%) were resistant to ciprofloxacin. 84.6% of *S aureus* isolates were resistant to ciprofloxaxin and moxifloxacin respectively. *Pseudomonas* spp. (80%) were highly susceptible to moxifloxacin whereas, highly resistant (80%) to vancomycin and chloramphenicol. Gram negative bacilli were highly susceptible to tobramycin.
Conclusions: Results suggest high isolation rate of fungal isolates causing sight threatening keratitis and endophthalmitis in the studied geographical area of Western India.

ABSTRACT BS-55

Prevalence of Multidrug Resistant *Pseudomonas aeruginosa* in Ocular Infections

Guru Prasad Manderwad[1], *Suma Nalamada*[1], *Archana Singh*[2], *Savitri Sharma*[3]
[1]Jhaveri Microbiology Centre, Prof. Brien Holden Eye Research Centre, Kallam Anji Reddy Campus, L.V Prasad Eye Institute, Banjara Hills, Hyderabad, India; [2]Cornea and Anterior Segment Services, L.V. Prasad Eye Institute, Banjara Hills, Hyderabad, India; [3]Ocular Microbiology Services, L.V. Prasad Eye Institute Patia, Bhubaneswar, India.

Purpose: Infection with multidrug resistant Pseudomonas *aeruginosa* is highly prevalent in hospitals, and it is one of the major contributing factors for the morbidity and mortality among patients. Multidrug resistance *Pseudomonas aeruginosa* (MDRPA) are resistant to atleast three drugs of the classes such as β-lactams, carbapenems, aminoglycosides and floronquinolones. Not much of information is available regarding the presence of multidrug resistance *P. aeruginosa* in ocular infections. This study aims to determine the prevalence of drug resistant *P. aeruginosa* in ocular infections.
Methods: Microbiology records were retrospectively reviewed for all patients seen between Jan 2010 and Dec 2010.The organisms were identified by conventional biochemical tests and by Vitek 2 compact system. Antimicrobial susceptibility testing of the isolates was performed by Kirby- Bauer disk diffusion method. Sensitivity pattern of 86 *P. aeruginosa* isolates from various ocular samples (corneal scraping -69, corneal button-7, vitreous-5, pus- 3, conjunctival swab-1, Scleral buckle -1) were tested. The drugs used for the sensitivity test included amikacin, ofloxacin, gentamicin, tobramycin, ceftazidime, moxifloxacin, gatifloxacin, cefuroxime, chloromphenicol, and ciprofloxacin.
Results: Different type of ocular diseases from which *P. aeruginosa* was isolated included Microbial keratitis-57, Graft infiltrate-16, Endophthalmitis-5, Panophthalmitis-3, orbital cellulitis-2, fungal granuloma-1, Pseudomembrane conjunctivitis-1, Scleral buckle infection-1. Out of 86 isolates, multidrug resistance was observed in 35 isolates (40.6%). The pan resistance was noted in 27 out of 35 isolates. Remaining 51 isolates (59.4%) were found to be sensitive to most of the drugs tested.
Conclusions: This study reports a high prevalence of multidrug resistance in ocular infections in our hospital and highlights the importance of considering antibiotic susceptibility results of *Pseudomonas aeruginosa* prior to therapy.

ABSTRACT BS-56

Comparison of Commercial Kits - IgM Microlisa and Leptocheck with MAT in the Serodiagnosis of Leptospiral Uveitis

Arun Kannan[1], Chidambaranathan Gowri Priya[1], Sivakumar R. Rathinam,[2] and Veerappan Muthukkaruppan[1]
[1]Dr G Venkataswamy Eye Research Institute, Aravind Medical Research Foundation, [2]Uvea Clinic, Aravind Eye Hospital, Anna Nagar, Madurai - 625020, Tamilnadu, India.

Purpose: Uveitis is an important late complication of systemic leptospirosis. The gold standard technique Microscopic Agglutination Test (MAT) is less sensitive and all the commercial kits available are for early detection of acute systemic leptospiral infection. Hence the purpose of this study is to evaluate whether two such commercial kits are efficient for serodiagnosis of Leptospiral uveitis, which occurs months to years after systemic infection.
Methods: Serum samples from leptospiral uveitis patients (MAT positive -15, negative - 15), 15 non-leptospiral uveitis patients and 5 controls were selected. These samples were tested for the presence of leptospiral IgM antibodies by (i) MAT using a panel of 19 serovars, (ii) LEPTO IgM MICROLISA (J.Mitra &Co.Pvt. Ltd, India) and (iii) Leptocheck (Zephyr Biomedicals, India). The statistical analysis was carried out using stata 11.0.
Results: Comparison of the results of commercial kits with MAT as the standard revealed the sensitivity of leptocheck (80%) to be higher compared to IgM Microlisa (53.3%), while the specificity of IgM Microlisa (71.4%) is higher compared to leptocheck (51.3%). However when the results of MAT and IgM Microlisa were combined the sensitivity and specificity increased to 73.3% and 85% respectively.
Conclusions: Commercial kits though sensitive and specific for systemic leptospirosis have limited diagnostic capacity for leptospiral uveitis in our geographic location. Combination of the gold standard MAT with IgM Microlisa is a better choice for serodiagnosis of leptospiral uveitis.

ABSTRACT BS-57

Oxidative Stress Markers in Patients with Primary Open Angle Glaucoma

Anjaleena Anthony[1], *Amita Goyal*[1], *Arpna Srivastava*[1], *Ramanjit Sihota*[2], *Jasbir Kaur*[1]
[1]Department of Ocular Biochemistry, [2]Glaucoma Research Facility and Clinical Services, Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi, India.

Purpose: To investigate the levels of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in aqueous humor of patients with primary open angle glaucoma.
Methods: Twenty patients with primary open angle glaucoma and twenty age- matched cataract patients (controls) were included in the study. Aqueous humor samples were collected at the beginning of surgery through paracentesis. Activities of antioxidant enzymes were measured spectrophotometrically.
Results: A significant increase in superoxide dismutase and glutathione peroxidase enzyme activities was observed in aqueous humor of primary open angle glaucoma patients as compared to cataract patients ($p<0.001$). No significant difference was observed in the catalase activity.
Conclusions: These results suggest that oxidative stress may lead to an induction of antioxidant enzymes. Superoxide dismutase and glutathione peroxidase activities may be used as oxidative stress markers in aqueous humor of primary open angle glaucoma patients.

ABSTRACT BS-58

Seropositivity Rate Among Cornea Donors at Eye Donation Centres Attached to Ramayamma International Eye Bank

Brinda P Ramachandran[1], Usha Gopinathan[1], Prashant Garg[1,2] Virender S Sangwan[2]
[1]Ramayamma International Eye Bank, [2]Cornea and Anterior Segment, Ocular Immunology and Uveitis Services, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To study the seropositivity rate among cornea donors at Ramayamma International Eye Bank
Methods: A retrospective review of donor records of Eye Donation Centres attached to Ramayamma International Eye Bank from 2006-2010. The donor blood samples received from Eye Donation Centers were screened for HIV, HBsAg, HCV & Syphilis in accordance with the NPCB Medical standards for Eye Banking. The serological screening was done by certified technicians of the Eye Bank with NACO/NARI approved kits and the report is verified and signed by a Microbiologist of a NABL certified laboratory.
Results: Out of the total 3353 donors donated during the 5 year period, serology screening was done for 2843 donors (85%). 510 donors (18%) were not screened for the following reasons – No blood sample received (49%) inadequate blood sample (36%), and sample hemolysed (15%). 41 donors reported positive for HIV (1%), 57 donors (2%) reported positive for HBsAg, 17 donors (0.6%) reported positive for HCV and 3 donors reported positive for Syphilis Antibody.
Conclusions: The seropositivity rate among cornea donors is 4%. Roughly one fifth (18%) of donor corneas were not available for transplantation due to lack of non availability & inadequate blood sample. This warrants further investigation.

ABSTRACT CS-1

Economic Burden of Diabetes in Urban Indians

Pablo Chandra, Parikshit Gogate
Gogate's Eye Clinic, Pune, India.

Purpose: To find out the average economic burden of medical care on a patient with diabetes in Pune, India
Methods: A semi-open ended questionnaire followed by interview was conducted with patients attending diabetes and ophthalmic out patient departments. They were asked regarding the duration of diabetes, methods undertaken for blood sugar control and the amount they spend on consultations, laboratory tests, medicines and procedures if any within past year. Data was collected regarding the socioeconomic status according to Kuppaswamy classification.
Results: 219 patients participated of whom 129 were males (58.9%). The average age was 48.6 yrs. Average annual income of respondents was Rs 28064 (India's per capita income Rs 38084 in 2010). Average annual direct cost of diabetes treatment was Rs 8,822 of which 52.1% was spend on medicines, 3.2% was spend on lasers, 12.6% was spend on surgical procedures, 11.6% spent on investigations and 10.4% was spend on clinician fees. Average indirect cost was Rs 3949 of which 3.4% was spend on travelling purpose, 0.4% was spent on health classes, 4.9% was spent on diet control and 91.3% was loss of wages. Average expenditure done by lower middle class was 22.7% of their income. Average percentage of income for direct cost was 3.6% and 1.4%. The cost increased with the increase in duration of diabetes ($p=0.013$), but was not related to use of insulin ($p=0.464$) compared to oral agents.
Conclusions: Diabetes is a significant economic burden on individuals, especially in the lower middle class. The cost is recurrent and increases over years.

ABSTRACT CS-2

Causes of Blindness and Visual Impairment in Sindhudurg, Coastal Maharashtra

Shailbala Patil, Parikshit Gogate, Siddharth Vora, Sachin Ainapure, Ashok Mahadik, Anil Kulkarni
Gogate's Eye Clinic, Pune, India.

Purpose: To gauge the prevalence and causes of blindness and visual impairment in western coastal district of India.
Methods: Extended rapid assessment of avoidable blindness was conducted in the district of Sindhudurg with 0.9 million population and 2747 people were examined with stratified cluster random sampling.
Results: The mean age was 61.8 yrs with 50.6% males. Amongst those examined 1415 (51.7%) had visual acuity (VA) >6/18, 924 (33.8%) had VA 6/60-<6/18(visual impairment), 266 (9.7%) had VA <6/60-3/60 (severe visual impairment) and 132 (4.8%) had VA <3/60 (blindness by WHO standards). Blindness was more in 70 + age group and in persons who were not working. Causes of presenting VA < 6/60 were cataract 82.4%, diabetic retinopathy in 3.5%, corneal scars 9.7%, glaucoma 0.9%. 51.5% of visual impairment was due to refractive errors. Corneal scarring was more common in the elderly ($p<0.01$). 58.2% of population had access to presbyopia spectacles, but of them only 79% were N6.
Conclusions: Cataract and refractive errors remained the most important causes of visual impairment. This is the first population based study which shows no gender difference in blindness and diabetes as a significant cause.

ABSTRACT CS-3

Long Term Outcome of Pediatric Cataract Surgery in Western India

Parikshit Gogate, Shailbala Patil, Mitali Shah, Mohini Sahasrabudhe, Anil Kulkarni, G V Rao
Gogate's Eye Clinic, Pune, India.

Purpose: To study the long term outcome of pediatric cataract surgery in western India
Methods: 332 non-traumatic pediatric cataracts of 205 children operated in 2005-8 underwent a comprehensive eye examination in 2011. Demographic data, visual acuity and causes of poor outcome were recorded.
Results: The average age was 13.1 years (range 2mon-17 years, SD 5.02) and 64.2 % were male. 37.8% had best corrected visual acuity >= 6/18. 20 (8.3 %) needed more than one surgery for an eye. Only 12% had attended regular follow-up. Of those who underwent primary posterior capsulotomy and anterior vitrectomy (PPC+AV) (all aged <7yrs), 21.4% developed posterior capsular opacification (PCO); amongst those who did not 140/179 (78.2%) had PCO ($p<0.001$). Poor outcome (vision <6/60) was associated with congenital cataract ($p=0.002$), not doing primary posterior capsulotomy ($p<0.001$), but was not associated with age at surgery (0.098), delay between presentation and surgery ($p=0.32$), non-use of phaco machine (0.41) and gender (0.263).
Conclusions: Long term outcomes are good for developmental, but not congenital cataract. PPC+AV and better follow-up needs to be popularized for improved outcomes.

ABSTRACT CS-4

Uncorrected Refractive Errors, Presbyopia and Spectacle Coverage in South India

Srinivas Marmamula[1,2], *Saggam Narsaiah*[1], *K Sekhar*[1], *D Susheel*[1], *Gullapalli N Rao*[1]
[1]International Centre for Advancement of Rural Eye Care, [2]Bausch and Lomb School of Optometry, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To investigate the 'unmet need' and spectacle coverage for refractive errors & presbyopia in subjects aged ≥40 years in Vijayawada region in South India state of Andhra Pradesh.
Methods: A population-based cross-sectional study was conducted using a novel Rapid Assessment methodology. Cluster random sampling method was used to enumerate 2500 subjects from 55 clusters. Unaided, aided and pinhole visual acuity (VA) was assessed using a Snellen chart at a distance of 6 meters. The VA was re-assessed using a pinhole, if it was <6/18 in either eye. Near vision was assessed using N notation chart. Uncorrected Refractive Error was defined as presenting VA <6/18 and improving to 6/18 or better on using a pinhole. Presbyopia is defined as binocular near vision worse than N8 in subjects with binocular distance ≥VA 6/18.
Results: Of the 2600 subjects enumerated, 2455 (response rate-94.4%) subjects were available for examination. Of these, 55% were females and 52% were aged 40-49 years. Prevalence of unmet need for refractive errors was 7.7% (95% CI, 6.6 –8.8%) and unmet need for presbyopia was 53.4% (95% CI, 51.3 – 55.5%). Spectacle coverage for refractive error was 39% and for presbyopia it was 25%. Prevalence of spectacle use was 27.6% (95% CI, 25.8 – 29.4%) and bifocals were the most commonly used type of spectacles.
Conclusions: There is a high unmet for refractive errors and presbyopia in Vijayawada region in Andhra Pradesh. Strategies need to be formulated to address this huge unmet need.

ABSTRACT CS-5

Is Rod Function Normal in Subjects with Rod Monochromatism?

Naga Chandrika, Vijay Kiran, Padmaja K Rani, Subhadra Jalali
Smt. Kannuri Santhamma Centre for Vitreo Retinal Diseases, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To evaluate the changes in Electroretinography responses in subjects with rod monochromatism over a period of follow up
Methods: It is a Prospective study in which a total of three (6 eyes) previously diagnosed rod monochromatism subjects satisfying the inclusion criteria were recruited in the study. All Subjects underwent a comprehensive eye examination (vision assessment. slit lamp and dilated Fundus examination) and for obtaining ERG responses they were dark adapted for 30 minutes followed by light adaptation for 10mins. This study is done using Lvpei zari electrode following guidelines given by ISCEV.The Amplitude and the implicit time of all the responses were recorded after a comparable, reproducible and satisfactory wave form was noticed.
Results: There was no significant change in Mean VA (20/233 vs 20/200) at baseline and follow-up .Two out of three subjects had photophobia and nystagmus. Amplitudes at baseline and follow up (80±25 vs 69±26) showed no significance difference (p-value 0.51) and the implicit time at baseline and follow up (101.03±7.78 vs104±9.25) which also showed no significance difference (p-value 0.44).
Conclusions: The Electroretinography responses, amplitude and implicit time in subjects with rod monochromatism showed no significant difference at baseline and follow up visits.

ABSTRACT CS-6

Revised NEI-VFQ or IND-VFQ in Visually Impaired: A Conundrum

Shailaja P Reddy, Vijaya K Gothwal, Seelam Bharani, Rebecca Sumalini, Deepak K Bagga
Meera and L B Deshpande Centre for Sight Enhancement, Vision Rehabilitation Centres, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To compare the psychometric properties of Visual Function Scale (VFS) of the Revised NEI-VFQ and Indian Vision Function Questionnaire (IND-VFQ). Second objective was to test validity of the VFS of revised NEI-VFQ, in the visually impaired (VI) Indian population using Rasch analysis.
Methods: 120 VI patients (mean age, 45 years) recruited from Vision Rehabilitation Centres, LVPEI were administered both questionnaires. Rasch analysis of the VFS of revised NEI-VFQ resulted in a valid 10-item scale (VFS_VI). The VFS_VI and revised IND-VFQ were assessed using Rasch analysis for the following properties: person separation reliability (PSR), targeting of item difficulty to patient ability, item separation, differential item functioning (DIF).
Results: Both the VFS_VI and IND-VFQ possessed good PSR. However, targeting of the VFS_VI was biased towards greater disability as compared to IND-VFQ (0.22 vs. -0.48 logits). Item separation was larger for IND-VFQ than VFS_VI indicating that it covered a wider range of visual disability (12.21 vs. 3.71 logits). Large DIF (>1.0 logit) was observed for VFS_VI, but IND-VFQ was relatively free from DIF. Both questionnaires showed significant relationship with VA (r=0.50 for VFS_VI and 0.49 for IND-VFQ, P<0.0001).
Conclusions: Results indicate that both the VFS_VI and IND-VFQ are psychometrically robust measures of visual disability in VI. Both these questionnaires provide interval-level scores of visual disability so will be useful tools as patient- reported outcome measures of low vision interventions. However, users should be aware that the IND-VFQ does not contain reading-related items so may not be responsive to all low vision interventions.

ABSTRACT CS-7

Changes in Astigmatism Components with Accommodation

Pesala Veerendranath[1,2], *Mukesh Taneja*[2], *Virender S Sangwan*[2]
[1]Prof. Brien Holden Eye Research Centre[a], [2]Cornea and Anterior Segment Services, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To evaluate the changes in the Astigmatism components with accommodation.
Methods: 208 eyes of 208 subjects (9 to 62 years) were enrolled in the study with mean age 30.88 ± 11.22 years. All the subjects had best corrected distance visual acuity of 20/25 or better with mean refractive error of 0.11± 0.50 D. Subjects had clear ocular media for auto-refraction and can steadily fixate targets. Accommodation was induced by instructing subjects to fixate on an N8 target for near and a 4/40 letter for distance measurements. A Shin-Nippon NVision- K5001 auto-refractor was used to measure the refractive state in both eyes in the unaccommodated (4 m) and two accommodated states (40 and 33 cm). The spherical and cylindrical refractive errors obtained from the auto-refractor were converted in to power vectors and the astigmatism components (J45 and J180) with and without accommodation were calculated and compared.
Results: Mean J45 Astigmatism component for distance and with accommodation at 40 cm was -0.02±0.19D and 0.03±0.23D respectively and this difference was statistically significant (p=0.01). With Accommodation there was no significant difference in mean J45 at 33 cm (p=0.25) and J180 at 40 cm (p=0.82) and J 180 at 33 cm (p=0.81).
There was no statistical significant change in J45 (p = 0.24) and J180 (p = 0.34) astigmatism components with accommodation at 40 cm and 33 cm.
Conclusions: Our study concludes that there was no change in astigmatism components with accommodation except J45 astigmatism component at 40 cm.

Support: Hyderabad Eye Research Foundation

ABSTRACT CS-8

Assessment of Retinal Nerve Fiber Layer and Ganglion Cell Complex Thickness in Amblyopic Eyes

N Srividya, Ajit B Majji
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To assess the thickness of retinal nerve fiber layer and ganglion cell complex in Amblyopic eyes
Methods: Optical coherent tomography (OCT) measurements of retinal nerve fiber layer, ganglion cell complex and macular thickness were performed on 50 patients of anisometropic , strabismic amblyopia and normals
Results: Mean global RNFL thickness of the strabismic amblyopes and normals was 97.7 µm and 108.5 µm, respectively (mean difference, 10 µm thinner in the amblyopes). The global RNFL thickness of the anisometropic amblyopic and normal eyes were 112.5 µm and 110 µm, (mean difference , 2.46 µm thicker in the amblyopes). There is no significant difference in the RNFL thickness between amblyopes and normals.
In ganglion cell complex , the global and focal loss volumes showed significant difference in the strabismic amblyopes($p = 0.01$) compared to the normals. The mean foveal thickness (158 µm) was significant in the anisometropic group ($p = 0.026$)
Conclusions: Our findings indicate that there is no significant RNFL thickness variation among the amblyopes and the normals. But the foveal thickness was statistically significant in the anisometropic amblyopes.The global loss and focal loss volumes of the ganglion cell complex showed statistical significance indicating ultrastructural degenerative changes in the Strabismic amblyopes.

ABSTRACT CS-9

Assessment of Inter-Observer Variability in the Clinical Measurements of Ptosis

Mohd Zeeshanuddin[1], Winston D Prakash[2], Milind N Naik[2], Mohd J Ali[2]
[1]Bausch and Lomb School of Optometry, Hyderabad, [2]Department of Ophthalmic Plastics and Facial Aesthetics, L.V. Prasad Eye Institute, Hyderabad, India.

Purpose: The report inter-observer variability in the clinical measurement of blepharoptosis.
Methods: Prospective comparative study involving 100 eyes of 50 patients who presented to Oculoplasty service for clinical evaluation . Three examiners consecutively measured relevant biometric parameters in the ptosis patients using standard lighting, and a millimetre ruler. Parameters measured were: palpebral fissure height (PFH), Marginal reflex distance 1 &2(MRD), Levator function(LPSA), Lid crease distance(LCD), Lateral limbus to Lateral canthus distance(LL-LC) and medial limbus to medial canthus distance(ML-MC). The values of each examiner were masked to the other. Inter-observer variability was analysed using intra-class correlation coefficient (ICC).
Results: The inter class correlation for the PFH values was 0.816 (0.743- 0.871) p< 0.0001, for MRD1 values was 0.830 (0.752- 0.890) p< 0.0001, for MRD2 was 0.822 (0.693-0.904) p< 0.0001, for LPSA was 0.869 (0.818-0.908) p< 0.0001, for LCD was 0.871 (0.820- 0.910) p< 0.0001, for LL to LC was 0.747 (0.604- 0.810) p< 0.0001, for ML to MC was o.730 (0.610- 0.804). This shows that 82 to 84% of the times all the measurements except LL to LC and ML to MC correlate between the three observers. The LL to LC and ML to MC values correlates 74% and 73% of the times. The Bland and Altman graphs show that all the values in different sets of measurements fall in 95% class interval.
Conclusions: An acceptable correlation was observed between clinical measurements for ptosis.

ABSTRACT CS-10

Corneal Erosions with Silicone Hydrogel Lens Wear: Clinical Features and Outcome

Preeji Mandathara[1], Padmaja Sankaridurg[2], Pravin Krishna[1], Virender Sangwan [1]
[1]L V Prasad Eye Institute, Hyderabad, India; [2]Brien Holden Vision Institute, Sydney, Australia.

Purpose: To describe the incidence and the characteristics of corneal epithelial erosions seen with the use of silicone hydrogel contact lenses on a continuous wear (CW) basis and to report on the management and course of the events.
Methods: The events of corneal erosions from a prospective clinical trial involving the use of silicone hydrogel lenses on a 29 night CW basis in 210 subjects for 6 months were included in the study. The incidence of events was calculated as the number of events per 100 patient eye years.
Results: 16 events of corneal erosions were seen in 11 subjects. Two subjects had 2 recurrent episodes each. The incidence for first events was 7.0 events per 100 patient eye years. All events excepting one were symptomatic and in 60% of cases the symptoms were experienced on awakening. Excepting two events that were foreign body related, the remaining erosions were central or paracentral in location with a characteristic inverted teardrop or linear lesion. The erosions were small to moderate in size (largest lesion measured 1.6mm vertical and 0.3 mm horizontal) and multiple lesions were common. All events were managed with discontinuation of lens wear and topical lubrication.
Conclusions: Corneal erosions appeared to be predominantly as a result of mechanical interaction of the lens with the cornea. While the condition was acute and symptomatic, the events were easily managed by discontinuation from lens wear, did not result in any sequelae and required no other treatment or intervention.

ABSTRACT CS-11

Clinical Outcomes of Allogenic Cultivated Limbal Epithelial Transplantation for Bilateral Limbal Stem Cell Deficiency

Sayan Basu,[1,2], Sujata Das[3], Mark M Fernandez[4], Subhas Gaddipati,[2] Geeta K Vemuganti[2], Virender Sangwan[1,2]

[1]Cornea and Anterior Segment Services, [2] Sudhakar and Sreekanth Ravi, Stem Cell Biology Laboratory, L V Prasad Eye Institute, Hyderabad, [3]Cornea and Anterior Segment Services, L V Prasad Eye Institute, Bhubaneswar, India, [4]Duke University School of Medicine, Department of Ophthalmology, Durham, NC, USA.

Purpose: This study reports the clinical outcomes of allogenic cultivated limbal epithelial transplantation for the treatment of bilateral limbal stem cell deficiency (LSCD).

Methods: This retrospective study included 28 eyes of 21 patients with bilateral clinically diagnosed total LSCD who were treated between 2001 and 2010. All patients developed corneal blindness after the age of eight years. A limbal biopsy was obtained from the eye of a living related donor. The limbal epithelial cells were expanded ex-vivo on human amniotic membrane for 10-14 days using a xeno-free feeder cell-free explant culture system. The resulting cultured epithelial monolayer and amniotic membrane substrate was transplanted on to the patient's eye. A penetrating keratoplasty (PK) was performed if post-operatively visual impairment persisted due to corneal scarring.

Results: At a mean follow up of 58±33 months 20 eyes (71.4%) had either maintained a healthy corneal surface or underwent penetrating keratoplasty (PK). The corneal allograft survival rate (n=13) was 76.9±12% at 12 months with median survival of 40 months. No donor or recipient eyes developed serious ocular complications. Visual acuity improved to 20/60 or better in 7 eyes after limbal transplantation alone, and in 11 other eyes following PK.

Conclusions: Ocular surface stability and visual restoration can be successfully achieved in eyes of patients with bilateral total LSCD by performing allogenic cultivated limbal epithelial transplantation followed by PK.

ABSTRACT CS-12

Computerized Graphical Representation of the Zone of Clear and Single Binocular Vision

Kamalpreet Dhaliwal[1], Baggam V Preethi[1], Shrikant R Bharadwaj [1,2]
[1]Bausch & Lomb School of Optometry, [2]Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Clear and single visual experience of our three-dimensional environment is maintained through ocular accommodation and vergence eye movements. As these two systems are neurally coupled, any imbalance in the stimulus demands or coupling strengths can lead to abnormal binocular vision. Graphical analysis is a tool that maps the overall zone of clear and single binocular vision (ZCSBV) of the patient and allows customized management strategies to be devised. The purpose of this study was to design and validate a custom-written computer software for mapping the ZCSBV, allowing rapid and comprehensive diagnosis and management of binocular vision disorders.
Methods: 41 healthy asymptomatic subjects (22 males and 19 females; mean±1SD age: 21.61± 2.46) with best-corrected visual acuity ≥ 20/20 in both eyes participated. The near point of accommodation, interpupillary distance, dissociated phoria and positive and negative relative vergence (PRV, NRV) were measured using standard clinical techniques to map the ZCSBV.
Results: The mean±1SD of most output variables measured here were comparable to Morgan's normative values. Significant difference (>2Δ) was found between PRV distance blur and recovery; PRV near recovery; NRV distance break; NRV near blur and break. These parameters were plotted to map the ZCSBV successfully in patients.
Conclusions: The ZCSBV could be mapped rapidly using the custom-designed software. The pattern of ZCSBV obtained across asymptomatic individuals with normal binocular vision parameters can be used as a normative database to compare against those with binocular vision symptoms and aesthenopia.

ABSTRACT CS-13

The Quality of Life Associated with Glaucoma

Deepak K Bagga,[1] Vijaya K Gothwal,[1] Shailaja P Reddy,[1] Rebecca Sumalini,[1] Seelam Bharani,[1] Garudadri Chandrasekhar,[2] Sirisha Senthil,[2] Vanitha Pathak-Ray,[2] Harsha B L Rao,[2] Anil K Mandal[2]
[1]Meera & L B Deshpande Centre for Sight Enhancement, [2.] VST Centre for Glaucoma Care, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To determine the health related quality of life (QoL) in adults with glaucoma.
Methods: 222 adults with glaucoma (mean age 59.6 years; 66% males) recruited from glaucoma clinic of a tertiary eye care centre, were administered 2 questionnaires: Glaucoma Quality of Life (GQoL) and Standardized, validated time trade off (TTO). Negative GQoL score indicate lower visual disability. TTO score range from 0 to 1; 0 indicating worst health condition (death) and 1 indicating perfect health. Factors associated with higher or lower utility scores were investigated.
Results: Mean utility score of the patients (n=209) was 0.82 (SD=0.22; 95% confidence interval [CI], 0.79 to 0.85). Only 55.7% of the patients were ready to trade some part of their life for perfect vision and the mean utilities of these patients was significantly lower compared to entire sample (0.67, SD=0.20; 95% CI, 0.64 to 0.71). Approximately one quarter (24%) of these were ready to trade half of their remaining life. Patients who were willing to trade, were significantly younger (mean age 56.5 years), had significantly decreased contrast sensitivity (1.12 log contrast sensitivity), worse visual field (mean mean deviation in better eye -12.09 dB) and greater visual disability (-1.17logits) as compared to group with perceived perfect health.
Conclusions: Glaucoma is associated with significant decrease in QoL, similar to as angina (0.74 to 0.80) and amputation (0.73 to 0.82) for the average person with the condition. Early detection may help preserve field loss and reduce visual disability thereby improving QoL.

ABSTRACT CS-14

Bilateral Granular Dystrophy: A Clinicopathogenetic Correlation after Alcohol Assisted Debridement with Phototherapeutic Keratectomy

Alok Sati[1], Muralidhar Ramappa[1], Sunita Chaurasia[1], Geeta K Vemuganti[2], Prithija Protala[2], Chitra Kannabiran[3], Virender S Sangwan[1]
[1]Cornea and Anterior Segment Services, [2]Ophthalmic Pathology Services, [3]Department of Genetics, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India.

Purpose: To report the clinical, histopathological and genetic features of bilateral granular dystrophy and the outcome after alcohol assisted debridement with phototherapeutic keratectomy.
Methods: Two siblings presented with the complaint of painless, progressive diminution of vision in both the eyes, since childhood. The best corrected visual acuity in elder sibling was 20/400 in both the eyes and younger sibling had 20/200 in both eyes. Slit lamp examination showed greyish white, discrete, breadcrumb to ring shaped opacities predominantly in anterior stroma with clear corneal periphery. Anterior segment OCT revealed normal corneal thickness. The elder sibling underwent alcohol assisted phototherapeutic keratectomy in both eyes whereas younger one has undergone in right eye. The debrided epithelia were sent for histological examination. Blood samples were collected and sent for genetic analysis.
Results: After surgery, the elder sibling appreciated marked improvement in visual acuity i.e 20/50 in right eye and 20/30 in left eye whereas younger appreciated it as 20/40 in right eye. Postoperatively both corneas of both siblings were clear except a few opacities in deeper stroma. Histopathologically, the amorphous material attached to debrided epithelium that was stained eosinophilic with H&E stain showed brilliant red stain with Massons´ trichrome thereby confirming hyaline deposits in stroma. Genetic analysis revealed mutation at 12 exon of *TGFB1* gene. This mutation is of heterozygous in nature as revealed by three bands on gel electrophoresis.
Conclusions: Visually symptomatic superficial variant granular dystrophy can be successfully treated with alcohol assisted debridement combined with phototherapeutic keratectomy and achieving a good visual outcome.

ABSTRACT CS-15

Effect of Room Lighting on the Visual Performance of School-Age Children

Swetha Tirumala[1], *Asmitha Koruttla*[1], *Shrikant R Bharadwaj* [1,2]
[1]Bausch & Lomb School of Optometry, [2]Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: Many fundamental visual functions critically depend on the ambient light level. The lower socio-economic population of India often performs these tasks under the low light levels emitted by kerosene wick-lamps. A novel LED based lighting system provides uninterrupted and non-polluting light to this section of the population. This study determined the efficacy of this device by studying the effect of ambient light levels on visual performance of school-age children.
Methods: 50 children (10 to 17yrs), with best-corrected visual acuity of 20/20 participated in the study. Visual parameters including best-corrected monocular and binocular distance and near visual acuity, low contrast acuity and reading speed was assessed under three different LED light levels (60Lux, 90Lux and150Lux), fluorescent (890Lux) room lighting and kerosene lamp lighting (2–3Lux) using standard clinical techniques.
Results: Distance and near high-contrast visual acuity, low-contrast acuity and reading speed gradually increased with ambient light levels, with these parameters significantly higher with 60Lux light levels than with kerosene lamps ($p<0.001$). High-contrast acuity and reading speed reached fluorescent lamp light levels by 90Lux ($p=0.05$), while low-contrast visual acuity reaching fluorescent lamp light levels by 150Lux ($p=0.05$).
Conclusions: The results demonstrate the superiority of the LED based lighting system to that of kerosene wick lamps in aiding visual functions. The results of visual performance with 150Lux light levels being similar to that of fluorescent lamp lighting suggests that this novel device can be used as a viable alternative to the kerosene wick-lamps to perform fine visuomotor tasks.

ABSTRACT CS-16

Clear Corneal Tunnel Infection after Uneventful Phaco Emulsification

Archana Singh[1], Muralidhar Ramappa[1], Jagadeesh C Reddy[1], Sunita Chaurasia[1], Geeta K Vemuganti[2]
[1]Cornea and Anterior Segment Services, [2]Ophthalmic Pathology Services, LV Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India.

Purpose: To report the outcomes of patch keratoplasty in a case of clear corneal tunnel infection after an uneventful phaco emulsification. Also to emphasize the role of histopathological examination for the successful management of such cases.
Methods: A 36 year old lady presented with progressive decrease in her vision associated with redness, watering, pain and appearance of a white spot on the black portion in the right eye of 3 months duration. Prior to this, patient had under gone uneventful phaco emulsification. The clinical features of tunnel infections are non-specific and are often non-contributory to the possible etiologic agent. Therefore, a detailed microbiological workup is mandatory. Corneal scraping, biopsy were inconclusive .Patch graft was planned in the right eye. Histopathological examination confirmed the presence of septate fungal filaments present in mid to deep stroma. The patient was treated accordingly.
Results: A successful patch graft was performed. Post-operative course was uneventful. The visual acuity improved to 20/50 and the patient became symptomatically better.
Conclusions: The diagnosis and management of such cases is challenging. Histopathological examination is an important diagnostic tool and patch graft may be a good option for the ultimate management of these challenging cases.

ABSTRACT CS-17

Analysis of Visual Parameters in Subjects with and without Diabetic Retinopathy

P Hari Kumar, Ajit B Majji
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To compare the difference in visual acuity, contrast sensitivity, color vision, perimetry values in diabetic patients with different stages of diabetic retinopathy.
Methods: Non- interventional, prospective, comparative study was conducted on patients attending the retina-vitreous center. Visual acuity, contrast sensitivity, visual fields and color vision were determined using EDTRS chart, Pelli-Robson chart, Humphrey Visual Field analyzer, Ishihara chart respectively.
Results: The data of 82 eyes of 52 patients was analyzed, and the visual fields mean MD was found to be highest in group IV and lowest in group I. For contrast sensitivity, the contrast was highest in group I and lowest in group IV. In terms of vision, group I was the highest and least in group IV. The values of contrast sensitivity in group IV (P=0.00) showed statistically significant. The visual fields values were statistically significant in group IV (p=0.00) compared other 3groups. Color vision showed statistically significant between groups (p=0.005)
Conclusions: This study showed the decrease in contrast in patients with NPDR and Lasered PDR and change in visual fields for patients with Lasered PDR. Diabetes also has effect on color vision.

ABSTRACT CS-18

Clinicopathological Analysis of Repeat Descemet Stripping Endothelial Keratoplasty or Penetrating Keratoplasty for Failed Descemet Stripping Endothelial Keratoplasty

Shivani Pahuja, Jatin Ashar, Muralidhar Ramappa, Pravin Vaddavalli, Sunita Chaurasia, Somashiela Murthy, Prashant Garg, Virender Sangwan, Geeta Vemuganti
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To evaluate outcome of repeat (re) DSEK/penetrating keratoplasty (PK) after DSEK & histology of failed DSEK
Methods: Retrospective analysis of reDSEK/ PK for failed DSEK from Jan 2006-June 2010
Results: 18 eyes / 17 patients underwent DSEK for endothelial dysfunction. Grafts failed in median 85(1-622) days. Re DSEK was performed in 14 while 4 had PK after 136.5 (13-622) days. Air through venting incisions helped to detach lenticule. Follow up was 1 week to 16 months. Vision was <0.05 in PK; improved to 0.4 in 7, 0.4-0.05 in 3 and < 0.05 in 4 of re DSEK. Endothelial loss in re DSEK was 22.81+9.35%.1 reDSEK graft failed, 2 PK cases had rejection. Histology showed endothelial loss, retained host descemet's membrane
Conclusions: Re DSEK has good outcome after failed DSEK.

ABSTRACT CS-19

RGP Contact Lens Fitting in Keratoconus Using Fitscan Technology

Mariya Khan, Sobia Taureen, Srikanth Dumpati, Preeji Mandathara
Bausch and Lomb School of Optometry, L V Prasad Eye Institute, Hyderabad, Kismatpur, Hyderabad, India.

Purpose: To assess and compare the contact lens parameters that were calculated by Fit scan using corneal topography (orbscan IIz) with the parameters that were calculated by the diagnostic contact lens fitting method in Keratoconus.
Methods: A prospective comparative study of 85 Keratoconic eyes that were referred for RGP contact lens trial at Bausch & Lomb contact lens centre, L V Prasad Eye Institute, Hyderabad, India, was conducted. Two masked observers calculated the contact lens parameters of RGP lens by diagnostic fitting method and using Fitscan technology. The base curve was calculated by the two methods were compared using Wilcoxon signed rank test and the agreement between two methods were analysed using Bland Altman plot.
Results: Eighty five eyes from 65 patients were included in the study. The mean age was 17.63±2.78 (range: 12 – 23) years and 46 were males. The study revealed that the fitscan calculates the base curve of RGP contact lens on average 0.22mm flatter than the diagnostic method ($p=<0.0001$). The bias between the two methods was found to be 2.7% in Bland Altman plot.
Conclusions: Fitscan is a helpful tool in selecting the contact lens parameters in RGP fitting in Keratoconus which will reduce the need for multiple contact lens trials. Though there is a bias of 2.7% between the two methods, selecting the base curve of the initial trial lens base curve 0.20mm flatter than the Fitscan predicted value will help in reducing the chair time in contact lens fitting in Keratoconus.

ABSTRACT CS-20

Role of Corneal Topography in RGP Contact Lens Fitting in Keratoconus

Preetam Kumar, Preeji Mandathara, Srikanth Dumpati, Varsha Rathi
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To report the parameter selection of RGP lens in Keratoconus based on corneal topography data.
Methods: Retrospective study of Keratoconus patients who were fitted with RGP contact lenses was done. The data on patient demographics, unaided and best spectacle corrected visual acuity, refraction, corneal topography, contact lens parameters, lens fitting characteristics and contact lens corrected visual acuity were collected. Multivariate linear regression analysis was performed to find the association between different topographic indices and base curve of the contact lens and multivariate logistic regression analysis was done to study the association of cone size and location with diameter selection.
Results: 41 eyes of 24 keratoconus patients who fitted with RGP lenses were included in the study. Among them 13 were males. The mean age was 20.9 ± 8.0 years. The minimum simulated keratometry was 50.04 ± 7.14, maximum keratometry was 56.45 ± 9.05, average 3mm kertometry was 52.33 ± 7.62 and average 5mm keratometry was 48.39 ± 5.14. The size of the cone was 3mm in 12% eyes , 5mm in 44% eyes and >5mm in rest. The average 5mm keratometry found to be the best predictor of the base curve of the RGP contact lens in Keratoconus fitting ($p<0.001$). Also lens diameter of >9.00 mm was found to fit in Keratoconus where the cone size is larger than 5mm(p0.06).
Conclusions: Corneal topography is an important tool in contact lens fitting in Keratoconus. The average 5mm keratometry is the best predictor for the base curve selection. As the size of the cone is larger, we require large diameter contact lenses.

ABSTRACT CS- 21

Inter and Intra Subject Variability of Luminanace - Slope Calibration in Eccentric Photorefraction

Asa Narasaiah[1], Geeta Sravani[1], T. Rowan Candy[2], Shrikant R Bharadwaj[1,3]
[1]Bausch & Lomb School of Optometry, [3]Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India.[2]Indiana University School of Optometry, Bloomington, Indiana, USA.

Purpose: Refractive power measured using eccentric photorefraction depends on the calibration of luminance slope formed across the pupil into dioptric units. This study determined two aspects of this defocus calibration factor: Its inter and intra-subject variability and the impact of experimental set-up on the calibration factor.
Methods: 57 emmetropic adults (20.3 to 31.3yrs) fixated on the photorefraction unit at 75cm with their left eye. Anisometropia was induced by placing trial lenses (+8D to -8D in 1D step) before the IR filter occluded right eye and the defocus calibration factor was calculated by plotting luminance slope for each lens against the induced anisometropia. This paradigm was repeated (n=24) twice within 5 minutes and twice within two weeks to determine intra-subject variability and it was repeated (n=15) with an IR reflecting hot-mirror and an IR transmitting cold-mirror to determine variability due to experimental set-up.
Results: Mean (+/-95% CI) calibration factor obtained within the +/-5D linear range was 0.69+/-0.28Ls/D. Mean calibration factors in sessions one (0.64+/-0.22Ls/D) and two (0.66+/-0.25Ls/D) were not statistically significantly different from each other (p=0.38).Mean calibration factor with hot mirror (0.56+/- 0.13Ls/D) and with cold-mirror (0.51 +/- 0.19Ls/D)were not significantly different from each other (p=xx) but both were significantly different from direct viewing of the camera(0.621 +/- 0.17Ls/D)(p<0.02).
Conclusions: Large variability in the defocus calibration factor across subjects and across experimental set-up suggests that individual calibration factors should be used to convert luminance slope in to diopetrs in each subject and with each experiment

ABSTRACT CS-22

Reasons for Loss to Follow-Up and Visual Outcomes after Cataract Surgery at Secondary Eye Centre in Adilabad District of Andhra Pradesh

N Rajashekar Rao[1], *Giridhar Pyda*[2]
[1]Government of Andhra Pradesh, [2]ICARE, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To find out the reasons for loss to post operative follow-up and to measure the visual outcomes among the cataract surgeries at secondary eye centre over a period of one year.
Methods: 1953 cases operated for cataract with ECCE/SICS/Phaco with PCIOL were studied out of 2240 surgeries performed in one year (2008-09) at Bhosle Gopalrao Patel Eye Centre, Mudhole. The study was retrospective, quantitative by convenient sampling with the sample of 147 subjects within 227 dropouts.
Results: 11.6% (n=227) were lost to follow-up, among them 69.16% (n=157) are non-paying category. Out of 87 subjects studied, 43 (49.4%) got examined elsewhere and rest had reasons as, (i) can see well or happy with vision, (ii) can't afford, (iii) no escort available. The visual outcomes among 87 lost to follow up cases are 70.1% (presenting), 75.9% (pin-hole) in good, 27.6% (presenting), 21.8% (pin-hole) in borderline and 2.3% were in poor category. Among those 1726 completed follow-up at 4-11 weeks were 56.2% (presenting), 88.9% (pin-hole) in good and 5.8% (presenting), 4.1% (pin-hole) are in poor category.
Conclusions: The uptake of follow-up services following cataract surgery is considerably good and more dropouts observed among non-paying Category. Good outcome range can be improved by limiting borderline proportion and poor outcomes are within the range, <5% of WHO guidelines.

ABSTRACT CS-23

Axial Length Measurement Using B-Scan Vs A-Scan Ultrasonography

Rajeev K Reddy, Vasundhara, G Sushma
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To evaluate the agreement between standard A-scan biometry and B-scan ultrasonography for axial length (AL) measurements in high myopic eyes.
Methods: In a prospective study, AL measurements in 38 eyes of 38 subjects (high myope) were obtained by A-scan and B-scan ultrasonography by independent observers. AL on B-scan ultrasonography was also separately done by two independent observers to evaluate interobserver agreement.
Results: The interobserver agreement for AL measurement on B-scan ultrasonography in high myopic eyes (mean difference=0.06 mm, 95% LoA: -0.25 to 0.37) was good. Mean difference between AL measurements on A and B-scan ultrasonography in high myopic subjects was -0.44 mm (95% LoA:-0.78 to -0.10). AL measurement on B-scan was always greater than that on A-scan ultrasonography. Proportional bias in agreement was not detected in high myopic eyes (regression coefficient $\beta = -0.03$, $p = 0.12$).
Conclusions: There were significant differences in AL measurements by A-scan and B-scan ultrasonographies and they cannot be used interchangeably for AL measurements.

ABSTRACT CS-24

Axial Length Measurement Using B-Scan Vs A-Scan Ultrasonography

Rajeev K Reddy, G Sushma, Vasundhara
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To evaluate the agreement between standard A-scan biometry and B-scan ultrasonography for axial length (AL) measurements in emmetropic eyes.
Methods: In a prospective study, AL measurements in 34 eyes of 34subjects (emmetropes) were obtained by A-scan and B-scan ultrasonography by independent observers. AL on B-scan ultrasonography was also separately done by two independent observers to evaluaton interobserver agreement.
Results: The interobserver agreement for AL measurement on B-scan ultrasonography in emmetropic (mean difference=0.07 mm, 95% LoA: -0.45 to 0.59) was good. Mean difference between AL measurements on A and B-scan ultrasonography in emmetropic subjects was -0.32 mm (95% LoA: -0.88 to 0.2). AL measurement on B-scan was always greater than that on A-scan ultrasonography. Proportional bias in agreement was not detected either in emmetropic (regression coefficient $\beta = -0.08$, $p = 0.18$).
Conclusions: There were significant differences in AL measurements by A-scan and B-scan ultrasonographies and they cannot be used interchangeably for AL measurements.

ABSTRACT CS-25

Retrospective Analysis of Choroidal Neovascular Membranes Secondary to Central Serous Chorio Retinopathy

Rajeev R Pappuru, Vivek Pravin Dave, Mudit Tyagi
Smt. Kannuri Santhamma Centre for Vitreo Retinal Diseases, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To describe the optical coherence tomography features of choroidal neovascular membranes (CNV) secondary to central serous chorioretinopathy (CSC) and its response to intravitreal bevacizumab
Methods: Nineteen eyes of 19 patients of CNV secondary to central serous chorioretinopathy imaged with high resolution OCT were evaluated for intra retinal and sub retinal changes. Retrospective chart review of 7 eyes of 7 patients with CNV associated with chronic CSC diagnosed by fluorescein angiogram and optical coherence tomography was done. Patients who received Photodynamic therapy previously were excluded. Response to intravitreal bevacizumab 1.25 mg was assessed by visual acuity and optical coherence tomography.
Results: Nineteen patients were evaluated on OCT. The mean best corrected visual acuity (BCVA) was 20/50 (range, 20/20 to 20/200). All the CNVM were of the classic type, predominantly subfoveal (63%). All patients showed presence of sub retinal fluid and thickened RPE-CC complex. 6 eyes (32%) had a serous pigment epithelial detachment (PED). No fibrovascular PED was noted. Post bevacizumab, BCVA ranged from 7/200 to 20/40 at presentation. Improvement or stabilization of Snellens visual acuity noted in all patients. Subretinal fluid resolved in all patients.
Conclusions: High resolution spectral OCT well demonstrates various structural changes occurring in CNV with chronic CSCR. Intravitreal Bevacizumab for CNV associated with chronic CSC showed anatomical and visual improvement.

ABSTRACT CS-26

Outcomes of Cataract Surgery Following Treatment for Retinoblastoma (Rb)

Tarjani V Dave, Pravin K Vaddvalli, Ramesh Kekunnaya, Santosh G Honavar
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To analyze the results of cataract surgery in patients treated for Rb and to assess the ocular and systemic outcomes.
Methods: Retrospective interventional non-comparative case series of nineteen consecutive patients who underwent cataract surgery after treatment for retinoblastoma. Main Outcome Measures were: (1) Recurrence of retinoblastoma, (2) need for enucleation and (3) systemic metastasis. Overall outcome was defined as favorable in the absence of any of these measures and unfavorable in the presence of 1 or more.
Results: Thirteen patients (68%) underwent cataract surgery with lens implantation and 6(32%) without lens implantation. None had a primary posterior capsulotomy. One required membranectomy and 2(11%) yag capsulotomy at a median duration of 8 months following cataract surgery. 6 patients (32%) achieved final visual acuity better than 20/200. One eye of 3 with recurrent Rb was enucleated while 2 regressed with chemotherapy. None developed systemic metastasis. Interval between completion of Rb treatment and cataract surgery was <12 months in patients who recurred. Median interval between completion of treatment for Rb and intraocular surgery was 10 months in patients with unfavorable outcome vs 32 months in those with a favorable outcome.
Conclusions: Cataract surgery may be justified beyond 12 months after documented Rb regression. Clear corneal approach with preservation of posterior capsule is ideal. While local tumor recurrence is a possibility, risk of metastasis is remote.

ABSTRACT CS-27

Profile of Patients Presenting for 1-Week Postoperative Visit Following Uncomplicated Phacoemulsification for Senile Cataract

Nazia Begum[1], *Ashik Mohamed*[2], *Virender Sangwan*[3]
[1]Bausch and Lomb School of Optometry, [2]Hyderabad Eye Research Foundation, [3]Cornea and Anterior Segment Services, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To describe the postoperative profile of the patients following an uncomplicated phacoemulsification for senile cataract.
Methods: A review of case records of 180 patients who underwent phacoemulsification was performed. The profile of patients presenting with one day, one week and one month postoperative visit which includes patient`s symptoms, visual acuity, anterior chamber reaction, intraocular pressure and pupillary status was obtained.
Results: Following the cataract surgery, there appears to be no significant difference (P = 1.00, "Tukey's method with Bonferroni Correction") between one week and one month visits in visual acuity (in log MAR, 0.17 ± 0.17 & 0.16 ± 0.16 respectively) and intraocular pressure [IOP] (in mm Hg, 13.3 ± 2.4 & 12.8 ± 2.0 respectively). Minimal and expected transient IOP spike was seen which is not clinically deleterious. The severity of occurrence of anterior chamber reaction decreased post operatively. At one week and one month postoperative visits, no complications were observed and no clinical and emergency services were required.
Conclusions: The intervention rate in routine clinical review after uncomplicated cataract surgery is almost low. Our study indicates that the one week review after uneventful phacoemulsification gives a mutual reassurance and can be safely deferred until one month.

ABSTRACT CS-28

Part Time Occlusion Therapy for Anisometropic Amblyopia Patients in 4 to 15 Year Old Children

Joshef Mollah, Amit Gupta, Monica Samant
Department of Pediatric Ophthalmology, Strabismus and Neuro-ophthalmology, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India.

Purpose: To determine the effectiveness of part time patching therapy in treating anisometropic amblyopia in 4 to 15 year old children
Methods: It was a retrospective study.58 patients were included. We compared the pre and post patching best corrected visual acuity (BCVA) in 4 to 8 years of age (group 1- 24 subjects, BCVA ranging from 20/50 to 20/200) and 9 to 15 years of age (group 2- 34 subjects, BCVA ranging from 20/40 to 20/400) separately. Then we compared both group together. Every patient having more than two, 2monthly follow up. We have collected the data from 3rd visits- almost 1 year follows up.
Results: In group 1 we compared mean line improvement between pre and post therapy BCVA, It was 3.44 and in group 2 it was 3.03 (>2 line is clinical significant) Now in log mar pre BCVA is 0.75±0.24 and post therapy BCVA is 0.37±0.17 and when we compared the pre post therapy BCVA the p value is <0.0001 that statistically significant. Similarly in group 2 pre log mar BCVA is 0.70±0.25 and post is 0.40±0.22, and p value is <0.0001. When we compared the post BCVA between the two groups (group 1 3.32±1.8; group 2 = 3.03±1) the p value is 0.442, statistically not significant means both group respond in similar fashion. Paired t test is used for statistical analysis
Conclusions: Both groups respond well and visual outcome was significant in spite of different age groups.

ABSTRACT CS-29

A Case Series of Posterior Microphthalmos with Pappilomacular Folds

Joshef Mollah, Amit Gupta, Shaistha Parveen
Department of Pediatric Ophthalmology, Strabismus and Neuro-ophthalmology, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad.

Purpose: The most common clinical features of Posterior microphthalmos are small axial length, high hyperopic with macular fold, sometimes macular schisis with normal anterior segment structures .To report a case series of three Posterior microphthalmos siblings of a family having parental consanguinity.
Methods: Three kids aged 7, 8,9yrs respectively were presented with complaint of difficulty in vision both eyes, underwent complete ophthalmic evaluation that included vision assessment, cycloplegic refraction (1% Cyclopentolate hydrochloride), slit lamp evaluation, ophthalmoscopy, OCT (optovue), A-scan, AC depth (IOL Master) measurement and keratometry.
Results: The ocular manifestations reviled bilateral high hyperopia with reduced visual acuity with best correction also. (log mar BCVA-OU=0.73±0.26).Anterior chamber (OD=3.68±0.27; OS=3.70±0.33), lens thickness (OD=4.01±0.01; OS=4.02±0.03) & IOP (OU=14±2), were normal whereas corneal curvature was steep with normal diameter (OD=10.99±0.10; OS=10.97±0.06) along with significant shortened axial length (OD=16.24±0.55; OS=16.10±0.53).Extra ocular motility & Ocular alignment was normal along with deeply set eyeball. On retinal examination C: D ratio was normal, On OCT all patients had of supero-nasal papilomacular folds with increased macular thickness (OD=373±68.51; OS=385.33±39.00).
Conclusions: Posterior microphthalmos and high hyperopia and pappilomacular retinal folds has been unrecognised. Although multifactorial inheritance is possible, this pedigree is consistence with an autosomal recessive disorder.

ABSTRACT CS-30

Measurement of Retinal Nerve Fibre Layer (RNFL) Thickness by Spectral Domain Optical Coherence Tomography (OCT)

Asra Fatima, Uday Kumar, Sirisha Senthil, BL Harsha
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To measure the retinal nerve fibre layer (RNFL) thickness in different stages of glaucoma and compare them using Spectral Domain optical coherence tomography (OCT).
Methods: The discriminating power of OCT parameters for detection of different stages of glaucoma was determined using three parameters – retinal nerve fibre layer (RNFL), optic nerve head (ONH), and ganglionic cell count (GCC). 89 glaucoma subjects were included in the study of which are classified into 3 groups based on Hodapp-Anderson-Parrish criteria- early, moderate and advanced. Individual groups were measured using Spectral Domain OCT and then analyzed.
Results: One way analysis of variance (ANOVA) was used for multiple comparisons. The SSI used for different groups was similar in all the groups ($p > 0.13$). The average RNFL thickness, inferior hemisphere thickness, inferior and nasal quadrant thickness showed significant difference between all the groups except moderate and advanced ($p<0.03$). Other parameters like superior hemisphere, superior and temporal quadrant thickness showed difference only between early and moderate groups ($p>0.05$). Cup area, disc area, rim area, nerve head volume and other parameters in ONH scan did not show any significant difference between the groups ($p>0.40$). Inner average thickness, inner superior and inferior average thickness, foveal and ganglionic cell loss volume (FLV and GLV) showed significant difference only between early and advanced groups ($p<0.03$).
Conclusions: OCT parameters can be useful for determining glaucomatous damage and its severity.

ABSTRACT CS-31

Outcomes of Endothelial Keratoplasty (EK) without Stripping Descemet's Membrane (DM)

Sunita Chaurasia, Muralidhar Ramappa, Virender S Sangwan
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To study the outcomes of endothelial keratoplasty without stripping the Descemet's membrane.
Methods: Retrospective, consecutive case series of 23 eyes from 2009 to 2010, where EK was done without stripping DM.
Results: Mean age 53.7 yrs, M: F=15:8, with endothelial dysfunction due to Pseudophakic/aphakic corneal edema (65.2%), ICE syndrome (8.6%), failed graft (21.7%), primary congenital glaucoma (4.6%) underwent EK without DM removal. Two patients had partial graft detachment and one had secondary angle closure. Clear grafts were achieved in all patients. Visual acuity ranged from 20/200 to 20/40 (8 patients had co-morbid factors responsible for poor vision).
Conclusions: Endothelial keratoplasty without DM stripping is a viable alternative when the DM is expected to be normal and optically clear.

ABSTRACT CS-32

Bilateral Congenital Hyper Plastic Pupillary Membrane: A Clinical and Histopathological Correlation after Successful Surgical Excision

Muralidhar Ramappa, Somasheila I Murthy, Sunita Chaurasia, Rushmika Singhla, Varsha Rathi, Geeta K Vemuganti, Virender S Sangwan
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To report the clinical and histopathological features of congenital bilateral persistent hyperplastic pupillary membrane and the outcome after surgical removal.
Methods: A 12 -year old boy was presented with the complaints of severe glare and blurring of vision in the daylight in both the eyes, for the past 5-6 years. The best corrected visual acuity was 20/80 in both the eyes. Slit lamp examination showed a Medusa-head like appearance of the persistent pupillary membrane, which appeared to be fused to the anterior lens capsule in the pupillary axis, and there were multiple iris strands extending to the collarette. The patient underwent surgical excision of the membranes in the both the eyes. The excised membrane was sent for histological examination.
Results: During surgery, the numerous iris strands were first severed from the iris collarette and the opaque hyperplastic pupillary membrane was then meticulously peeled off from the anterior lens capsular. The underlying anterior lens capsule was intact with a ring of pigmentation at the site of the insertion of the pupillary membranes and the lens was clear. Histopathology of the excised membranes from both the eyes showed similar features of numerous adherent iris strands to a thick multilayered PAS positive basement membrane like material, highly reminiscent of lens capsule. Post-operative course was uneventful and crystalline lens remained clear at the last follow up. The visual acuity improved to 20/30 and the patient's symptoms of glare and photophobia disappeared.
Conclusions: Visually symptomatic persistent pupillary membrane can be successfully removed without damaging the underlying crystalline lens and achieving a good visual outcome

ABSTRACT CS-33

Boston Scleral Contact Lens in Coexisting Steven Johnson Syndrome and Keratoconus

Srikanth Dumpati, Varsha Rathi, Preeji Mandathara
Bausch and Lomb Contact Lens Centre, L V Prasad Eye Institute, Hyderabad

Purpose: To study the role of Boston Scleral Contact Lens in patients with co-existing Steven Jonhson Syndrome and Keratoconus.
Methods: Retrospectively 3 patients were identified and reviewed from computerized medical record database that shows Steven Jonhson Syndrome (SJS) and Keratoconus (KC). Patient's data such as age, gender and visual acuity before and after lens wear and symptoms and before and after lens wear and follow up was recorded.
Results: 6 eyes of 3 patients had SJS and KC. The symptoms of prelens wear photophobia decreased with lens wear. Patients were able to tolerate light and were able to open eyes. Visual acuity improved from 0.9 ± 0.6 Log MAR to 0.4 ± 0.5 Log MAR with scleral contact lens wear in these patients over follow up of 24 months. The patients were using the lenses on an average of 9 hours per day. With these lenses limitation were debris formations after 4.5 hours of lens wear in all 3 patients. One patient showed bulbar congestion and pain in both eyes.
Conclusions: The visual acuity and symptoms were improved with scleral contact lens in coexisting SJS and KC.

ABSTRACT CS-34

Impact of Lens Induced Anisometropia on Accommodative Responses to Step and Ramp Stimuli in Adults

Vaishnavi Raghuram[1], Vivek Labishetty[1], Shrikant R Bharadwaj [1,2]
[1]Bausch & Lomb School of Optometry, [2]Prof. Brien Holden Eye Research Centre, L V Prasad Eye Institute, Hyderabad, India.

Purpose: While anisometropia causes inter-ocular differences in retinal image quality, its impact on the accommodative performance of adults remain unknown. This study compared the accommodative performancewith transiently induced myopic and hyperopic anisometropia to step (abrupt changes in stimulus demand) and ramp (gradual changes in stimulus demand) changes in near-visual demand.
Methods: Individually calibrated accommodative responses from both eyes of 12 emmetropic adults (19 to 28yrs) were recorded using a dynamic (30Hz) infrared photorefractor. Both pupils were dilated using 5%PHCl. Subjects watched a high contrast visual target under four conditions (i) binocular (ii) monocular (RE covered with IR filter) (iii) & (iv) with +3.25D or -3.25D lens before right eye (induced myopic and hyperopic anisometropia, respectively). In ramp condition, the LCD screen moved between 66cm and 33cm (1.5D demand) at 0.2D/sec with 4sec stable period at each extreme. In step condition, the stimulus switched electronically between two LCD screens placed at 66cm and 33cm with a 4sec stable period at each extreme.
Results: Mean(±1SD) left eye's accommodative response was largest under binocular (ramp:1.08D ±0.22D;step:1.10D ±0.23D), then monocular (ramp:0.96D ±0.29D; step:1.00D±0.29D), and then with +3.25D(ramp:0.88D±0.32D; step:0.95D±0.34D) and -3.25D (ramp:0.93D±0.26D; step:1.01D±0.26D). 2-factor ANOVA showed no statistical significant main effect of viewing condition (P=0.20), or stimulus presentation (P=0.65), with no interaction (P=0.99). Accommodative responses of all subjects, except one, were consensual in nature.
Conclusions: Accommodative performance of adults seems immune to the presence of induced anisometropia for both abrupt and gradual changes in near-visual demands.

ABSTRACT CS-35

Comparison of Accuracy of Intraocular lens (IOL) Power Calculation Formulae in Children < 2 years

Amit Gupta,[1] Ramesh Kekunnaya,[1] Virender Sachdeva,[2] BL Harsha,[3] Pravin V Krishna[4]
[1]Department of Paediatric Ophthalmology, Strabismus and Neuro-ophthalmology, LV Prasad Eye Institute, Kalllam Anji Reddy Campus, [2]Department of Paediatric Ophthalmology, Strabimsus and Neuro-ophthalmology, LV Prasad Eye Institute, GMRVaralakshmi Campus, [3]Department of Glaucoma, [4]Cornea and Anterior Segment Services, L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, India.

Purpose: To assess the accuracy of IOL power calculation formulae in children < 2 years.
Methods: We retrospectively analyzed records of patients with congenital cataract less than 2 years who underwent primary IOL implantation Data was analysed to look at the target refraction using various IOL power calculation formulae (SRKII, SRK T, Holladay, Hoffer Q) and actual post-operative refraction. We calculated the absolute prediction error with each of the formulae.
Results: 128 eyes of 84 children with congenital/ developmental cataracts underwent surgery. The mean age at surgery was 11.7 ± 6.2 months. Absolute prediction error was found to be 2.27 ± 1.69D with SRK II , 3.23 ± 2.24 with SRK T ,3.62 ± 2.42 D with Holladay formula and 4.61 ± 3.12D with Hoffer Q formulae. Number of eyes with absolute PE within 0.5 D was 27 (21.1%) with SRK II, 8 (6.3%) with SRK T, 12 (9.4%) with Holladay and 5 (3.9%) with Hoffer Q. Comparison between different formulae showed absolute prediction error with SRK II formula was significantly better than other formulae ($p<0.001$). Number of eyes with absolute prediction error within 2 D was 57 % with SRK II formula. Prediction error with the SRK II formula was not affected by age ($p = 0.31$), mean Keratometry ($p= 0.32$) and axial length ($p=0.27$)
Conclusions: Although absolute prediction error tends to remain high with all modern generation formulae, SRK II was the most predictable formula in our series.

ABSTRACT CS-36

Hyperhomocystenemia in Isolated Sixth Cranial Nerve Palsy: Culprit or Bystander

Virender Sachdeva[1], Vaibhev Mittal,[1] Merle Fernandes,[1] Avinash Pathengay,[1] Ramesh Kekunnaya[2], Amit Gupta[2]
[1]Department of Pediatric Ophthalmology, Strabimsus and Neuro-ophthalmology, LV Prasad Eye Institute, GMR Varalakshmi Campus, [2]Department of Pediatric Ophthalmology, Strabismus and Neuro-ophthalmology, L V Prasad Eye Institute, Kallmam Anji Reddy Campus, Hyderabad, India.

Purpose: To investigate association between hyperhomocystenemia and isolated sixth cranial nerve palsy
Methods: A retrospective analysis of all patients who presented with isolated sixth cranial nerve palsy between November 2008 to December 2010 was done.
Results: A total of 27 patients presented during the period. Mean amount of esotropia in primary gaze was 20 prism diopters (range 12 PD-30 PD, SD = 7.48 PD). Mean age of the patients was 25 years. Mean duration of symptoms as 29 days (range 3 – 90 days). Imaging showed ischaemic lesions in two and in other two imaging showed no abnormality. Further laboratory evaluation did not show abnormality in ESR, C-reactive protein, lipid profile, echocardiogram and carotid Doppler.All patients except 4 had one or other risk factor for ischaemia like diabetes, hypertension or coronary artery disease. These 4 patients (14.8%) were found to have hyperhomocysteinemia as only risk factor. Mean serum homocysteine level was 19.20 μmol/L (range 16.07– 20.50, SD = 2.09 μmol/L, Normal: 3.9-13.9μmol/L). All patients were treated with vitamins B6 (250mg), B12 (1500μg) and folic acid (5mg). 2 patients were given Injection Botulinum toxin in the medial rectus muscle of same eye. All patients showed a complete resolution with orthotropia in primary gaze and complete recovery of extra-ocular motility by the end of the two months.
Conclusions: Hyperhomocysteinemia, a known vaso-occlusive risk factor may be associated with Isolated abducens nerve palsy. We recommend it should be investigated in patients with nontraumatic cranial nerve palsies.

ABSTRACT CS-37

Topical Brimonidine and Phenylephrine in Achieving Homeostasis During Strabismus Surgery

Amit Gupta[1], Ramesh Kekunnaya,[1] Virender Sachdeva,[2] BL Harsha,[3] Vaibhev Mittal[2]
[1]Department of Paediatric Ophthalmology, Strabismus and Neuro-ophthalmology, [3]Department of Glaucoma, LV Prasad Eye Institute, Kalllam Anji Reddy Campus, Hyderabad, India; [2] Department of Paediatric Ophthalmology, Strabimsus and Neuro-ophthalmology, LV Prasad Eye Institute, GMR Varalakshmi Campus, Vishakapatnam, India.

Purpose: To compare the effect of phenylephrine and brimonidine on intraoperative and subconjuctival hemorrhage.
Methods: The subjects were divided into 3 subgroups depending on the eye drops used to control bleeding during the procedure: Group A: Hydroxypropyl methyl cellulose (HPMC, control group), Group B: Phenylephrine 10% and Group C: Brimonidine 0.15%. Intraoperative bleeding and postoperative subconjunctival hemorrhage were graded on a scale of one to three. The scores were compared among the study groups.
Results: A total of 97 strabismus procedures were performed in each of the 3 subgroups. The mean age of patients at surgery was 18.2 $\pm$ 8.2 years. The number of patients with severe bleeding (score 3) was 48.5% in the control group, while it was 8.2% and 5.2% for the Phenylephrine and Brimonidine groups respectively. The number of patients with a severe score for subconjunctival hemorrhage was significantly lesser in Phenylephrine and Brimonidine groups compared to control group ($p<0.0001$). The scores for intraoperative bleeding were similar in the two treatment groups ($p=0.46$) while subconjunctival bleeding was significantly less with Brimonidine ($p=0.005$). None of the patients showed any significant post-operative adverse events.
Conclusions: Topical Phenylephrine and Brimonidine significantly reduced the incidence of intra-operative bleeding during strabismus surgery compared to the control group (Hydroxypropyl methylcellulose). Topical Brimonidine was more effective than Phenylephrine in reducing post-operative subconjunctival hemorrhage.

ABSTRACT CS-38

Natural History of Macular Telangiectasia

Raja Narayanan,[1] Shantanu Neravetla,[2] Mudit Tyagi,[1] Jay Chhablani,[3] Rajeev Reddy,[1] Benjamin Nicholson[4]
[1]L V Prasad Eye Institute, Kallam Anji Reddy Campus, Hyderabad, [2]University of Louisville, Kentucky, [3]Shiley Eye Center, University of California, San Diego, [4]Cole Eye Institute, Cleveland, Ohio, USA.

Purpose: To describe the baseline characteristics of eyes with idiopathic juxtafoveal retinal telangiectasia (Mactel) in a tertiary care center in South India.
Methods: Ninety eyes of 45 patients were evaluated retrospectively. Patients with a minimum of 2 years follow-up were included in the study.
Results: The mean age at baseline was 60.4 years. There were 25 females and 20 males in the study. Ten patients had diabetes at baseline. At baseline 33 eyes showed intraretinal pigments, 18 eyes showed perifoveal graying, 15 eyes had subretinal neovascularization. Average follow up period was 33 months (range 18 – 223 months). The mean visual acuity at baseline was 20/40 and at last follow-up was 20/50. At final follow up, 31 eyes maintained the same visual acuity as baseline. Three eyes out of the 75 non-neovascular mactel eyes at baseline developed subretinal neovascularization during follow up period (incidence of 1.3% per year). Photodynamic therapy or intravitreal anti-vascular endothelial growth factor agents or a combination of both were the mode of treatment for the subretinal neovascularisation.
Conclusions: This is the largest study of macular telangiectasia describing the natural history of the disease over a 3-year period. Visual acuity remained stable over the study period.

ABSTRACT CS-39

Ganglion Cell Complex (GCC) in Normal and Macular Dystrophies - A Spectral Domain Optical Coherence Tomography (SD OCT) Study

Deepika Kommanapalli, Uday Kumar Addepalli, Shankar Kalmuri, A Bichapa, Rajeev Reddy
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To characterize the thickness of ganglion cell complex in eyes with macular dystrophy using high-speed, high resolution Spectral-domain optical coherence tomography (SD-OCT).
Methods: Patients with macular dystrophy in both eyes and normal age and gender matched controls underwent SD-OCT imaging using the RTVue (Optovue Inc.), which has an axial resolution of 5 microns, GCC scan was obtained. Based on boundaries automatically obtained on computer displays of OCT cross-sections, the thicknesses of the ganglion cell complex were averaged over both 5 anf 1.5 millimeters regions centered at the fovea. The ganglion cell complex (GCC) was the sum of the nerve fiber layer(NFL),ganglion cell layer(GCL) and inner plexiform layer(IPL) thicknesses.
Results: Thirty three patients (sixty six eyes) with macular dystrophy and 33 normal controls underwent high resolution OCT imaging. Subjects ranged from 10 to 55 years old. Macular dystrophy diagnoses included Stargardt disease (11), Cone dystrophy (4), Cone-rod dystrophy (10), Central retinitis Pigmentosa (1), Best disease (2) and 7 other miscellaneous Macular dystrophies. The following thickness values reported are mean ± standard deviation. Mean GCC inner average thickness (GIAT) averaged over the central 5mm area, was 99.05± 7.78 µ for normal subjects, 80.24± 15.68 µ for patients with macular dystrophies (p< 0.0001). Mean GCC inner superior Average thickness (GISAT) is 98.30±7.67 µ for normal subjects, 40.12±41.66 µ for patients with macular dystrophies (p< 0.0001).Mean GCC inner inferior average thickness (GIIAT) is 99.83±8.30 µ in normal subjects and 114.05±88.99 µ(p=0.1977)
Conclusions: Eyes with macular dystrophy had a significant decrease in inner average ganglion cell complex thickness and inner average superior ganglion cell complex thickness compared to normal. The higher resolution and definition of the FD-OCT technology facilitated measurements of the thickness of retinal sub layers.

ABSTRACT CS-40

Scleral Thickness: A Normative Data using Ultrasound

Priyangshu Chandra, Padmaja Kumari Rani
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: to assess the mean scleral thickness in the different region of the globe
Methods: 100 eyes of 50 patients with no ocular pathology underwent both ultrasound biomicroscopy (UBM) and B scan. Scleral thickness was measured in 3 region of the globe in 4 primary quadrants. Anteriorly 2 mm away from the limbus, in the equator region at the muscle insertion site and posteriorly 2 mm away from the disc, to make all the measurement uniform statistical analysis was used to see the mean scleral thickness in different region and to see the correlation between different variables like age, gender, axial length with scleral thickness
Results: 50 patients had a mean age with ± SD of 47.4 ±15.035 years. Mean scleral thickness with ± SD was 0.62 ± 0.038 mm at the posterior pole, 0.61 ± 0.039 mm at the equator and 0.48 ± 0.04 mm anteriorly. a positive correlation exist between gender and anterior scleral thickness (P > 0.0165) . Whereas other variables does not have any correlation with scleral thickness in different region.
Conclusions: Scleral thickness gradually increase from the anterior (0.48 ± 0.04 mm) to the equator (0.61 ± 0.039 mm) and posteriorly (0.62 ± 0.038 mm) the scleral thickness remained almost same and posteriorly scleral thickness is more in female than male.

ABSTRACT CS- 41

Wavefront Aberrations in Normal Indian Aging Population

Apoorva Karsolia, Ramdas Gore, G Rekha, Merle Fernandes
L V Prasad Eye Institute, GMR Varalakshmi Campus, Visakhapatnam, India.

Purpose: To investigate the magnitude and distribution of monochromatic aberrations in a Indian population and to correlate these with increase in age.
Methods: The study was approved by the Institute review board. We included subjects over 18 years who visited the out patient department and were willing to give consent to undergo the following investigations: retinoscopy, slit lamp biomicroscopy, applanation tonometry, indirect ophthalmoscopy, topography, pachymetry and aberrometry. Lower and higher order aberrations (till the 6^{th} order) were analyzed with respect to their magnitude and distribution in various age groups, correlation with age and pupil size. Statistical analysis was done using Pearsons product moment correlation for non-parametric data and SPSS software Version 13.0.
Results: 181 eyes of 124 subjects were included, with mean age of 41.13 yrs (range 18 – 75 yrs). The mean spherical equivalent was 0.3812 D (range -4.75 D to +3.125D). There was a positive correlation between age and spherical equivalent ($r=0.57$, $p<0.0001$). The mean root mean square (RMS) of higher order aberrations (HOA) was 0.41 microns (Range 0.018 to 2.81 microns). The RMS of HOA showed a weak negative correlation with age ($r=-0.23$, $p=0.002$) and there was a weak negative correlation of RMS coma with age ($r=-0.14$, $p=0.049$). There was no correlation with RMS spherical aberrations, 4^{th}, 5^{th} or 6^{th} order aberrations with age. There was a positive correlation between RMS HOA, 3^{rd}, 4^{th}, 5^{th} and 6^{th} order aberrations with increase in pupil size.
Conclusions: There is a reduction in higher order aberrations and coma with age with an increase in aberrations related to pupil size.

ABSTRACT CS-42

Collagen Cross Linkage – One Year Review

Swathi Chary, K Madhukar Reddy
Drishti Eye Centre, Hyderabad, India.

Purpose: To review collagen cross linkage patients of progressive keratoconus for safety and complications over a period of a year.
Methods: A retrospective study was done of collagen cross linkage patients. In this clinical study advanced keratoconus patients were considered with corneal pachymetry being >400 microns and all types of cones for collagen cross linkage. Bupivacane was used for epithelial debridement and isotonic riboflavin (0.1%) was applied for 30minutes. Cornea was exposed to UVA radiation of 365nm for 30 minutes.
Results: 19 eyes in the age group of 11-26 yrs were treated with collagen cross linkage in progressive keratoconus. Best corrected visual acuity dropped by 1 line. The average spherical power remained the same and an increment of 1 dipotre of cylinder was observed. In 4 eyes there was a reversal and flattening of the keratoconus by 3.6 diopters cylindrical power.
Conclusions: Collagen cross linking is an effective means for stabilizing the cornea in keratoconus. Collagen cross linking has become a standard therapy for progressive keratoconus.

ABSTRACT CS-43

Shivani Pahuja, Jatin N Ashar, Muralidhar Ramappa, Pravin Vaddavalli, Sunita Chaurasia, Prashant Garg, Somashiela Murthy, Virender Sangwan
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To evaluate outcomes of deep anterior lamellar keratoplasty (DALK) in children
Methods: Retrospective analysis of pediatric DALK from 2001 - 2010
Results: 26 eyes of 26 children, 7.82+4.64 years, m: f 13:13, underwent DALK for keratoconus (8),microbial keratitis (6), corneal scar(6), corneal keolid (3), chemical injury with limbal stem cell deficiency (2) and dermoid (1). Big bubble achieved in 5, while manual dissection done in 21. Patients followed up for 30 days-6 years. Final vision ranged from counting fingers to 20/30 (mean sphere 2.32D, mean cylinder -2.5D). Complications were graft infiltrate (3), graft dehiscence (3), Descemetï¿½ï¿½â„¢s detachment (2)
Conclusions: DALK is feasible in children but does not offer advantage over PK in terms of complications

ABSTRACT CS-44

Documentation of Keratoconus Progression in Patients with Vernal Keratoconjunctivits Using Orbscan

Jatin Ashar, Mukesh Taneja, Varsha Rathi, Somasheila Murthy, Virender Sangwan
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To document changes on Orbscan over a period of 24 months in patients with keratoconus and VKC
Methods: Retrospective analysis of clinical and Orbscan data of 22 eyes of 11 patients with keratoconus and VKC with atleast 2 follow up over 2 years period was done. The parameters studied included patients demography, clinical features, visual acuity, refraction and Orbscan. The changes in various Orbscan parameters were studied.
Results: Mean age was 14 + 4.1years. 20 eyes had keratoconus, while 2 had forme fruste keratoconus. 8 eyes of 22 showed evident progression (>1 diopter change in mean K over 24 months). There was no significant difference in the visual acuity or clinical features over followup. In patients with progression, statistically significant change ($p<0.05$) was found in posterior float curvature and radius, sim K astigmatism and maximum astigmatism. Rest of the parameters did not show significant change. Among the patients without evident progression, none of the parameters showed significant change. One patient with FFKC progressed to keratoconus over 2 years.
Conclusions: Serial topographic analysis provides information about progression of keratoconus in patients with VKC.

ABSTRACT CS-45

Outcomes of Penetrating Keratoplasty in Mooren's Ulcer

Anurag Mathur, Jatin Ashar, Virender Sangwan
L V Prasad Eye Institute, KAR Campus, Hyderabad, India.

Purpose: To report anatomical and functional outcomes of penetrating keratoplasty (PK) in Mooren's ulcer
Methods: From a database of 320 Mooren's ulcer, patients who had undergone Penetrating keratoplasty were identified and data was collected for demographics, duration of symptoms, , treatment, systemic illness, visual acuity on presentation, severity, medical and surgical treatment, histopathology and visual acuity at final visit. Kaplan â€“ Meier survival plots, Wilcoxon rank sum test and descriptive statistics were used to analyse the data.

Result: 19 eyes of 18 patients were included in the study. A total of 26 keratoplasties were done including 15 tectonic (10 crescentric patch grafts, 5 large grafts), 8 optical and 3 for therapeutic purpose. Overall 23 of 26 grafts were tectonically stable at last visit. Two patch grafts and one large graft showed recurrence of Mooren's ulcer with perforation. Secondary infection was the most common complication followed by recurrence of Mooren's ulcer in the graft. Optical PK showed poor visual outcome.

Conclusions: Penetrating keratoplasty in mooren's ulcer can reactivate the disease process. Good anatomical result can be obtained by appropriate use of immunosuppression. Visual outcomes may not be good due to host of factors.

ABSTRACT CS-46

Intrasession and Intersession Repeatability of the Orbscan System on Corneal Topography Assessment in the Normal Human Eye

Pesala Veerendranath[1], T V Amarnath[2], Pravin V Krishna[1]
[1]Cornea & Anterior Segment, [2]Smt. Kannuri Santhamma Centre for Vitreo Retinal Diseases, L V Prasad Eye Institute, Hyderabad, India.

Purpose: The Purpose of the study is to determine Inter and Intra observer repeatability of corneal topography using Orbscan IIz Corneal topography system.
Methods: 100 eyes of 50 healthy volunteers in which both right and left eyes of 25 males and 22 females respectively, right eye of 1 male and left eye of 3 females were investigated with Orbscan IIz by two investigators for intersession and interobserver variability. The subjects are taken from L.V.Prasad Eye Institute.
Results: Intersession repeatability scores were (>0 .9) for all parameters of Orbscan except for 3mm, 5mm zone irregularity and Sim 'K' astigmatism axis(0.633,0.777 and 0.837) respectively. Inter session test re test variability values were Sim K astigmatism-0.123037, ABFS (mm)- 0.030759, ABFS (D)- 0.248194, PBFS(mm) - 0.044901, PBFS(D) - 0.386787, 3mm zone irregularity (D) -0.351432, 5mm zone irregularity (D) -0.290621, Max K (D) -0.262337, Min K (D) -0.24678, Central pachymetry(µm)-4.772971, 3mm zone Mean power(D)-0.228395, 5mm zone mean power(D)-0.191626, White-to-White diameter(mm)-0.106066 respectively. Intrasession repeatability scores for investigator 1 were also (>0.9). Intra session test re test variability by investigator 1 were Sim K astigmatism-0.124451, ABFS (mm)- 0.023355, ABFS (D)- 0.135765, PBFS(mm) - 0.035214, PBFS(D) - 0.41295, 3mm zone irregularity (D) -0.323855, 5mm zone irregularity (D) - 0.257387, Max K (D) -0.25173,Min K (D) -0.206475, Central(µm)-3.50725, 3mm zone Mean power(D)-0.181019, 5mm zone mean power(D)-0.141421, White-to-White diameter(mm)-0.09051 respectively.
Conclusions: The results of this study indicate that Orbscan shows excellent performance in assessing repeatability scores (>0.9) in both Inter and Intrasessions in all the parameters except for 3mm and 5mm zone irregularity and Sim k astigmatism axis in which they showed moderate to poorer repeatability scores.

ABSTRACT CS-47

Spatial Profile of Macular Pigment Density and Correlation with Central Foveal Thickness in Normal Asian Indian Eyes

Koushik Sargod, Rohit Shetty, Padmamalini Mahendradas, Kavitha Avadhani, Rajesh S Kumar, Sriharsha Nagaraj, Bhujang K Shetty
Narayana Nethralaya Post Graduate Institute of Ophthalmology, Bangalore, India.

Purpose: To determine macular pigment optical density (MPOD) using the heterochromatic flicker photometry in normal Asian Indian eyes and to investigate its correlation with central foveal thickness.
Methods: We determined MPOD using the Tinsley macular pigment densitometer (Tinsley Ophthalmic, Surrey, UK) which measures macular pigment absorption density using the log sensitivity to 460nm light for the fovea and parafovea after normalizing with respect to 540nm . Foveal thickness was obtained using the spectral domain optical coherence tomography (Spectralis, Heidelberg Engineering, Germany).
Results: 89 eyes (44 subjects) were analyzed (23 males and 21 females); mean age was 32.5 ±12.3 (range, 18-70) years. The mean MPOD was 0.415 (95% C.I, 0.387-0.444) and the mean foveal thickness was 227.2 μ (95% C.I, 221.9-232.5). MPOD correlated poorly with foveal thickness ($r=0.07$, $p=0.5$). Males showed higher MPOD values compared to females ($r=-0.30$, 95% C.I, -0.55-0.01, $p<0.05$); however, there was no inter-eye asymmetry ($r=0.61$, 95% C.I, 0.38-0.75, $p<0.0001$).
Conclusions: MPOD does not appear to correlate with central foveal thickness in Asian Indian eyes.

ABSTRACT CS-48

Management of Post-Lasik Keratectasia with Contact Lenses

Parthasarathi Kalaiselvan, Preeji Mandathara, Varsha Rathi
L V Prasad Eye Institute, Hyderabad, India.

Purpose: To study the management of Post-Lasik Keratectasia with Gas Permeable (GP) Contact lenses at a tertiary eye care centre.
Methods: A retrospective study of patients diagnosed with Keratectasia following Lasik surgery and referred to Bausch and Lomb contact lens clinic at L V Prasad Eye Institute, Hyderabad, from January 2003 to December 2009 was done. Data on patient demographics, refraction, pre contact lens fitting best spectacle corrected visual acuity, corneal topography, contact lens parameters and visual acuity with contact lens were obtained.
Results: 27 eyes of 16 patients were included in the study. The mean age was 28.2 ± 4.3 years and among them 43.75% were males. The mean pre-contact lens fitting refractive error was -3.50 ± 4.80 D sphere, -3.53 ± 3.05 D cylinder. The corneal topography showed mean simulated keratometry reading in the steeper and flatter meridians were 50.13 ± 8.12D and 47.09 ± 6.88D respectively. Rigid gas permeable contact lenses were fitted in all eyes. Seventeen eyes were fitted with standard tricurve design GP lenses and 10 eyes with Rose K2 lens. The average base curve and power of the contact lenses were 7.13 ± 0.62mm and -8.24Ds ± 5.49D. The diameter ranged between 8.7 to 10.40mm. The average best spectacle corrected visual acuity improved from 0.3 ± 0.2 to 0.1 ± 0.1 in log MAR chart with contact lenses ($p<0.000$)
Conclusions: The post-lasik keratectasia corneas resemble the topography pattern of keratoconus. GP lenses improve the visual acuity significantly in these eyes.

ABSTRACT CS-49

Assessing the Ability of High School Children as Key Informants in Detection of Childhood Blindness

Ashwin Mallipatna, Charles Baik, Ravindra R Battu, Jyoti Matalia, Vasudha K, Mathew Kurian, Pradeep Banandur, Helen Dimaras, Brenda Gallie

Narayana Netralaya, Bangalore,India.

Purpose: We studied the ability of school children to identify normal and abnormal eyes to assess their capability as key informants for childhood blindness.

Methods: School children were trained using images of eye diseases in children. Before and after training, we tested their ability to identify normal or abnormal eyes in photographs.

Results: 123 tenth-standard children were trained. Correct responses increased from 62.8% to 77.1%. The first version of the training module produced the lowest scores (pre-training: 53.0%; post-training: 62.8%). The second and third versions showed improved scores (pre-training 67.4%, 68.1%; post-training 85.1%, 83.3%).

Conclusions: School children may be effective key-informants.

ABSTRACT CS-50

Comparison of Macular Hole Measurement between Spectral Domain Cirrus SD OCT and Digital Photography

Souvik Mandal, Mukesh Singh, Soumava Basu, Taraprasad Das
L V Prasad Eye Institute Bhubeneswar, India.

Purpose: To estimate the agreement between spectral domain Optical Coherence Tomography (SD OCT) and Digital fundus I photography for macular hole measurement.
Methods: Twenty eyes with idiopathic full-thickness macular hole were taken for this study. Eyes with cataract,corneal opacity and vitreous opacity(signal strength < 5) were excluded. Measurement of macular hole size was done by Cirrus HD OCT and digital photograph (Visupac 450, Zeiss). Two examiners (MS and SM) masked to each other measured macular hole.
Results: Both the measurement shows good intra-observer repeatability (ICC for OCT= 0.98, ICC for digital photograph= 0.81). Average size of macular hole was more for digital photography (655±233.1 µm) than OCT (632.35±211.7 µm) but it was not statistically significant (p=0.06). Agreement between two instrument shows a wider interval (Limits of agreement= 98.75).
Conclusions: SD- OCT and digital fundus photograph showed good intra-observer repeatability. There was a poor agreement between two instruments.

ABSTRACT CS-51

Corneal Endothelial Cell density and Morphology of Indian Population in Different Age Groups

Souvik Mandal, Sujata Das, Santanu Jana
L V Prasad Eye Institue, Bhubaneswar, Orissa, India.

Purpose: To calculate the corneal endothelial characteristics in Indian population this includes the pediatric age groups.
Methods: Specular microscopy done on 240 normal volunteers, aged from 3-70 years, among which 136(56.5%) were male and 104(44.5%) were female. The analyzed parameters were mean cell density, coefficient of variation of cell area, central corneal thickness and hexagonality.
Results: The mean cell density of the group was 2807±338.8(range 3891-1986). Mean CV was 42.8±9.2(range 21-72). Mean hexagonality of endothelial cell were 49.8±9.2 (range 25-78). Average number of cell were analyzed in each eye was 147.9±21.3 (range 120-178). The mean central pachymetry was 521.2±34 ranged 490-581 µm. There was a statistically significant decrease in MCD with increase in age ($p<0.001$, $r= -0.58$). Regression analysis indicated a cell loss rate of 0.5% per year. A significant decrease in mean hexagonally($r= -0.38$, $p<0.0001$) was noted. No significant difference between hexagonality and CV were noted between each age group. MCD between different sex showed a significant difference. The endothelial cell counts were compared with presently available literature.
Conclusions: Endothelial cell characteristics were analyzed for pediatric age groups and younger children (3-20). MCD and Hexagonality of cells are negatively correlated with age.

ABSTRACT CS-52

Macular Folds after Topiramate Induced Angle Closure

Mukesh Kumar, Siddharth Kesarwani
L V Prasad Eye Institue, Bhubaneswar, Orissa, India.

Purpose: To report 2 rare cases of bilateral angle closure glaucoma with macular folds after topiramate therapy.
Methods: Case 1-A 24-years-old girl, with no prior history of glasses, initiated on topiramate for chronic headaches presented with decreased vision. On examination, she was found to have a myopic refractive error of -5DS was found with an intraocular pressure of 50mm Hg and 55mm Hg in the right and left eye, respectively, closed angles on gonioscopy, ciliary effusion on UBM with ILM folds in the macula in both eyes.
Case 2- A 20 year female presented with acute headache and decreased vision following administration of topiramate. On examination, her IOP was 25mm Hg in both eyes with closed angles on gonioscopy, a myopic refractive error of -4.5DS and prominent, macular folds with no fluid in both eyes.
Results: There was complete resolution of the ciliary effusion and macular folds after discontinuation of topiramate and conservative treatment with topical steroids and cycloplegics in both eyes.
Conclusions: Topiramite may be associated with macular folds at the macula which may resolve with conservative treatment alone.

ABSTRACT CS-53

Comaprison of Central Corneal Thickness Measurement in Keratoconus with Specular Microscopy, Spectral Domain OCT and Orbscan-II vs Ultrasound Pachymetry

Mukesh Kumar, Ramesh Kumar, Bhaktipada Sahoo, Souvik Mandal, Srikant Sahoo
L V Prasad Eye Institute, Bhubaneswar, Orissa, India.

Purpose: To compare central corneal thickness measurement in Keratoconus with Orbscan □, Specular microscopy, SD-OCT versus Ultrasound pachymetry.
Method: 38 keratoconic eyes were examined and analyzed in our study. Eyes with apparent corneal opacity excluded. CCT was measured by using all these four instruments- orbscan □, specular microscope, SD-OCT and US pachymetry. The acoustic equivalent correlation factor (0.92) was used for orbscan reading
Results: The mean CCT was compatible among SD-OCT (429.89±63.1 [SD]), specular microscope (419.31±64.7 [SD]) and US pachymetry (437.18±61.6 [SD]). However orbscan□ significantly smaller measurement than the other 3 instruments (399.3±67.38 [SD]). Linear regression between instrument was high (Pearson's correlation > 0.7) for all instrument. But SD-OCT showed the better correlation to the US pachymetry (r=0.98, P<0.001) than the other 2 instruments.
Conclusions: CCT measurement was comparable among US pachymetry, SD-OCT and specular microscope while orbscan □ gave significantly lower measurement in keratoconic eyes. The measurement all the four instruments showed high linear correlation with one another.

ABSTRACT CS-54

Retinal Nerve Fiber Layer Thickness in Normal Indian Children Measured with Spectral Domain Optical Coherence Tomography

Bhaktiprasad Sahoo, Gopal Varshney, Mukesh Kumar, Siddharth Kesarwani, Aparna Rao
L V Prasad Eye Institute, Bhubaneswar, Orissa, India.

Purpose: To obtain the retinal nerve fiber layer (RNFL) thickness normative values of Indian children by using optical coherence tomography (OCT) and to investigate the effect of axial length (AL) on RNFL thickness.
Methods: Fifty Nine normal children aged between 6 and 17 years were recruited for this study. The RNFL thickness was measured by OCT (Cirus OCT). AL was measured by the IOL Master.
Results: The median age of our subjects was 11.18 years. The mean full circle peripapillary RNFL (RNFLFC) was 93.66mm (SD 10.36) and 92.77mm (SD 10.51) in the right and left eyes, respectively. Linear regression analysis showed a significant negative correlation between AL and RNFL-FC in both the right ($r = -0.38$, $P<0.05$) and left eyes ($r = -0.32$, $P<0.05$). The effect was the strongest in the superior quadrant. Every 1mm increase in AL would result in a decrease in RNFL-FC by 2.7 to 2.9 mm. Age did not show significant effect on RNFL-FC.
Conclusions: This study provides normative RNFL values for Indian children. It can be used as a reference for the screening of glaucoma in children. AL demonstrates a significant negative correlation with RNFL thickness.

ABSTRACT CS-55

Preferential Hyperacuity Perimeter in AMD

Sharmila Pawar
Aditya Jyot Eye Hospital Mumbai, India.

Purpose: To compare the efficacy of preferential hyper acuity perimetery (PHP) with amslers grid in detection of wet AMD in cases of intermediate AMD.
Methods: Study period : 6 months, Patients: 50 eyes. Prospective, non randomized study. Inclusion criteria: Age > 50 yrs.Vision not less than 6/18.Patients having intermediate AMD , other eye armd and patients not included in the exclusion criteria.
Exclusion criteria: Patients having macular and optic nerve disease other than armd.
Any signficant media opacity. Patient underwent refraction ,BCVA examination, PHP evaluation, amslers grid examination, dilatation, biomicroscopic examination, fundus photo, OCT and fundus fluroscein angiography (FFA) in some cases.
Results: Total of 50 eyes, dry AMD was seen in 36 eyes and wet AMD was diagnosed in 14 eyes. Out of 14 cases of wet AMD, amslers was positive in 8 and PHP was positive in 12. Out of Dry AMD cases amslers was positive in 4 and PHP was positive in 6. EPR validity test calculator was used. Amslers showed Sensitivity =58%(95% CI), Specificity=88%(95% CI). PHP showed Sensitivity =92% (95% CI), Specificity = 83% (95% CI) Sensitivity of amslers was 58% and of PHP was 92%. Specificity of amslers was 88% and of PHP was 83% Accuracy of amslers was 78% and of PHP was 86%.
Conclusions: This study demonstrated that the PHP had a greater sensitivity than the Amsler grid in detecting AMD related lesions and is also a good tool for monitoring of the patients.

ABSTRACT CS-56

"EPI" On versus "EPI" Off Corneal Collagen Crosslinking- An Intraoperative Study

Chintan Malhotra
Narayana Nethralaya, Bangalore, India.

Purpose: To intraoperatively study the penetration of riboflavin 0.1% aqueous solution into human corneas during collagen cross linking using the hand held spectral domain OCT.
Methods: One eye each of 6 patients undergoing collagen cross linking of the cornea using 0.1% aqueous solution of riboflavin and UVA light (370 nm), were imaged intraoperatively using the hand held spectral domain OCT. In 3 eyes the epithelium was removed completely(Group 1) while in the other 3 the epithelium was removed in blocks in a grid pattern, leaving behind intact islands of epithelium (Group 2). The depth of penetration of riboflavin (seen as a band of increased reflectivity) was measured at the end of the 1 hour procedure.
Results: In group 1 the riboflavin penetration was homogenous extending upto a depth of 200 microns into the corneal stroma. In group 2 greater depth of penetration was seen in the epi off areas, compared to the epi on areas, where the penetration was minimal. This uneven penetration was reflected post operatively as a grid like haze of the cornea.
Conclusions: Corneal epithelium blocks adequate penetration of riboflavin into the stroma as directly imaged intraopertively and thus may lead to an inhomogenous crosslinking effect.

ABSTRACT CS-57

Delayed- Onset Endogenous *Salmonella typhi* Endophthalmitis Following Typhoid: A Case Report and Review of literature

Manish Kumar Sinha[1], *Subhadra Jalali*[1], *Suma Nalamada*[2]

[1]Smt Kanuri Santhamma Centre for Vitreo-Retinal Diseases, [2]Jhaveri Microbiology Centre, Kallam Anji Reddy campus, L V Prasad Eye Institute, Hyderabad, India.

Purpose: To report a case of endogenous endophthalmitis caused by gram negative bacterium *Salmonella typhi* as a delayed complication of systemic typhoid and review of literatures.
Methods: Case description and literature review.
Results: A 4-year-old male child presented with acute endophthalmitis in the left eye of four days duration. His past history was significant for typhoid fever two months back. Vitreous biopsy on culture, revealed *Salmonella typhi*. Despite aggressive treatment with vitrectomy and systemic and intravitreal antibiotics, the affected eye did not improve and went into phthisis. Literature showed only one previous case in association with typhoid and eight cases of *salmonella* species (non-typhoid) causing endogenous endophthalmitis.
Conclusions: *Salmonella* infections in the eye are fulminant and can lead to loss of the eye, inspite of vigorous treatment. Endogenous endophthalmitis due to *Salmonella typhi* can present as a delayed complication of systemic typhoid and should be incorporated into standard literature on typhoid.

ABSTRACT CS-58

Optic Disc Size and its Correlates in High Myopia in Central India

Vinay Nangia, Rajesh Gupta, Ajit Sinha
Suraj Eye Institute, 559, New Colony, Nagpur, India.

Purpose: The disc size is known to vary significantly. High myopes are thought to have large discs. Myopic discs are also thought to be more predisposed to glaucoma. Data on the size of the optic disc in myopia from India re not commonly available. It was the purpose of this study to determine the size of the optic disc in myopes in Central India and to determine its relationship with ocular parameters.
Methods: 73 eyes of 45 subjects (15 males) with a myopia of -8.00D or more underwent an eye examination including vision, refraction, slit lamp evaluation, keratometry, Heidelberg Retina Tomography, applanation tonometry, central corneal thickness, pupillary dilation, optic disc photography. Confocal Scanning Laser Ophtalmoscopy was done using the HRT 2 (software version 3). The optic disc contour line was drawn using the optic disc photograph as a guide. With the automatic reference plane, the computerized algorithm provides several values of optic disc morphometric parameters including optic disc area, cup area, cup disc ratio and retinal nerve fiber layer. Data were analysed using (PASWstatistics V.18)
Results: The mean age was 33.00+-13.96 yrs. The mean spherical equivalent was -11.15+-2.96D, optic disc area was 2.20+- 0.69 mm2. (range 0.86-4.53). The mean axial length was 27.42±1.9mm. The Optic disc area showed significant correlations in high myopic eyes with rim area ($p<0.001;r=0.829$), cup area ($p<0.001;r=0.44$), with cup volume ($p=0.007;r=0.34$), with cup shape measure ($p=0.034;r=0.271$) and with horizontal and vertical corneal diameters. ($p=0.01;r=0.34$) and ($p=0.04;r=0.27$) respectively. It showed no correlations with spherical equivalent (p=0.53+-0.079)axial length ($p=0.548;r=0.08$), anterior chamber depth (p 0.351; r= -0.128)lens thickness ($p=0.31;r=-0.13$) and central corneal thickness ($p=0.31;r=0.13$). Multivariate regression analysis with disc area as dependant parameter and rim area, cup area, cup volume, cup shape measure, vertical and horizontal corneal diameters, spherical equivalent and axial length showed significant correlations with rim area ($p<0.001$;CI 95% -0.997,1.002) and with cup area ($p<0.001$;CI95% 0.999,1.011). Other variables did not show any significant correlation.
Conclusions: Several studies have found the optic disc to be significantly larger in eyes with high myopia. The Mean optic disc area has been found to be 2.25+-0.51mm2 in Central India. This study found the mean optic disc area in a clinic based population with high myopia of more than -8.00 D (mean sph. Equ. = -11.5+--2.96) to be 2.2+-0.69mm2. It may be important to study the relevance of this finding, specially since myopic disc are considered to be more predisposed to being glaucomatous and a smaller myopic disc may be even harder to diagnose.

In case of any help please contact the following

Oraganising Committee:

Dr Inderjeet Kaur		9391623090
Mr Jai Ganesh		9908604305
Ms Preeti Tornal		9618044168
Accommodation:	Mr K Nageshwera Rao	9959623176
	Mr Praveen K Balne	9937646364
	Mr Ganeshwer Rao M	9032231905
Transport:	Dr Ramesh Babu	9492660160
	Mr Subhash Gaddipati	9642769880
	Mr V V Pulla Rao	9490648720

Shuttle Bus Service Timings:

Arnold Bhavan Guest House to LVPEI	07.30 AM (29,30 & 31 July 2011)
CCMB/IICT Guest House to LVPEI	07.30 AM (29,30 & 31 July 2011)
LVPEI to Arnold Bhavan Guest House	09.30 PM (29, July 2011)
LVPEI to CCMB/IICT Guest House	09.30 PM (29, July 2011)
LVPEI to Taj Banjara Hotel	07.30 PM (30, July 2011)
Taj Banjara to Arnold Bhavan Guest House	10.00 PM (30, July 2011)
Taj Banjara to CCMB/IICT Guest House	10.00 PM (30, July 2011)
LVPEI to Arnold Bhavan /CCMB/IICT Guest House	5.00 PM (31 July, 2011)
LVPEI to Railway Station, Secunderabad	5.00 PM (31 July, 2011)

25 YEARS LVPEI
So that all may see

LV Prasad Eye Institute
Kallam Anji Reddy Campus
LV Prasad Marg, Banjara Hills
Hyderabad 500034, India
Tel: +91 40 30612519, 30612511
Email: ierg@lvpei.org
Website: http://www.lvpei.org