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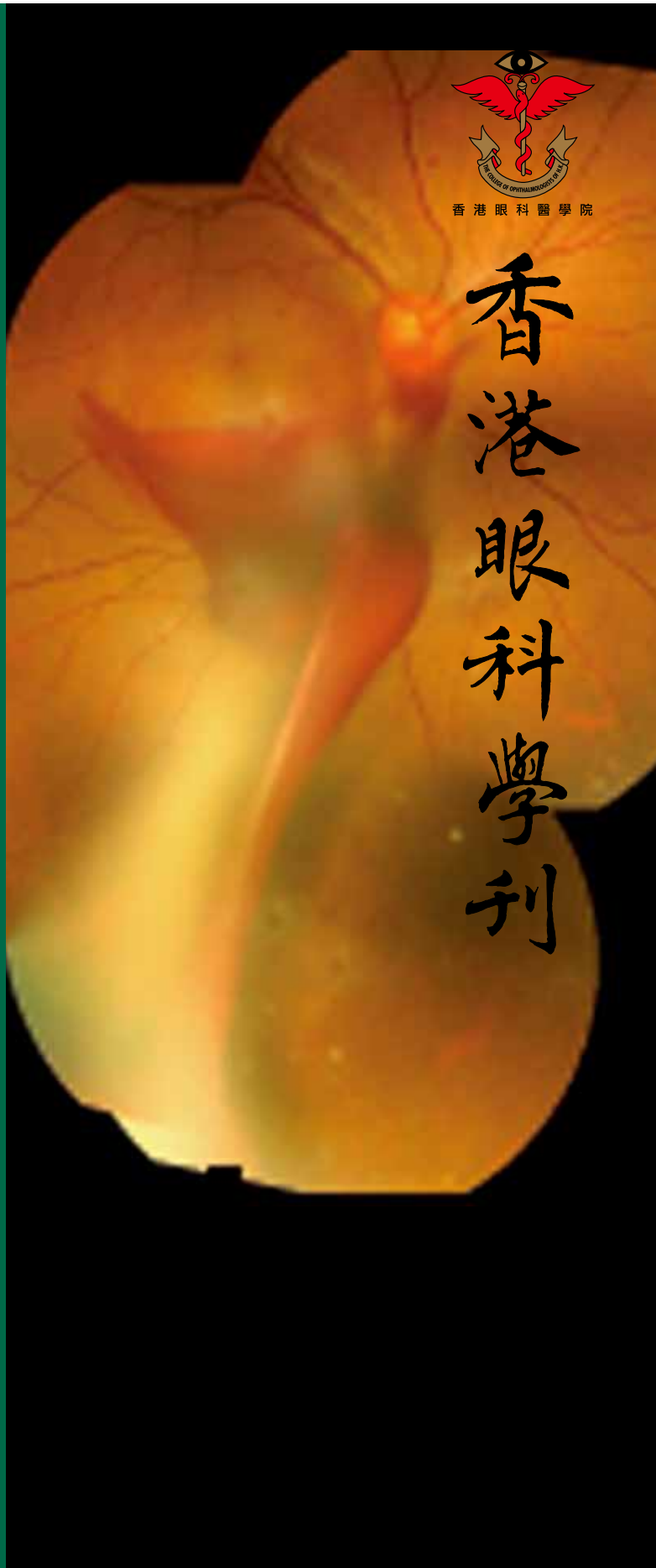

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EDITORIAL

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Cytomegalovirus retinitis – a local perspective

Ian Y. H. Wong, MMed (Ophth, NUS), FCOphth HK, FHKAM (Ophthalmology), FRCSEd (Ophth), FRCOphth
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Cytomegalovirus retinitis (CMVR) can cause blindness and affects mainly immunocompromised patients. It is most frequently associated with human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome (AIDS). In patients with HIV infection/AIDS, the risk of developing CMVR increases when the CD4+ count falls below 50 cells/mm^3.[1] The lifetime risk of developing CMVR is estimated to be 25% to 40%.[2] The typical presentation is reduced vision in eyes that lack inflammation. Characteristic fundal findings include whitish retinal infiltrate and prominent retinal hemorrhages, assuming the 'cheese-on-ketchup' appearance.[3,4] Other features include frosted branch angiitis and retinal detachment in late stages.

According to the latest report on the global trend of HIV infection published by the World Health Organization in 2013, cytomegalovirus retinitis remained to occur most commonly in Africa. In Asia, there were 4,967,000 HIV infected patients in 2001 and 6,131,000 in 2012 (23.4% increase).[5]

In Hong Kong, the Department of Health reported 1,672 HIV-infected individuals in Hong Kong as of May 2016.[6] In Singapore, the HIV-infected population was 6,829 in 2015, according to the Ministry of Health in Singapore.[7] Although the prevalence of HIV infection remains relatively low in Hong Kong, efforts should be made to prevent this dreadful disease from spreading and its complications from arising.

In a cohort of 18,733 HIV-infected subjects in the United States, the annual incidence of CMVR was reported to be 45.3% during 1993 to 1995 prior to the introduction of the highly active antiretroviral therapy, and subsequently dropped to 8.8% during 1996 to 2000 and further to 1.5% during 2001 to 2008.[8] Successful suppression of the HIV virus enables infected patients to remain disease-free and at lower risk of developing CMVR.

The focus for CMVR has shifted to non-HIV infected individuals who are immunosuppressed because of treatment for diseases/conditions such as cancer, chemotherapy, organ transplantation, and autoimmune diseases.[9] In Hong Kong, the seropositivity rate for CMV has been reported to be 90%,[10] which is comparable with other Asia-Pacific countries. In China, the rate was reported to be as high as 98.7% among a group of pregnant women in the Jiangsu Province.[11] Other nearby countries/regions have also reported high seropositivity rates.[12,13] The rate in Asia-Pacific is much higher than that in Europe[14-16] or the US.[17] The differences between HIV- and non-HIV-infected patients with CMVR in terms of clinical features and visual prognosis have been reported.[18]

Presence of CMVR is not necessarily associated with CMV seropositivity.[18,19] This implies the possibility of end-organ infection/reactivation in the absence of seropositivity.[18] This occurs most commonly in the eye, presenting as CMVR, and the gut, presenting as CMV enteritis.[20] CMV also affects the corneal endothelium in the form of endotheliitis.[21] In view of this, the use of local anti-CMV therapy may be more

effective than systemic therapy, although the importance of eradicating CMV systemically cannot be overlooked.[18]

In this issue, Cheung and Kwong[22] reviewed the various causes, clinical presentations and treatments for CMV infection. This provides a reference for CMV infection beyond CMVR and enables ophthalmologists to formulate treatment plans for such patients.

In addition, Shih et al[23] and Ng et al[24] each wrote a perspective article to report the major research focuses and achievements of the 2 local ophthalmology institutions. This year marks the 10th and 22nd anniversary of the establishment of the Department of Ophthalmology of The University of Hong Kong and the Department of Ophthalmology and Visual Science of The Chinese University of Hong Kong, respectively. Readers can appreciate the efforts made by local scientists in vision research, from bench to bedside. Both institutions have together put Hong Kong in the forefront of ophthalmology. With the years to come, it is undoubted that more achievement will be accomplished. On behalf of the Editorial Board, I would like to take this opportunity to congratulate both institutions on their achievements, and wish them many fruitful years to come.

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Establishing research in ophthalmology and visual sciences at the University of Hong Kong: an institution profile

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Abstract

Established in 2006, the Department of Ophthalmology of the Li Ka Shing Faculty of Medicine, University of Hong Kong became the territory's second clinical academic unit in ophthalmology and visual science. Under the leadership of Professor David Sai Hung Wong (recently succeeded by Professor Jimmy Shiu Ming Lai), the department has focused on clinical excellence and research in vitreoretinal disease, glaucomatous optic neuropathy, and cornea and external eye disease. Through collaboration with leading ophthalmic institutions locally and abroad, the department aims to become one of the top academic institutions in Southeast Asia.

Key words: Anterior eye segment; Glaucoma; Retina

Introduction

The Department of Ophthalmology was established in 2006 as the Eye Institute of the Li Ka Shing Faculty of Medicine, University of Hong Kong and subsequently recognized as an independent department in 2013. It became the territory's second clinical academic unit in ophthalmology and third visual science research centre. Professor David Wong, a renowned vitreo-retinal surgeon and clinician-scientist from the United Kingdom, was selected as the founding Chair Professor and head. Professor Wong was consultant ophthalmologist at the Royal Liverpool Hospital and chairman of the Scientific Committee of the Royal College of Ophthalmologists as well as its Senior Vice President. In 2005 he was made an honorary Professor of the University of Liverpool in recognition of his outstanding contribution to ophthalmic research. He was also the founding member of the British Association of Vitreoretinal Surgeons and board member of the European Society of Retinal Specialists. Professor Wong's appointment at the University of Hong Kong would not have been possible without Mrs Felicia Young and family's very generous contribution through the Albert Bing Ching Young Endowed Chair Professor in Ophthalmology.

In 2007, a team comprising Professor Wong, Dr. Wico Wai Kwan Lai and Dr. Kenneth Kai Wang Li was formed at the Department of Ophthalmology of the Queen Mary Hospital to initiate translational research in visual sciences. Dr. Amy Cheuk Yin Lo, a neuroscientist from the university's Department of Anatomy, was recruited to head the department's research postgraduate studies and laboratory research. Not long afterwards, the department added Professor Jimmy Shiu Ming Lai and Dr. Carol Shan Yu to the ranks, thereby broadening the scope of research specialties.

Over the last 10 years, an aging population in Hong Kong has increased the burden on public specialist ophthalmic services, and there is an increasing demand by patients for advanced technological solutions and increased opportunities to expand our work into Mainland China. In 2012, the HKU-Shenzhen Hospital in Shenzhen, China was opened, with our department providing training and clinical services in

ophthalmology. To meet the demands of a broader scope of service, a number of young clinician-scientists were recruited, including Dr. Raymond Lai Man Wong, Dr Marcus Marcet, Dr Jacky Lee, Dr. Ian Yat Hin Wong, Dr. Kendrick Co Shih, Dr. Bonnie Nga Kwan Choy, Dr. Alex Lap Ki Ng and Dr. Jennifer Wai Huen Shum. The laboratory team was expanded with the addition of Dr. Bin Lin, Dr. Qizhou Lian and Dr. Catherine Chiu as principal investigators.

This paper reviews their significant contributions over the years.

Vitreoretinal disease

Led by Professor Wong, much of the department's initial research focused on 3 main areas of vitreoretinal disease: surgical retina, diabetic retinopathy screening and retinal neuroprotection.

In 2009, we established Hong Kong's first territory-wide centralised diabetic retinopathy screening program in the Hospital Authority. Through the use of telemedicine technology, we were able to provide cost-effective screening and arbitration services for public primary care facilities all over Hong Kong. The introduction of the screening program also resulted in a number of published collaborative epidemiological studies on the subject.[1,2] Another major research area was surgical retina,[3,4] especially with regard to the use of silicone oil and the emulsification process, a troublesome and potentially devastating long-term complication.[5-7] Working with university's mechanical engineering department, our team further developed *in-vitro* models (Eye-on-a-Chip) to further study the emulsification process in different conditions using silicone oils of different molecular weights.[8-10] Last but not least was our department's significant contribution to retinal neuroprotection research. This area was laboratory-based with the use of both animal experimental models and *in-vitro* models using cell culture. The neuroprotective agents studied during this period included supplements like lutein[11-15] and lycium barbarum[16,17] as well as stem cell therapy techniques.[18] Our group also investigated the effect of aldose reductase deficiency on ameliorating retinal ischemia-reperfusion injury in animal models.[19-21]

With the addition of more academic staff with special interests in the field of vitreoretinal diseases, the scope of research interests was extended to medically treated retinal diseases and posterior segment imaging, while continuing in the established themes such as surgically treated retinal diseases.[22,23] For instance, the utilization of optical coherence tomography, in particular enhanced-depth-imaging to measure choroidal thicknesses, and its relation to important pathologies.[24-28] Participation in international multicenter clinical trials by the clinical trials team for retinal diseases of the University of Hong Kong was another major leap forward. With Dr. Ian Wong as the principal investigator, the team has participated in numerous trials and studies, including the VIVID EAST, EVEREST 2, PLANET, CEDAR and UNCOVER, to name a few.

Another focus in recent years was the development of new treatment regimens for medically treated retinal diseases such as diabetic macula edema, central serous choroidoretinopathy, polypoidal choroidal vasculopathy and retinal vein occlusive disease.[29-37] The vitreoretinal service also involved in the management of infectious uveitis, especially cytomegalovirus retinitis among immunocompromised patients. The affiliated teaching hospital — Queen Mary Hospital — is a designated transplant center for bone marrow, liver, lungs and kidneys. Close collaboration between our staff and other medical colleagues has enabled the development of expert care and research in this field.[38-40]

Glaucomatous optic neuropathy

With the arrival of Professor Jimmy Shiu Ming Lai in 2009, our department was blessed with one of the region's renowned experts in glaucomatous optic neuropathy. Already a well-published author in the field, Professor Lai led a young team to develop new areas of research, including epidemiology, therapeutic lasers, minimally invasive glaucoma surgery and most recently the use of cross-linking techniques in glaucoma. He also collaborated with a number of laboratory-based researchers to develop neuroprotection in glaucoma.

Much of the epidemiological research conducted at the department was based on estimating prevalence, disease burden and risk factors of glaucomatous optic neuropathy. This was led by a number of ongoing collaborators, chiefly Dr. Jacky Wai Yip Lee, who is now in private practice. Our research group utilized new technologies, including optical coherence tomography and 24-hour intraocular pressure (IOP) monitoring, to conduct a number of epidemiological studies.[41-50] Professor Lai's team has also been actively involved in the refinement of primary angle closure glaucoma management. This is especially important in the context of Southeast Asia, where this subtype represents up to 80% of all glaucoma cases.[51-54] Amongst these publications, the most important contribution was the team's involvement in the multi-national, multi-centre randomized controlled trial comparing early lens extraction for angle closure glaucoma and conventional therapy.[55] Another area of research that was initiated by Professor Lai and greatly expanded after his arrival at the department was the use of selective laser trabeculoplasty for glaucoma. The technique utilizes a frequency-doubled N: YAG laser and minimizes permanent damage to the trabecular meshwork, compared with conventional argon laser trabeculoplasty techniques. This allows the procedure to be repeated. While the initial research work was on its effectiveness in primary open angle glaucoma, Professor Lai's team successfully demonstrated its effectiveness in normal tension glaucoma as well, with a reduction in IOP fluctuation over 24-hours.[43,52,56-65] The university's glaucoma team also utilized minimally invasive surgical techniques, including trabectome and exPRESS

shunt implantation, to expand available treatment options for lowering IOP in glaucoma patients.[66,67] Ultimately as IOP lowering therapy remains the only proven treatment in the context of glaucomatous optic neuropathy, Professor Lai's team has also worked towards introducing IOP-independent neuroprotective strategies to glaucoma. His laboratory team is currently investigating the effectiveness of trans-corneal electrical stimulation to improve retinal ganglion cell survival using both acute and chronic ocular hypertensive injury models in animals.[68,69]

Most recently Professor Lai's team came up with new ways to utilize cross-linking technology. This was a method to rapidly and safely strengthen covalent bonds within tissue and was first used for cornea ectatic disease. It is currently approved by the US Food and Drug Administration for the treatment of keratoconus. Professor Lai's team successfully used this technique to treat leaking blebs after glaucoma surgery. With a single treatment using the conventional protocol, bleb leakage was halted in a preliminary study of 5 eyes.[70]

Cornea and external eye disease

Our department's newest area of research is that of cornea and external eye diseases. While still in the early stages, the focus is on cornea cross-linking therapy and regenerative therapy. We have also initiated epidemiological work in microbial keratitis[71,72] and in the prevention of myopia progression with topical atropine.[73] Our department recently began conducting research into corneal cross-linking. The research work is led by Dr. Alex Lap-Ki Ng, in collaboration with colleagues from the Department of Ophthalmology and Visual Sciences at the Chinese University of Hong Kong, Hong Kong Sanatorium and Hospital as well as Hong Kong Laser Eye Centre.[74-76] Dr. Ng's group concentrated on the different protocols of cross-linking, and extending its application to laser refractive procedures including LASIK and ReLEx small-incision lenticule extraction. The aim is to reduce ectatic complications and myopia regression after laser vision correction.

One of the most topical areas in corneal research is currently wound healing and regenerative therapy. Our laboratory team, led by Professor Wong and Dr. Qizhou Lian, have developed techniques to promote corneal epithelial wound healing after chemical injury.[77] Furthermore, they have identified and characterized progenitor cells in the cornea endothelium and trabecular meshwork that may serve as a source of replenishment for an aging cornea.[78,79] Most recently, Dr. Catherine Chiu's team has been working to characterize lens epithelial proliferation and migration after injury in order to understand its potential in wound healing.[80]

Future plans

Looking forward to the next 10 years, our department's focus is on the understanding and treatment of eye diseases relevant to patients in Asia. We aim to consolidate our current strengths and develop new research areas, all the while fueling it with the enthusiasm of a youthful team. We remain greatly in debt to the established clinical and academic institutions in Hong Kong for their support throughout our department's infancy. To secure Hong Kong's future as a leading epicentre for ophthalmic research, the way forward is through collaboration.

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The Chinese University of Hong Kong Ophthalmic Research Centre: from the past to the future

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Abstract

The Department of Ophthalmology and Visual Sciences of the Chinese University of Hong Kong was established in 1994 as the first academic ophthalmology department in Hong Kong. The department involves patient services, research, professional training, medical student teaching, post-graduate education, community services, international services and project development. For research, we conducted programs to advance ophthalmic surgery, clinical trials, epidemiology studies, investigative ophthalmology and basic sciences. A comprehensive ophthalmic research laboratory system is established for microscopies, genomics, molecular biology, cell biology, histopathology, analytical chemistry, experimental animals and bioinformatics. The Clinical Drug Trial Centre has been accredited by the China Food and Drug Administration since 2006. We work closely in clinical ophthalmology, research, training and education with the Shantou University / Chinese University of Hong Kong Joint Shantou International Eye Center. We have built a collaborative network in ophthalmic research with institutions in Hong Kong, mainland China and overseas. In 2015, we incorporated our Lim Por-yen Eye Genetics Research Centre, Pao So Kuk Macular Diseases Treatment and Research Centre and Lee Wing Kit Advanced Ophthalmic Training and Education Centre with the research laboratories to form the Chinese University of Hong Kong Ophthalmic Research Centre. While the existing Chinese University of Hong Kong Eye Centre mainly conducts clinical services and research, the 2 centers work together on patient orientated research to advance the investigation and treatment of common and rare eye diseases.

Key words: Cell biology; Genetics; Universities

Introduction

The Department of Ophthalmology and Visual Sciences of the Chinese University of Hong Kong (CUHK) is devoted to clinical services, research, training and education. The personnel and resources are shared by both clinical and laboratory research (**Figure**). Clinical research involves major subspecialties including corneal and external eye diseases, refractive surgery, cataracts, glaucoma, vitreoretinal diseases, orbital diseases, oculoplasties, neuro-ophthalmic diseases and pediatric eye diseases. Clinical research is conducted according to the good clinical practice and in collaboration with the Hong Kong Eye Hospital, Prince of Wales Hospital, Alice Ho Nethersole Hospital and other local, national and overseas hospitals. We study disease mechanisms including degeneration, inflammation, ocular nerve cells, cellular dysfunctions, oxidative stress, neoplasm, tumor, endocrine complications and genetics. We also conduct research using state-of-the-art equipment in ophthalmic imaging, electrophysiology and angiography. Some of our facilities in clinical research are depicted in **Table 1**.

For basic research, specialized equipment is installed such as

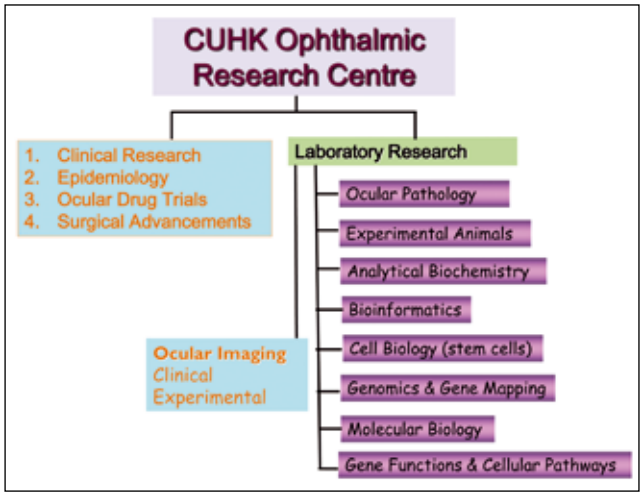

Figure. Clinical and laboratory research at the Chinese University of Hong Kong Ophthalmic Research Centre.
Clinical research includes refractive surgery, cataract surgery, corneal transplantation, ocular imaging, ocular epidemiology, visual electrophysiology, as well as different retinal and corneal diseases. Laboratory research includes etiology, molecular genetics, histopathology, biochemistry and therapeutic treatment of various eye diseases.

a multi-photon confocal microscopy, fluorescence-activated cell sorter and a high-throughput genotyping machine (**Table 2**). Research equipment designated for animal studies includes multi-focal electroretinogram and optical coherence tomography.

Our research has received much support from individual and institutional benefactors. In 2003, a generous donation by the late Mr Lim Por Yen enabled establishment of the Lim Por-yen Eye Genetics Research Centre to facilitate basic and translational research of genetic eye diseases. Another generous donation in 2005 by Mr Chang Bei Ming and Madam Chang Pao So Kok enabled establishment of the Pao So Kuk Macular Diseases Treatment and Research Centre to strengthen research and education in detection and treatment of macular diseases, including age-related macular degeneration (AMD), polypoidal choroidal vasculopathy (PCV), diabetic retinopathy, diabetic macular edema and retinitis pigmentosa (RP). In 2012, the establishment of the Lee Wing Kit Advanced Ophthalmic Training and Education Centre (AOTEC) raised the standard of our training, research and education to the next level. The AOTEC, named in memory of the late Mr. Lee Wing Kit, beloved father of the late Mr. Peter T.C. Lee, was built through a generous donation from Mr. Peter T.C. Lee and Mrs. Nancy Lee, with support from the University Grants Committee, the Hospital Authority, and the Food and Health Bureau of the HKSAR Government. Facilities in AOTEC include the Telemedicine Centre for international exchange of educational programs, grand rounds and clinical consultations, the Microsurgical Training Centre and the Virtual Reality Eye Surgery Training Laboratory. The latter 2 provide hands-on and virtual reality-enhanced training in ophthalmic microsurgery. The Hospital Authority Lions Eye Bank is also housed at AOTEC. Recently, we were awarded the Addition, Alteration and Improvement grant by the University Grants Committee to upgrade and enhance our teaching, education and training facilities in clinical ophthalmology and visual sciences.

Since 1999, we have contributed more than 1,200 scientific articles to science citation index peer-reviewed international journals, including *Ophthalmology*, *Archives of Ophthalmology*, *American Journal of Ophthalmology*, *Investigative Ophthalmology & Visual Science*, *Scientific Reports*, *Human Molecular Genetics*, *Proceedings of the National Academy of Sciences*, *American Journal of Human Genetics*, *Nature Communications*, *Nature Genetics* and *Science*. This has been achieved through the concerted efforts of staff and students, as well as collaboration with our local, national and international colleagues.

In this review, we describe the development of our laboratory-based research with emphasis on genetics, cell and molecular biology, and animal studies, with a brief discussion of future research direction.

Genetic studies

We have studied the genomics and molecular genetics of common complex eye diseases and rare Mendelian diseases. Our strategies include hypothesis-driven candidate gene screening and association studies, as well as the hypothesis-free familial linkage analysis and genome-wide association studies. Our goals are to identify the disease-causing and disease-associated genes for eye diseases so as to facilitate early-stage clinical diagnosis, provide guidance for clinical treatment, and offer genetic testing and counseling.[1]

Gene screening study

A genetic disease can be caused by disease-causing mutations in one or multiple genes, or by the interaction of multiple associated gene variants and environmental factors. A candidate gene study aims to determine the mutation spectrum and/or the association pattern of a specific gene for a disease in a population.[2] This strategy is comparatively low cost, and the candidate gene sequences and polymorphic changes can be resolved by Sanger's DNA sequencing and allelic discrimination (e.g. TaqMan genotyping) assays.[3]

Whole gene mutational screening aims to identify disease-causing mutations. Our first candidate gene screening study was the sequence analysis of the *PAX6* gene in a small number of Hong Kong Chinese patients with aniridia, in which 5 nonsense mutations and 1 frameshift mutation were discovered.[4] In addition, we identified a novel mutation (V53A) in the *myocilin* (*MYOC/TIGR*) gene in a Chinese patient with primary angle closure glaucoma and 3 other disease-causing *MYOC* mutations for primary open angle glaucoma (POAG).[5,6] In contrast, we confirmed that nonsense truncation mutations in *MYOC* are not disease-causing,[7] and we found no disease-causing mutation in

Table 1. Facilities at the Chinese University of Hong Kong Eye Centre.

Clinical equipment	Feature
Laser surgery / treatment	
1. Allegretto wave® Eye-Q Excimer Laser	Ophthalmic laser system for refractive surgery of the cornea
2. AMO Intralase IFS Femtosecond Laser Machine	Ophthalmic laser system for refractive surgery of the cornea
3. Alcon Yag Laser	Capsulotomy
4. Lumenis OMNI Laser	Fundal or glaucoma laser treatment
5. Topcon PASCAL Photocoagulator Laser	Fundal or glaucoma laser treatment
Anterior segment	
1. KONAN Specular Microscopy	Endothelial cell density and cell morphology measurement
2. Zeiss Anterior Segment Optical Coherence Tomography	Non-contact cross-sectional and 3D imaging for anterior segment of the eye
3. Bausch and Lomb's Orbscan II	Corneal shape measurement
4. NIDEK Pachymeter	Noninvasive ultrasonic technique for corneal thickness measurement
5. Zeiss IOL Master	Eyeball length, surface curvature and intraocular lens power measurement
6. Reichert Ocular Response Analyser	Intraocular pressure and corneal biomechanical property measurement
7. Lumenis Allergo Topolyzer	Corneal surface topographic measurement
8. NIDEK Non-contact Tonometer	Intraocular pressure measurement, the fluid pressure inside the eye and central corneal thickness
9. OCULUS Corvis® ST Tonometer	Intraocular pressure and corneal thickness measurement
Posterior segment	
1. Stratus Optical Coherence Tomography System	A noncontact, noninvasive diagnostic imaging technique for cross-sectional retinal structure analysis
2. Cirrus High-definition Optical Coherence Tomography System	A noncontact, noninvasive diagnostic imaging technique for cross-sectional retinal structure analysis
3. Heidelberg Spectralis Optical Coherence Tomography System	A noncontact, noninvasive diagnostic imaging technique for cross-sectional retinal structure analysis
4. Heidelberg Retinal Tomography III	3D imaging of human optic nerve head and retina.
5. Heidelberg Retinal Angiography II	For red free, fluorescein angiography, fundus auto-fluorescein and indocyanine green angiography imaging
6. Topcon Fundus Camera	Retinal image capture, including color, red free, fluorescein angiography, fundus auto fluorescein and indocyanine green angiography
7. Zeiss GDx VCC Scanning Laser Polarimetry	Retinal nerve fiber layer thickness measurement
Ocular function	
1. Espion Electroretinography System	Ocular electrophysiological test including visual evoked potential, electroretinography and electro-oculogram
2. Veris Multifocal-electroretinography System	Electrophysiological test of multifocal retinal function
3. Zeiss Humphrey Visual Field Analyzer	Non-intrusive evaluation of visual field
4. Zeiss Frequency Doubling Technology Perimeter	Visual field screening and full threshold testing
Other ophthalmic investigation	
1. NIDEK Autorefractor	Objective measurement of refractive error
2. TOMEY Ultrasound B-scan UD-1000	Producing a 2D image inside of the eye
3. TOMEY Ultrasound A-scan	Intraocular lens power calculations, axial length, anterior chamber depth, lens thickness and central corneal thickness measurements

the *oculomedin* (*OCLM/TISR*) gene in Chinese POAG.[8] Moreover, we identified differential mutation patterns of the *optineurin* (*OPTN*),[9] *WD repeat domain 36* (*WDR36*)[10] and *neurotrophin-4* (*NTF4*)[11] genes in Chinese POAG.

Our work on rare Mendelian eye diseases includes congenital cataract, corneal dystrophies, and inherited retinal degenerative diseases (such as RP, Bietti's crystalline dystrophy, and Best vitelliform macular dystrophy). For different forms of congenital cataract, we identified multiple novel mutations in 3 different crystallin genes (αA-crystallin gene, *CRYAA*, c.34C>T and c.292G>A;[12,13] βB-crystallin, *CRYBB2*, c.92C>G;[14] and γD-crystallin, *CRYGD*, c.43C>A and c.494delG[15,16]). For late-onset Fuchs endothelial corneal dystrophy, 4 mutations (E399K, G709E, T754M and c.99-100delTC) in the solute carrier family 4, sodium borate

Table 2. Facilities at the Chinese University of Hong Kong Ophthalmic Research Centre.

Laboratory equipment	Feature
Analytical chemistry	
1. Waters Solid phase extraction system	A high throughput platform to purify complex samples for liquid and gas chromatographic analysis
2. Waters High performance liquid chromatography - Fractionating system	Giving preparative separation and fractionating of a biological or herbal complex mixture
3. Agilent Gas chromatography mass spectrometry	Perform good sensitivity and reliability of routine analysis in a test sample
4. Waters Ultra-performance Liquid Chromatograph - FLR, Pda and ECD	Good resolution and accuracy for the identification and quantification of complex samples
5. Waters Liquid Chromatography tandem mass spectrometry	Highest quality nano to microscale 2-D separation for proteomics and metablomics investigation
6. Agilent Liquid Chromatography tandem mass spectrometry	Highest quality nano to microscale 2-D separation for proteomics and metablomics investigation
Animal laboratory	
1. Heidelberg Spectral-domain Optical Coherence Tomography System	A noncontact, noninvasive retinal imaging technique to take cross-section pictures of retina for live animals
2. Heidelberg Retinal Angiography II	For red free, fluorescein angiography, fundus auto-fluorescein and indocyanine green angiography imaging.
3. Espion Electroretinography System	Electrophysiological test of retinal function in animal eye diseases models
4. Zeiss surgical microscopes	Surgical microscopes for animal surgery
Cell biology	
1. Beckman Coulter MoFlo™ Astrios cell sorter	Cell sorting with specific marker
2. Beckman Coulter Cytomics FC500 flow cytometer	Cell marker analysis
3. Thermo Scientific steri-cycle carbon dioxide incubator	Cultured cell incubation
4. Forma Scientific class IIA/B3 biological safety cabinet	Cultured cell manipulation
5. NU-201-330E laminar fumehood	Tissue dissection
6. Millipore Milli-Q Alo	Water filtration
Genomics and molecular biology	
1. Fluidigm EP1 genetic analysis System	High throughput genotyping analysis
2. Roche LightCycler 480II	Real-time polymerase chain reaction
3. Applied Biosystems 3130xl automated sequencer	Direct DNA sequencing
4. BioRad ChemiDoc	Immunoblotting imaging
5. BioRad PCR machines	Polymerase chain reaction and molecular reactions
6. Beckman Coulter Allegra X-22R centrifuge	Sample unification
7. TaiTec Hybridization Incubator	Molecular reactions
8. Grant-bio orbital shaker-incubator ES-20	Bacterial cloning
Microscopy laboratory	
1. Nikon A1R MP multiphoton confocal microscopy	Precise fluorescence imaging
2. Nikon Eclipse Ni fluorescence microscopy	General fluorescence imaging
3. Nikon Eclipse Ti inverted fluorescence microscope	Live cell imaging
Pathology	
1. Thermo Shandon Excelsior automatic tissue processor	Preparation of tissue samples from chemical fixation to paraffin infiltration
2. Thermo Scientific CryoStar Nx50 cryostat	Preparing frozen tissue sections
3. Leica RM2135 microtome	Preparing paraffin sections
4. Leica EG1160 tissue embedding center	Paraffin block assembling
5. Leica DMLS light microscope	General slide
6. Leica M28 dissection microscope	Tissue dissection

transporter, member 11 (*SLC4A11*) gene,[17] and 2 mutations (D64D and N696S) in zinc finger E-box-binding homeobox 1 (*ZEB1*/*TCF8*) gene were identified.[18] In inherited retinal degenerative diseases, we found that disease-causing mutations in the rhodopsin (*RHO*) and retinitis pigmentosa 1 (*RP1*) genes account for approximately 2.0% and 2.2% of our Hong Kong Chinese RP patients respectively,[19-23] a lower percentage than in Caucasians. We identified the

first compound heterozygous truncation mutations in *RP1* causing autosomal recessive RP.[22] In Hong Kong Chinese RP patients, 2.9% carry the photoreceptor-specific nuclear receptor (*NR2E3*) mutations, compared with ~1% in Caucasian RP patients.[24] Furthermore, we identified novel mutations in the U5 small nuclear ribonucleoprotein 200 kDa helicase (*ASCC3L1*/*SNRNP200*) gene,[25,26] the eyes shut (*EYS*) gene,[27] the adenomatous polyposis coli (*APC*) gene in familial adenomatous polyposis with congenital hypertrophy of the retinal pigment epithelium,[28] the cytochrome P450, family 4, subfamily V, polypeptide 2 (*CYP4V2*) gene in Bietti's crystalline dystrophy,[29] the Bestrophin 1 (*BEST1*) gene in Best vitelliform macular dystrophy,[30] the tyrosinase (*TYR*) gene for type 1 oculocutaneous albinism,[31] and the sal-like 4 (*SALL4*) gene for Duane retraction syndrome.[32]

Familial linkage analysis

Family linkage analysis, based on the theory of linkage disequilibrium between known genetic markers with the disease-causing mutations, is a useful strategy to identify unknown disease-causing genes for inherited diseases that show a clear family history. The techniques have evolved from genome-wide linkage scan with microsatellite markers or single nucleotide polymorphisms (SNPs) to whole genome or exome sequencing.

Our first family linkage analysis of autosomal dominant high myopia on chromosome 18p revealed linkage at the D18S476 marker with a maximum LOD score of 2.4 in 5 Hong Kong Chinese families.[33] A novel locus for autosomal dominant high myopia (*MYP16*) on chromosome 5p15.33-p15.2 (17.45-cM interval) was mapped in 3 Hong Kong Chinese families with maximum LOD score of 4.81 at D5S2505, and the *IRX2*, *IRX1*, *POLS*, *CCT5* and *CTNND2* as disease-causing genes were excluded.[34] For glaucoma, in a 27-member Filipino family with autosomal dominant juvenile-onset POAG, we excluded mutations in the *MYOC*, *OPTN* and *WDR36* genes.[35] Our genome-wide scan identified the chromosomal region 5q22.1-q32 flanked by the D5S2051 and D5S2090 markers as a novel locus for POAG (*GLC1M*), with a maximum LOD score of 4.82.[36] We then excluded the neuregulin 2 (*NRG2*) and secreted protein acidic and rich in cysteine (*SPARC*) genes as the disease-causing gene in this locus.[37,38] In another study, chromosome 15q22-q24, a 16.6-Mb region flanked by D15S1036 and rs922693 with a maximum LOD score of 3.31, was mapped as a novel locus for autosomal dominant juvenile-onset POAG in a Hong Kong Chinese family.[39] The *NR2E3*, SMAD family member 6 (*SMAD6*) and ceroid-lipofuscinosis, neuronal 6 (*CLN6*) genes were excluded as disease-causing in this locus. In collaboration with University College London, we confirmed in 3 Hong Kong Chinese autosomal recessive RP families the *RP25* locus on chromosome 6 mapped by the 10K GeneChip Mapping Array.[40]

With the advent of next generation sequencing, including whole or targeted genome or exome sequencing, discovery of gene mutations is quick and cost-effective.[41] We have identified novel disease-causing genes and mutations for inherited retinal dystrophies,[42-44] including the U4/U6 small nuclear ribonucleoprotein Prp4 (*PRPF4*) and secreted phosphoprotein 2 (*SPP2*) genes for autosomal dominant RP,[45,46] Usher syndrome 2A (*USH2A*) mutations for Usher syndrome,[47] as well as mutations in Bardet-Biedl syndrome 2 (*BBS2*), McKusick-Kaufman syndrome (*MKKS*), ADP-ribosylation factor-like protein 6 (*ARL6*) and Meckel syndrome, type 1 (*MKS1*) genes for Bardet-Biedl syndrome.[48]

Genetic association study

Most common eye diseases such as AMD and POAG are late-onset, and their etiology is complex and a result of the interaction of multiple genetic and environmental risk factors, rather than a single mutation. It is difficult to have large pedigrees for linkage analysis. Association studies are conducted to identify the susceptibility gene variants that are associated with the disease phenotype, based on the proportional differences of a genetic polymorphism between patients and control subjects.[49] Association studies can be performed on a small scale by direct sequencing or allelic discrimination assay, or on a genome-wide scale by microarray or next generation sequencing.

Our first association study was on the apolipoprotein E (*APOE*) gene in AMD, where we found no significant association.[50] In contrast, we discovered a link between the *APOE* ε4 allele and normal tension glaucoma,[51] and its interaction with *MYOC* and *OPTN* polymorphisms in normal tension glaucoma,[52] while the *MYOC* promoter polymorphisms alone were not associated with POAG.[53] Moreover, we investigated other glaucoma-related loci, including lysyl oxidase-like protein 1 (*LOXL1*),[54,55] tumor necrosis factor (*TNF*),[56] tumor protein p53 (*TP53*),[56] atonal homolog 7 (*ATOH7*),[57] raftlin lipid raft linker 1 (*RFTN1*),[57] and chromosome 2p16.3,[58] in POAG. For AMD, we investigated formyl peptide receptor 1 (*FPR1*),[59] pigment epithelium-derived factor (*PEDF*),[60] and chromosome 8p21 and 4q12,[61] as well as the complement pathway and the high-density lipoprotein pathway. We have reported association of AMD with complement factor H (*CFH*; I62V), complement component 2 (*C2*; rs547154), and cholesteryl ester transfer protein (*CETP*; rs3764261).[62-66] We also compared the genetics of AMD with PCV and found that most AMD genes are associated with PCV, some with different effect sizes, e.g. SNPs at the *ARMS2-HTRA1* locus. We also found a PCV-specific association with the complement component 3 (*C3*) gene and the ATP-binding cassette, subfamily G, member 1 (*ABCG1*) gene.[66] Since uveitis and AMD share a similar pathogenesis of inflammation, we tested the association of complement pathway genes in uveitis and identified an association of anterior uveitis with complement factor B (*CFB*; rs1048709),[67] complement factor I (*CFI*; rs7356506),[68] and *CFH* (I62V),[69,70] but not *C2*.[67] The manganese superoxide dismutase (*SOD2*), chemokine (C-C motif) ligand 2 (*CCL2*) and *KIAA1109* genes are also associated with uveitis.[71,72] In addition, we confirmed the association of the paired box 6 (*PAX6*) promoter dinucleotide repeats with

high myopia,[73,74] the endothelin 1 (*EDN1*) K198N variant with diabetic retinopathy,[75] the transcription factor 4 (*TCF4*) and protein tyrosine phosphatase, receptor type G (*PTPRG*) genes with corneal dystrophies,[76,77] as well as the cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*) and interleukin 13 (*IL13*) genes with Graves' ophthalmopathy in the Hong Kong Chinese population.[78,79]

Since the first report of genome-wide association study (GWAS) for AMD was published in 2005, it has become the most successful technology for mapping genes associated with complex diseases. We reported our first GWAS, which was also the first Chinese GWAS in eye genetics, in 2006 on AMD, where we discovered the high temperature requirement factor A1 (*HTRA1*) promoter polymorphism rs11200638 to be associated with exudative AMD in the Hong Kong Chinese population.[80] Further fine mapping analysis revealed functional variants related to the disease pathogenesis and confirmed *HTRA1* to be the strongest associated gene in Chinese.[81,82] Our second GWAS identified the association of caveolin genes (*CAV1/CAV2*) with POAG in a multi-institutional collaborative project.[83] Since then, we have collaborated with the Singapore Eye Research Institute and the Sichuan Provincial Key Laboratory for Human Disease Gene Study to discover 8 novel associated genes and variants for primary angle closure glaucoma (PACG; *PLEKHA7*, *COL11A1*, *PCMTD1/ST18* and *ABCC5*)[84,85] as well as POAG (*ABCA1*, *PMM2*, *CDKN2B-AS1* and *TGFBR3*).[86,87] For myopia, our collaboration identified 4 novel genes and loci associated with high myopia: chromosome 13q12.12,[88] vasoactive intestinal peptide receptor 2 (*VIPR2*),[89] syntrophin, beta 1 (*SNTB1*),[89] and zinc finger E-box binding homeobox 2 (*ZFHX1B*).[90] We also worked with the Consortium on Refractive Error and Myopia (CREAM), which comprised 37,382 individuals from 27 studies of European ancestry and 8,376 from 5 Asian cohorts. Genome-wide meta-analysis has deciphered 21 genes and loci associated with refractive errors (*CD55*, *PRSS56*, *CHRNG*, *CACNA1D*, *BMP3*, *LAMA2*, *CHD7*, *TOX*, *ZMAT4*, *RORB*, *CYP26A1*, *BICC1*, *GRIA4*, *RDH5*, *PCCA*, *ZIC2*, *GJD2*, *RASGRF1*, *MYO1D*, *KCNJ2* and *CNDP2*),[91] and 9 with axial length (*ALPPL2*, *CD55*, *C3orf26*, *GJD2*, *LAMA2*, *MIP*, *RSPO1*, *ZC3H11B* and *ZNRF3*).[92] In the GWAS on central corneal thickness and keratoconus, we identified 16 associated genes and loci (*USP37*, *GPR15*, *TIPARP*, *CWC27-ADAMTS6*, *RXRA-COL5A1*, *LCN12-PTGDS*, *FGF9-SGCG*, *FOXO1*, *TJP1*, *AKAP13*, *LRRK1*, *CHSY1*, *BANP-ZNF469*, *HS3ST3B1-PMP22*),[93] as well as on Vogt-Koyanagi-Harada syndrome, identifying 3 susceptibility loci (*IL23R-C1orf141* on 1p31.2, *HLA-DRB1/DQA1* on 6p21.3 and *ADO-ZNF365-EGR2* on 10q21.3).[94] With the development of the next generation sequencing technique, novel coding variants were identified for neovascular AMD, including the cholesteryl ester transfer protein (*CETP*) D442G variant,[95] and the ubiquitin protein ligase E3D (*UBE3D*) V379M variant.[96]

Although we have identified more than 70 loci/genes/variants associated or linked with different ocular diseases, the role of these genes in the disease pathogeneses has not been determined. Biological studies would be an important approach to decipher the functional roles of these genetically identified genes and variants.

Biological studies

Biological studies at CUHK Ophthalmic Research Centre comprise cell and molecular biology projects and animal studies, providing support for genetics analysis and supplementing clinical investigation. We aim to (1) characterize the function of the disease-causing/susceptible genes, (2) delineate the disease mechanisms, and (3) provide pre-clinical platforms for drug/treatment testing and disease modeling.

Characterization of disease-causing/susceptible genes

Genetic studies, such as candidate gene screening and association analysis, can identify disease-causing mutations and susceptible variants, respectively. To delineate the role of pathogenic mutations or to confirm the biological relevance of the associated variants to the diseases, *in vitro* experiments for gene function analysis are performed.

In the γD-crystallin gene (*CRYGD*; c.43C>A and c.494delG) for congenital cataract,[15,16] the c.494delG mutation deletes 1 nucleotide in the *CRYGD* open reading frame and is predicted to cause a frameshift and an early termination of translational product (G165fs).[16] Recombinant CRYGD mutant protein overexpressed in lens epithelial cell line (B3) leads to a reduced solubility of γD-crystallin protein and localization on the nuclear envelope (co-localized with lamin A/C), where the wildtype protein exists in both the nucleus and cytoplasm. The c.43C>A change is a missense mutation, causing the substitution of Arg at residue 15 to Ser (R15S). The recombinant mutant protein has similar solubility in detergent to the wildtype protein, but is predicted to raise the local hydrophobicity and create a hypothetical casein kinase II phosphorylation site.[15] Similarly, in the αA-crystallin gene (*CRYAA*, c.34C>T and c.292G>A),[12,13] the c.292G>A mutation causes the substitution of Gly at residue 98 to Arg (G98R). Compared to the cytoplasmic localization of wildtype CRYAA protein, the G98R mutant forms aggregates inside the endoplasmic reticulum (ER) and is predominantly detergent-insoluble.[13] Overexpression of CRYAA G98R mutant protein induces ER stress in B3 cells, and in turn leads to cell apoptosis. The c.34C>T change in *CRYAA* is a missense mutation, causing the substitution of Arg at residue 12 to Cys (R12C). The R12C mutant protein has similar detergent-solubility and cytoplasmic localization to the wildtype protein, but mutant-expressing cells exhibit a delayed expression of HSP70, compared with wildtype-expressing cells.[12]

We examined the effect of high myopia-associated dinucleotide repeat $(AC)_m(AG)_n$ polymorphism at the *PAX6* P1 promoter on its transcriptional activity.[73] Luciferase assays showed elevated transcriptional activity with

increasing length of $(AC)_m$ repeats ($(AC)_{Below20-22}$ vs $(AC)_{20-22}$: 1.27 folds, and $(AC)_{Below20-22}$ vs $(AC)_{Above20-22}$: 1.50 folds) although the transcriptional activity was not significantly elevated for increasing length of $(AG)_n$ repeat. At the same length of combined repeats, transcriptional activity of $(AC)_{23}(AG)_6$ was similar to that of $(AC)_{21}(AG)_8$, suggesting that both AC and AG repeats contributed to the transcriptional activity of the *PAX6* P1 promoter. This phenomenon may be due to influence of transcription factor binding sites within this region.[73] Gene function analysis of *NTF4*, in which we discovered mutations of POAG, showed that only the G157A variant protein, not the A182V variant, was detected by immunoblotting, while both wildtype and mutant *NTF4* mRNA were detected after transfection.[11] Compared to wildtype NTF4 protein, the G157A variant is less soluble in detergent but did not affect protein trafficking. Both NTF4 variants impaired NTF4 protein function, in which HeLa cells expressing NTF4 mutants were less stimulated to migrate than those expressing wildtype protein.[11]

Identification of disease mechanisms

Retinoblastoma (RB) is one of the most common causes of cancer in children.[97] Its etiology is the loss of function of retinoblastoma gene (*RB1*) alleles by loss of heterozygosity and gene mutations,[98] but this accounts for only 80 to 90% of RB patients. To search for novel RB-causing mechanisms, we identified promoter hypermethylation and impaired expression in O6-methylguanine-DNA Methyltransferase (*MGMT*),[99] MutL homolog 1 (*MLH1*),[100] and RAS-associated domain family 1A (*RASSF1A*) genes.[101] Loss of heterozygosity was detected in RB on chromosome 19, 20, 21, 22 and X,[102] whereas allelic loss at chromosome 13q31 with reduction in glypican 6 (*GPC6*) gene expression was found in 92% of RB patients.[103] In addition, B lymphoma Mo-MLV insertion region 1 (BMI1) is widely expressed in human RB, and it stimulates RB cell (Y79) proliferation and suppresses apoptosis with reduced expression of p14ARF and p16INK4 and upregulation of cyclin D1 and D2 as well as CHX10 and RAX.[104]

Ocular hypertension is the major risk factor for glaucoma. We established an experimental animal model to mimic ocular hypertension and retinal ganglion cell degeneration. We found that the JAK/STAT pathway, PI3K/AKT pathway and macrophages had a detrimental effect on RGC survival in rodents after acute ocular hypertension.[105-107] Acute intraocular pressure (IOP) elevation activates JAK/STAT pathway in RGCs, and blockage of JAK/STAT pathway by pathway inhibitor (AG490) further enhances RGC death when IOP is elevated.[105] Similarly, acute IOP elevation activates PI3K/AKT pathway in the inner nuclear layer and ganglion cell layer, where inhibition of PI3K/AKT pathway by inhibitors (LY294002 and KY12420) leads to RGC loss in rats after acute IOP elevation.[106] Interestingly, there are different responses of macrophages on RGC survival in rats with different autoimmune backgrounds. A significant increase in the number of macrophages and extensive RGC loss occurred in experimental autoimmune encephalomyelitis (EAE)-vulnerable Lewis rats after acute IOP elevation, but not in EAE-resistant Fischer 344 and Sprague Dawley (SD) rats. Removal of macrophages in Lewis rats but not in Fischer and SD rats reduced the extent of RGC loss.[107] Macrophage activation by zymosan had a detrimental effect on RGC survival in Fischer and SD rats after acute IOP elevation, confirming the negative effect of macrophages on RGC survival under ocular hypertension. Moreover, ocular hypertension can also be induced by the use of steroid, such as dexamethasone and triamcinolone that disrupts the trabecular meshwork (TM) cells.[108] To delineate this pathogenic mechanism, we performed global gene expression profiling on dexamethasone and triamcinolone acetonide-treated TM cells in culture by microarray.[109,110] We found 29 genes differentially expressed in dexamethasone-treated cells and 57 genes differentially expressed in triamcinolone-treated cells. *MYOC*, *GAS1*, *SENP1*, *ZNF343* and *SOX30* are the common genes regulated by both dexamethasone and triamcinolone.

The etiology of AMD is multi-factorial and influenced by environmental factors such as cigarette smoking and diabetes mellitus.[111,112] Vascular endothelial growth factor (VEGF), one of the initiators of choroidal neovascularization in exudative AMD, is highly expressed in aqueous humor of exudative AMD patients compared with controls, whereas pigment epithelial-derived factor (PEDF), acting antagonistically to VEGF,[113] also shows increased levels in exudative AMD patients.[114] Higher baseline aqueous VEGF level is associated with persistent angiographic leakage in exudative AMD patients.[115] In exudative AMD patients with 3 monthly intravitreal anti-VEGF agent (Avastin) injections, aqueous VEGF level reduced from 102.6 pg/mL at baseline to 18.3 pg/mL after 2 months, whereas PEDF level increased from 11.2 ng/mL at baseline to 38.7 ng/mL.[116] There was also significant central foveal thickness reduction at 6 months after Avastin injection.[115] Although the *HTRA1* gene shows a strong association with AMD,[117] the HTRA1 protein in vitreous humor is associated with VEGF ($r = 0.650$), especially in patients with retinal detachment ($r = 0.835$).[118] In cultured human fetal RPE cells, upon stress induction by tunicamycin and dithiothreitol, *HTRA1* and *VEGFA* expression is upregulated, but the 2 genes do not mutually regulate each other.[118]

MicroRNAs (miRNAs) are important in regulating cellular physiological functions, including stem cell differentiation and regenerative properties. We conducted global microRNA profiling for human limbal stem cells and central corneal epithelial cells and found that miRNA-145 promotes limbal stem cell differentiation by suppressing integrin β8 (ITGB8) expression.[119] In addition to miRNA-145, there are 36 microRNAs enriched in the limbal region, including miRNA-10b, 126, 143 and 155.[120] The target genes regulated by these miRNAs are involved in cell survival, cell apoptosis, cell movement, cell-matrix interaction, cell-cell adhesion as well as pathways of immune response and cellular protection. Apart from corneal cell differentiation, we also found miRNAs regulated retinal differentiation from

stem cells.[121] We have identified 71 differentially expressed human miRNAs in the retinal differentiation process of human periodontal ligament-derived stem cells (PDLSCs). The predicted miRNA target genes are closely related to neuronal differentiation processes, and 2 of them (VEGF and PTEN) are significantly upregulated during the retinal differentiation process. Furthermore, we have delineated the miRNA profile of nicotine-treated PDLSCs and identified 16 differentially expressed human miRNAs.[122] Among them, miRNA-1305 and miRNA-18b were significantly upregulated in PDLSCs obtained from cigarette smokers compared with those from non-smokers.[123]

Pre-clinical drug testing and experimental disease modeling

We have been working on herbal chemicals, small molecules and stem cells. We have selected herbal molecules with known chemical structure and reported benefits to vision. We have shown that green tea extract and its biological active components (catechins) can be easily taken up by the digestive system and distributed to different organs through circulation within 1 hour.[124,125] Although epigallocatechin gallate, the most abundant catechin in green tea extract, is a powerful antioxidant,[126] it can induce caudal retardation with abnormal axial flexion and delayed hind-limb formation in rat embryos.[127] A high dose (550 mg/kg) of green tea extract can increase oxidative stress in plasma, aqueous humor, vitreous humor, cornea and retina, but decrease it in the lens and choroid-sclera.[128] Oral administration of a high dose of green tea extract can alleviate ocular inflammation in a rat model of endotoxin-induced uveitis by reducing infiltrating leucocytes and macrophages, protein exudation in aqueous humor, the production of TNF-α, IL-6 and MCP-1 in aqueous humor, as well as downregulation of Toll-like receptor 4 (TLR-4), CD14 and nuclear factor-kappa B (NF-κB p65) in the iris and ciliary body.[129] In addition to anti-oxidation and anti-inflammation, epigallocatechin gallate, together with isoliquiritigenin from licorice and resveratrol from grapes, are anti-angiogenic. They can suppress VEGF-induced human umbilical vein endothelial cell proliferation and migration by activating focal adhesion kinase and upregulating PEDF.[130,131] Topical application of ginkgo biloba extract can result in steroid-induced changes in trabecular meshwork and lower the IOP in rabbits.[132]

Small molecules can reduce mutant protein misfolding and alleviate the ER stress response. We identified D384N mutation in *MYOC* gene from a POAG family with reduced protein solubility, aggregation-prone and nonsecreted.[133] Treatment with trimethylamine N-oxide (TMAO) improved the solubility of the D384N mutant, reduced its distribution in ER, alleviated ER stress, and rescued the cells from apoptosis. Meanwhile, TMAO treatment can induce moderate upregulation of heat shock protein 70 (HSP70). Coherently, TMAO suppressed corneal dystrophy-causing R124C transforming growth factor beta-induced (*TGFBI*) mutant-induced amyloid-beta (Aβ) fibrillar aggregation.[134] Another chemical chaperone, sodium 4-phenylbutyrate (4-PBA), can ameliorate cataract-causing G165fsX8 mutant of γD-crystallin protein insolubility and relieve its mislocalization from nuclear envelope. In addition, 4-PBA treatment can reduce cell apoptosis and upregulate HSP70 expression.[135] We investigated the therapeutic effect of neuropeptides in different ocular diseases and reported the extra-pituitary effect of growth hormone releasing-hormone receptor antagonist to alleviate endotoxin-induced ocular inflammation in an experimental animal model.[136]

Stem cells participate in disease pathogenesis and can be used in regenerative medicine.[137-139] Our center has a strong interest in tissue-specific stem cells (human adult stem cells), especially limbal progenitor cells, retinal progenitor cells and mesenchymal stem cells. We studied the *ex vivo* expansion of human limbal epithelial cells,[140] and the nuclear matrix changes in long-term culture of limbal epithelial cells.[141] Subsequently, we delineated the miRNA profile of human limbal progenitor cells and central corneal cells.[119,120] We also investigated the application of stem cells for retinal diseases.[142-144] We used a novel approach to study retinal cells by the trans-differentiation ability of human mesenchymal stem cells. In collaboration with the University of Miami, we directed human mesenchymal stem cells obtained from periodontal ligament to a retinal fate, expressing retinal lineage markers, including PAX6, RAX and rhodopsin.[145] We modified our initial induction protocol to direct human PDLSCs into retinal ganglion-like cells.[123] The differentiated PDLSCs expressed neuronal and retinal ganglion cell markers (ATOH7, POU4F2, β-III tubulin, MAP2, TAU, NEUROD1 and SIX3), formed synapses and gave glutamate-induced calcium responses with spontaneous electrical activity, indicating that the trans-differentiated stem cells exhibited the characteristics of functional neurons.

Future directions

The CUHK Ophthalmic Research Centre is equipped with different advanced research technologies. We are one of the pioneers in the field of *in vivo* imaging to monitor the progression of disease phenotypes and treatment responses in animals.[146] We have established animal models of glaucoma, optic nerve injury and AMD to serve as a platform for *in vivo* pre-clinical drug testing and disease pathogenesis studies.[147-150]

In eye genetics, although we have identified more than 70 loci/genes/variants associated or linked with different ocular diseases, the role of these genes/variants in the disease pathogenesis or protein function is yet to be characterized. *In vitro* cellular and molecular studies as well as animal experiments are important strategies to decipher the disease-causing mechanisms of these genetically identified genes and variants. Our research direction is disease-orientated and translational. We will focus on disease mechanism, drug discovery, molecular disease diagnosis, biomarker identification and advancing therapeutic treatments.

We find it a great privilege to have clinical ophthalmologists, visual scientists, and research students working together at

the CUHK Ophthalmic Research Centre. We look forward to more collaborative research projects with colleagues to advance ophthalmology and visual sciences in Hong Kong and regions beyond.

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Declarations

The authors declared no competing financial interests.

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Review Article

Cytomegalovirus infection in non–human immunodeficiency virus–infected patients: a hematologist perspective

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Abstract

Cytomegalovirus is an opportunistic pathogen in immunocompromised patients, particularly those infected with the human immunodeficiency virus. In non–human immunodeficiency virus–infected patients, including patients with hematological malignancies and those undergoing hematopoietic stem cell transplantation, cytomegalovirus infection also leads to significant morbidity and mortality. The high cytomegalovirus seroprevalence in our locality explains the vulnerability of immunocompromised subjects to cytomegalovirus reactivation. Recipients of allogeneic hematopoietic stem cell transplantation and patients treated with certain chemotherapeutic drugs for hematological malignancies are at high risk of cytomegalovirus reactivation. Cytomegalovirus disease is associated with a wide range of clinical manifestations, as almost any organ can be involved. Histopathology remains the gold standard to confirm end-organ cytomegalovirus involvement, although pp65 antigenemia assay and quantitative polymerase chain reaction of viral DNA are useful tools in daily practice. Nevertheless, peripheral blood-based assays may not be positive in patients with cytomegalovirus involving organs including the gastrointestinal tract, brain and eye. Different treatment approaches are used in the management of cytomegalovirus infection, including prophylactic, preemptive and therapeutic strategies. Ganciclovir and its oral prodrug valganciclovir remain the cornerstone treatment of cytomegalovirus disease, albeit at the risk of increased myelosuppression. Alternatively, foscarnet may be used in cytopenic patients. Clinical vigilance and multidisciplinary collaborations are of paramount importance in the management of cytomegalovirus infection.

Key words: Cytomegalovirus infections; Hematopoietic stem cell transplantation; Immunosuppressive agents; Virus activation

Introduction

Cytomegalovirus (CMV) is a double-stranded DNA virus in the herpesvirus family. The majority of the human populations have been exposed to CMV, as judged by seroprevalence studies. CMV establishes a lifelong latent state in the host once infection has occurred.

Cytomegalovirus infection, reactivation and disease

Primary CMV infection is asymptomatic in immunocompetent individuals, and this is followed by a latent state without active viral replication. In immunocompromised subjects, however, primary CMV

infection may lead to an infectious mononucleosis-like syndrome with atypical lymphocytosis, lymphadenopathy, and widespread organ involvement of the brain, liver, lung and gut; which is potentially fatal. In most instances, clinical problems arise from CMV reactivation in seropositive subjects due to advanced age, or diseases and treatment that result in an immunocompromised state. The latter category includes infection by the human immunodeficiency virus (HIV), autoimmune diseases treated with immunosuppressants and high-dose corticosteroids, cancers treated with chemotherapy, treatment with lymphoablative drugs, and organ transplantations including hematopoietic stem cell transplantation (HSCT). Although reinfection with a CMV strain different from the endogenous latent strain in seropositive subjects is also possible, there is differentiation between endogenous reactivation and exogenous reinfection in clinical practice. Notably, CMV infection/reactivation and CMV disease are not synonymous. CMV infection/reactivation refers to the presence of CMV replication regardless of symptoms. It might allude to CMV DNA- or RNA-emia, CMV antigenemia, or CMV viremia, depending on the detection method used. Conversely, CMV disease is defined as CMV infection/reactivation with presence of clinical signs and symptoms compatible with CMV end-organ damage.[1] Tissue-invasive CMV disease can affect various organs and cause significant morbidity and mortality.

Among cancer patients and those who have undergone HSCT, Asians have been shown to have the highest CMV antigenemia rate and viral burden.[2] In fact, CMV seroprevalence varies in different populations, being highest in South America, Africa and Asia.[3] The seropositive rate of our local population is over 90%.[4,5] Therefore, the importance of recognizing this disease entity and managing it properly cannot be overemphasized.

Hematological patients at risk

Patients with hematological malignancies are very often immunocompromised, due to the underlying diseases and their treatment. Allogeneic HSCT recipients are at the highest risk of CMV reactivation. Without anti-CMV prophylaxis, about 80% of CMV-seropositive allogeneic HSCT recipients have CMV infection; the incidence of CMV disease in the early post-HSCT period being as high as 35%.[6-8] Other risk factors include graft-versus-host disease (GVHD), increased or prolonged courses of immunosuppression, human leukocyte antigen–mismatched or –unrelated HSCT, and administration of T-cell depleting agents.[7,9] Autologous HSCT recipients are at a lower risk of CMV infection. Because CMV infection is a major post-transplantation complication, different strategies of prophylactic and preemptive therapies have been incorporated into standard HSCT protocols to reduce the risk of CMV disease.

For non-HSCT patients, susceptibility to CMV infection is increased with particular types of drug. Fludarabine, a purine analogue, is a potent suppressor of T cells and increases the risk of opportunistic viral and fungal infections. It is mainly used to treat patients with chronic lymphocytic leukemia. Rituximab is an anti-CD20 monoclonal antibody widely used in the treatment of B-cell lymphomas.[10] In our population with a high seropositive rate, combination of fludarabine-based chemotherapy regimens with rituximab is associated with a significantly higher risk of CMV retinitis.[11] Alemtuzumab is a monoclonal antibody against CD52. It is active against chronic lymphocytic leukemia and other low-grade B- and T-cell lymphoproliferative diseases. As CD52 is expressed on all B-cells and T-cells, treatment with alemtuzumab leads to profound lymphoablation. Hence, alemtuzumab strongly predisposes to CMV reactivation, with reported frequencies varying from 10% to 100%.[12,13] Therefore, it is recommended that regular virologic surveillance and/or anti-CMV prophylaxis are needed in patients treated with these drugs.[14,15]

Clinical manifestations of cytomegalovirus infection

CMV infection without any clinical manifestations is specified as 'asymptomatic CMV infection'.[1] Patients with CMV disease, however, have a wide range of clinical manifestations, as almost any organ can be involved. CMV pneumonia is the most serious complication, with a high mortality of >50%.[16] CMV retinitis is sight-threatening and affected patients might present with non-specific, subtle blurring of vision. It is most often associated with HIV infection, and is the most common manifestation of CMV end-organ disease in HIV-infected patients. CMV retinitis is increasingly recognized in hematological patients.[11] CMV gastrointestinal disease is becoming more prevalent in recipients of allogeneic HSCT.[17] Because it often overlaps with GVHD, which is itself an important predisposing factor to CMV reactivation, the differentiation between the 2 conditions is challenging. Other rare manifestations of CMV diseases include hepatitis and encephalitis.

Laboratory workup and pitfalls

The diagnosis of CMV infection requires judicious application of laboratory tests in the appropriate clinical contexts. Commonly available tests for the diagnosis of CMV infection involve detection of a component of or the whole virus in blood or other bodily fluid/specimen. These tests include the pp65 antigenemia assay, molecular tests for detection of DNA or mRNA, viral culture and histopathology. The CMV antigenemia assay detects pp65 proteins in CMV-infected peripheral blood leukocytes. It is a semi-quantitative and rapid test, with results generally available the same day. Thus it is the preferred tool for regular surveillance when a preemptive strategy is adopted, and is useful for monitoring treatment response. Yet, it has limited utility in neutropenic patients as an adequate number of polymorphonuclear leukocytes is a prerequisite for the test.[18] Quantification of viral DNA by polymerase chain reaction (PCR) has become more popular, due to its high sensitivity and ability to measure viral load. The burden and the rate of increase of viral load are useful parameters to

assess risks of CMV disease.[1]

Histopathology remains the gold standard for diagnosing end-organ CMV disease, although its application is limited by the risk of the invasive diagnostic procedure and availability of other non-invasive methods for documentation of CMV infection in blood. Nevertheless, histopathology is still needed in certain clinical scenarios, for example, to exclude other concomitant pathology or co-pathogens, or when patients do not respond to anti-CMV treatment.

There are 2 major pitfalls in laboratory diagnosis of CMV infection. Firstly, absence of CMV in blood does not exclude CMV disease.[19] It has been well reported that peripheral blood-based CMV assays might be negative in CMV disease affecting various organs, including the gastrointestinal tract, lungs and eyes.[8,11,16] Circulating pp65 and CMV DNA can remain undetectable even in CMV infection affecting multiple anatomic sites.[20] Given the limitations of blood-based CMV assays, clinicians should always be vigilant and maintain a high index of suspicion when managing at-risk patients.

Conversely, it is important to understand that detection of CMV in blood or other body fluid does not necessarily indicate CMV disease. High sensitivity and good negative predictive value of PCR make it a useful test to exclude CMV disease.[7,8,21] Nevertheless, detection by PCR alone in bodily fluid or biopsy specimen is insufficient for the diagnosis of most CMV end-organ diseases.[22] When neurologic symptoms are present, detection of CMV by PCR in cerebrospinal fluid samples is diagnostic of central nervous system involvement. For CMV retinitis, its diagnosis is conventionally based on characteristic retinal lesions, and an ophthalmoscopic examination performed by an experienced ophthalmologist remains indispensable.[22-24] PCR-based analysis of ocular fluids serves as a valuable confirmatory tool, as opportunistic infection by other herpesviruses including varicella zoster virus and Epstein-Barr virus may give similar appearance on fundoscopic examination.[25] CMV DNA may be detected in the vitreous humour in approximately 80% of CMV retinitis, and a positive result is highly suggestive of the pathogenic role of CMV.[26]

Management

There are 3 major strategies in the management of CMV infection, namely prophylactic, preemptive and therapeutic.[9] The prophylactic approach is used in patients at high risk, such as recipients of allogeneic HSCT from unrelated donors and patients prescribed alemtuzumab therapy. Antiviral drugs are routinely administered to at-risk patients without any evidence of CMV reactivation. Preemptive therapy refers to initiation of antivirals when there is evidence of CMV replication in blood, by the presence of CMV pp65 antigen, CMV DNA or mRNA. This strategy is commonly used in standard-risk allogeneic and autologous HSCT recipients. The threshold to initiate antiviral treatment, however, varies across different centers. In patients diagnosed with CMV end-organ disease, a therapeutic dose of systemic anti-CMV treatment should be commenced.

Antiviral agents

Intravenous ganciclovir remains the first-line agent in the treatment of CMV infection. Induction dose is 5 mg/kg every 12 hours for 2 to 3 weeks, followed by 5 mg/kg/day as maintenance. The duration of therapy depends on the severity of disease and resolution of signs and symptoms. The most significant side-effect of ganciclovir is marrow suppression, and neutropenia can occur in up to 30% of HSCT recipients during ganciclovir treatment. Hence extra caution is needed for ganciclovir therapy in patients with hematological malignancies, with or without prior HSCT. Ganciclovir can also be given as an intravitreal injection, resulting in a high intraocular concentration of the drug. Based on the experience of treating HIV-infected patients, a combination of systemic and intravitreal anti-viral drugs should be used in patients with CMV retinitis.[26]

Valganciclovir is an alternative to intravenous ganciclovir for the treatment of CMV infection in patients who tolerate oral medication with normal or minimally impaired gastrointestinal absorption. It is an orally available prodrug of ganciclovir, achieving blood concentrations equivalent to intravenous ganciclovir. The usual regimen of valganciclovir for induction therapy is 900 mg twice daily. Similar to ganciclovir, valganciclovir is also myelosuppressive and may lead to profound neutropenia. Both ganciclovir and valganciclovir require dose reduction in patients with impaired renal function.

For patients who cannot tolerate ganciclovir or valganciclovir, particularly those with significant pre-existing cytopenia, the less myelosuppressive but also less-effective drug foscarnet can be used. It is administered intravenously. Nephrotoxicity and electrolyte disturbances are the most important side-effects. It chelates divalent cations, causing hypocalcemia, hypomagnesemia and hypokalemia. Monitoring of renal function and electrolytes is mandatory for patients treated with foscarnet. The recommended regimen is 90 mg/kg every 12 hours.

At the beginning of preemptive treatment, viral load might continue to rise due to replication of virus, underlying immunosuppression and delayed onset of action of the drug.[9] Unless there is clinical progression to CMV disease, or rapidly rising viral copy numbers, switching of drugs for preemptive therapy earlier than 2 weeks is generally not recommended. In patients unresponsive to or intolerant of ganciclovir and foscarnet, other antivirals such as cidofovir and maribavir can be considered as third-line treatment. The novel antiviral drug letermovir has been shown in a phase II study to provide effective prophylaxis against CMV reactivation in HSCT patients,[27] but it is not widely available.

In addition to antiviral therapy, intravenous immunoglobulin is often given to immunocompromised patients with CMV

infection. Reduction in immunosuppressant(s), if any, is strongly recommended. Chemotherapy and/or monoclonal antibodies should also be withheld until CMV infection is under control. Dose reduction or even cessation of therapy will have to be considered, especially in severe/refractory cases.

Conclusion

CMV infection is increasingly recognized in non-HIV subjects. CMV retinitis, once considered exceptionally rare outside the clinical context of HIV infection and organ allografting, can be found in hematological patients, particularly those prescribed potent chemotherapy or lymphoablative monoclonal antibodies. As CMV infection can involve different organs, a multidisciplinary approach to its management is essential. Goal-directed investigations with appropriate microbiological workup depend on which organs are affected. Systemic antiviral drugs are warranted in the treatment of CMV disease. As such, clinicians from different disciplines should be familiar with their toxicity profile.

Declaration

All authors have disclosed no conflicts of interest.

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Review Article

Understanding Alzheimer's disease in Hong Kong: can regular retinal checkups be used to diagnose, monitor and study disease pathogenesis?

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Abstract

Recently, interest in using the retina to represent simple brain neurobiology has increased, particularly in regard to neurodegenerative disorders such as Alzheimer's disease. Various laboratories and clinics worldwide are currently evaluating the use of novel techniques to monitor retinal health in patients with Alzheimer's disease. Unfortunately many of these tools are still at the development stage, while others may be unavailable or expensive in the Asia Pacific region, including cities like Hong Kong. In this review we address the current state of Alzheimer's disease retinal research in Hong Kong, emphasizing the recent literature, the use of Alzheimer's disease mouse models in retinal/brain research, and the potential implementation of retinal checkups to diagnose and monitor neurodegeneration in patients with Alzheimer's disease. We believe that the combined efforts of local researchers and clinicians will not only advance our understanding of Alzheimer's disease pathogenesis in both the brain and retina, but will also enhance the diagnosis and treatment options available for patients with Alzheimer's disease in Hong Kong.

Key words: Alzheimer disease; Amyloid beta-peptides; Retinal neurons; tau proteins; Translational medicine research

Introduction

Alzheimer's disease (AD) is recognized as the most common cause of dementia, a neurodegenerative disease affecting a continually increasing number of patients worldwide. In Hong Kong alone, dementia was estimated to affect approximately 103,433 people over the age of 60 years in 2009 and is expected to increase 222% by 2039.[1] It was also specified as the cause of an estimated 1112 deaths in 2014, up from just 999 in 2013 and 252 in 2001,[2] firmly placing it in the city's top 10 leading causes of death. Furthermore, of those diagnosed with dementia, approximately 65% are related to late-stage AD pathogenesis.[3] Thus, as the size of the elderly population in Hong Kong is expected to increase up to an estimated 33% by 2064 (compared with 15% of the population in 2014[4]), the amount of interest in local AD

research and funding should also expand in order to advance our understanding of this debilitating disease and provide better patient care.

A vast amount of research has been published on the causes and symptoms of AD as well as possible treatment options and curative agents. Notably, even with our knowledge of the various risk factors, diagnosis of AD pre-mortem is limited.[5] Clinicians currently rely on a list of AD criteria[6] that in 85% to 90% of cases are enough to predict AD.[7-9] A non-invasive technique to definitively provide a positive diagnosis would be invaluable, particularly for early detection. In response to this need, many laboratories have evaluated the use of positron emission tomography imaging[10,11] and magnetic resonance imaging[12,13] techniques to visualize the AD-induced changes that occur in the brain. These techniques, however, still do not properly address our lack of understanding about the causes of the disease, and many are expensive with limited availability in this region of the world. Furthermore, treatment options for these patients are also hindered by the inadequate number of specific drug targets.

Thus, it is the focus of several laboratories at the University of Hong Kong to address the underlying causes of AD by investigating AD-related retinal neurodegeneration in various animal models as a proxy for overall disease pathogenesis with the hope of not only identifying possible drug targets, but also developing potential diagnostic tools to use in conjunction with the current AD cognitive criteria. In this review, we have evaluated the potential use of regular retinal heath examinations to monitor AD-related neurodegeneration in relation to AD prognosis in Hong Kong patients. We have briefly analyzed what is currently known concerning disease development and pathogenesis in both the brain and retina as well as the role of various AD animal models in our search for diagnostic biomarkers and treatment options.

The pathogenesis of Alzheimer's disease in the brain

AD is a progressive neurological disorder characterized by amyloid beta (Aβ) plaque and subsequent neurofibrillary tangle (NFT) formation in the brain.[14-16] These pathological indicators are accompanied by decreased synaptic density, increased neuroinflammation, and neuronal cell death, ultimately leading to memory loss and learning deficits.[17-20] Aβ plaques build up in the brain as a result of excessive formation and reduced clearance of Aβ leading to extraneuronal aggregation.[21-23] The soluble oligomers formed are then thought to activate microglia- and astrocyte-related inflammatory responses, processes that subsequently cause oxidative stress, mitochondrial dysfunction and disturbed cellular homeostasis.[24-26] NFTs are formed via the intraneuronal accumulation of hyperphosphorylated forms of the microtubule-associated protein tau.[27-29] This type of extensive post-translational modification ultimately causes increased soluble tau, protein aggregation, and formation of NFTs that can block normal cellular transport.[30,31] Tau hyperphosphorylation and aggregation are likely triggered by upstream Aβ aggregation and appear to be required for Aβ-dependent cognitive dysfunction.[32-36] Therefore, all AD patients are found on autopsy to have some level of Aβ and tau accumulation, and while the symptoms and progression of the disease vary greatly among individual patients, the level of Aβ aggregate- and NFT-induced neurodegeneration is likely related to the progressive decline in cognitive and functional ability observed during the life of the patient.[12,37-39]

Notably, there are 2 types of AD: the sporadic age-related form (late-onset) and the genetic familial form (early-onset). Late-onset AD development has been linked to a wide range of genetic and environmental factors, including diet,[40] physical activity,[40,41] microbiome,[42] and obesity/cardiovascular diseases,[43] but the underlying causative mechanisms are largely unknown. In fact, late-onset AD patients are likely to have functional changes in their brain over 10 to 20 years before their manifestation.[44] This further obscures the key factors involved. Recent evidence suggests that there may be various genetic risk factors associated with sporadic AD. For example, both the ε4 allele of apolipoprotein E (*APOE*)[45-47] and the R47H allele of the triggering receptor expressed on meyleoid cells 2 (*TREM2*)[48-50] have been associated with a higher risk of developing AD. Although these genes have been linked to AD-related processes including amyloid clearance and blood brain barrier integrity, animal studies indicate that other genetic and/or environmental factors are necessary for the development of the full AD phenotype observed in humans.

Although the vast majority of AD patients are diagnosed with the late-onset form, close to 1% of cases are early-onset.[51,52] The histological features of the Aβ plaques and NFTs, as well as the clinical symptoms to some extent, in early-onset AD are identical to those of the late-onset AD.[53-56] Despite this, they appear to stem from different causative factors, with familial AD primarily involving mutations in amyloid precursor protein (*APP*),[57-59] presenilin 1 (*PS1*),[60,61] presenilin 2 (*PS2*),[62,63] and/or tau genes.[31,64] *APP*, a single transmembrane glycoprotein gene that is the precursor of Aβ, can be enzymatically cleaved by α-, β- and γ-secretases; but it is the sequential actions of the β-, and γ-secretases at the endoplasmic reticulum that generate the harmful AD-related form of Aβ.[65] Previous work has shown that mutations in the *APP* gene not only encourage cleavage by these secretases to generate more Aβ fragments, but they also increase their self-aggregation properties.[66] Alternatively, *PS1* and *PS2* are both multitransmembrane proteins that function in the γ-secretase complex.[67] Loss-of-function mutations in these genes also appear to promote Aβ generation and aggregation, with *PS1* mutations being more common in AD patients than *PS2* mutations.[68-70] Finally, tau, encoded by the *MAPT* gene, is known to play an essential role in microtubule stabilization and the regulation of axonal transport processes in neuronal cells, with 6 isoforms being expressed in the central nervous system at different times during development.[71-77] Various missense and

alternative splicing mutations of the tau pre-mRNA appear to play a significant role in microtubule-binding ability, whereby increased levels of unbound tau may be due to the aberrant phosphorylation and self-aggregation observed in neurodegenerative diseases.[78,79] Although some behavioral impairments have been observed,[80] tau knockout mice do not display any significant neurodegenerative defects.[81]

The pathogenesis of Alzheimer's disease in the retina

Although multiple ocular tissues may be involved in the vision deficits associated with AD,[82,83] changes in the retina likely play a significant role. The retina develops from the neural tube, similar to other regions of the brain and central nervous system. While the mammalian retina has a unique physical structure, including 5 groups of neurons responsible for transmitting light signals to the brain (photoreceptors, bipolar cells, horizontal cells, amacrine cells and retinal ganglion cells [RGCs]), many of the signaling molecules, cytokines and immune responses in this tissue are similar to those utilized in the brain. Furthermore, visual deficits — such as problems with depth perception, reading, motion perception, color recognition and impaired spatial contrast sensitivity — are common in AD patients.[84-89] These visual symptoms may be noticeable in the very early stages of AD, even prior to diagnosis, and have been linked with the level of cognitive decline in patients.[19,90] These disturbances in an AD patient's vision have also been associated with a substantial loss of RGCs and decreased thickness of the retinal nerve fiber layer and macula as well as optic nerve degradation and decreased venous blood flow.[91-97] Further, amyloid plaques have also been detected in the retina of AD patients as well as patients with mild cognitive impairment.[98] Thus, it would appear these changes in the retina may mirror the extent of neurodegeneration in the brain during AD, making further investigation of these AD-linked retinal changes essential. Pathologic changes observed in the retina of AD patients appear to be closely related to those observed during glaucoma and age-related macular degeneration.[99-101] It is likely that common signaling pathways are functioning during these neurodegenerative processes; additional work is necessary to elucidate these factors.

There are numerous advantages using the retina to study AD pathology. For clinicians, the retina can be directly imaged in living patients without invasive measures, providing a novel means of diagnosing AD. For research scientists, using the retina to study AD has the added benefits of being easily accessible for macro-histologic analysis as well as single neuron imaging. Retinal tissue extraction is also relatively straightforward, allowing researchers to use standard gene expression techniques in addition to primary retinal cell culture for in-vitro studies of mechanisms and/or drug responses. A number of Hong Kong–based clinicians, in association with research scientists at the University of Hong Kong, have already applied various techniques, including optical coherence tomography (OCT), electroretinograms (ERGs) and contrast vision testing, to evaluate retinal neurodegeneration in animal models as well as AD patients. These retinal examinations, however, are not yet used regularly in the clinical evaluation of AD.

Transgenic mice as a tool to study Alzheimer's disease–linked neurodegeneration

Numerous transgenic animals have been used to study brain development and disease. For AD alone, 118 mouse models have been created using single or multiple genetic manipulations resulting in various AD-related physiological changes.[102] Most of these mouse models and studies focus on AD pathogenesis in the early-onset form because of their ease of use in terms of genetic manipulation and reproducibility, while late-onset/sporadic models are more challenging. As various review articles have already comprehensively summarized many of the transgenic mouse models currently in use,[103,104] in the present review we focus on the use of 3 primary models (Tg2576, tau P301L, and 3xTg) to highlight the significant role of transgenic mice in AD research, particularly in Hong Kong–based laboratories.

Tg2576 mice

Tg2576 mice harbor a mutation in the *APP* gene (K670N/M671L) that was derived from a Swedish family with an extensive history of AD under the control of hamster prion promoter.[105,106] These single mutation transgenic mice overexpress *APP* and have age/disease progression–related Aβ deposition, and the postmortem histology of the brain appears to similar to that of AD patients.[107,108] Further, a reduction in the number of neurons was also detected, as were glial activation and increased expression of pro-inflammatory cytokines.[109-112] Cognitive defects are also apparent, with age-dependent deficits in working and spatial memory as well as contextual and cued fear learning ability.[106,113,114] Notably, the onset of AD appears to vary in this model, ranging from 6 to 10 months of age,[115] and may be a factor of genetic background.

While Tg2576 mice model distinct AD symptoms, there is a noticeable difference in their magnitude. For example, the cognitive defects and neuronal cell loss are not as extensive as those observed in their human counterparts.[116] *APP* transgenic mice also do not develop tau pathologies on their own, meaning that while these models may express hyperphosphorylated tau in some tissues, they do not present any of the downstream NFT-dependent defects observed in AD patients.[117]

tau P301L mice

To study the specific role of NFT formation in AD neuropathology, tau mutant mice have also been generated. One such model that expresses tau with a leucine replaced for the proline at position 301 (P301L)[118] has shown to result in the production of NFTs in both the brain and spinal cord, resulting in significant spinal cord atrophy and a limited life expectancy.[30] Notably, in animals with late-onset spinal cord deterioration, NFT formation is observed to significantly

impair basic motor behavior.[118,119] These findings are supported by those obtained using a conditional expression model, whereby the expression of tau P301L is controlled using a tetracycline expression system.[120] Tau pathology is rapid following tetracycline introduction, with NFTs forming as early as 2.5 months followed by a marked decrease in the number of neurons as well as spatial memory impairment in as little as 4 months.[120]

As with most animal models, the limitations of tau P301L mice primarily stem from promoter-dependent expression bias (tissue/cell type localization as well as transgene expression levels/dose) in addition to the widespread effects of genetic background that can limit the use of this murine model for translational research. Furthermore, these animals did not form the hallmark Aβ plaques observed in AD patients,[29,118] indicating that they do not mimic the full human AD phenotype.

3xTg mice

While the 2 previous mouse models allow AD-related amyloidosis and tau pathology to be separately investigated, in order to evaluate the combined effects of these processes, double and even triple transgenic mice have been produced. 3xTg mice, for example, incorporate the Swedish *APP* mutation, the tau P301L mutation, as well as a mutation in the *PS1* gene (M14V),[33] a combination that appears to result in phenotype changes that mimic human AD.[121] Notably, while *PS1* homozygous knockout mice have lethal developmental defects in their central nervous system,[122] mice harboring non-lethal mutations in the *PS1* gene do not appear to develop the same pathology as mice with *APP* mutations, although altered Aβ processing is evident.[123] These mice do not form Aβ plaques. Nonetheless, when used in conjunction with an *APP* mutation, the rate of amyloid deposition and plaque formation is accelerated.[124] Further, recent research has indicated that while increasing the expression and phosphorylation of tau has no effect on the development of Aβ aggregates, meaning NFT formation is likely a downstream event,[125] endogenous murine tau is necessary for *APP*-/Aβ-mediated neurodegeneration.[126] Indeed, tau knockout mice appear to be resistant, at least in terms of cognitive decline, to Aβ-induced toxicity.[127]

These findings indicate that in order to fully appreciate the full spectrum of AD pathogenesis, animal models presenting both Aβ plaque and NFT formation should be used. The triple transgenic 3xTg mice appear to be an ideal option, with the onset of AD pathology being observed at 3 months of age, beginning with Aβ deposition (mediated by the *APP* and *PS1* mutations) followed by tauopathy (mediated by the tau P301L mutation).[32,128,129] The progressive development of plaques and tangles also appears to continue in an age-dependent manner and are accompanied by cognitive deficits as well as upregulated microglial activity.[121,128,130-132] Although some neuronal loss was observed in these mice, this loss was not detected in the specific region of the hippocampus known to be the main area of cell loss in AD patients.[133,134]

Retinal changes in Alzheimer's disease models

Importantly, many of the mouse models demonstrate AD-related visual dysfunction and retinal neurodegeneration.[135] For example, Tg2576 mice appear to have an age-dependent increase in both *APP* and Aβ deposition in various retinal cell types as well as a reduction in retinal thickness.[136,137] These mice also appear to have increased expression of hyperphosphorylated tau and reduced axonal transport, although the formation of tangles is not described.[138,139] An age-dependent decrease in retinal thickness is also observed in tau P301L mice, primarily in the peripheral region.[140] Alternatively, in the triple 3xTg AD mouse model, retinal Aβ plaques are observed in addition to glial activation.[141]

General limitations

Although there are many advantages using mouse models to mimic human AD, such as genome manipulation, controlled genetic background, rapid disease maturation, cost, etc., there are also a number of drawbacks. For example, most of the models are considered incomplete as they do not mimic the full spectrum of human AD pathogenesis, including the 3xTg mouse model, as they lack substantial cell and synaptic loss[42,142] and appear to have slightly different biochemical and morphologic characteristics, particularly when compared with the sporadic form of AD.[143] Further, the use of inbred mouse strains eliminates interference from genetic background during laboratory investigation, but this is not an option when treating AD patients, whose genetic variability may play a significant role in the progression of the disease.[144] Nonetheless, these mouse models still offer the most direct means of studying AD outside of AD patients. In fact, the lack of complete AD-related structural changes and cognitive decline in these models[145] implies that they may better represent the prodromal phase of the disease, suggesting that they may be of the most value when evaluating upstream causative biomarkers as well as early diagnostic tools.[146] With this in mind, these models can be used not only to investigate early changes in gene expression and function in the brain, but also evaluate the role of the retina as a means to diagnose AD.

Translating basic science to clinical application

These transgenic models of AD have enabled researchers to potentially develop better treatment and diagnostic options for AD patients, particularly the use of retinal checkups as a proxy for evaluating AD-related neurodegeneration in the brain. Laboratory models allow testing of AD drugs prior to clinical trials; many animal models have retinal changes similar to those found in AD patients and are ideal for determining the applicability of various retinal scans, such as OCT, ERGs and contrast vision testing. These technological advances can elucidate the upstream biomarkers of this disease and detect expression and functional changes in the early stages of the disease prior to the irrevocable structural changes in both the retina and the brain. Discovering these biomarkers is useful in AD diagnosis and provides additional options for drug targeting during disease treatment.

While various conventional drugs — including acetylcholinesterase inhibitors, N-methyl-D-aspartate receptor antagonists, monoamine oxidase inhibitors, metal chelators, anti-inflammatory drugs and antioxidants[147,148] — are often used in an attempt to treat or slow down the cognitive decline in AD patients, researchers have failed to produce a viable option for the treatment, cure and/or prevention of AD. Interestingly, a number of traditional Chinese medicines (TCMs) have recently been evaluated for their use as a therapy for AD and other dementias, either alone or in conjunction with other conventional drugs.[149,150] Some of the naturopathic compounds found in TCMs have also been shown to protect and combat retinal neurodegeneration.[151,152] The amount of active research in the field of TCM in the Asia Pacific region, including Hong Kong, in conjunction with the AD focus of various laboratories at the University of Hong Kong provides a foundation for continued research into natural AD treatment options. These researches evaluate these compounds in terms of their characteristics and in-vitro/in-vivo effects in local basic science laboratories as well as their translation to clinical use. The relationship between the University and the Hong Kong Alzheimer's Disease Association[153] also provides the opportunity for enhanced discussion between research scientists and clinicians in the search for optimal translational research models, both in vivo and in vitro, and ultimately the best treatment options for each patient.

Conclusion

The increasing prevalence of AD and the aging population in Hong Kong necessitate continued studies of this disease such as AD-related brain and retinal changes using both animal models and clinical subjects in order to develop additional diagnostic and treatment options. It is hoped that regular retinal health checkups will be used to diagnose, monitor and study AD in Hong Kong patients, and that new methodologies for drug development and AD diagnosis will be implemented in both basic science laboratories for AD animal models as well as in clinical settings in Hong Kong.

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Declaration

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ORIGINAL ARTICLE

Safety and efficacy of dexamethasone intravitreal implant injection for macular edema associated with retinal vein occlusion

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Abstract

Purpose: To evaluate the safety and efficacy of dexamethasone intravitreal implant injection for retinal vein occlusion.

Methods: Twenty-two patients with central or branch retinal vein occlusion who were treated with dexamethasone intravitreal implant (Ozurdex) injection for macular edema at the Hong Kong Eye Hospital between December 2011 and December 2013 were retrospectively reviewed. Best-corrected visual acuity, proportion of patients with 3-line gain in visual acuity, central macular thickness and intraocular pressure before and after implant were recorded, as were any complications.

Results: Of the 22 patients, 11 each had central or branch retinal vein occlusion. The mean duration of follow-up was 9.6 months. The mean overall logMAR visual acuity improved from 1 to 0.67 ($p < 0.0001$) at 3 months and to 0.88 ($p = 0.048$) at final follow-up. The mean gain in visual acuity was 3.6 lines at 3 months, and 1.5 lines at final follow-up; 63.6% and 45.5% of patients at the respective follow-ups had gained ≥3 lines of vision. Nonetheless, 2 eyes lost ≥3 lines of vision at final follow-up. The mean central retinal thickness decreased from 589 μm to 327 μm at 3 months ($p < 0.0001$), and to 409 μm at final follow-up ($p = 0.0001$). Fifteen eyes had recurrence of macular edema after a mean of 4.5 months; recurrence was earlier in central than branch retinal vein occlusion by 1.1 months ($p = 0.031$). Three eyes had intraocular pressure >21 mm Hg. Five eyes out of 8 phakic patients developed cataract progression, of whom 2 subsequently underwent cataract extraction. Two eyes developed ischemic central retinal vein occlusion and had lost vision at final follow-up. There were no retinal tear, retinal detachment or endophthalmitis.

Conclusion: Ozurdex is efficacious in treating macular edema associated with retinal vein occlusion in terms of the gain in visual acuity and reduction in central retinal thickness, even for patients with a long duration of macular edema and poor baseline visual acuity. It has a good safety profile. The main complications are cataract progression and increased intraocular pressure (IOP). The duration of effect may be shorter than 6 months, especially in patients with central retinal vein occlusion. Earlier retreatment may be considered in order to maximize visual benefits.

Key words: Dexamethasone; Macular edema; Retinal vein occlusion; Treatment outcome; Visual acuity

Introduction

Retinal vein occlusion (RVO) is a common retinal vascular disorder, with an age and sex standardized prevalence of 5.2 per 1000.[1] It is the second most common cause of vision loss amongst retinal vascular diseases in developed countries, second only to diabetic retinopathy. The main cause of vision loss in RVO is macular edema (ME),[2,3] secondary to non-perfusion and leakage of the retinal capillaries. Current treatment for ME in RVO includes the use of anti-vascular endothelial growth factor (VEGF) agents such as ranibizumab[4,5] and aflibercept,[6] intravitreal injection of steroids such as triamcinolone[7] and dexamethasone,[8,9] and focal laser for ME secondary to branch retinal vein occlusion (BRVO).[10]

Ozurdex (Allergan, Irvine, CA, USA) is a sustained-release dexamethasone implant that was approved in June 2009 by the US Food and Drug Administration for the treatment of ME secondary to RVO. Its efficacy and safety have been demonstrated in a large randomized controlled trial with over a thousand patients followed up over 1 year (the GENEVA study).[8,9] It has a longer duration of effect (3-6 months), compared with monthly ranibizumab (Lucentis; Genentech, South San Francisco, CA, USA).[8,9,11] Its main complications are cataract progression and IOP rise.[8,9]

Ozurdex has been shown to have similar safety and efficacy to intravitreal triamcinolone in the treatment of ME in RVO in terms of visual acuity gain and decrease in central macular thickness (CMT). Nonetheless, dexamethasone has a longer duration of effect than triamcinolone and therefore requires fewer re-injections.[12]

In this study we report the efficacy and safety of Ozurdex implants in patients with ME associated with BRVO and central retinal vein occlusion (CRVO) at the Hong Kong Eye Hospital.

Methods

The study was approved by the Research Ethics Committee (Kowloon Central/ Kowloon East) of the Hong Kong Hospital Authority (rec no: KC/KE-13-0218/ER-3).

Twenty-two eyes in 15 women and 7 men aged ≥18 years with ME secondary to BRVO or CRVO who were treated with Ozurdex implant injection and followed up for >3 months at the Hong Kong Eye Hospital between December 2011 and December 2013 were retrospectively reviewed.

Clinical data collected included pre-existing eye diseases, prior treatment for ME, best-corrected visual acuity (BCVA), pre- and post-injection central subfield thickness measured by spectral domain optical coherence tomography (OCT), slit lamp and indirect ophthalmoscopy findings, IOP and any associated complications. Fundus fluorescein angiography (FFA), if performed, was also noted.

The efficacy outcome measures were BCVA, OCT central subfield retinal thickness and proportion of patients with gain/loss in BCVA at 3 months post injection and at final follow-up. The safety outcome measures were the rates of cataract progression and IOP increase, and any serious complications such as endophthalmitis, retinal detachment, suprachoroidal hemorrhage.

Statistical analysis

All statistical analysis was performed using SPSS 17.0 statistics software. Qualitative variables were described using percentages, and quantitative variables were described using mean and standard error. For univariate analyses, Student's *t* test was used for independent data, and paired *t*-test was used for paired observations. Statistical significance was defined as $p < 0.05$.

Results

Demographics and clinical characteristics

The patient demographics and clinical characteristics are shown in **Table 1**. The mean patient age was 73 years (standard deviation [SD], 11; range, 51 to 97). The mean duration of follow-up was 9.6 months (SD, 4.9; range, 4 to 23). Of the 22 eyes, 11 each had CRVO or BRVO for a mean of 9.7 months (SD, 13.7; range, 0.5 to 54). Five eyes had pre-existing ocular disease. Five patients with CRVO and 1 patient with BRVO underwent concomitant phacoemulsification and intraocular lens insertion. Eight eyes were pseudophakic and 8 eyes were phakic.

Mean change in visual acuity

The mean visual acuity changes are shown in **Figure 1**. The mean ± SD logMAR score improved from 1.03 ± 0.07 to 0.67 ± 0.06 at 3 months, with a gain of 0.36 ± 0.06 ($p < 0.0001$), to 0.88 ± 0.08 at final follow-up, with a gain of 0.15 ± 0.07 ($p = 0.048$).

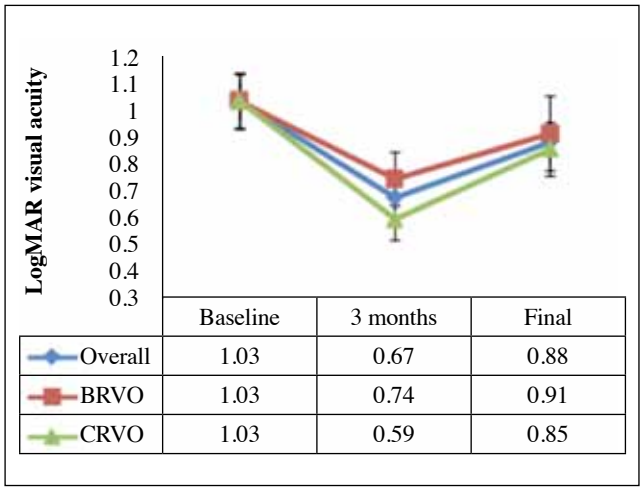
In the BRVO group, the mean ± SD logMAR score improved from 1.03 ± 0.11 to 0.74 ± 0.1 at 3 months, with a gain of 0.29 ± 0.07 ($p = 0.002$), to 0.91 ± 0.14 at final follow-up, with a gain that was not significant ($p = 0.29$). In the CRVO group, the mean ± SD logMAR score improved from 1.03 ± 0.1 to 0.6 ± 0.08 at 3 months, with a gain of 0.12 ± 0.11 ($p = 0.002$), to 0.85 ± 0.1 at final follow-up, with a gain that was also not significant ($p = 0.1$).

Proportion of patients with visual acuity changes at 3 months and final follow-up (Figure 2)

At 3 months, 63.6% of patients had a visual gain of ≥3 lines, 13.6% had a gain of 2 lines, 4.5% had a gain of 1 line and none lost vision. At final follow-up, 45.5% of patients had a visual gain of ≥3 lines, 4.5% had a gain of 2 lines, 4.5% had a gain of 1 line, 36% had no change and 9% (2 patients) had a vision loss of ≥3 lines owing to progression to ischemic disease, with neovascularization of pre-retinal hemorrhage and vitreous hemorrhage. The 2 patients presented initially with blurring of vision for months with a VA of 6/60 and 6/30 and were diagnosed with non-ischemic CRVO;

Table 1. Demographics and clinical characteristics of patients.	
Characteristic	**Data**
No. of patients	22
No. of eyes	22
Age (years)	73 ± 11
Gender	
Male	7 (32%)
Female	15 (68%)
Pre-existing eye disease (n = 5)	
Dry age-related macular degeneration	2 (9%)
Diabetic retinopathy (non-proliferative)	1 (4.5%)
Narrow angle with peripheral iridotomy	1 (4.5%)
Retinal arteriole macroaneurysm	1 (4.5%)
Type of retinal vein occlusion	
BRVO	11 (50%)
CRVO	11 (50%)
Prior treatment for ME secondary to retinal vein occlusion (n = 6)	
Anti-VEGF	3 (13.6%)
Anti-VEGF plus focal/grid laser	3 (13.6%)
Concomitant cataract extraction (n = 6)	
BRVO	5 (45%)
CRVO	1 (9%)
Lens status	
Pseudophakic	8 (36.4%)
Phakic	14 (63.6%)
Mean duration of ME prior to Ozurdex injection	9.7 ± 13.7 (0.5-54)
BRVO	9.6 ± 11.9 (1-44)
CRVO	9.8 ± 15.9 (0.5-54)
Mean follow-up duration	9.6 ± 4.9 (4-23)

Abbreviations: BRVO = branch retinal vein occlusion; CRVO = central retinal vein occlusion; ME = macula oedema; VEGF = vascular endothelial growth factor.
** Data are shown as No., No. (%), or mean ± standard deviation (range).*



	Baseline	3 months	Final
Overall	1.03	0.67	0.88
BRVO	1.03	0.74	0.91
CRVO	1.03	0.59	0.85

Figure 1. Change in mean visual acuity (LogMAR) after Ozurdex injection at baseline, 3 months and final follow-up.

baseline FFA was not performed. Their clinical findings included no relative afferent pupillary defect, diffuse retinal hemorrhage without cotton wool spots, tortuous retinal vessels, hard exudates and macula edema without signs of retinal neovascularization. FFA was subsequently performed in 1 patient and revealed capillary drop-out of >10 DD confirming ischemia.

Mean central subfield thickness

The change in mean ± SD CST is shown in **Figure 3**. The decrease in mean ± SD CST from baseline to 3 months was 265 ± 44 μm ($p < 0.0001$) overall, 256 ± 80 μm ($p = 0.01$) in BRVO patients and 273 ± 50 μm ($p < 0.0001$) in CRVO patients. At final follow-up, the decrease in CST in the overall and CRVO subgroups remained significant by 175 ± 67 μm ($p = 0.02$) and 247 ± 108 μm ($p = 0.046$), respectively, whereas the decrease in CST in the BRVO subgroup was not significant.

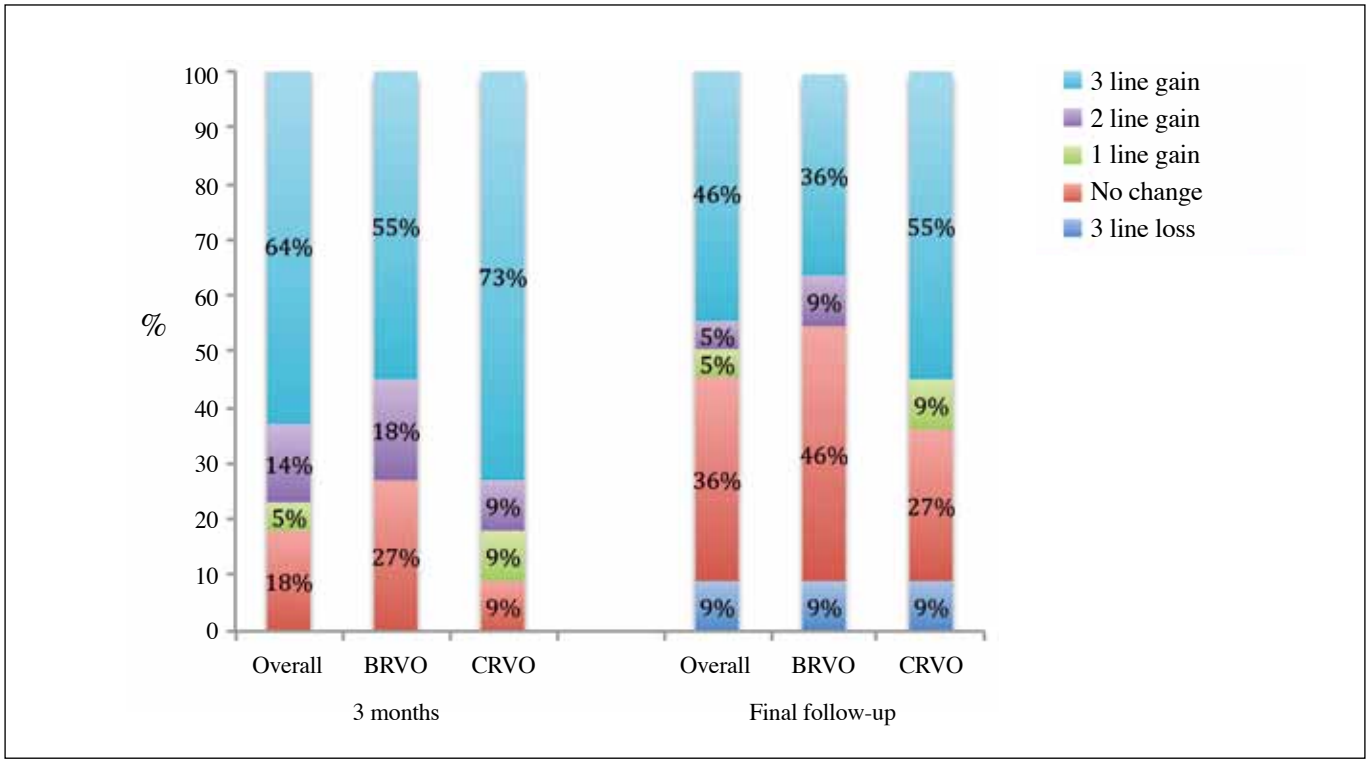
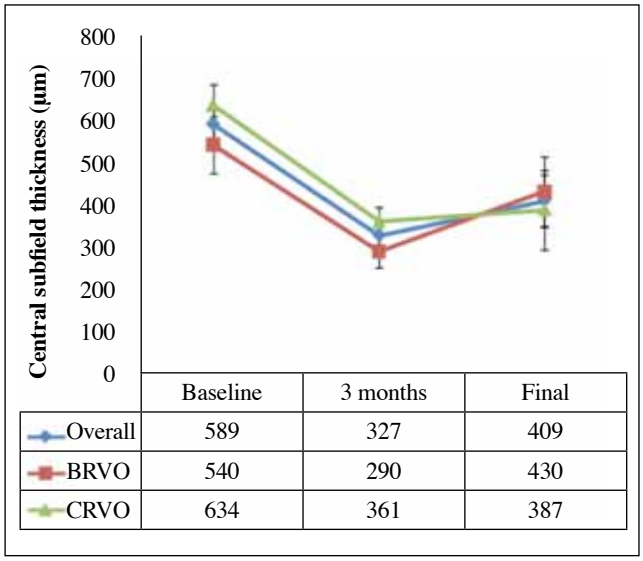


Figure 2. Proportion of patients with different changes in visual acuity (LogMAR) compared with baseline after Ozurdex injection at 3 months and at final follow-up.



	Baseline	3 months	Final
Overall	589	327	409
BRVO	540	290	430
CRVO	634	361	387

Figure 3. Change in mean central subfield thickness after Ozurdex injection at baseline, 3 months and final follow-up.

Non-responder and recurrence

Two eyes (9%) showed a poor response to Ozurdex, as evidenced by no decrease in OCT or an increase in CRT after injection. Fifteen eyes (75%) had recurrence of ME after a mean of 4.5 ± 1.1 months; 8 of them (80%) opted for repeat Ozurdex injections, with a mean of 1.1 ± 0.8 extra injections. Of the 15 eyes, 7 (46.6%) had BRVO and 8 (53.3%) had CRVO. Recurrence of ME was earlier in eyes with CRVO (4 ± 0.8 [range, 3 to 5] months) than BRVO (5 ± 1.1 [range, 3 to 6] months) by a mean of 1.1 months (p = 0.031).

Complications

Complications are shown in **Table 2**. Five eyes (63%) in 8 phakic patients developed cataract progression; 2 of them (40%) subsequently underwent cataract extraction. The rise in IOP was mild and none required surgical intervention. Two eyes (9%) had ischemic conversion and ultimately vision loss despite panretinal photocoagulation. There were no cases of retinal tear, retinal detachment or endophthalmitis.

Table 2. Complications after Ozurdex injection.

Complication (n = 22)	No. of eyes (%)	Treatment
Raised IOP (mm Hg)	3 (13.6%)	
>21	2 (9.1%)	Topical IOP lowering medication
>25	1 (4.5%)	Topical IOP lowering medication
Cataract (posterior subcapsular cataract) progression (n = 8)	5 (63%)	Two (40%) eyes underwent cataract operation

Abbreviations: IOP = intraocular pressure.

Discussion

In our study, Ozurdex injection was effective in the treatment of ME associated with RVO in terms of VA improvement and CST. Although these findings echo those of the GENEVA study,[9] there are some important differences. First, our study included patients with very poor baseline VA (worse than 6/60) compared with the GENEVA trial that included only patients with VA between 6/60 and 20/50. The mean baseline VA was also worse in our patients. We had a greater proportion of eyes with CRVO (50%) compared with 38% in the GENEVA study that may partially explain the poorer baseline VA. The duration of ME prior to Ozurdex injection was also longer in our patients, with a mean of 9.7 months compared with 5.2 months in the GENEVA study. The mean baseline CST was slightly higher (589 μm vs 562 μm) in our study. Overall, our patients had a longer duration of ME with poorer VA and thicker macula at baseline. Despite this, we had a greater proportion of patients with 15-letter gain at 3 months overall (63.6% vs 22%) and in BRVO (54.5% vs 24%) and CRVO (72.7% vs 18%) subgroups. There was also a greater mean change in VA from baseline (overall gain of 3.6 lines vs 1.4 lines) at 3 months. This contrasts with the post hoc analysis of pooled data from the GENEVA trial at 12 months that revealed longer duration of ME was associated with a lower likelihood of achieving a gain of ≥15 letters in BCVA.[13] One reason for the difference may be that our sample was confounded by concomitant cataract extraction performed in 6 patients (5 in the CRVO group and 1 in the BRVO group). The improvement in vision was 2.9 lines, which was comparable to the 3-line improvement in the Geneva study. Our findings suggest that Ozurdex is effective even in patients with a longer duration of ME and poorer baseline VA, especially in those with BRVO.

The duration of effect of Ozurdex has been reported to be 3 to 6 months, peaking at month 2.8,14 In our study, the mean duration of effect overall was 4.5 months. ME recurred in eyes with CRVO significantly earlier than eyes with BRVO after a mean of 4 and 5 months, respectively. Similar results were reported in the SOLO study where early reinjection at 16 weeks was required for 50% and 41% of eyes with CRVO and BRVO respectively.[14] Mathew et al. using monthly OCT monitoring to evaluate retreatment strategy also reported 4 months to be an optimal time for re-treatment.[15] Similarly, our study found that early re-treatment (4 months for CRVO and 5 months for BRVO) is useful in maximizing visual benefits in these eyes.

The safety profile was similar to that reported in other studies,[5,8] with a mean follow-up of 10 months. The most common complications were cataract progression and increased IOP. The long-term safety profile of Ozurdex has been shown to be good.[16-18] Despite this, physicians should keep in mind the potential for steroid-related complications when using Ozurdex, especially in patients who receive repeated injections.

This current study was limited by its small sample size, recruitment of patients from a single center and the lack of a control arm. Concomitant cataract surgery in a number of patients, especially in eyes with CRVO, acted as a confounding factor for the gain in VA.

Conclusion

Ozurdex is effective and safe in the treatment of ME associated with RVO, with significant gain in VA and reduction in macular thickness, even in patients with poor baseline VA and a long duration of ME. Its effect may be shorter than previously reported, especially in CRVO patients. Earlier follow-up with repeat injections of Ozurdex may maximize visual benefits.

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CASE REPORT

Sudden vision loss after repeated retching: a case of Valsalva retinopathy

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Abstract

We report on a patient who developed unilateral retinal and vitreous hemorrhages after repeated retching and vomiting. A 22-year-old man of South Asian descent was seen at the Accident and Emergency Department, Queen Mary Hospital, for sudden loss of vision in his right eye. The episode was acute, painless and preceded by repeated retching and vomiting after a spicy curry meal. Examination revealed fresh intra- and pre-retinal hemorrhage involving the inferior macula. The condition resolved spontaneously with conservative measures and did not require specific treatment or intervention. Repeated vomiting as a result of a poorly tolerated spicy meal resulted in unilateral Valsalva retinopathy. This case report details the course of recovery and what patients and physicians should expect and rule out. It is important to reassure patients during this course that they will regain full visual function.

Key words: *Eye hemorrhage; Retinal diseases*

Introduction

Valsalva retinopathy is a form of premacular hemorrhage that typically occurs after strenuous physical activity. Initial presentation is usually a unilateral, painless, acute vision loss that is preceded by a sudden increase in intra-abdominal pressure, hence the term 'Valsalva' retinopathy. The name may be misleading in that it is not a disease of the retina, but simply a pressure-induced hemorrhage caused by ruptured sub-retinal capillaries. The hemorrhage tends to obscure vision severely and suddenly, and is alarming for most patients. The advantage of this is that patients tend to seek help early, and this prevents complications of prolonged blood-trapping in the premacular space.

Case report

In September 2015, a 22-year-old man of South Asian decent, with good past health, presented with sudden onset loss of vision in his right eye. The episode was immediately preceded by repeated retching and vomiting, as he had been unable to tolerate a particularly spicy curry meal that evening. There was no associated ocular pain or headache and no preceding head or ocular trauma. Vision in his left eye vision was unaffected. The patient denied any history of easy bruising, or use of anticoagulants or antiplatelet medication. There was also no family history of bleeding tendencies or disorders although both of his parents had type II diabetes mellitus.

On physical examination, the patient had unaided Snellen Chart visual acuity of hand movements at 1 m in his right eye and 0.7 m in his left eye. Intraocular pressure was measured at 14 mm Hg in both eyes via Goldmann

applanation tonometry. His pupils were equal and reactive, with no relative afferent pupillary defect. He had full-range extraocular muscle movements and there was no proptosis. Anterior segment examination of both eyes using a slit lamp revealed no abnormalities. A dilated fundus examination of both eyes revealed a large pre-retinal hemorrhage of at least 5 disc-diameters over the inferior macula in the right eye. The disc was pink with a cup-disc ratio of 0.5. Multiple intra-gel clots along with minor dot and blot hemorrhages were present. Retinal vessels were not tortuous and there was no evidence of retinal neovascularization (**Figure**). The left eye fundus examination was unremarkable, with no evidence of vasculopathy or retinal neovascularization.

Differential diagnoses

In considering the differential diagnoses, common causes of intraocular hemorrhage include proliferative diabetic retinopathy, retinal artery macroaneurysm, and retinal vein occlusion.[1] Other causes include bleeding tendency, posterior vitreous detachment, retinal break, vasculitis, Terson's syndrome, trauma etc. It is important to note that these conditions are typically found in middle-aged to elderly patients. With the clinical history and lack of clinical features to indicate underlying vasculopathy or intracranial hemorrhage, it was most likely that the repeated vomiting had caused a retinal arteriole to rupture, leading to premacular hemorrhage. The patient was noted to have approximately -1.0D of uncorrected myopia in his right eye and -0.5D in his left eye that explained the suboptimal vision for his age even in the unaffected eye.

Management

To look for possible underlying vasculopathy, blood was taken for complete blood count, clotting profile (prothrombin time and activated partial thromboplastin time), erythrocyte sedimentation rate, C-reactive protein, fasting glucose and lipid profile. The blood tests were normal with no evidence of bleeding tendencies or diabetes mellitus (**Table 1**).

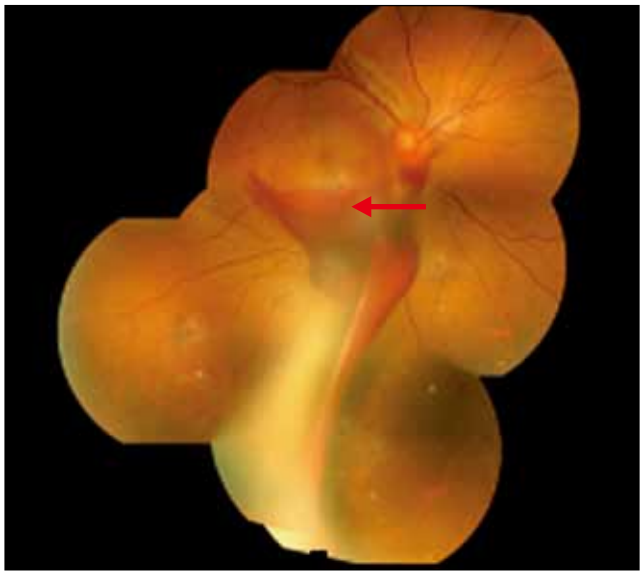

Figure. Fundus photograph of the right eye showing a large well circumscribed area of pre-retinal hemorrhage (arrow) involving the macula.

In view of the above findings, the patient was advised to allow time for the blood to resolve spontaneously. He was instructed to avoid physical exertion, and to prop his head up with 3 pillows while in bed. No further ocular treatment was recommended. Follow-up sessions were scheduled for the following morning, in 3 days and in 10 days. His vision began improving as early as day 2 with no complications (**Table 2**). Initially, a large pre-retinal hemorrhage covering the inferior macula was seen and there were no breaks or holes found in the peripheral retina. Minor dot and blot hemorrhages were present, as expected with sudden increases in intraretinal vascular pressure. Many intra-gel clots also contributed to obscuring the patient's vision. The blood spread out into the vitreous by the 3rd day and was seen to be resolving well by day 10, with a corresponding visual acuity of 0.5 in his right eye. After this, the patient defaulted from further follow-up visits.

Discussion

Vision is the most dominant sense for humans. Sudden changes in vision, especially loss, can be intimidating for patients. Nonetheless not all causes of vision loss need specific ocular treatment or intervention. This case provides a timeline for which Valsalva retinopathy spontaneously resolves. It is important for physicians to offer security and reinforce the patient's trust in conservative management.

Valsalva retinopathy occurs when a sudden increase in intrathoracic or intra-abdominal pressure causes an abrupt increase in peripheral intraocular venous pressure, leading to rupture of sub-retinal capillaries. It is often seen in younger patients with no history of retinal disease.[2] Detailed history taking is essential for diagnosis as a wide range of activities that involve forced expiration can lead to Valsalva retinopathy. These may include heavy lifting, labor, constipation, sneezing, sexual activity and vomiting.[3,4] It is also important to exclude risk factors for ocular hemorrhage such as diabetes and bleeding disorders.

Vision loss is often unilateral, acute and painless.[2] It is a result of the macula being obscured by hemorrhage that may be sub-retinal, intra-retinal, pre-retinal, sub-hyaloidal, or in the intra-vitreal cavity.[5] In our case the presentation was predominantly a pre-retinal hemorrhage that covered the inferior macula. The prognosis of Valsalva retinopathy is excellent. Patients usually return to baseline visual function after resolution although the recovery process can take several months.[6,7]

In our case, although the patient presented with a severe loss of vision, he recovered as early as the 3rd day after presentation. Vision is regained as the pre-macular blood is absorbed. It is important during this time to reassure the patient that the symptoms are transient and will resolve

Table 1. Baseline blood results.

Blood parameter	Data	Reference range
Metabolic profile		
Sodium (mmol/L)	138	136-148
Potassium (mmol/L)	4.3	3.6-5.0
Chloride (mmol/L)	100	100-109
Urea (mmol/L)	5.9	2.5-6.3
Creatinine (μmol/L)	66	67-109
R-glucose (mmol/L)	5.0	
Total protein (g/L)	84	68-84
Albumin (g/L)	49	39-50
Globulin (g/L)	35	24-37
Total bilirubin (μmol/L)	14	4-23
Alkaline phosphatase (U/L)	65	42-110
Alanine transaminase (U/L)	29	8-58
Aspartate transaminase (U/L)	27	15-38
Coagulation		
Prothrombin time (sec)	13.3	11.3-13.5
Activated partial thromboplastin time (sec)	29.8	25.9-33.7
Complete blood count		
White blood cell count (x 10^9/L)	7.99	3.89-9.93
Red blood cell count (x 10^{12}/L)	4.59	4.46-5.71
Hemoglobin (g/L)	133	133-171
Hematocrit (%)	40.1	39.5-48.8
Mean corpuscular volume (fL)	87.4	82.0-95.5
Mean corpuscular hemoglobin (pg)	29.1	27.0-32.4
Mean corpuscular hemoglobin concentration (g/dL)	33.2	32.6-35.7
Red cell distribution width (%)	13.0	11.2-13.9
Platelets (x 10^9/L)	231	154-371
Differential count		
Neutrophil (x 10^9/L)	5.20	2.01-7.42
Lymphocyte (x 10^9/L)	1.93	1.06-3.61
Monocyte (x 10^9/L)	0.48	0.18-0.65
Eosinophil (x 10^9/L)	0.18	0.02-0.45
Basophil (x 10^9/L)	0.03	0.01-0.07

Table 2. Physical examinations findings.

Physical examination	Day 1	Day 3	Day 10
Snellen Chart visual acuity (unaided)	RE: hand movements only; LE: 0.7	RE: 0.3; LE: 0.7	RE: 0.5; LE: 0.7
Intraocular pressure (mm Hg)	RE: 14; LE: 14	RE: 12; LE: 14	RE: 11; LE: 10
Pupillary reflex	Pupils equal and reactive to light, no relative afferent pupillary defect		
Anterior segment exam	Clear corneas with formed and quiet anterior chambers; clear crystalline lenses		
Dilated fundus examination of the RE	Large pre-retinal hemorrhage covering posterior pole, at least 5 disc diameters in size tracking towards inferior retina; right supra-temporal arcade blot hemorrhage; no retinal breaks or holes	Large pre-retinal hemorrhage covering posterior pole; no retinal breaks/holes seen	Pre-retinal hemorrhage resolving and shifting inferiorly; no more intra-retinal hemorrhage; no retinal breaks/holes seen

Abbreviations: LE = left eye; RE = right eye.

with conservative management, as loss of vision can be debilitating and alarming for most. Patients should be informed that no permanent damage or further complications should be expected. To help with the recovery process, they should be advised to prop the head up and avoid strenuous physical activity.

Conservative management with close observation is the primary initial treatment for Valsalva retinopathy and is followed with good recovery.[8] In patients with very large and dense hemorrhages, blood trapped in the subhyaloid or sub-inner limiting membrane space for prolonged periods of time can cause visual impairment. This can be due to toxic damage to the retina by hemoglobin and iron, pigmentary macular changes or formation of epiretinal membranes. These complications, however, are typically uncommon unless the hemorrhage persists for over 6 weeks.[9]

Should the pre-macular hemorrhage persist after a reasonable period of observation, alternative treatments can be considered. Laser therapy is advised when conservative management is unsuccessful.[8] Nd:YAG laser treatment has been reported to help diffuse the hemorrhage into the vitreous gel, and can speed up its clearance.[1] However, the procedure comes with risks such as unsealed internal limiting membrane and epiretinal membrane formation.[10,11] In more severe cases of Valsalva retinopathy, vitreoretinal surgery has also been cited to be useful when spontaneous resorption is incomplete and visual acuity is significantly affected.[9]

Conclusion

Valsalva retinopathy is a self-limiting condition associated with sudden severe loss of vision in the affected eye, leading patients to seek immediate medical attention at accident and emergency departments. This case report provides a natural history of Valsalva retinopathy caused by repeated vomiting after a spicy meal; patient reassurance and conservative measures are effective first-line strategies for Valsalva retinopathy.

Declaration

All authors have disclosed no conflicts of interest.

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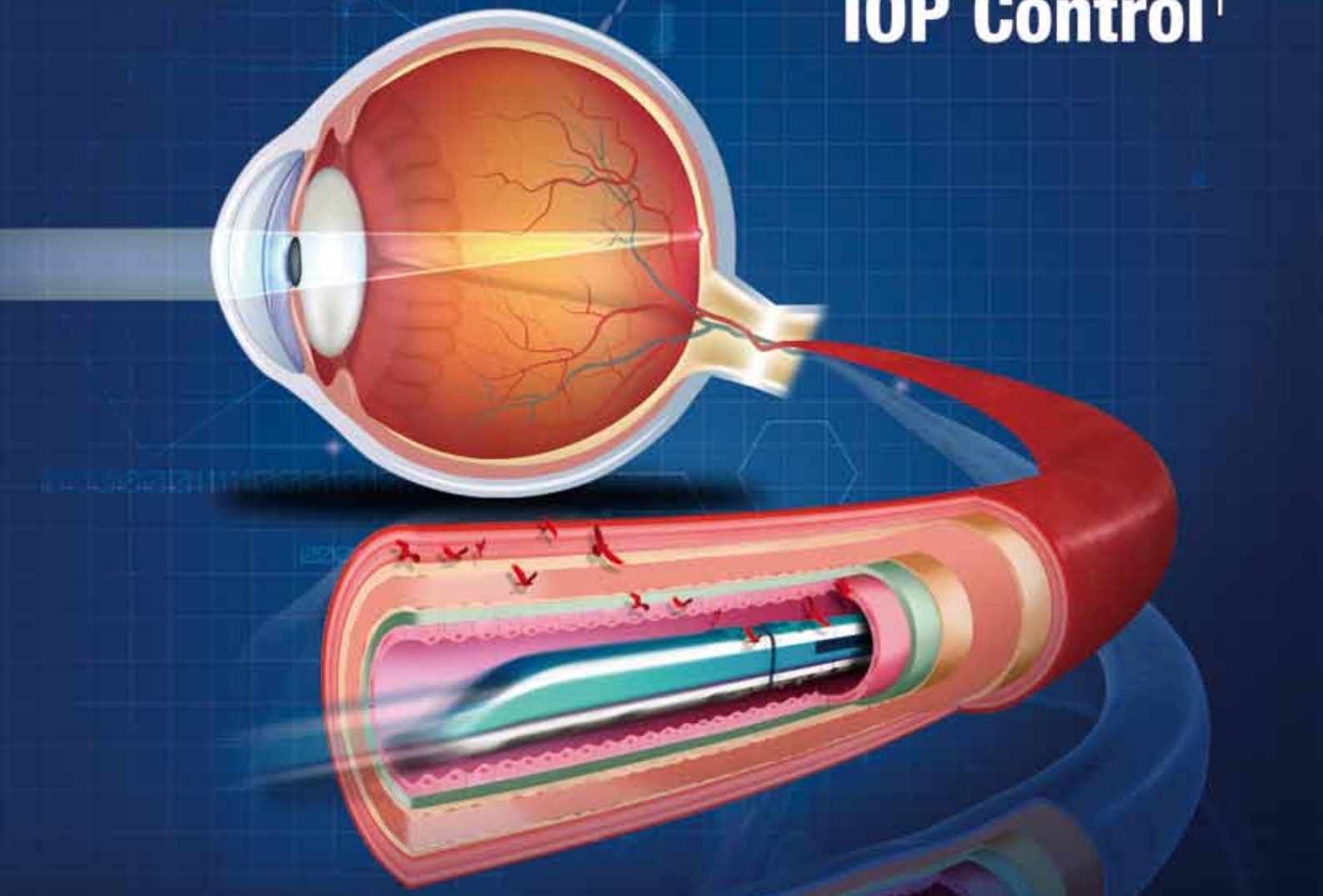

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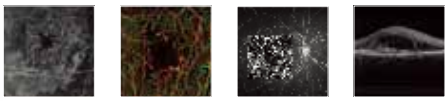

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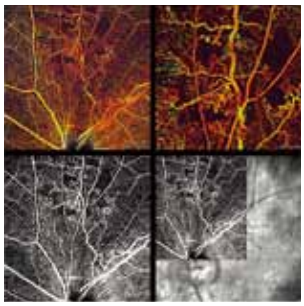

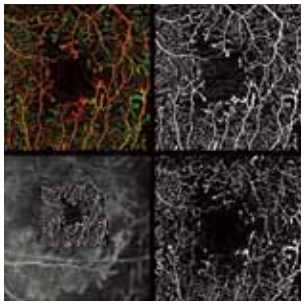

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