Research Article: The Effect of Simulated Cataract Light Scatter on Retinal Vessel Oximetry

Sunni R Patel[1], Chris Hudson[1,2,3], John G Flanagan[1,2,3], Rebekka Heitmar[4]

Affiliations

1. Vision Science Research Program, Toronto Western Research Institute, University Health Network, Toronto Western Hospital, Toronto, Canada

2. Department of Ophthalmology and Vision Science, Faculty of Medicine, University of Toronto, Toronto, Canada

3. School of Optometry and Vision Science, University of Waterloo, Waterloo, Canada

4. Aston University, School of Life and Health Sciences, Aston Triangle, Birmingham, UK

*Corresponding authors: Dr Sunni R Patel, FP 6-206, Toronto Western Hospital, Department of Ophthalmology and Vision Sciences, VSRP/UHN, 399 Bathurst St, Toronto, Canada, M5T 2S8. Tel: 001 416 603 5694 Email: sunni_patel@hotmail.com, Dr Rebekka Heitmar, Aston University, School of Life and Health Sciences, Aston Triangle, B4 7ET, Birmingham, UK, phone: 0044 (0)1212043853, email: R.Heitmar1@aston.ac.uk

Abstract

To assess the impact of light scatter, similar to that introduced by cataract on retinal vessel blood oxygen saturation measurements using poly-bead solutions of varying concentrations. Eight healthy, young, non-smoking individuals were enrolled for this study. All subjects underwent digital blood pressure measurements, assessment of non-contact intraocular pressure, pupil dilation and retinal vessel oximetry using dual wavelength photography (Oxymetry Modul, Imedos Systems, Germany). To simulate light scatter, cells comprising a plastic collar and two plano lenses were filled with solutions of differing concentrations (0.001, 0.002 and 0.004%) of polystyrene microspheres (Polysciences Inc., USA). The adopted light scatter model showed an artifactual increase in venous optical density ratio ($p=0.036$), with the 0.004% condition producing significantly higher venous optical density ratio values when compared to images without a cell in place. Spectrophotometric analysis, and thus retinal vessel oximetry of the retinal vessels, is altered by artificial light scatter.

Keywords : Light scatter; retinal oximetry; retinal oxygenation; extraneous factors; cataract models

1. Introduction

In vivo, non-invasive retinal vessel oxygen saturation measurements are relatively novel. Due to the non-invasive nature of this technology and its good reproducibility (Hammer et al., 2008, Lasta et al., 2012), it has attracted interest in the clinical community for the assessment of systemic and ocular disease with a vascular component. Diseases investigated so far include diabetic retinopathy (Hammer et al., 2009, Hardarson and Stefansson, 2012b), glaucoma (Hardarson et al., 2009, Traustason et al., 2009) and retinal vein occlusion (Hardarson and Stefansson, 2010, Hardarson and Stefansson, 2012a). Ease of use and low variability paired with high sensitivity and specificity are essential for any diagnostic technology. Although the eye offers a unique possibility to non-invasively observe the retinal microcirculation, it also requires certain prerequisites in order to obtain good quality images, including clear media (cornea, lens, vitreous and anterior chamber) and an adequate ability to fixate by the individual under observation.

With increasing age and in the presence of systemic or ocular disease, media transmission is invariably altered (Wuerger 2013, Sakanishi et al. 2012, Artigas et al. 2012, Bron et al., 2000, Polo et al., 1996). This change in ocular media transparency and scattering effect (light diffusion) produced by a cataract can affect both spectral transmission and morphology. By degrading the spatial resolution of the retinal features the detection of the vessel lumen and exterior can thus impact the measurement of optical density of the retinal vessels (Mita et al. 2012). Optical density contributes to the calculation of retinal vessel blood oxygen saturation measurements. Despite this, the impact of lens opacity on the assessment of blood oxygen saturation is unknown. Previous studies from our group have quantified the influence of artificial light scatter on various retinal imaging instruments (Azizi et al., 2007, Burke et al., 2006, Venkataraman et al., 2005). In order to mimic the change in lens morphology, we introduced an additional plano lens, filled with a polybead solution to simulate light scatter.

We hypothesised that light scatter, typical in an ageing lens, could alter retinal vessel oxygen saturation measurements as it might impact upon fundus and vessel reflection which form the basis of the retinal vessel blood oxygen saturation calculation.

2. Materials and Methods

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Aston University institutional review board. Written informed consent was provided. We included eight young healthy participants (mean age 32+/-4 years). All participants were drug free with no systemic disease, no ocular abnormalities, and no history of any ocular surgery. Participants had no lens opacity, exhibited intraocular pressures less than 21 mmHg, a logMAR (logarithm of the minimum angle of resolution) visual acuity of 0.0 or better, and a refractive error ≤ ±6.00 DS and ≤ ±2.50 DC.

All measurements were taken in the morning between 9-11am with the participants having abstained from caffeinated and carbonated beverages, alcohol, chocolate, red meat, vitamin C or participated in any forms of exercise for a minimum of 4 hours. Intra ocular pressure was measured using the Keeler IntelliPuff (Keeler Instruments, UK) prior to instillation of one drop of Tropicamide 1% (Minims, Chauvin Pharmaceuticals Ltd, UK) to dilate the pupil. After resting in a sitting position and acclimatizing to a temperature of 22ºC for 15-20 minutes blood pressure (BP) was measured using a digital BP monitor (UA-779, A7D Instruments, UK) according to best practice guidelines (Williams et al., 2004).

2.1. Artificial Light Scatter Model

The details of the artificial light scatter model have been published elsewhere (Azizi et al., 2007, Burke et al., 2006, Venkataraman et al., 2005). In brief, cells comprising a plastic collar (inner diameter 25 mm) and two 35 mm removable CR39 plano parallel lenses (with a

spacing of 4.5 mm between the lenses and a thickness of 2.04 mm each) were filled with solutions of differing concentrations of polystyrene microspheres (Polybead© Polysciences Inc., USA). The total volume of each cell was 2.2 ml. The diameter of the microspheres was chosen to be similar to the mean diameter of aggregated lens proteins (500 nm) that are thought to produce intraocular light scatter in the normal aging lens. Microsphere concentrations of 0.001%, 0.002%, and 0.004% were made up from a 0.16% stock solution. A cell filled with distilled water only was also used as an additional control. Cells filled with a solution of 0.008% were tested for imaging but proved to produce images which could no longer be analysed.

The cells were re-filled with solution for each subject as the microsphere solution is not constantly homogenous (i.e. the microspheres can deposit and settle with gravity if left over time). Furthermore, cells were checked regularly with a spectrophotometer to ensure consistency of the optical transmission and absorption characteristics throughout the course of the study.

2.2. Image acquisition:

The cells were mounted on the objective lens of the Zeiss FF450+ using a custom made adaptor that incorporated a 20˚ tilt to minimise surface reflections. After full pupil dilation was reached, we obtained a minimum of 5 images per condition (i.e. no lens, distilled water, microsphere polybead concentrations of 0.001%, 0.002% and 0.004%) with the camera angle set at 30 degrees and the optic nerve head centered. A minimum of 5 minutes resting time between conditions was given (Figure 1).

Oxygen saturation measurements were performed using the “oxygen tool” (Imedos Systems, Imedos GmbH, Jena, Germany) as described elsewhere (Hammer et al., 2008). In brief, fundus images were taken using a customized dual wavelength filter (transmission bands at 548 and 610nm; bandwidth 10 nm each). Optical densities of the vessels were measured as

the logarithmic ratio of the fundus reflection at the vessel center and its surrounding. The optical density ratio (ODR) at 610 and 548nm has been found to be inversely proportional to the vessel hemoglobin oxygen saturation when compensating for the vessel diameter and fundus pigmentation (Hammer et al., 2008).

2.3. Image analysis:

For analysis purposes we selected the three best images per condition. Using the Visualis software (Imedos Systems, Jena, Germany), we used a predefined template to measure one retinal arteriole and one retinal venule approximately half a disc diameter (DD) from the ONH and of one DD in length. This distance and length was chosen in order to obtain results which could be used for comparison to earlier publications using the same device. The vessel diameter, optical density ratio, pigmentation (numerical value output from the software) and oxygen saturation were obtained for all three images (per condition) of each participant, using the “multi measurement tool”.

2.4. Statistical analysis:

Statistical analysis was performed using Statistica version 6.0 (StatSoft, Tulsa, OK). Analysis of variance (ANOVA) was used to establish whether the three repeated measures were comparable; following this we obtained averaged values for ODR, SO_2, pigmentation and vessel diameter for further analyses. Due to the small sample size and changes in variance with changes in solution density we employed a non-parametric (Mann-Whitney-U) test and compared the “no-lens” condition to the highest solution strength as well as comparing the “distilled water” condition to each of the three solutions (0.001%, 0.002% and 0.004%). Furthermore we used the “no-lens” condition to normalize our ODR values in order to

evaluate the fractional change in ODR occurring with increasing solution strength for each arteriolar and venular ODR.

3. Results

The cohort consisted of 8 healthy, normotensive (average systolic blood pressure: 112 ± 10mmHg, average diastolic blood pressure: 73±8mmHg) non-smoking individuals (4M; mean age 32 ± 4 years) with mean intraocular pressures of 12 ± 2mmHg. All values for ODR, SO_2, and vessel diameter (D) per condition (i.e. solution strength) are shown in Table 1.

There were no statistically significant differences between ODR, oxygen saturation, pigmentation and vessel diameter values as analysed from the three consecutively taken images (per condition). Subsequently the average values for ODR, oxygen saturation, pigmentation and vessel diameter per condition were used for further analyses.

To illustrate the effect of light scatter upon the ODR measurement which is the “raw” output of the oximeter we compared only the “no-lens” condition with the highest solution strength using the Mann-Whitney-U test. Arterial ODR for “no-lens” compared to “highest solution strength (0.004%)” was not statistically significant different (p=0.592); venous ODR as compared for the same conditions was significantly different (p=0.036); see Table 1 and Figures 2-4. In addition we compared arterial and venular ODR values of the three solution strengths to the “distilled-water” condition and found no statistically significant change (arterioles: 2-3: p=0.645; 2-4: p=0.613; 2-5: p=0.623; venules: 2-3: p=0.754, 2-4: p=0.966, 2-5:p=0.109).

From the results in Table 1 it is visible that all components used to calculate oxygen saturation are affected by the introduction of light scatter, to get a better understanding of the relative change in ODR we normalized each individuals values using the no lens condition as their 100% level. The results of this are shown in Figure 5 which highlights that despite a

more significant “absolute” change in venous ODR, the arteriolar ODR is affected about a factor of 2 (compared to venous relative change in ODR) when analysing the relative change.

4. Discussion

The results of this study demonstrate that simulated light scatter alters retinal vessel oximetry parameters.

ODR is assumed to have an inverse linear relationship with oxygen saturation but here the saturation calculations also include correction factors for vessel diameter and pigmentation as they have been found to alter saturation values in a linear manner too (Beach et al. 1999, Hammer et al. 2008).

These correction factors for vessel width and fundus pigmentation were derived in a sample of twenty healthy individuals with clear media (Hammer et al. 2008). However, when looking at Table 1 it becomes clear that the measured ODR, saturation and diameter are far from linearly correlated. This highlights that the correction factors for diameter as well as pigmentation have considerable effects on the calculated saturation and brings up the question whether they are appropriate even under “ideal” imaging conditions.

The vessel diameter D which shows increased values with increasing scatter can be explained by a loss of contrast at the vessel/ surrounding tissue boundary leading to blurred edges giving an increased diameter value. A same factor: change in contrast is leading to the change in pigmentation observed; the value given for pigmentation here is calculated as $log \frac{\mathrm{I\,610\,(out)}}{\mathrm{I\,548\,(out)}}$ which is a simple ratio/ contrast value of the red and green tissue reflectance.

Why do ODR and pigmentary values change and why is the relative change in ODR larger in retinal arterioles than venules?

The change in ODR, pigment and D as stated in Table 1 is the product of a number of different factors; namely: (1) wavelength and particle size dependence of light scatter (van Bree et al. 2011, van Bree et al 2012, Ginis 2013), (2) loss of contrast, interference and optical density.

Although the scatter cells introduced have all been prepared using the same polybead stock solution by diluting it with distilled water this does not guarantee that there is only a single particle size influence which could have contributed along with the wavelength and angle dependency of scatter light in changing ODR. Theoretically the solution should act partly as a neutral density filter which in turn would explain the blurring and change in contrast of the vessels as seen in Figure 1, but as the ocular stray light is wavelength (for wavelengths longer than 600nm) and fundus pigmentation (in eyes with lighter pigmentation) dependent (Ginis 2013) this could have contributed to the changes in ODR as measured. The relative difference in magnitude of the change in ODR as seen in Figure 5 between retinal arterioles and venules can be partly explained by the aforementioned detailed factors.

Clinical importance of these findings:

Despite retinal oximetry development in the early sixties by Hickam and colleagues it has first found wider use in recent years due to the improvements in optics and computer power. More recently this technology has seen great interest in evaluating diabetic, glaucomatous and respiratory disease patients (Hammer et al. 2009, Hardarson et al. 20012, Hardarson et al. 2009, Trautason et al. 2009, Palkovits et al. 2013). In humans, the crystalline lens undergoes a number of changes with advancing age which include the aggregation of lens crystallins forming high molecular weight aggregates of up to 300–500nm diameter in size and referred to as cataract. These aggregates introduce intraocular light scatter due to differences in refractive index manifesting as opacification of the crystalline lens which

subsequently lead to degradation of retinal image quality (Moss and Wild, 1994). The resulting degradation of retinal images in a cataractous eye is primarily caused by forward light scatter.

The light scatter model used in this study incorporated polybead microspheres, mimicking a forward light scatter similar to the ultra-structural features of human lens fiber cells (Costello et al., 2007). The results derived, however, must be interpreted with caution since the model may not simulate the impact of true cataract as it only introduces light scatter and does not mimic the effects of lens yellowing.

Many patients, in particular those suffering from Diabetes Mellitus develop lenticular opacities earlier than their healthy counterparts, without taking this factor into consideration when using retinal oximetry, this might lead to false conclusions in patients with early cataract. Future studies on pre and post cataract patients will investigate the impact of cataract on retinal oximetry measurements, while taking into account the artificial lenses' properties in regards to scatter, color, transmission and lens material.

Although the sample presented was small, this study demonstrated for the first time the effect of light scatter on dual wavelength retinal vessel oximetry. It provides preliminary evidence of the effects of light scatter, irrespective and independent to the effect of ageing, which has been suggested to result in reduced venous saturations (Geirsdottir et al., 2012). These results further highlight the need for future clinical studies to incorporate lens grading and possibly transmission measurements when establishing normative data. This will enable more accurate comparisons between age related and disease related retinal vessel blood SO_2 values.

References

Artigas J.M., Felipe A., Navea A., et al. 2012. Spectral Transmission of the Human Crystalline Lens in Adult and Elderly Persons: Color and Total Transmisssion of Visible Light. Invest Ophthalmol Vis Sci. 53(7),4076-84.

Azizi B., Buehler H., Venkataraman S. T. et al. 2007. Impact of simulated light scatter on the quantitative, noninvasive assessment of retinal arteriolar hemodynamics. J Biomed Opt. 12(3),034021.

Beach JM, Schwenzer KJ, Srinivas S. et al. 1999. Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. J Appl Physiol. 86(2):748-58.

Bron A.J., Vrensen G.F.J.M., Koretz J.,et al., 2000. The Ageing Lens. Ophthalmologica 214,86-104.

Burke M. A., Khanna C. J., Miller A. et al. 2006. The impact of artificial light scatter on scanning laser tomography. Optom Vis Sci. 83(4),222-7.

Costello M. J., Johnsen S., Gilliland K. O. et al. 2007. Predicted light scattering from particles observed in human age-related nuclear cataracts using mie scattering theory. Invest Ophthalmol Vis Sci. 48(1),303-12.

Geirsdottir A., Palsson O., Hardarson S. H. et al. 2012. Retinal vessel oxygen saturation in healthy individuals. Invest Ophthalmol Vis Sci. 53(9),5433-42.

Ginis HS, Perez GM, Bueno JM, et al. 2013Wavelength dependence of the ocular straylight. Invest Ophthalmol Vis Sci. 54(5):3702-8.

Hammer M., Vilser W., Riemer T. et al. 2008. Retinal vessel oximetry-calibration, compensation for vessel diameter and fundus pigmentation, and reproducibility. J Biomed Opt. 13(5),054015.

Hammer M., Vilser W., Riemer T. et al. 2009. Diabetic patients with retinopathy show increased retinal venous oxygen saturation. Graefes Arch Clin Exp Ophthalmol. 247(8),1025-30.

Hardarson S. H., Gottfredsdottir M. S., Halldorsson G. H. et al. 2009. Glaucoma filtration surgery and retinal oxygen saturation. Invest Ophthalmol Vis Sci. 50(11),5247-50.

Hardarson S. H., Stefansson E. 2010. Oxygen saturation in central retinal vein occlusion. Am J Ophthalmol. 150(6),871-5.

Hardarson S. H., Stefansson E. 2012a. Oxygen saturation in branch retinal vein occlusion. Acta Ophthalmol. 90(5),466-70.

Hardarson S. H., Stefansson E. 2012b. Retinal oxygen saturation is altered in diabetic retinopathy. Br J Ophthalmol. 96(4),560-3.

Hickham JB, Frayser R, Ross JC. 1963. A study of retinal venous blood oxygen saturation in human subjects by photographic means. Circulation. 27:375-85.

Lasta M., Palkovits S., Boltz A. et al. 2012. Reproducibility of retinal vessel oxygen saturation measurements in healthy young subjects. Acta Ophthalmol. 90(8):e616-20.

Mita N., Hatsusaka N., Shibuya E., et al. 2012. Change in retinal image contrat with age in eyes with transparent lenses. J Cataract Refract Surg 38(10), 1783-87.

Moss I. D., Wild J. M. 1994. The influence of induced forward light scatter on the normal blue-on-yellow perimetric profile. Graefes Arch Clin Exp Ophthalmol. 232(7),409-14.

Palkovits S, Lasta M, Boltz A, et al. 2013. Measurement of retinal oxygen saturation in patients with chronic obstructive pulmonary disease. Invest Ophthalmol Vis Sci. 54(2):1008-13.

Polo V., Pinilla I., Abecia E. et al. 1996. Assessment of the ocular media absorption index. Int Ophthalmol. 20(1-3),7-9.

Sakanishi Y., Awano M., Mizota A., et al. 2012. Age-Related Changes in Spectral Transmittance of the Human Crystalline Lens in situ. Ophthalmologica 228,174-180.

Traustason S., Hardarson S. H., Gottfredsdottir M. S. et al. 2009. Dorzolamide-timolol combination and retinal vessel oxygen saturation in patients with glaucoma or ocular hypertension. Br J Ophthalmol. 93(8),1064-7.

van Bree MC, van der Meulen IJ, Franssen L, et. al. 2011. Imaging of forward light-scatter by opacified posterior capsules isolated from pseudophakic donor eyes. Invest Ophthalmol Vis Sci. 29;52(8):5587-97

van Bree MC, van der Meulen IJ, Franssen L, et al. 2012. In-vitro recording of forward light-scatter by human lens capsules and different types of posterior capsule opacification. Exp Eye Res. 96(1):138-46

Venkataraman S. T., Hudson C., Harvey E. et al. 2005. Impact of simulated light scatter on scanning laser Doppler flowmetry. Br J Ophthalmol. 89(9),1192-5.

Williams B., Poulter N. R., Brown M. J. et al. 2004. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. J Hum Hypertens. 18(3),139-85.

Wuerger S.2013. Colour Constancy Across the Life Span: Evidence for Compensatory Mechanisms. PLOS ONE 8(5),e63921.

Figure Legends

Figure 1: The lens set up and images taken at each condition for a participant.

Figure 2: The average optical density ratio (ODR) values for arterioles and venules for each lens condition. Data represented as mean and SD bars.

Figure 3: The average vessel oxygen saturation (SO_2) values for arterioles and venules for each lens condition. Data represented as mean and SD bars.

Figure 4: The average vessel diameter (D) values for arterioles and venules for each lens condition. Data represented as mean and SD bars.

Figure 5: Illustration of the fractional change in ODR with increasing solution strength (red trace: arterioles; blue trace: venules).