Retinal oximetry in health and disease

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Retinal oximetry in health and disease

Dissertation

To obtain the degree of Doctor at Maastricht University,
on the authority of the Rector Magnificus

PROF. DR. RIANNE M. LETSCHERT

in accordance with the decision of the Board of Deans,
to be defended in public on

THURSDAY 29TH APRIL 2021
at 12:00 hours

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There is nothing as glamorous as
a brilliant achievement of the
human mind

- Ayn Rand

The studies presented in this thesis were performed at Narayana Nethralaya, Bangalore, India. The analysis and manuscript preparation were done at University Eye Clinic, Maastricht University, Netherlands.

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CHAPTER 1

General Introduction

Chapter 1

The retina is the light sensitive layer at the back of the eye. It is very metabolically active and has one of the highest metabolic demands in the body. The outer segments of the photoreceptors form the most metabolically active layer of the retina. (Kur 2012) Alterations in oxygen supply can alter normal physiology or can be a surrogate marker for deranged function in the retina. The blood vessels of the retina can be easily visualized as the vitreous, lens, aqueous humor and cornea that lie anterior to it are transparent (figure 1, right).

Fig 1 – left – Schematic 3D illustration of the cross section of the eye. The retina lies at the back part of the eye. Right- color photo of the retina of the right eye that shows the blood vessels running through it (by Häggström, Mikael (2014).

"Medical gallery of Mikael Häggström 2014". WikiJournal of Medicine 1 (2). DOI:10.15347/wjm/2014.008. ISSN 2002-4436. Public Domain)

Blood carries oxygen to the various tissues of the body primarily bound to hemoglobin. The percentage of hemoglobin that is saturated with oxygen is known as the oxygen saturation. When the saturation of the hemoglobin changes, the way it interacts with light also changes. Due to this alteration in spectral behavior, saturated hemoglobin does not absorb or reflect light, but transmits it almost completely. As the saturation decreases, it starts to absorb more light thus transmitting less light.

Fig 2 – a graph depicting the absorption of light as a function of wavelength for completely oxygen bound (HbO₂) and oxygen free or de-oxygenated haemoglobin (Hb). The wavelengths where the absorption is the same are known as isosbestic points and where they are different are known as non-isosbestic points.

The optical density or absorbance of a material is a logarithmic intensity ratio of the light falling upon the material, to the light transmitted through the material. This optical density changes related to the saturation of hemoglobin. In the retina, the light reflected from the side of the vessel is assumed to be the light that falls upon the vessel and the reflected light intensity from the vessel is assumed to be the light that is transmitted through the blood. Hence the logarithm of the ratio of the light intensity on the side of the vessel to the light intensity on the vessel is calculated and this ratio is called the optical density. The ratio of the optical density at a non-isosbestic wavelength (light absorbance dependent on saturation) to the optical density at an isosbestic wavelength (light absorbance independent of saturation) is the optical density ratio. This optical density ratio is inversely and linearly related to the oxygen saturation. The constants for the equation can be obtained by calibration for each population.

All measurements in this thesis have been acquired using the Oxymap T1 retinal oximeter (Oxymap hf., Reykjavik, Iceland). The camera captures two images – at 570nm and 600nm simultaneously. Fig 3 shows the two images captured and it can be seen that in the 570nm image the arterioles and venules appear of equal intensity, in the 600nm image the arterioles are nearly transparent because oxy hemoglobin does not absorb much light, de-oxyhemoglobin still absorbs light. It is this difference in light absorption based on oxygen saturation that is the core principal of dual wavelength retinal oximetry.

Fig 3 – The Oxymap T1 retinal oximeter on the left with the different parts labelled. The 600nm and 570nm images are labelled from which the oximetry reading are obtained.

The optical density (OD) is determined for a point on the vessel (green dots in Fig 4) by obtaining the $\log_{10}$ of the ratio of intensity on the side of the vessel (red dots – I_0) to the intensity on the vessel (green dot - I). The ratio of the OD for a point in the 600nm image to the 570 nm image gives the optical density ratio (ODR). This ODR is inversely and linearly related to the saturation.

Fig 4 – a – optic disc centered image that shows two concentric circles that mark the region of interest within which segments are selected, the white line on the infero-temporal venule showing the selected segment. b (570nm image) and c (600 nm image) show the points considered for I (reflectance on the vessel – green dots) and I_0 (reflectance from the side of the vessel – red dots)

HISTORY OF RETINAL OXIMETRY

First Application

Retinal oximetry was first described by Hickam et al. in 1959 (Hickam 1959) as an investigative modality by which the oxygen saturation can be determined in the retinal vessels. They used a red wavelength of about 640 nm where reduced or de-oxygenated hemoglobin absorbed more light than oxygenated hemoglobin and an infrared wavelength at about 800 nm where the relative absorption was the same for the two types of hemoglobin. The negatives of the fundus images obtained were projected onto a screen mounted with a photocell that could measure the density of any portion of the image. They hypothesized that the percent oxygen saturation of the blood sample bears a linear relationship to the ratio of its optical density in red light to that in infrared light. In addition, they hypothesized that if the disc was a light source then the optical density of the vessel crossing it is proportional to difference in the optical density between the images of the disc and the vessel. To confirm the linear relationship, they used a model

eye containing glass capillaries filled with blood samples with saturations ranging from 24 to 100%. Photographs were taken and subsequently measurements were performed followed by linear regression. The relationship between the saturation and optical density was thus established. Similar work was also done a decade later by Laing et al. (Laing 1975), however their method depended on external calibration by means of independent arterial measurements. Apart from being laborious this method was also limited by non-linearity and variable reproducibility of the photographic film. (Beach 1999).

Three Wavelengths

In the mid-1980s Delori et al. (Delori 1988) used a three wavelength (558, 569 and 586 nm) photoelectric oximetry method that could operate continuously. Unlike from film recordings as described earlier optical densities now were calculated from its defining relationship which is the log of the ratio of incident light and transmitted light. We saw that Hickam et al. assumed that the light measured on the disc was that of the incident light or the light source; Delori et al assumed the light returning from just outside the vessel to be the incident source and the drop in brightness inside the vessel to be the absorption. It stands to reason that these estimates, especially for incident light can be subject to changes due to the level of fundus pigmentation and other optical factors and thus reduce the accuracy of the measurements. However, most of the problem was eliminated due to the use of three wavelengths – two insensitive to the saturation of blood and one sensitive. In other words, the three-wavelength method offered a way of internal calibration. Although the earlier film recordings were replaced by a continuous photoelectric sensor, the method could not easily capture images across the retina. This was because that the filter wheel was too slow to keep up with the eye motion. (Beach 2014).

First Reference Values

A decade later Schweitzer et al. (Schweitzer 1999) replaced the continuous light scan with the entrance slit (1.5mm x 40 μ m) of an imaging spectrograph which provided a continuous spectrum of light reflected from inside and outside a short section of the vessel. They then fitted their spectral curves with the known absorption of oxy and deoxy hemoglobin and that of the baseline retinal pigment they obtained a calibrated measurement. They used 76 different wavelengths and established normative values in

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human eyes; 92.2% in arterioles and 57.9 in venules. They made 265 measurements at 193 locations in 30 eyes. Their model was valid between the wavelengths of 510 to 586 nm which basically assumed a blood layer to be present in front of a diffusely reflecting background. They described three components of the reflected light as specularly reflected, internally reflected and transmitted light. They also showed that with increasing blood layer thickness the absorption increased or transmission decreased, internal reflectance remained relatively constant irrespective of layer thickness below wavelengths of 600 nm and internal reflectance increased with increasing velocity to reach a constant at velocities above 6.4mm/sec.

A major drawback of both techniques, however, is the limitation on measurements at one single cross section of one or two vessels. By contrast, a complete, two-dimensional mapping of the SO_2 in the retinal vascular tree is needed for clinical diagnostics. (Hammer 2008)

Return of dual wavelength with digital imaging

Beach et al. (Beach 1999) then introduced an image splitter from right angle prisms that could take simultaneous digital images at two different wavelengths. One wavelength was sensitive to changes in oxygen saturation or non-isosbestic (569 nm) while the other was insensitive or isosbestic (600 nm). They used a vessel tracking algorithm to calculate the optical density of blood in the retinal vessels from reflectance on the vessel and in the surrounding retinal tissue. They claimed that this was a simpler method in theory than those previously described requiring less complex instrumentation and adaptable to routine fundus cameras. It established the empirical relationship between the ratios of vessel density (optical density ratio or ODR) and systemic SO_2 .

They also showed that it was possible to compensate for the variability of fundus pigmentation among races and individuals by correcting for extravascular or choroidal pigmentation. The hypothesis is that the optical densities may be underestimated at 569 nm because pigmentation in the surrounding tissue causes strong light absorption. Extravascular reflectance (EVR) at 569 nm and 600 nm near the temporal vessels was measured and the ratio obtained. This ratio was then used to modify the value of incident light at 569 nm to compensate for the influence of pigmentation. This technique resulted in reduced variability in the calculated saturations. In another attempt to correct for the effect of choroidal pigmentation they attempted another model where only the reflectance inside the vessel was compared at 569 nm and 600

nm and replacing the reflectance outside the vessel with an estimate of reflectance from an area of unpigmented fundus; this however did not result in any advantages of the above described EVR method. Both methods did not however compensate for the choroidal pigmentation below the vessel.

Commercially available machines

Based on the methods described by Beach et al. (Beach 1999) a commercially available machine called the Oxymap was made available. Hardarson et al. used automation of the image processing to dramatically improve the repeatability of the measurements and was shown to be sensitive to changes in oxygen saturation. (Hardarson 2006) This machine though does not use any of the above mentioned compensations for fundus pigmentation. It does however use an in-built vessel width correction to compensate for artefactual changes due to the vessel diameter. Using this setup a number of studies have been published both in healthy (Geirsdottir 2012; Jani 2014; Mohan 2015) and diseases subjects (Olafsdottir 2014; Vandewalle 2014; Battu 2015; Stefansson 2017)

Another commercially available oximeter is the Retinal Vessel Analyzer (DVA; Imedos Systems UG, Jena, Germany) (Hammer 2012) which is based on the work by Hammer et al. (Hammer 2008) It uses the equations described by Beach et al (Beach 1999) with compensation for vessel diameter and fundus pigmentation.

Summary

Retinal oximetry was initially practiced in the 1960s with the use of photographic films, two wavelengths and the use of negatives to measure optical densities. Later in the 1980s three wavelengths were introduced which enabled internal calibration however these were either too slow to keep up with eye motion or scanned a very small area of the retina. In the late 1990s, 76 wavelengths were used to define normal values for oxygen saturation in the human retinal circulation and the effect of physiological changes like blood layer thickness, hematocrit and velocity were described. However, these were tedious and could not be adapted clinically. We then saw the return of dual wavelength oximetry very similar to the initial description in the 1960s but with the use of digital photography using a right-angle prism as a beam splitter and the use of automatic segmentation for vessel tracking. Soon we saw the development of two commercially available oximeters. A vast number of studies have been published using

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them however there were no studies in an Indian population. It can be hypothesized that the fundus of a person of Indian ethnicity may be more pigmented and hence may alter observed retinal saturation values. There were also diseases where retinal oximetry was neither done or if it was done, the results were inconclusive.

Fig 5 – Timeline depicting the evolution of retinal oximetry and the cardinal events in history.

RESEARCH QUESTIONS

We have seen that dual-wavelength oximetry considers the light intensity on the vessel as well as the neighboring retina. Reflectivity of the background can be affected not only by the pigmentation of the RPE, but also the thickness of the retinal nerve fiber layer (RNFL). Hence a thicker RNFL may alter background reflectivity thus altering the measured saturation. This brings us to the first two of our research questions – how does retinal oximetry differ in a more pigmented fundus of an Indian population as compared to reported values in other normative databases (Geirsdottir 2012; Jani 2014) and how does measured oxygen saturation vary with changes in RNFL thickness.

We noticed with our initial pilot analysis that retinal oximetry carries with it a large subjective component – manual segment selection. The segments selected have rough guidelines (Geirsdottir 2012), however it can still lead to subjective variations. Our third study question was thus can segment selection be automated in order to have uniform objective readings.

It has also been established that angle of gaze (covered in more detail in Chapter 12 – Pitfalls in oximetry and mathematical modelling) can have an impact on measured saturations (Geirsdottir 2012). Majority of the published studies have reported values using an optic disc centered method of acquiring images. Our fourth question was can a different image acquisition protocol impact measured values.

Retinal oximetry measures a functional parameter and hence we wondered how it was altered in eyes with significant functional changes but nil to minimal anatomical changes like amblyopic eyes. We also were curious to know how the saturations were altered in eyes with retinal dystrophies. The family of vascular diseases like diabetic and hypertensive retinopathy are characterized by chronic derangements in the vasculature while vascular occlusions are more acute characterized by an abrupt interruption of blood supply; the common endpoint however is ischemia. Where fluorescein angiography gives us great insight into the structural organization and integrity of the retinal vasculature; retinal oximetry can further our understanding by elucidating the physiological alterations occurring in the tissue. Inflammatory conditions like uveitis too can lead to an altered metabolic state and thus alter oxygen consumption. Diseases of the optic nerve head can compress on the central retinal arteriole and venule and affect the measured saturation values. Our next set of study questions were thus how oxygen saturation was altered in the above mentioned disease states.

There are a number of pitfalls in retinal oximetry (saturations exceeding 100%, variability within the eye, variability due to the angle of gaze and rattling artefacts) and anatomical factors (effect of RNFL thickness, of the central light reflex and of the background reflectance) which need to be understood thoroughly to correctly interpret oximetry readings. We wondered how these factors can affect measured saturations and how we can overcome them.

Chapter 1

AIMS OF THE THESIS

Our aims were to answer the above described research questions. In summary we aimed to answer

1. What is the normal range of retinal saturation in pigmented Asian Indian eyes and is it different from other published studies?
2. Can segment selection be completely automated in order to avoid subjective bias?
3. Does RNFL thickness affect measured saturation values?
4. Would a change in imaging protocol from optic disc centered imaging change measured values?
5. How is oximetry altered in
 - a. Amblyopia
 - b. Retinal dystrophies
 - c. Arterial occlusions
 - d. Venous occlusions
 - e. Retinal vasculitis
 - f. Diabetic retinopathy
 - g. Optic disc edema
6. To describe the pitfalls in retinal oximetry and ways to overcome them

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CHAPTER 2

Normative database

PLoS One 2015

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Chapter 2

ABSTRACT

Objective

To study the oxygen saturation profile in normal Asian Indian eyes

Design

A cross sectional prospective study

Subjects

Ninety-eight consecutive patients presenting to our hospital with best corrected distance visual acuity (BCVA) of 20/20 and a normal ophthalmic examination were included in the study. Patients having any ocular or systemic disease were excluded from the study.

Materials and Methods

Oximetry was performed on all subjects with the Oxymap T1 retinal oximeter (Oxymap hf, Reykjavik, Iceland).

Main Outcome Measures

The images were analysed for oxygen saturation and diameter.

Results

The mean age was 33 years (Range: 18-63; SD: 12.4). The average arteriolar saturation was $90.3 \pm 6.6\%$ and the venous saturation was $56.9\% \pm 6.3$. The average A-V (arterio-venous) difference was $33.2\% \pm 5.2$. There was an increase in arteriolar ($R^2 = 0.264$; $p=0.001$) and venous saturation ($R^2 = 0.151$; $p=0.001$) with age. There was no significant change in the arterio-venous saturation difference (AVSD). The inferotemporal quadrant had the lowest saturations. Age correlated positively with ocular perfusion pressure (OPP) ($R^2 = 0.07$; $p=0.007$). OPP correlated positively with global arteriolar saturation ($R^2=0.057$, $p=0.018$).

Conclusion

This study provides the normative database for an Indian population and is comparable to previous studies. Age, vessel diameter and OPP were the significant factors that influenced the saturation. Arteriolar and venous saturations increased with age while the AVSD did not change significantly.

Chapter 2

INTRODUCTION

Oxygen is important for the normal physiology and metabolism in the body. The retina has the highest metabolic demands of any tissue in the body with the photoreceptors having the highest demand in the retina. (Kur 2012) Inadequate delivery of oxygen or altered utilization of oxygen can either trigger disease or be a marker for underlying disease activity respectively. (Hammer 2012; Hardarson & Stefansson 2012; Geirsdottir 2014; Vandewalle 2014)

Hemoglobin bound to oxygen has differential absorption of light at different wavelengths. This is the basic principle used in dual wavelength oximetry. Fundus photographs at 2 different wavelengths are taken and the relative oxygen concentration can be determined using photo spectrometry. (Hammer 2002; Hardarson 2006) Jani et al (Jani 2014) in their work state that this machine is based on a similar principle as the finger pulse oximeter .

Previous studies (Geirsdottir 2012; Jani 2014) have established a normative database using the Oxymap T1 Retinal Oxymeter (Oxymap, Reykjavik, Iceland). We have used the same equipment in our study. Jani et al (Jani 2014) showed in their multiethnic study that race and pigmentation when uniform do not play a role. We wanted to evaluate this in an Indian population.

It has been shown that narrower retinal vessels show higher oxygen saturations (Geirsdottir 2012). In contrast, in another study (Jani 2014) the diameter had no impact on the measured saturation as the software compensated for the optical artifacts caused by the diameter. In view of the conflicting results, we intend to establish our own results.

We aim to study averages and variability of oxygen saturation in healthy individuals in an Indian population. We also aim to study the interocular variability, quadrant values, relationship with diameter and age.

METHODS

Healthy subjects presenting to our outpatient department at Narayana Nethralaya, Bangalore were enrolled in the study with their consent. The study follows the tenets of Helsinki. The Institutional Review Board and Ethics Committee approved the study.

Inclusion - Enrolled subjects had a normal ophthalmic examination, BCVA 20/20 – 20/30, with spherical refractive error less than ± 6.00 diopters and cylindrical error of less than ± 2.00 diopters.

Exclusion – Patients with BCVA vision less than 20/30, cataract greater than Lens Opacification Classification System – Nuclear sclerosis Grade 2/Posterior Subcapsular Cataracts (NS2/PSC) or other significant media opacities, history of ocular conditions such as diabetic or hypertensive retinopathy, macular degeneration, or retinal vascular occlusions or chronic systemic conditions like diabetes or hypertension that can confound retinal oximetry measurements were excluded. Patients regularly taking any systemic drugs or smokers were also excluded.

Image Acquisition

All patients underwent dilation with 1% tropicamide and 10% phenylephrine and subsequent retinal imaging with the oximetry using the Oxymap T1 Retinal Oximeter (Oxymap, Reykjavik, Iceland). The procedure was explained to them in detail and a written informed consent was obtained. They were allowed to rest for 5 minutes before the images were captured. This was done to eliminate exercise induced fluctuations in readings. Resting blood pressure and pulse oximetry (H5, Silicon Labs, India) readings were taken for all patients. None of the subjects had consumed caffeine within 2 hours of the examination.

The aiming light was set at the lowest setting, flash intensity was 50W, small aperture and large pupil settings were applied to the TRC 50DX (Topcon, Japan) Fundus camera.

One experienced photographer obtained standardized images for all the subjects. We obtained 2 images per eye in all the subjects – 50° - disk centered (Fig. 1) and macula centered. Defocus can cause decrease in contrast and hence alterations in the measured values. We ensured that all the images analysed were in sharp focus.

Fig 1 – A normal Oxymap image with the pseudo - colour saturation overlap map (Background image - 570 nm)

Segment Selection and Analysis

The software used was version 2.4.2. The oxygen saturation was given by the equation: $\text{saturation} = (a \times \text{ODR} + b)100$; $a = -1.28$ and $b = 1.24$. Segments of vessels (Fig. 2) were then chosen according to the protocol described by Geirsdottir et al[9]. The vessels were at least 8 pixels wide ($72 \mu\text{m}$), at least 100 pixels long, were minimum 50 pixels from the optic disc margin and at least 100 pixels from the edge of the image. In cases of vessel branchings which were less than 150 pixels from the optic disc, segments after the branching were taken. All images had uniform background pigmentation. Arteriolar and venous saturations were determined separately for superior, inferior, nasal and temporal quadrants. For analyzing the quadrant values, we selected the thickest arteriole and venule in each quadrant without any weighted averaging. Global values were also determined by averaging the quadrant values.

Fig 2 – Method of analyzing images – a. 570nm image; b. Masking of vessel branchings and A-V crossings along with marking of 2 concentric circles within which vessel segments would be analysed; c. Pseudocolor map along with previous markings; d. Vessel segment markings (thickest arteriole and venule per quadrant)

Statistical Analysis

Statistical analysis was done using the IBM SPSS v22 software. All continuous variables were tested for normality using the Shapiro-Wilk test. The difference between means was tested using independent t-test for normally distributed data and the Mann-Whitney U test for non-parametric data. For normally distributed data the Pearson's correlation was used, for non parametric data the Spearman's correlation was used. The level of significance was $p < 0.05$. Linear regression was performed after identifying significant correlations.

RESULTS

There were a total of 98 eyes (98 subjects). The mean age was 33 years (18-63) (SD=12.4) (Fig. 3). There were 44 males and 54 females. No statistically significant bilateral differences were noted and hence only the right eyes were used for the statistical data, hence avoiding duplication. The arteriolar and venous saturations were normally distributed (Shapiro-Wilk Test). The average arteriolar saturation was 90.3 (95% C.I 88.9-91.6) (Standard deviation (SD): 6.57, skewness: 0.086, kurtosis: -0.257) and the venous saturation was 56.9 (95% C.I 55.7-58.2) (SD: 6.31, skewness: -0.004, kurtosis: -0.678). The average A-V (arterio-venous) difference was 33.2 (95% C.I -32.2 - 34.3) (SD: 5.18, skewness: 0.369, kurtosis: 0.864). The other parameters like diameter and quadrant wise saturation are summarized in Table 1

Effect of age - There was an increase in arteriolar ($R^2 = 0.263$, $p=0.001$) and venous saturation ($R^2 = 0.151$, $p=0.001$) with age. The A-V difference did not show a significant change with age. The trends have been summarized in Fig. 3. The average ocular perfusion pressure (OPP) was 43.6 (95% C.I 42.1-45.1) (SD: 7.3, skewness: 0.019, kurtosis: -0.526). This showed a positive correlation with age ($R^2 = 0.073$; $p=0.007$) and with the global arteriolar saturations ($R^2=0.057$, $p=0.018$)

Temporal vs Nasal analysis - The average arteriolar saturation (%) was 87.1 (95% C.I 85.9-88.3) temporally and 93.5 (95% C.I 92.2-94.8) nasally ($p=0.001$); whereas the venous saturation was 53.7 (95% C.I 52.7-54.7) temporally and 60.1 (95% C.I 59.0-61.3) nasally ($p=0.001$). The average arterial and venous diameters (μm) were 135 and 177 temporally; whereas they were 111 and 142 nasally ($p=0.001$, $p=0.001$). Both the temporal ($R^2=0.04$, $p=0.007$) and nasal ($R^2=0.03$, $p=0.026$) saturations correlated positively with the OPP. The inferotemporal quadrant had the lowest saturations with 86% and 51%, highest diameters with 136 μm and 182 μm , arteriolar and venous respectively.

Fig 3 – Relationship of Arteriolar, Venous and A-V Difference Saturations with age.(x-axis - Age [Years]; y-axis - Saturation [%])

Eye Laterality - There were no statistically significant bilateral differences noted in either the quadrants or the global arteriolar, venous or A-V difference saturations.

Multivariate regression - A multiple linear regression was performed for the dependent variables namely the arteriolar, venous and A-V difference saturations by taking the independent variables as age, sex, ocular perfusion pressure and spherical equivalent. The results have been summarized in Table 2. The naïve analysis of all the variables in the table yielded a significant correlation; age was the only significant variable in the multivariate model for global arteriolar and venous values.

Effect of diameter - We found a negative correlation between diameter and saturation for individual vascular segments (arterioles: $R^2=0.06$, $p = 0.001$; venules: $R^2=0.05$, $p = 0.001$). Globally average diameter and saturation showed a weak negative correlation which was not statistically significant.

Global			Quadrant - Arteriolar					Quadrant - Venous				
	Arteriolar	Venous	AV Difference	ST	SN	IN	IT	ST	SN	IN	IT	
Saturation (%)	90.3 ± 6.6	56.9 ± 6.3	33.2 ± 5.2	88.0 ± 7.9	93.9 ± 8.8	93.0 ± 9.2	86.2 ± 8.6	56.0 ± 6.7	60.0 ± 7.6	60.2 ± 8.8	51.4 ± 6.8	
Diameter (μm)	123.4 ± 11.2	159.7 ± 12.7		134.3 ± 16.8	111.3 ± 16.8	111.1 ± 15.6	136.4 ± 17.7	171.4 ± 21.7	142.9 ± 19.8	141.7 ± 21.2	182.2 ± 20.6	

AV difference – arterio-venous difference; ST – supero-temporal; SN – supero-nasal; IN – infero-nasal; IT – infero-temporal.

Table 1 - Summary of the Global and Quadrant saturations and diameters.

Arteriolar			Venous			A-V Difference		
Standardised Co-efficient	P Value	Standardised Co-efficient	P Value	Standardised Co-efficient	P Value	Standardised Co-efficient	P Value	P Value
Intercept	76.64	<0.001	50.90	<0.001	<0.001	33.66	<0.001	<0.001
Age	0.41	<0.001	0.263	0.028	0.028	-0.077	0.542	0.542
Spherical Eq	0.066	0.507	0.064	0.548	0.548	-0.007	0.950	0.950
Systolic	0.039	0.810	0.088	0.614	0.614	-0.077	0.678	0.678
Diastolic	0.048	0.821	-0.232	0.314	0.314	0.017	0.944	0.944
OPP	0.053	0.832	0.220	0.412	0.412	0.108	0.706	0.706

only those parameters significant on univariate analysis were included in the multivariate analysis

Table 2 - Multivariate analysis for arteriolar, venous and A-V difference saturations

DISCUSSION

Our results on normative Asian Indian eyes using the Oxymap T1 retinal oximeter yields an average saturation of 90.3% in the arterioles and 56.9% in the venules which is similar to previous studies (Geirsdottir 2012; Jani 2014) which give an average arteriolar value of 90.4-92.2% and a venous value of 55.3-55.6%.

Pulse Oximetry and the eye

The difference between the average pulse oximetry readings and the average arterial SO_2 is 7%, but no statistical correlation was found between the two parameters. The standard deviation of the pulse oximetry readings is only 2 as compared to 7 with oximetry.

Perfusion pressure

Geirsdottir et al have in their study showed the ocular perfusion pressure increases with age. (Geirsdottir 2012) We found a similar trend in our study as well. This would cause the blood to course faster through the retinal vasculature.

Effect of aging

Tissue loss with aging (Bonnell 2003) should result in lower oxygen extraction and hence higher saturations. There is also a decrease in diameter with age. This too can result in optical artifacts, artificially raising the saturations. Aging can also result in subtle changes in the lens resulting in decreased contrast in the acquired images. This can cause a lower measured saturation.(Hardarson 2013) We found an increase in arteriolar, and venous saturations with age, leaving the A-V difference unaffected. We feel that decreased diameter, decreased retinal nerve fiber layer thickness and increased perfusion pressure would lead to this trend. We attribute the increased saturation in arterioles and venules found in our study to multiple factors. An opposite trend was noted in the previously mentioned studies. Retinal tissue is lost with aging, hence there are 2 important events that occur. The first being that the oxygen requirement and extraction decreases, hence increasing saturation. In the setting of decreased tissue A-V difference should decrease. But here we are looking at oxygen extraction which is a product of A-V difference x blood flow. Blood flow is heavily

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dependent on diameter which decreases with age, as a result of age-related arteriolosclerosis. This means the total amount of blood flowing through the human retina decreases as age advances. Hence a decrease in extraction with an associated decrease in blood flow may explain the unaffected A-V difference with age. The second important event occurring with advancing age is a theoretical decrease in the reflectivity due to loss of RNFL. This might not manifest significantly in Caucasians, but in more heavily pigmented fundi like in Indians, more melanin is exposed, hence absorbing more light. This could alter the final measured Optical Density Ratio (ODR), hence altering saturation measurements. These are hypotheses that need to be tested. There is also a possibility that the Retinal Nerve Fiber Layer (RNFL) responds differently at 570nm and at 600nm. Jani et al state that fundus pigmentation did not affect measurements, but maybe all those subjects had a good RNFL thickness not allowing the complete role of melanin to manifest.

The vessel walls change with age owing to sclerosis, which may alter the amount of light that interacts with the blood column and thus prevents us from measuring the true saturation. Our patients were methodically screened, and we ensured that none of them had systemic hypertension or diabetes. It would be interesting to see how saturations vary with varying grades of hypertensive retinopathy. On one hand increased perfusion pressure and blood flow velocity may alter saturation and altered vessel wall characteristics may change saturation on the other.

Conclusion

This study provides the normative database for an Indian population and is comparable to previous studies. Age was the most significant factor that influenced the saturation. We also see that diameter and saturation of individual vessel segments are inversely correlated. Arteriolar and venous saturations increased with age whereas A-V difference saturations remained unchanged in this study. It is essential to understand the normal variations in the saturation to be able to pick up subtle changes in pathology.

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CHAPTER 3

Automatic Analysis

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ABSTRACT

Retinal oximetry is an important screening tool for early detection of retinal pathologies due to changes in the vasculature and also serves as a useful indicator of human-body-wide vascular abnormalities. We present an automatic technique for the measurement of oxygen saturation in retinal arterioles and venules using dual-wavelength retinal oximetry images. The technique is based on segmenting an optic-disc-centered ring-shaped region of interest and subsequent analysis of the oxygen saturation levels. We show that the two dominant peaks in the histogram of the oxygen saturation levels correspond to arteriolar and venular oxygen saturations from which the arterio-venous saturation difference (AVSD) can be calculated. For evaluation, we use a normative database of Asian Indian eyes containing 44 dual-wavelength retinal oximetry images. Validations against expert manual annotations of arterioles and venules show that the proposed technique results in an average arteriolar oxygen saturation ($SatO_2$) of 87.48%, venular $SatO_2$ of 57.41%, and AVSD of 30.07% in comparison with the expert ground-truth average arteriolar $SatO_2$ of 89.41%, venular $SatO_2$ of 56.32%, and AVSD of 33.09%, respectively. The results exhibit high consistency across the dataset indicating that the automated technique is an accurate alternative to the manual procedure.

INTRODUCTION

The retina is a light-sensitive tissue layer at the posterior inside of the eye. Millions of nerve axons running all over the retina convert the incident light into neural signals, which are carried to the brain by the optic nerve for visual perception. Oxygen is important for normal functionality and metabolism in the retina. The retinal arteries carrying oxygenated hemoglobin (HbO_2) enter the retina, and veins carrying deoxygenated hemoglobin (Hb) leave the retina through the optic nerve head. In a retinal fundus image, the optic nerve head appears as a disc, referred to as the optic disc (OD). The retina has the highest metabolic demand than any other tissue in the body (Kur 2012). Inadequate delivery and abnormal utilization of oxygen alter the normal functioning of the retina and trigger diseases such as diabetic retinopathy, glaucoma, age-related macular degeneration, retinal vein occlusions, and retinal detachment, leading to vision impairment (Hammer 2012; Hardarson & Stefansson Oxygen saturation in branch retinal vein occlusion 2012; Geirsdottir 2014; Vandewalle 2014; Mohan Retinal Oximetry: The Indian Experience in Healthy and Diseased Eyes 2015; Mohan Normative database of retinal oximetry in Asian Indian eyes 2015). Early detection of the pathological condition followed by suitable treatment may improve the retinal blood flow and oxygenation and restore vision (Hammer 2012; Hardarson & Stefansson Oxygen saturation in branch retinal vein occlusion 2012; Geirsdottir 2014; Vandewalle 2014; Mohan Retinal Oximetry: The Indian Experience in Healthy and Diseased Eyes 2015).

The retinal fundus image provides a direct view of the vasculature and can be used to compute oxygen saturation levels. The quest for retinal metabolic analysis through measurement of oxygen saturation resulted in a non-invasive dual-wavelength spectrophotometric retinal oximeter (Oxymap Retinal Oximeter ; Geirsdottir 2012). The Oxymap T1 (Oxymap, Reykjavik, Iceland) is a non-invasive dual-wavelength oximeter, which consists of an optical adapter, two high-resolution digital cameras, an image splitter, and two narrow band-pass filters. An Oxymap T1 mounted on top of a Topcon TRC-50DX (Topcon Corporation, Tokyo, Japan) fundus camera is shown in Fig. 1.

In a dual-wavelength oximeter, retinal fundus images are simultaneously captured with two wavelengths of light, one at 600 nm, which is sensitive to oxygen saturation, and the other at 570 nm, which is not oxygen sensitive, but is used to calibrate the light intensity. The images are processed by the Oxymap Analyzer software to detect the blood vessels and estimate the light absorbance (optical density) at each point along the vessels for

each wavelength and generate a pseudocolor map of the oxygen saturation (Oxymap Retinal Oximeter). The principle of retinal oximetry image acquisition and a representative image with the pseudocolor map is shown in Fig. 1.

Fig 1 – The Oxymap T1 mounted on top of a Topcon TRC-50DX retinal fundus camera and an illustration of the principle of retinal oximetry (Oxymap Retinal Oximeter).

Prior Art

Hickam et al. (Hickam 1959; Hickam & Frayser 1966) were the first to propose non-invasive retinal oximetry using special filters. The objective of their study was to investigate retinal blood circulation and metabolism in humans. They developed techniques for the measurement of diameter changes in retinal vessels for various stimuli and also oxygen saturation of retinal venous blood using fundus images. Several authors have contributed to the analysis of retinal oxygen saturation under healthy and diseased conditions of the retina and also development of the multi-wavelength oximetry system. Delori et al. (Delori 1988) proposed a noninvasive spectrophotometric technique and determined the oxygen saturation after compensating for the effect of light scattering by the red blood cells. Sebag et al. (Sebag 1989) studied the effect of optic atrophy on retinal blood flow and oxygen saturation in humans. Denninghoff et al. (Denninghoff 1997) reported the oxygen saturation in retinal vessels during hypoxia. Tiedeman et al. (Tiedeman 1998) studied the retinal oxygen consumption in patients with diabetes. Beach et al. (Beach 1999) proposed a method for noninvasive measurement of oxygen saturation using digital imaging techniques. They recorded images at 600 nm and 569 nm (oxygen-sensitive and oxygen-insensitive wavelengths, respectively) by using a modified fundus camera with an image splitter coupled to an 18-bit digital camera. Schweitzer et al. (Schweitzer 1999) considered wavelengths between 510 nm and 586 nm. They used the experimentally determined transmission spectra and the spectra of the internal reflection of saturated blood at a large number of wavelengths to calculate the oxygen saturation. Crittin et al. (Crittin 2002) developed a reflectance oximeter and showed that the optical density ratio could be used for relative oxygen saturation measurements. Harris et al. (Harris 2003) reviewed the literature on the advancements in retinal oximetry methods until the year 2003. Hardarson et al. showed the reliability of retinal oximeter (Hardarson 2006) and also analyzed the oxygen saturation under retinal vein occlusion (Hardarson & Stefánsson 2010) and diabetic retinopathy conditions (Hardarson & Stefánsson Retinal oxygen saturation is altered in diabetic retinopathy 2012). Michelson et al. (Michelson & Scibor 2006) and Olafsdottir et al. (Olafsdottir 2011) examined the oxygen saturation in retinal arterioles and venules simultaneously by imaging spectrometry and measured the oxygen saturation in patients with glaucoma. Schweitzer et al. (Schweitzer 2007) showed that healthy subjects and diabetics in the early stages of diabetic retinopathy exhibit comparable changes of oxygen saturation during breathing of 100% oxygen. Kagemann et al. (Kagemann 2007) proposed Fourier-domain optical coherence tomography to assess spectral oximetry. Three-dimensional disc-centered retinal tissue volumes were

assessed in healthy subjects. In this study, a two-wavelength optical density ratio approach was employed. Johnson et al. (Johnson 2007) presented a snapshot imaging spectrometer that acquires a complete spatial-spectral image cube in approximately 3 ms from 450 nm to 700 nm with 50 bands. The setup coupled to a fundus camera gave a true color retinal image. Hammer et al. (Hammer 2008) used a fundus camera equipped with a special dual-wavelength transmission filter and a color charge-coupled device camera. Two monochromatic fundus images recorded at 548 nm and 610 nm were used for the analysis. Ramella-Roman et al. (Ramella-Roman 2008) introduced a multi-aperture camera system based on a lenslet array architecture and captured images in six spectral bands. They reported *in vivo* testing on healthy volunteers.

Hammer et al. (Hammer 2009) showed increased retinal venous oxygen saturation in diabetic retinopathy patients. Denninghoff et al. (Denninghoff 2011) reported the first noninvasive *in vivo* application of blue-green oximetry to retinal vessels using a modified confocal scanning laser ophthalmoscope. Li et al. (Li 2011) used an adaptive optics-based confocal scanning laser ophthalmoscope to measure oxygen saturation in small retinal vessels. Images with a diameter smaller than 50 microns were recorded at 680 nm (oxygen-sensitive) and 796 nm (oxygen-insensitive) wavelengths. They showed that the oxygen saturation in the artery is higher than that in the vein and also that the oxygen saturation in small vessels can be affected by the metabolic activity in the retina. Mordant et al. (Mordant Spectral imaging of the retina 2011; Mordant Validation of human whole blood oximetry, using a hyperspectral fundus camera with a model eye 2011) used images obtained at wavelengths 500 nm and 650 nm (oxygen-sensitive and oxygen-insensitive, respectively) acquired from a hyper-spectral fundus camera and analyzed them with an oximetry model to measure oxygen saturation.

There have been a few manual attempts to measure retinal oximetry values from fundus images (Schweitzer 1999; Hardarson 2006). According to the study by Schweitzer et al. (Schweitzer 1999), the mean oxygen saturation levels for retinal arterioles and venules in healthy individuals are 92.2% and 57.9%, respectively. Oximetry analysis revealed that the oxygenation levels change in many retinal pathologies such as glaucoma (Michelson & Scibor 2006; Olafsdottir 2011), diabetic retinopathy (Schweitzer 2007; Hammer 2009; Hardarson & Stefansson Retinal oxygen saturation is altered in diabetic retinopathy 2012), retinal vein occlusions (Hardarson & Stefansson 2010; Hardarson & Stefansson Oxygen saturation in branch retinal vein occlusion 2012), and systemic hypoxemia (Traustason 2011). Jani et al. (Jani 2014), Geirsdottir et al. (Geirsdottir 2012), and Mohan et al. (Mohan Normative database of retinal oximetry in

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Asian Indian eyes 2015) have established normative databases using the Oxymap T1 retinal oximeter. Their study on the variability of oxygen saturation in healthy individuals revealed that the age, vessel diameter, and ocular perfusion pressure are significant factors that influence the saturation. The findings were based on manual marking of the arterioles and venules in the OD-centered ring-shaped region of interest on the dual-wavelength retinal oximetry images (Geirsdottir 2012; Mohan Normative database of retinal oximetry in Asian Indian eyes 2015).

The current practice to calculate oxygen saturation from the fundus image requires considerable manual effort. To start with, one must manually outline the OD and segment a ring-shaped region of interest that is concentric with the OD. Subsequently, the arterioles and venules have to be identified, and the oxygen saturation level and arterio-venous saturation difference (AVSD) has to be estimated. This procedure is not only tedious but also introduces subjectivity. Hence, there is a need for an automated and consistent analysis methodology. To the best of our knowledge, a computer-aided technique for solving the problem has not been reported in the literature although there is a pressing clinical need. The objective of this paper is to fill this gap.

METHODS

We have created a normative retinal oximetry image database of Asian Indian population using the Oxymap-Topcon duo (Mohan Normative database of retinal oximetry in Asian Indian eyes 2015). We propose a fully automated technique for performing retinal arteriolar and venular oxygen saturation measurements as an efficient and robust alternative to manual assessment (Geirsdottir 2012; Mohan Normative database of retinal oximetry in Asian Indian eyes 2015). The reliability and accuracy of the proposed technique are also measured considering manual assessment as the baseline.

Automated segmentation of the region of interest

We segment the region of interest based on the *active-disc method* (Kumar 2015) introduced recently for the segmentation of optic disc and cup and subsequent measurement of glaucoma specific parameters such as the cup-to-disc ratio and the rim-to-disc ratio (Kumar 2019). The active-disc method is motivated by the techniques developed by Pediredla et al. (Pediredla & Seelamantula 2012) and Thévenaz et al. (Thevenaz & Unser 2008). The method is iterative and performs shape-constrained segmentation. In our analysis, the shape is constrained to be a circle. The initialization is automatic and is based on normalized cross-correlation. The parameters are the coordinates of the center of the disc and the radius, which are optimized to minimize a locally computed energy function. The optimization is carried out efficiently using gradient-descent technique (Chong & Zak 2004) and Green's theorem (Simmons & Simmons 1996).

The *active-disc* comprises two concentric circles centered at the origin and parameterized as follows:

$$\begin{pmatrix} x_i(t) \\ y_i(t) \end{pmatrix} = \begin{pmatrix} r_i \cos t \\ r_i \sin t \end{pmatrix}, \quad (1)$$

for $i=1,2$, and $\forall t \in (0, 2\pi]$, where r_1 and r_2 denote the radii of the outer and inner circles, respectively, which are set to 1 and $1/\sqrt{2}$, respectively. An example of such a template is shown in Fig. 2(a). The concentric circles with isotropic scaling and translation are given by

$$\begin{pmatrix} X_i \\ Y_i \end{pmatrix} = R \begin{pmatrix} x_i \\ y_i \end{pmatrix} + \begin{pmatrix} x_c \\ y_c \end{pmatrix}, \quad (2)$$

where $i=1,2$, and (X_1, Y_1) , and (X_2, Y_2) denote the outer and inner boundaries, respectively; R represents the scale parameter, and (x_c, y_c) are the translational parameters, amounting to a total of three degrees of freedom. For brevity of notation, we drop the variable t and replace $(x_i(t), y_i(t))$ and $(X_i(t), Y_i(t))$ with (x_i, y_i) and (X_i, Y_i) , respectively. The active disc is illustrated pictorially in Fig. 2(b). The active-disc energy is a normalized contrast function, which considers the area inside the inner disc as the foreground and the annular region as the background. For an image f , let R_1 and R_2 denote the regions enclosed by the outer and inner discs, respectively. The active-disc energy is chosen to be

$$\begin{aligned}
 E &= \frac{1}{R^2} \left(\iint_{R_1 \setminus R_2} f(X, Y) dXdY - \iint_{R_2} f(X, Y) dXdY \right), \\
 &= \frac{1}{R^2} \left(\underbrace{\iint_{R_1} f(X, Y) dXdY}_{E_1} - 2 \underbrace{\iint_{R_2} f(X, Y) dXdY}_{E_2} \right), \\
 &= \frac{1}{R^2} (E_1 - 2E_2),
 \end{aligned} \tag{3}$$

where E_1 and E_2 are the image energies in the regions R_1 and R_2 , respectively. Minimizing the active-disc energy gives a tight fit contour of the optic disc. Figs. 2(c) and 2(d) show an example of the OD in a dual-wavelength oximetry image and the optimal active disc fit to the OD boundary, respectively.

Fig 2 – (a) A circular template; (b) Circular active disc; (c) Optic disc image in dual-wavelength oximetry; and (d) Optimal active disc fit to the optic disc.

We perform optimization using gradient-descent technique (Chong & Zak 2004), which is a first-order approach. One starts with an initial guess P_0 , where P is a generic variable that is used to denote the parameters R , x_c , and y_c , and updates P as follows:

$$P_{n+1} = P_n - \gamma_n \nabla E[P_n],$$

where $E[P_0] \geq E[P_1] \geq E[P_2] \dots$, and ∇ denotes the gradient. The parameter $\gamma_n > 0$ is the step-size parameter.

The gradient-descent technique requires partial derivatives of the energy function. Since the integrals are two-dimensional and the contours are closed, one could compute the partial derivatives efficiently using Green's theorem (Simmons & Simmons 1996). In our optimization, we need partial derivatives of the energy function with respect to the parameters R, x_c , and y_c . We show the calculations for one of the parameters. Applying Green's theorem to E_2 gives

$$E_2 = \oint_{R_2} f^X dY = - \oint_{R_2} f^Y dX, \quad (4)$$

where $f^X(X, Y) = \int_{-\infty}^X f(\zeta, Y) d\zeta$ and $f^Y(X, Y) = \int_{-\infty}^Y f(X, \zeta) d\zeta$. E_2 is a function of (X, Y) , which are functions of the parameters of the disc. The partial derivative of E_2 with respect to R is given by

$$\frac{\partial E_2}{\partial R} = \frac{\partial E_2}{\partial X} \frac{\partial X}{\partial R} + \frac{\partial E_2}{\partial Y} \frac{\partial Y}{\partial R}. \quad (5)$$

Substituting (4) in (5) and simplifying gives

$$\begin{aligned} \frac{\partial E_2}{\partial R} &= \oint_{R_2} \frac{\partial f^X}{\partial X} \frac{\partial X}{\partial R} dY - \oint_{R_2} \frac{\partial f^Y}{\partial Y} \frac{\partial Y}{\partial R} dX, \\ &= \frac{R}{2} \int_{t=0}^{2\pi} f(X_2, Y_2) dt. \end{aligned} \quad (6)$$

The partial derivative of the energy E_1 with respect to R can be found similarly:

$$\begin{aligned} \frac{\partial E_1}{\partial R} &= \oint_{R_1} \frac{\partial f^X}{\partial X} \frac{\partial X}{\partial R} dY - \oint_{R_1} \frac{\partial f^Y}{\partial Y} \frac{\partial Y}{\partial R} dX, \\ &= R \int_{t=0}^{2\pi} f(X_1, Y_1) dt. \end{aligned} \quad (7)$$

The partial derivative of the energy E with respect to R can be obtained as follows:

$$\frac{\partial E}{\partial R} = \frac{1}{R^2} \left(\frac{\partial}{\partial R} (E_1 - 2E_2) \right) - \frac{2}{R^3} (E_1 - 2E_2). \quad (8)$$

Substituting (6) and (7) in (8) and simplifying, we get

$$\frac{\partial E}{\partial R} = \frac{1}{R} \left(\int_{t=0}^{2\pi} f(X_1, Y_1) dt - \int_{t=0}^{2\pi} f(X_2, Y_2) dt - 2E \right). \quad (9)$$

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The partial derivatives of the energy E with respect to the coordinates of the center of the disc can be found as follows:

$$\frac{\partial E}{\partial x_c} = \frac{1}{R^2} \left(\int_{t=0}^{2\pi} (\sqrt{2} f(X_1, Y_1) dt - 2f(X_2, Y_2)) \cos t dt \right), \quad (10)$$

$$\frac{\partial E}{\partial y_c} = \frac{1}{R^2} \left(\int_{t=0}^{2\pi} (\sqrt{2} f(X_1, Y_1) dt - 2f(X_2, Y_2)) \sin t dt \right). \quad (11)$$

After accurate localization and segmentation of the OD, we follow the protocol adopted by Mohan et al. (Mohan Normative database of retinal oximetry in Asian Indian eyes 2015) and Geirsdottir et al. (Geirsdottir 2012) for segmenting the OD-centered ring-shaped region of interest and subsequent oxygen saturation analysis. The region consisting of the annulus between two circles of radius $(50 + R)$ pixels and $2(50 + R)$ pixels (1 pixel = 9 microns) concentric with the OD is selected for computing the oxygen saturation. The region is partitioned into supero-temporal (ST), supero-nasal (SN), infero-nasal (IN), and infero-temporal (IT) quadrants. Fig. 3 illustrates the complete procedure.

Fig 3 – Illustration of OD-centered ring-shaped region-of-interest segmentation for oximetry analysis on the right eye (first row) and left eye (second row). (a1)-(a2): Detection (green +) and segmentation of the optic disc (white circle); (b1)-(b2): Delineation of the ring-shaped region of interest (white concentric circles); (c1)-(c2): Segmentation of the ring-shaped region of interest and partitioning into supero-temporal (ST), supero-nasal (SN), infero-nasal (IN), and infero-temporal (IT) quadrants.

The oxygenated blood enters the eye through the central retinal arteriole and gets divided into four main branches, which are the major vessels and have thick walls. The blood then flows through the smaller arterioles and the capillary bed where oxygen exchange takes place. The blood then enters the smaller venules and finally the central retinal venule through the four larger venules. These larger venules much like the arterioles, have done oxygen exchange due to the thick walls and as a result, the oxygen saturation would be relatively stable in the larger vessels where fundus oximetry measurements are made.

Oxygen saturation ($SatO_2$) is the percentage of hemoglobin that is bound to oxygen (Delori 1988) and is measured as follows:

$$SatO_2 = \frac{HbO_2}{HbO_2 + Hb} \times 100.$$

Since about 98.5% of the oxygen carried in blood is bound to hemoglobin, $SatO_2$ is an accurate measure of the amount of oxygen in blood. (Oxymap T1 Instruction Manual ; Mairbäurl & Weber 2011 ; Pittman 2011). The principle of retinal oximetry is based on differential light absorption of HbO_2 and Hb (Oxymap T1 Instruction Manual). Fully oxygenated blood appears as bright red and deoxygenated blood reflects in the green to violet color bands. Various oxygen saturation levels correspond to different colors from red to the violet band of the spectrum (Oxymap T1 Instruction Manual). The mean oxygen saturation for retinal arterioles and venules in healthy individuals is 92.2% and 57.9%, respectively (Schweitzer 1999) (cf. Fig. 4). The pseudocolor maps could be used to assess the saturation values. The resulting saturation values have a bimodal distribution, one corresponding to the arterioles and the other corresponding to the venules.

Fig 4 – Interpretation of the pseudocolor map for $SatO_2$ in retinal arterioles and venules together with their mean values in healthy individuals according to the clinical study reported in [17]. The relationship between oxygen saturation and the corresponding red/green/blue component contribution for pixel color is also shown.

The relationship between $SatO_2$ and the respective red/green/blue component for pixel intensity is shown in Fig. 4 and can be specified by the following:

Condition-1:

*if $r = 255, g \neq 255, b = 0$; then $SatO_2 = 100 - 0.1 \times g$; and
if $r \neq 255, g = 255, b = 0$; then $SatO_2 = 50 + 0.1 \times r$.*

Condition-2:

if $r = 0, g \neq 0, b \neq 0$; then $SatO_2 = 25 + 0.1 \times g$.

Condition-3:

$$\text{if } r \neq 0, g = 0, b \neq 0; \text{ then } SatO_2 = 25 - 0.2 \times r.$$

A histogram of oxygen saturation levels for an example oximetry image is shown in Fig. 5. The histogram has a bimodal distribution with peaks very close to the $SatO_2$ in the venules and arterioles. The bimodal peaks are identified using a least-squares polynomial fit of order twelve. The two dominant peaks represent the $SatO_2$ in the venules and arterioles, respectively. We follow the same procedure for the ST, SN, IN, and IT quadrant-based oxygen saturation analysis.

Fig 5 – A histogram representing oxygen saturation in the region of interest and a bimodal curve fit to it.

RESULTS AND DISCUSSION

We have created a normative database (Mohan Normative database of retinal oximetry in Asian Indian eyes 2015) of retinal oximetry in Asian Indian eyes for validating the proposed technique. The ground truth has been obtained by manually marking all the vessels in the region of interest that are above the cut-off of 8 pixels (equivalently, 72 microns) in diameter. The manual method accounts for the values in all the pixels in a selected vessel and averages them out. This is prone to error and

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skewing of the mean due to extremes. The algorithm reverse-calculates the values given on the exported retinal oximetry image with the help of the pseudocolor map. The bimodal fit on the histogram of oxygen saturation levels gives an accurate saturation estimate.

A total of 44 retinal oximetry images of healthy individuals of Asian Indian origin were used in the study. The bimodal peaks are identified and arteriolar and venular saturations and AVSD values are automatically calculated. In Table I, we compare the average of the manually obtained values against those computed by the proposed algorithm. We observe that the algorithm results are comparable to the manual measurement. We use the intraclass correlation coefficient (ICC) as a measure of reliability of the algorithm estimate of oxygen saturation in comparison with that of the ground-truth. We present the ICC for single and average measures with 95% confidence interval (CI). For arteriolar oxygen saturation, the ICC showed a good agreement for single measures (0.66; 95% CI: 0.51 to 0.78) and excellent agreement for average measures (0.85; 95% CI: 0.76 to 0.92). For venular oxygen saturation, the ICC showed a good agreement for single measures (0.61; 95% CI: 0.45 to 0.74) and excellent agreement for average measures (0.82; 95% CI: 0.71 to 0.90). For the AVSD, the ICC showed fair agreement for single measures (0.47; 95% CI: 0.29 to 0.64) and good agreement for average measures (0.72; 95% CI: 0.55 to 0.84). We compare the manual and algorithm measurements of arteriolar and venular oxygen saturation using the Bland-Altman difference plots. In this method, one plots the differences between the manual and algorithm results against the averages of the two techniques. The Bland-Altman plots for measured oxygen saturation in arterioles, venules, and AVSD versus manual assessment are shown in Figs. 6(a), (b), and (c), respectively. The plots show excellent agreement between the manual and algorithm results. There is only one estimate outside the limits of agreement for arteriolar and venular oxygen saturation and AVSD.

Fig 6 (a) – Bland-Altman plot for comparing the algorithm-derived arteriolar oxygen saturation levels versus manual measurements for 44 images.

Fig 6 (b) – Bland-Altman plot for comparing the algorithm-derived venular oxygen saturation levels versus manual measurements for 44 images.

Fig 6 (c) – Bland-Altman plot for comparing the algorithm-derived AVSD versus manual measurements for 44 images.

The boxplots for the measured oxygen saturation in arterioles, venules, and AVSD versus manual assessment are shown in Figs. 7(a), (b), and (c), respectively.

Fig 7 (a) – Boxplots for comparing the algorithm-derived arteriolar oxygen saturation levels versus manual measurements for 44 images.

Fig 7 (b) – Boxplots for comparing the algorithm-derived venular oxygen saturation levels versus manual measurements for 44 images.

Fig 7 (c) – Boxplots for comparing the algorithm derived AVSD versus manual measurements for 44 images.

The boxplots do not indicate the presence of outliers in the estimated oxygen saturation. The automated oximetry analysis on the complete ring-shaped ROI and all the four quadrants (ST, SN, IN, and IT) is provided in Table I. A comparison of algorithm results for arteriolar and venular oxygen saturation and AVSD with that of the ground-truth for each quadrant is also provided in Table I. The results show that the automated analysis exhibits a high degree of agreement with manual measurements. The standard

	Region of interest				Quadrant-Arteriolar				Quadrant-Venular			
	Arterioles	Venules	AVSD	ST	SN	IN	IT	IT	ST	SN	IN	IT
Ground-truth	89.41	56.32	33.09	88.00	93.50	91.40	85.50	55.90	58.70	60.10	51.10	
Algorithm	87.48	57.41	30.07	86.06	92.90	92.10	87.55	55.30	63.06	61.20	52.30	

Table 1 - Comparison of estimated oxygen saturation versus the ground- truth. The numbers reported are obtained after averaging over 44 images. *ST: supero-temporal; SN: supero-nasal; IN: infero nasal; IT: infero temporal.*

	Region of interest			
	Arterioles	Venules	AVSD	
Ground-truth	5.15	4.73	4.51	
Algorithm	6.13	5.13	6.09	

Table 2 - Comparison of standard deviation (computed from 44 images) of estimated oxygen saturation and the ground-truth for a ring-shaped ROI.

deviations between the ground-truth and algorithm determined arteriolar, venular oxygen saturation, and AVSD for the ring-shaped ROI over 44 images are provided in Table II.

From a clinical perspective, this study is important, because it shows that the algorithm could be used in place of manual measurements. It has multiple advantages. First, it is automatic and fast thus saving time and effort. Second, a trained grader would not be needed to measure the oxygen saturation from an oximetry image. Third, it would eliminate inter-observer variability as different observers may choose different vessel segments giving rise to different saturations. With the help of an automated technique, oximetry measurements can be standardized. Finally, since the proposed approach considers the bimodal peaks, it would not be affected by extreme values.

The supporting material available online (Kumar) comprises the ImageJ plugin (ImageJ ; Schneider 2012) for implementing the technique presented in this paper, exemplar oximetry images, and a video demonstrating the functionality of the plugin.

Conclusions

We have developed an automated, reliable, and accurate technique for performing retinal arteriolar and venular oxygen saturation measurements as an efficient alternative to manual or semi-automated procedures. The segmentation of OD and subsequent extraction of ring-shaped region of interest is performed using the active-disc technique. The oxygen saturation level estimated is on par with that obtained by manual assessment. A bimodal fit on the histogram of the oxygen saturation levels showed prominent peaks corresponding to the venular and arteriolar oxygen saturations. The technique was validated on a normative database of Asian Indian eyes containing 44 retinal oximetry images. The validation resulted in an average arteriolar and venular oxygen saturation of 87.48% and 57.41%, respectively, and AVSD of 30.07%. The results are close to those obtained from manual procedures and are also consistent across the dataset.

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CHAPTER 4

Oximetry and the Retinal Nerve Fiber Layer

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ABSTRACT

Title

Perivascular and quadrant retinal nerve fiber layer thickness and its relationship with retinal oxygen saturation.

Purpose

To study whether there exists a relationship between retinal nerve fiber layer thickness and oxygen saturation and to identify whether peri-vascular thickness (pRNFL) or quadrant thickness (qRNFL) has a stronger correlation

Methods

Patients without any detectable ocular abnormality on complete ophthalmic evaluation underwent non-invasive photospectrometric retinal oximetry on the Oxymap T1 retinal oximeter (Oxymap hf, Reykjavik, Iceland) and spectral-domain optical coherence tomography to determine if the pRNFL (Spectralis, Heidelberg, Germany) and the qRNFL (RiVue, Optovue) correlated with the measured light intensities and the calculated retinal oxygen saturations.

Results

30 eyes of 30 patients (120 arterioles, 120 venules) were included in the study. The mean arteriolar saturations (%) were temporal: 84.5 (95% CI 82.7-86.3) and nasal : 93.5 (95% CI 91.0-95.8); Venous saturations were temporal : 54.4 (95% CI 52.9-55.9) and nasal : 59.9 (95% CI 58.3-61.4). The mean pRNFL thickness (μm) around arterioles was temporal : 153.1 (95% CI 146-159) and nasal : 109.4 (95% CI 103-115); around venules it was temporal : 147.4 (95% CI 140-154) and nasal : 101.2 (95% CI 95-107). The oxygen saturations correlated significantly with pRNFL (arteriolar $r=-0.514$; $p<0.001$ and venous $r=-0.513$; $p<0.001$) but did not show a significant correlation with qRNFL values.

Conclusion

This relationship between perivascular RNFL could be physiological or artifactual. If physiological it would explain and form the basis of altered oxygen saturation in a multitude of diseases. If artifactual it would necessitate the incorporation of a pRNFL correction. However, the results of this study enable us to expect increased saturation in

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areas of thinner RNFL or vice-versa with the current methodology of retinal vessel oximetry as obtained by dual wavelength photo-spectrometric fundus imaging

Keywords: RNFL, retinal nerve fiber layer, oximetry, retinal oxygen saturation, metabolism

INTRODUCTION

Retinal vessel oximetry is the process of measuring the oxygen saturation of hemoglobin in the blood vessels of the retina. There exist invasive and non-invasive methods for measuring oxygen saturation. (Hardarson 2013) Non-invasive Oximetry was described for the first time by Hickam B et al in 1959. (Hickam 1959) While the principle of photo spectrometry (fundus imaging) for evaluating oxygen saturation (oximetry) has remained the same, advancements have been made in the way images are captured and analyzed. (Palsson 2012) Automatic computational algorithms for vessel detection and optical density calculations have further simplified the process. (Hardarson 2006)

We have used the non-invasive spectrophotometric method detailed by Geirsdottir et al (Geirsdottir 2012) where the device uses a dual wavelength simultaneous fundus photography to determine oxygen saturation. This is based on the principle of differential absorption of light by hemoglobin at different wavelengths. The built-in software calculates I_0 (light reflected from the neighboring area of the vessel) and I (light reflected from the vessel) at two wavelengths, 570nm and 600nm. 570nm is isosbestic (oxy and deoxy hemoglobin having equal absorption) whereas 600nm is non-isosbestic (oxy and deoxy hemoglobin having unequal absorption). From these, it calculates the optical density (OD) ($\log_{10}[I_0/I]$) at both wavelengths to get OD_{570} and OD_{600} . It then determines the optical density ratio ($ODR = OD_{600}/OD_{570}$) which is linearly and inversely related to oxygen saturation.

Retinal vessel oximetry is a measure of oxygen saturation in the retinal vessels which mainly supply the layers of the inner retina till the outer plexiform layer. (Kur 2012) A significant amount of the inner retinal thickness is due to the retinal nerve fiber layer (RNFL). (Alamouti & Funk 2003) The arterioles and venules are surrounded by a capillary-free zone where the oxygen supply is by diffusion from the large vessels and not capillaries. (Kur 2012) It is known that the infero-temporal quadrant has the highest RNFL thickness and it has been reported that the infero-temporal venules have the least saturation. (Dichtl 1999; Heitmar & Safeen 2012; Jani 2014) We hypothesized that a thicker RNFL may thus increase the oxygen loss from the vessels. The RNFL also being known to be reflective may have optical implications by altering the absorbance of incident light and hence the measured oxygen saturations. (Knighton & Qian 2000) As background pigmentation and reflectance plays a critical role in the calculation of the ODR, RNFL thickness could possibly cause artifacts.

Studying the relationship of perivascular RNFL thickness (pRNFL) and correlating it with the oxygen saturation could possibly tell us how accurately the algorithm compensates

for background reflectivity. The RNFL thickness in a quadrant (qRNFL) is also a measure of the total amount of retinal tissue. More tissue would imply higher metabolism and hence higher demand. Studying this relationship with oxygen saturation would not only give us an idea of the physiology but could potentially serve as a biomarker in disease state. We therefore attempted to determine the relationship between pRNFL and qRNFL with the measured vascular diameter and saturation in normal Asian Indian eyes.

MATERIALS AND METHODS

In this prospective non-randomized observational study, consecutive patients who attended a tertiary care hospital for a routine eye examination were recruited. The study was approved by the institutional review board and adhered to the Declaration of the Tenets of Helsinki. All patients gave a written informed consent.

The inclusion criteria were 20/20 vision on Snellen's acuity chart and a normal ocular exam. The exclusion criteria were any ocular pathology (except ametropia), any previous ocular surgery and any systemic disease like diabetes or hypertension.

Personal history of smoking and caffeine intake were elicited. After a comprehensive ophthalmic evaluation including dilated indirect ophthalmoscopy, oximetry [Oxymap T1 retinal oximeter (Oxymap hf, Reykjavik, Iceland)] was performed in ambient room light. The patients were dilated with 1% tropicamide and 10% phenylephrine drops. The patients were allowed to be seated for 5 minutes prior to oximetry. On an average, 3 disc-centered images were taken at 50W flash intensity by a single observer. Since defocus can alter contrast and hence the measured saturation values, we discarded any de-focused images. The best single well-focused good quality image was used for analysis and read by a single reader (different from observer). One second-order arteriole and venule per quadrant were chosen to give us a total of 8 segments per eye. Segments were chosen between the 2 concentric circles as indicated in Fig. 1. The inner circle was drawn by assuming the centre of the optic disc and a diameter 100 pixels greater than the optic disc. The outer circle was concentric and twice the diameter of the inner circle. For each segment, we obtained the vessel point data that gave the I_{0570} , I_{570} , I_{0600} , I_{600} , OD_{570} , OD_{600} , ODR (I_0 : measurements by the side of the vessel; I : measurements on the vessel; 570: 570nm – isosbestic wavelength; 600: 600nm – non-isosbestic wavelength; OD: optical density; ODR: optical density ratio), retinal vessel oxygen saturation corrected for vessel width (SatCor) and vessel width (μm) (Fig. 1)

Fig 1 – The process of segment selection: pseudocolour map showing selected infero-temporal segment (note red color of arterioles in nasal hemi-field and more orange color of arterioles in temporal hemi-field) (white indicates selected segment)(a), 570nm image showing selected vessel point data (b), 600nm image showing selected vessel point data (c) (green indicates I_{570} , yellow indicates I_{600} and red indicates $I_{0(570\&600)}$)

Vessel width is known to cause artifacts in the measurement of retinal vessel oxygen saturation.⁵ In an attempt to avoid this artifact we have used SatCor throughout the manuscript. This value is automatically calculated by the software.

The patients also underwent spectral domain optical coherence tomography (SD-OCT) to measure pRNFL (Spectralis, Heidelberg, Germany) and qRNFL (RtVue, Optovue). We measured pRNFL on the Spectralis SDOCT using the built-in digital caliper by positioning on either side of the vessel and then averaging the two values. The location of calipers coincided with the red dots seen in Fig 1c which represents the area where the Oxymap T1 calculates the I_0 . The software in the Spectralis presents qRNFL thickness of the superior, nasal, inferior and temporal (S, N, I and T) regions, but not of superotemporal, superonasal, inferonasal and inferotemporal (ST, SN, IN and IT). As the vessel segments of interest in this study were chosen from the latter set of quadrants (ST, SN, IN and IT), the Spectralis could not be used for qRNFL. Hence, we used the RtVue for qRNFL as it determines ST, SN, IN and IT values. (Fig. 2)

Fig 2 – A comparison of measurement of qRNFL by the Spectralis and OptoVue of the same patient

Statistical Analysis

Statistical analysis was done using the IBM SPSS (version22 for Mac) software. Arteriolar and venous values (I_0570 , $I570$, I_0600 , $I600$, OD_{570} , OD_{600} , ODR , $SatCor$, $pRNFL$ and $qRNFL$) were analysed separately. All groups were tested for normality using the Shapiro-Wilk test. Comparison of means was done using the Mann-Whitney U test. Naïve analyses was done using Spearman's correlation to assess which variables showed a significant correlation with $SatCor$. These variables were then used in the multivariate analysis. Thus a multivariate analysis was done, keeping $SatCor$ as the dependent variable and using $pRNFL$, $qRNFL$ and vascular diameter as the independent variables. Since $pRNFL$ and $qRNFL$ could be closely related, we performed two different multivariate analyses, one with diameter and $pRNFL$ and the other with diameter and $qRNFL$ as the independent variables.

RESULTS

Demographics

Thirty eyes (30 patients) were included. There were a total of 240 (120 arteriolar, 120 venous) vascular segments. The average age was 35.8 years (SD 13.6) years; majority were females (n=30; females = 77%). All patients had a best-corrected visual acuity of 6/6 for distance on Snellen's acuity chart and intra-ocular pressures below 20 mm Hg on non-contact tonometry.

Temporal vs Nasal Hemifields

There were statistically significant differences between the temporal and nasal hemifield values (I , I_0 and OD at 570 nm and 600 nm, ODR, SatCor, vessel width, pRNFL and qRNFL) that have been summarized in Tables 1 & 2. There were statistically significant differences for I_{0570} ($p=0.009$) and I_{0600} ($p=0.045$) for arterioles and I_{0570} ($p=0.026$) for venules. The I_{570} and I_{600} were not statistically different between the temporal and nasal hemifields.

Parameter	Temporal Mean (ST+IT) (95% C.I)	Nasal Mean (SN+IN) (95% C.I)	p Value
I_{0570}	755 (684-826)	631(570-692)	0.009
I_{570}	430 (383-476)	400 (356-444)	0.339
I_{0600}	947 (859-1035)	821(743-897)	0.045
I_{600}	792 (717-868)	736 (665-807)	0.329
OD_{570}	0.254 (0.239-0.269)	0.208 (0.196-0.219)	<0.001
OD_{600}	0.079 (0.073-0.086)	0.048 (0.044-0.053)	<0.001
ODR	0.308 (0.293-0.322)	0.222 (0.203-0.241)	<0.001
SatCor (%)	84.5 (82.7-86.3)	93.5 (91.0-95.8)	<0.001
Diameter (μm)	132.9 (128-137)	114.3 (110-118)	<0.001
pRNFL (μm)	153.1 (146-159)	109.4 (103-115)	<0.001
qRNFL (μm)	111.8 (107-115)	103.8 (99-107)	0.007

Table 1 - Comparison between temporal and nasal hemifield parameters for arterioles (Mann-Whitney U test)

Parameter	Temporal Mean (ST+IT) (95% C.I)	Nasal Mean (SN+IN) (95% C.I)	p Value
I_{0570}	718 (644-791)	603 (544-662)	0.026
I_{570}	312 (274-350)	299 (263-335)	0.646
I_{0600}	908 (816-1000)	792 (719-865)	0.123
I_{600}	559 (499-618)	552 (497-607)	0.950
OD_{570}	0.374 (0.358-0.389)	0.317 (0.302-0.333)	<0.001
OD_{600}	0.215 (0.206-0.224)	0.161 (0.152-0.170)	<0.001
ODR	0.575 (0.565-0.591)	0.508 (0.495-0.522)	<0.001
SatCor (%)	54.4 (52.9-55.9)	59.9 (58.3-61.4)	<0.001
Diameter (μ m)	177.0 (170-183)	143.8 (138-149)	<0.001
pRNFL (μ m)	147.4 (140-154)	101.1 (95-107)	<0.001
qRNFL (μ m)	111.8 (107-115)	103.8 (99-107)	0.007

Table 2 - Comparison between temporal and nasal hemifield parameters for venules (Mann-Whitney U test)

I_{0570} & I_{0600} – light intensities at neighbouring pixels at 570 and 600nm. I_{570} & I_{600} – light intensities on the vessel at 570 and 600nm. OD_{570} & OD_{600} – Optical density at 570 and 600nm. ODR – ratio of OD_{570} & OD_{600} . pRNFL – perivascular retinal nerve fiber layer; qRNFL – quadrant retinal nerve fiber layer thickness. ST: supero-temporal; SN: supero-nasal; IN: infero-nasal; IT: infero-temporal; T: temporal; N: nasal.

Correlation of pRNFL with other parameters

There were strong correlations between pRNFL and other parameters as summarized in Table 3. Fig 3 and 4 shows the correlation of arteriolar and venular SatCor with pRNFL and qRNFL. pRNFL showed good correlation with SatCor (arterioles: $r = -0.514$, $p < 0.001$; venules: $r = -0.513$, $p < 0.001$). Significant correlation was seen between pRNFL and I_0 values at 570nm (arterioles: $r = -0.514$, $p < 0.001$; venules: $r = -0.513$, $p < 0.001$) and 600nm (arterioles: $r = -0.514$, $p < 0.001$; venules: $r = -0.513$, $p < 0.001$). The same correlation of pRNFL was not observed with I_{570} and I_{600} values. There was also a significant correlation between pRNFL and qRNFL values (arterioles: $r = 0.486$, $p < 0.001$; venules: $r = 0.509$, $p < 0.001$)

There was a significant correlation between SatCor and vessel diameter (arterioles: $r = -0.264$, $p = 0.004$; venules: $r = -0.329$, $p < 0.001$) SatCor and qRNFL did not show a significant correlation.

Oximetry and the Retinal Nerve Fiber Layer

Parameter	Arteriolar		Venular	
	r Value	p Value	r Value	p Value
I_{0570}	0.324	<0.001	0.218	0.017
I_{570}	0.188	0.040	0.110	0.230
I_{0600}	0.271	0.003	0.149	0.105
I_{600}	0.172	0.060	0.033	0.722
OD_{570}	0.324	<0.001	0.277	0.002
OD_{600}	0.582	<0.001	0.469	<0.001
ODR	0.585	<0.001	0.573	<0.001
SatCor	-0.514	<0.001	-0.513	<0.001
Diameter	0.523	<0.001	0.430	<0.001
qRNFL	0.486	<0.001	0.509	<0.001

Table 3 – Correlation of arteriolar and venular values with pRNFL thickness

I_{0570} & I_{0600} – light intensities at neighbouring pixels at 570 and 600nm. I_{570} & I_{600} – light intensities on the vessel at 570 and 600nm. OD_{570} & OD_{600} – Optical density at 570 and 600nm. ODR – ratio of OD_{570} & OD_{600} . RNFL – retinal nerve fiber layer

Fig 3 – (left) - Correlation of arteriolar SatCor (%) with pRNFL (μm) and qRNFL (μm) and

Fig 4 – (right) - Correlation of venular SatCor (%) with pRNFL (μm) and qRNFL (μm)

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	Univariate Analysis		Multivariate Analysis 1		Multivariate Analysis 2	
	Standardised Coefficients	p Value	Unstandardised Coefficients	p Value	Unstandardised Coefficients	p Value
Intercept (a)			109.40	<0.001	107.98	<0.001
Diameter (b)	-0.264	0.004	-0.025	0.579	-0.135	0.003
pRNFL (c)	-0.514	<0.001	-0.132	<0.001		
qRNFL (c)	-0.168	0.067			-0.022	0.692

Table 4 - Multivariate Analysis for SatCor – Arterioles

	Univariate Analysis		Multivariate Analysis 1		Multivariate Analysis 2	
	Standardised Coefficients	p Value	Unstandardised Coefficients	p Value	Unstandardised Coefficients	p Value
Intercept (a)			73.93	<0.001	74.17	<0.001
Diameter (b)	-0.329	<0.001	-0.035	0.072	-0.076	<0.001
pRNFL (c)	-0.513	<0.001	-0.090	<0.001		
qRNFL(d)	-0.125	0.172			-0.045	0.194

Table 5 - Multivariate Analysis for SatCor– Venules

Multivariate Analysis 1 – SatCor = a + b x diameter + c x pRNFL; Multivariate Analysis 2 – SatCor = a + b x diameter + c x qRNFL

Multivariate Analysis

Since RNFL thickness had a strong correlation (Table 3) with all the measured light intensities for both arteriolar and venular segments, it was taken as an independent variable along with diameter that showed a significant correlation with SatCor. qRNFL was also included to enable a clearer distinction between the relationship of the two parameters with oxygen saturation. The analysis was performed separately for arterioles and venules using the formula:

Multivariate Analysis 1 - SatCor = Constant(a) + b x diameter + c x pRNFL

Multivariate Analysis 2 - SatCor = Constant(a) + b x diameter + c x qRNFL

The univariate and multivariate analysis values along have been summarized in Table 4 & 5

DISCUSSION

In this study, a strong positive correlation was observed between pRNFL and measured light intensities and a negative correlation with calculated oxygen saturations.

It has been known that diameter has an inverse correlation with oxygen saturation.¹ We also confirm a significant correlation between diameter and SatCor (negative) as well as diameter and pRNFL (positive) in our univariate analysis, but the multivariate analysis showed that pRNFL was significant whereas qRNFL and diameter was not. This could imply that part of the reason why diameter showed a correlation with SatCor was maybe due to it's association with pRNFL.

Hardarson et al (Hardarson & Stefansson 2012) have hypothesized that it is possible that oxygen escapes out directly from arterioles by diffusion. Hence thicker RNFL could mean more diffusion and loss, and hence lower saturation levels.

ODR is derived from the formula - $\log(I_{0600}/I_{600})/\log(I_{0570}/I_{570})$. Since we see a positive correlation of RNFL mainly in the measured light intensities in the neighboring pixels of the vessel (I_0), any change in RNFL thickness would cause a corresponding optical change in these values. We see the strongest correlation with OD₆₀₀ for both arterioles and venules. We also observed in Table 1 and 2 that there were statistical differences between temporal and nasal hemifields in the I_0 values but not in the I values. This means that when the blood interacts with the path of light there is not a significant difference in the measured light intensities. On the other hand when mainly RNFL

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interacts with the path of light the thicker RNFL causes increased reflection and hence a significant increase in the ODR. This would imply that any increase in pRNFL would cause an increase in ODR and hence a drop in measured saturation values. This could imply either that the relationship is optical or physiological. If physiological, the size of the capillary free zone (Kur 2012) could be an important determinant of the measured saturations.

Temporal vessel diameters and both pRNFL and qRNFL were noted to be more than the nasal values. This corresponds to the lower saturations seen in both arterioles and venules temporally compared to their nasal counterparts. Vessel diameter can change the I values but should not affect the ODR values unless the real saturation is physiologically related to vessel diameter. SatCor is obtained after correction for vessel diameter and hence any residual difference could be real or due to incomplete correction.

Oximetry studies in glaucomatous eyes have reported that while venous saturation increase, arteriolar saturations are not affected, thereby causing a decrease in A-V difference, that is consistent with tissue loss. (Vandewalle 2014) The increase in venous saturations could be possibly explained by the fact that we found a negative correlation between pRNFL and measured oxygen saturation values. But we would also expect to see an increase in arteriolar saturation values; however, this has not been reported in the studies published.

As shown in Tables 1 & 2, I_0 at 570 and 600nm were different for the temporal and nasal hemifields for either arteriole or venule, but comparable between the two types of vessels. The I_{570} and I_{600} was higher for arterioles compared to venules. The higher value at 600nm can be explained by the fact that this is a non-isosbestic wavelength and hence oxy-hemoglobin absorbs very less light, most of it being reflected back. At 570nm the difference can be explained by the difference in diameters.

One potential limitation is that we had to use 2 different OCT machines as the Spectralis enabled us to measure pRNFL thickness, but did not give us qRNFL by ST, SN, IN and IT; the RiVue, on the other hand, gave us the qRNFL in the desired manner, but not the perivascular thicknesses. A Bland Altman plot showed the 95% limits of agreement to be -21.97-43.87 between the RNFL (S,N,I,T) measured by the Spectralis and the Optovue. Another potential limitation could be the fact that the angle of gaze could render the inferior vessel to have less contrast and hence partly explain their oximetry readings being lower. However we have ensured that all our measured vessel segments are present at the same eccentricity from the center of the image making this unlikely.

To the best of our knowledge, there have been no studies detailing pRNFL thickness and its correlation to measured oxygen saturation and diameter values; earlier papers have correlated qRNFL to saturations and diameters. (Geirsdottir 2012; Jani 2014) This is the first paper that highlights the differences in retinal vessel oxygen saturation between the temporal and nasal segments, given the differences in RNFL thicknesses between these quadrants. It is also the first report to detail the measured light intensities and correlate them with RNFL thickness to enable a better understanding of the factors affecting the final measured oxygen saturation values.

In the past the negative correlation between diameter and retinal vessel oxygen saturation necessitated the incorporation of a vessel width correction. We feel that this relationship between perivascular RNFL thickness and retinal vessel oxygen saturation may compel us to further evaluate if this relationship is physiological or artifactual. If physiological it would explain and form the basis of altered oxygen saturation in a multitude of diseases. If artifactual it would necessitate the incorporation of a pRNFL correction. However, the results of this study enable us to expect increased saturation in areas of thinner RNFL or vice-versa with the current methodology of retinal vessel oximetry as obtained by dual wavelength photo-spectrometric fundus imaging

Abbreviations - I_{0570} – Measurement on the side of the vessel – isosbestic; I_{570} - Measurement on the vessel – isosbestic; I_{0600} - Measurement on the side of the vessel – non-isosbestic; I_{600} - Measurement on the vessel – non-isosbestic; OD_{570} - Optical density isosbestic; OD_{600} - Optical density non-isosbestic; ODR- Optical density ratio; SatCor – Retinal vessel oxygen saturation corrected for vessel width; pRNFL – Perivascular RNFL; qRNFL – Quadrant RNFL

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CHAPTER 5

Quadrant Imaging

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ABSTRACT

Purpose

We aimed to compare measured oxygen saturations in normal eyes between optic disc centered and quadrant imaging (supero-temporal - ST, supero-nasal - SN, infero-nasal - IN and infero-temporal - IT).

Methods

Forty-two consecutive healthy subjects were included in the study. They underwent retinal oximetry on the Oxymap T1 retinal oximeter (Oxymap hf. Reykjavik, Iceland). Fifty-degree images were taken with the optic disc centered and quadrant methods and the oxygen saturations for arterioles and venules were compared between the two methods.

Results

Optic disc centered images gave average arteriolar saturation (%) of 90, 94, 93 and 89 and venous saturation of 58, 60, 60 and 52 in the ST, SN, IN and IT respectively. For quadrant images the average arteriolar saturation was 94, 95, 94 and 91 and the venous saturation was 62, 60, 61 and 60 in the ST, SN, IN and IT respectively. Temporally (ST + IT), the saturation values were significantly different ($p < 0.001$) between optic disc centered and quadrant images. We found no differences nasally (SN + IN).

Conclusion

We see that the direction of gaze has a significant impact on the measured saturations which is more apparent temporally than nasally. These optical artefacts must be kept in mind while interpreting oximetry images.

INTRODUCTION

Retinal oximetry, first described by Hickam *et al.* in 1960, (Hickam 1959) is a technique of measuring relative oxygen saturation in the retinal vessels of the eye. It uses a photospectrometric method with a fundus camera to estimate the saturation. Since 1960, there have been numerous advancements in retinal oximetry (Hardarson 2006) and normative values in various populations have been established. (Geirsdottir 2012; Jani 2014; Mohan 2015)

Most of these papers and other published literature use an optic disc centered method of estimation. It has been shown that vessel location within an oximetry image, depending on the gaze of the subject, has a significant effect on measured oxygen saturation in arterioles and venules. (Geirsdottir 2012) Many diseases affect the retina globally and hence global saturations would be enough, however many other diseases are local pathologies like chorio-retinitis or vein occlusions and hence it is important to study various angulations of gaze.

Our normative database (Mohan 2015) earlier focused only on selecting the thickest vessel per quadrant with optic disc centered imaging. Since selecting a single vessel per quadrant might not always be perfectly representative of the entire quadrant, we wanted to see what happened when we chose more than one vessel and then averaged the values to obtain a single value for the quadrant.

With the above questions in mind we aimed at comparing the measurements in the same eye in the same setting with optic disc centered versus quadrant images.

METHODS

Subjects

Forty-two consecutive healthy subjects presenting to our hospital in Bangalore, India for a routine eye check-up were included in the study. They all had a corrected distance vision acuity more than 20/30, no cataract or other significant media opacities, no history of ocular or systemic disease or history of smoking. The study was approved by the institutional review board (Narayana Nethralaya Ethics Committee) and adhered to tenets of the declaration of Helsinki.

Imaging (Method)

The procedure was explained to them in detail and a written informed consent was taken. The subjects were requested to rest for 5 minutes before the images were taken. None of the subjects had consumed caffeine within 2 hours of the test. After dilatation with 1% tropicamide and 10% phenylephrine all patients had their oximetry images taken on the Oxymap T1 retinal oximeter (Oxymap hf., Reykjavik, Iceland).

The right eye was imaged for every subject for the two methods – optic disc centered and quadrant centered. Each subject had two 50 degree optic disc centered images (Fig 1 & 2) and one image in each of 4 quadrants (supero-temporal - ST, supero-nasal - SN, infero-nasal - IN and infero-temporal - IT) taken – a total of 6 images per eye (Fig 3). The quadrant images were taken such that the edge of the optic disc was at the corner of the image.

Fig 1 – This is the 570nm image of the optic disc centered method.

Fig 2 – First Protocol -This is the same image above with the superimposed pseudo color map. Each quadrant may have 2-3 selectable vessels of which we chose the thickest arteriole and venule per quadrant. The arterioles chosen can be seen highlighted.

Fig 3 – First Protocol - This shows the quadrant image where we can see the branches of the primary vessels in the optic disc centered image. In the quadrant images we selected 3 segments each for arterioles and venules and averaged them. These were smaller vessels than the ones chosen in optic disc centered images. This was done to choose the vessels near the centre of the image. In quadrant images since the optic disc is in one corner choosing the same segment will mean we chose a vessel at one corner of the image - hence we went further out and chose vessels.

Segment Selection (Protocol)

The Oxymap software version used was 2.5.1, which gives an automatic image quality score based on contrast and focus. Images below 7.5 on the automatic quality analysis were excluded from the analysis. Saturations and diameters of the selected vessels were automatically provided by the software. We used two protocols of segment selection to compare the measured saturations between optic disc centered and quadrant images.

In the first protocol (Fig 1-3) the thickest arteriole and venule, 100-150 pixels in length were chosen in each quadrant in the optic disc centered images. For quadrant images averaged values of 3 segments of thickest arterioles and venules each above 100 μm were chosen; the segments were between 100 to 150 pixels in length and were selected as much in the centre of the image as possible. The intra-eye range (maximum – minimum) between quadrants for arteriolar and venous saturation (%) was compared between optic disc centered and quadrant images. In this protocol the exact same vascular segments are not selected for the quadrant and optic disc centered method. This may lead to saturation differences because of the differences in diameters. Hence, we performed on 20 eyes a second protocol (Fig. 4-6) for arteriolar segments, choosing exactly the same vessel segment in both the optic disc centered and quadrant images – one arteriole per quadrant - and compared their diameters and saturations.

Statistical Analysis

All data was entered in Microsoft excel 2013 for Mac and analysed using IBM SPSS v24 for Mac. Values were tested for normality using the Shapiro-Wilk test. The paired t-test was used for comparison of means.

Fig 4 – Second Protocol - Our pilot study of choosing the exact same segment in both the optic disc and quadrant images only for arterioles. You can see we went a little further out than the previous optic disc centered image. This was done to not have the segment near one corner of the quadrant image.

Fig 5 – Second Protocol - The same segment marked in the quadrant image.

Fig 6 – Second Protocol - A side by side comparison of the selected segment in the optic disc centered (left) vs the quadrant image (right). My observations are that in the quadrant image everything seems vertically compressed as compared to the optic disc centered image. Also, the background always seems brighter than the optic disc centered image.

RESULTS

There was a total of 42 subjects with an average age of 35 ± 11 years (Range 20 – 57 years) and a male female ratio of 10:11.

First Protocol

Table 1 and 2 summarize the arteriolar and venous saturation and diameter values. Saturations differ temporally between the optic disc centered and quadrant methods and are comparable nasally. Diameters are different temporally and comparable nasally for arterioles; they are different in all quadrants for venules.

The saturations were then compared within each method. The temporal saturations were significantly lower than the nasal ones for arterioles within both optic disc (89.1 vs 93.7 ; $p < 0.001$) and quadrant imaging (92.3 vs 94.4 ; $p = 0.001$). For venules the temporal saturations were significantly lower than the nasal ones within optic disc centered imaging (55.0 vs 60.1 ; $p < 0.001$), however we found no within differences in quadrant imaging (60.8 vs 60.7 ; $p = 0.954$).

The intra-eye range (maximum – minimum) was 11.9 ± 5.7 in optic disc centered versus 7.1 ± 3.6 in quadrant images for arterioles ($p < 0.001$) and was 11.6 ± 4.5 in optic disc centered versus 7.5 ± 3.2 in quadrant images for venules ($p < 0.001$).

Second Protocol

We found that the arteriolar saturation differed in three quadrants, although the diameter did in only one (see Table 3).

	ST		SN		IN		IT	
	Sat (%)	Dia (µm)	Sat	Dia	Sat	Dia	Sat	Dia
Optic Disc Centered	89.6±6.4	131.9±13.0	94.3±6.8	110.0±17.2	93.1±8.3	110.1±12.8	88.6±6.6	132.5±13.8
Quadrant	93.6±3.0	120.3±9.7	94.7±4.1	110.1±9.5	94.1±3.8	106.3±7.7	91.1±4.3	119.2±9.9
p value*	<0.001	<0.001	0.74	0.951	0.461	0.057	0.007	<0.001

Table 1 – Arteriolar Saturation values Sat: saturation; Dia: diameter; *paired t-test

	ST		SN		IN		IT	
	Sat	Dia	Sat	Dia	Sat	Dia	Sat	Dia
Optic Disc Centered	57.8±6.1	173.1±22.4	60.4±6.4	142.9±18.7	59.7±7.9	143.6±21.4	52.1±5.9	180.7±21.7
Quadrant	61.7±7.0	148.8±11.2	60.3±6.3	133.5±7.8	61.2±6.2	133.3±13.9	59.6±5.4	145.0±11.5
p value*	<0.001	<0.001	0.893	0.001	0.242	0.001	<0.001	<0.001

Table 2 – Venous Saturation values *paired t-test

	ST		SN		IN		IT	
	Sat	Dia	Sat	Dia	Sat	Dia	Sat	Dia
Optic Disc Centered	85.9±5.7	130.7±17.1	95.0±7.4	105.5±15.3	91.2±5.7	106.8±12.2	86.6±8.6	124.8±17.8
Quadrant	89.6±6.2	133.9±15.2	91.4±9.4	110.2±19.0	87.4±6.9	110.2±14.1	85.1±6.0	127.0±13.4
p value*	0.016	0.128	0.029	0.034	0.011	0.069	0.209	0.288

Table 3 – Arteriolar saturation values - Second Method *paired t-test

DISCUSSION

Using the first protocol, arteriolar and venous saturation values were significantly different between the optic disc and the quadrant method for measurements made in the temporal hemisphere; they were comparable nasally. In the second protocol which involved only the arterioles and where the exact same segment of vessel was chosen, we found that the saturations were different in three quadrants; the diameters were different in only one.

It can be argued that the difference between the two methods is because different vascular segments were chosen and that the diameters were different. However, we see from the results of the second protocol that despite selecting the exact same vessel the saturations differed more while the diameters were comparable. Since the second protocol was done mainly to support the findings of the first protocol the discussion will henceforth focus on discussing the results of the first protocol.

It is interesting that the difference between the two imaging methods is mainly seen temporally. Our within the group analysis showed that while optic disc centered imaging had significant temporal vs nasal differences for both arterioles and venules the quadrant imaging group had differences only in arterioles. It must also be noted that the difference in arterioles is much lesser in the quadrant imaging versus optic disc centered imaging.

It has been previously established by published normative databases (Mohan 2015) using the optic disc centered method that lower saturations are seen temporally. Jani *et al.* (Jani 2014) hypothesised that the proximity to the macula could be the cause for the lower saturations. Our study (Mohan 2016) which aimed to study the relationship between perivascular RNFL thickness and oxygen saturations found an inverse relationship. We found that the RNFL was thicker temporally than nasally and hence hypothesised that the thicker RNFL may be extracting more oxygen and hence resulting in the lower saturation. We can argue that if the measured saturations were low due to increased oxygen extraction due to proximity to the macula or due to increased RNFL thickness – i.e. a true decrease in saturations then it must persist even in the quadrant imaging. However, we see that this is not true and hence we are led to believe that this could be artefactual.

We also hypothesised in the same study (Mohan 2016) that it may be possible that this could be an optical artefact due to altered background reflectance due to RNFL. The RNFL thickness would remain the same be it optic disc centered or quadrant imaging

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and hence the effect should persist. This led us to question if there was some characteristic other than the thickness of RNFL that was influencing reflectance.

An interesting paper by Jeppesen *et al.* (Jeppesen & Bek 2019) report a significant negative association between linear velocity of blood in the vessels and the measured saturations. This can explain why we see lower saturations in the optic disc centered images temporally as the temporal vessels being larger will have a higher linear velocity of blood flow. However, this effect disappearing in the quadrant images indicates that linear velocity is only a part of the explanation for the unexplained values seen in retinal oximetry.

Knighton *et al.* (Knighton 1992) performed a study on toad eyes and have established that the reflectance of the RNFL arises from the light scattering of the cylindrical structures of the RNFL. This in turn is dependent on the its orientation relative to the imaging system. In a later study they (Knighton & Qian 2000) confirmed that the observed reflectance to a great extent depends on the configuration of the illuminating and viewing apertures and on the retinal position and orientation of the nerve fiber bundles. They found that in optic disc centered images the temporal “ribbons” were brighter than the nasal ones. This apparent brightness can interfere with the background reflectance thus altering measured saturation values in optic disc centered images, since the algorithm used to calculate the saturation assumes a retina based on a limited number of healthy subjects, that ignores and cannot take into account these differences. (Hardarson 2006) Note that in quadrant images each quadrant is centered. Hence the arguments put forward by Knighton *et al.* as described above do not apply and the RNFL is uniformly reflective. This can explain why in this configuration the measurements between the temporal and nasal hemispheres were comparable.

We also considered whether the reflectance of the RNFL changes spectrally, thus offering different reflectance at the isosbestic (570nm) and non-isosbestic (600nm) wavelengths. However, two studies by Knighton *et al.* (Knighton 1989; Knighton & Huang 1999) showed only small differences between 560 and 680 nm.

The results of the oxy-hemoglobin saturation curve offer another argument to support the theory that such large intra-eye variations seen in optic disc centered imaging especially with arterioles must be artefactual. In this curve which is sigmoid in shape we can see that for a haemoglobin saturation (SO_2) of 97.5% the partial pressure of oxygen (PO_2) must be 100 mm Hg, however at a saturation of 80% the PO_2 drops down to 45%. We can also hypothesise that since the blood comes from the central retinal arteriole and divides into the four major vessels the saturation in the four major vessels should be

nearly the same. Each percent difference in saturation requires a change of more than 3 mm Hg in PO_2 ; this means that the average 12% difference seen in arteriolar saturations would need a 40 mm Hg difference in PO_2 which is unexplained physiologically especially in normal eyes. The 12% variation in venules is still more plausible physiologically as firstly the differences in PO_2 are not as large in the physiological saturation zone of the venules (40-70%) and secondly each major venule would be draining an unequal area of the retina and hence oxygen extraction can differ.

The intra eye range was less in the quadrant imaging as compared to the optic disc imaging. This could be explained by the fact that three vessels were averaged in the quadrant images whereas only one vessel was chosen for the optic disc centered image. The quadrant images allow us to select more segments for each quadrant and thus may be more accurate and representative of the metabolic activity in that quadrant. The lower intra eye range also helps us get more physiologically acceptable values.

Hence, we may conclude that there are significant optical factors that are responsible for the final measured saturation values. These must be kept in mind when interpreting a retinal oximetry image. Future research should be focused on eliminating these optical artifacts possibly through multispectral imaging or in conjunction with OCT images. The results of this study also indicate that quadrant imaging may be a better method than optic disc centered imaging which is the standard imaging method in most published literature today.

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CHAPTER 6

Oximetry in Amblyopia

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ABSTRACT

Purpose

To study the retinal vascular oxygen saturation in amblyopic eyes and compare them to unaffected fellow eyes and eyes of normal subjects

Methods

32 amblyopic, 24 normal and 13 fellow eyes of patients below age of 18 were enrolled in this prospective observation study. Retinal oximetry was performed using the Oxymap T1 retinal oximeter. Retinal vascular oxygen saturations and diameters were compared between amblyopic eyes, normal eyes and unaffected fellow eyes.

Results

The average age was 8.6 years in the amblyopia group (M:F 16:16) and 10.9 years in the normal group (M:F 7:5) ($p=0.024$). Median CDVA in the amblyopia group was 20/50; it was 20/20 in the other groups ($p<0.001$). The average arteriolar and venous saturation in the amblyopia, normal and fellow group was 84.5% (95% CI 82.6-86.4), 83.2% (95% CI 80.7-85.6) and 80.8% (95% CI 78.6-82.9) and 51.9% (95% CI 50.4-53.4), 50.8% (95% CI 48.2-53.4) and 49.3% (95% CI 45.7-52.9). There was no statistically significant difference between the saturation values of the amblyopia group and the controls however significantly higher values were found in the amblyopia group compared to the fellow group for arteriolar and venous saturations ($p=0.013$; $p=0.005$). Arteriolar and venous diameters showed no significant difference between groups.

Conclusion

Amblyopic eyes showed higher mean oxygen saturations than the fellow eyes. This observation could be due to altered neuronal activity or could be due to a measurement artefact due to alterations in retinal reflectivity.

Keywords

Amblyopia; Retinal Oximetry;

INTRODUCTION

Amblyopia has been traditionally defined as decreased vision in one or both eyes caused by abnormal binocular interaction and/or pattern vision deprivation which in some cases can be corrected by treatment. (Noorden & Gunter 1985) Amblyopia results from degradation of the retinal image during a sensitive period of visual development. The degradation of the image, and subsequent central suppression that leads to amblyopia, results from one of three causal processes namely – strabismus, anisometropia and/or stimulus deprivation. Hence the disorder (amblyopia) is not the cause but it is the effect of an underlying pathological process. (Holmes & Clarke 2006)

Although amblyopia has been defined as low visual acuity in one or both the eyes with a structurally normal globe, there are many studies that show that the amblyopic eye may have some structural anomalies including changes in retinal ganglion cells, the retinal nerve fibre layer (RNFL), and the optic nerve (ON) (Lempert 2000; Baddini-Caramelli 2001; Yen 2004; Repka 2006; Wu & Hunter 2006; Dickmann 2009; Huynh 2009; Pineles & Demer 2009; Wu 2013); but there are also studies which have found no significant differences in the RNFL in amblyopic and normal eyes. (Delint 1998; Kee 2006; Repka 2006; Miki 2010; Al-Haddad 2011; Altintas 2020)

Retinal oximetry is a new tool which helps in measuring the oxygen saturation of haemoglobin in the blood vessels of the retina. A number of retinal diseases have been studied and significant differences have been found from normal. (Hardarson & Stefansson 2012a; Hardarson & Stefansson 2012b; Hardarson 2013; Geirsdottir 2014 ; Olafsdottir 2014; Vandewalle 2014; Battu 2015) Since it is believed that in amblyopia there is a structurally normal globe and it is mainly the visual function which is decreased, it would be interesting to see if there is a change in a quantitative functional parameter like retinal vessel oximetry.

Aim of the study

1. To compare the retinal vessel oxygen saturation between amblyopic eyes and healthy control eyes of children.
2. To compare the inter ocular difference in retinal vessel oxygen saturation in children with unilateral amblyopia between the amblyopic and non-amblyopic fellow eye
3. To compare the retinal vessel oxygen saturation between healthy fellow eyes of unilateral amblyopic patients and healthy control eyes.

MATERIALS AND METHODS

Children and adolescents (<18 years of age) presenting to the outpatient department of a tertiary eye care centre (Bangalore, India), were enrolled in the study with their written consent. The study follows the tenets of Helsinki. The Institutional Review Board and Ethics Committee approved the study. This was a comparative observational cross sectional non-interventional study.

Inclusion and Exclusion Criteria

Subjects diagnosed clinically to have treatment naive amblyopia (ametropic, anisometropic or strabismic) were included in the amblyopia group; subjects with bilateral amblyopia had the worse eye included. Clinically, amblyopia was defined as a corrected distance visual acuity (CDVA) of $\leq 20/30$ or a difference of the CDVA between the two eyes of at least 2 Snellen chart lines in the absence of any clinically detectable ocular pathology. The control group comprised of children who presented for a routine annual checkup with normal ophthalmic exam and CDVA of 20/30 – 20/20 and their right eye was included. The fellow eyes of subjects with unilateral amblyopia were analysed separately in the fellow group. Patients with significant cataract or any identifiable ocular pathology were excluded from the study.

Image Acquisition and Segment Selection

All patients underwent dilation with 1% tropicamide and 10% phenylephrine and subsequent retinal imaging (Fig. 1) with the oximetry using the Oxymap T1 retinal oximeter (Oxymap hf., Reykjavik, Iceland) running the software version 2.5.1. They were allowed to rest for 5 minutes before the images were captured. This was done to eliminate exercise induced fluctuations in readings. None of the subjects had consumed caffeine within 2 hours of the examination.

Fig 1– Retinal oximetry pseudocolor map showing a comparison between a normal (left) and amblyopic (right) eye.

The aiming light was set at the lowest setting, flash intensity was 50Ws, small aperture and large pupil settings were applied to the TRC 50DX (Topcon, Japan) Fundus camera.

One experienced photographer obtained standardized images for all the subjects which were 50° disc centered. The right eyes of all patients were imaged first and then the left eye; two images were taken per eye and the one with the better image quality was selected. If both the images had inadequate image quality then the eye was excluded. Image quality was decided based on the automatic objective image quality score with cut-off of 7.

Segments were selected as described in our previous study.(Mohan et al. 2015) We analysed global arteriolar and venous saturation and diameter and arterio-venous saturation difference (AVSD) and compared them between the amblyopic and control (normal and fellow eyes) arms.

Statistical Analysis

Statistical analysis was done using the IBM SPSS v22 software. All continuous variables were tested for normality using the Shapiro-Wilk test. The difference between means was tested using independent t-test for normally distributed data and the Mann-Whitney U test (unpaired) or the Wilcoxon signed rank test (paired) for non - parametric data. For normally distributed data the Pearson's correlation was used, for non-parametric data the Spearman's correlation was used. The level of significance was $p < 0.05$.

When significant differences in age were found between groups, a multivariate analysis was done keeping the saturations as the dependent variable and the age and the group as the independent variable to correct for age.

RESULTS

There were a total of 32 amblyopic eyes, 24 normal eyes and 13 fellow eyes of patients with unilateral amblyopia. The image quality of the groups were comparable ($p=0.192$) with the mean quality being 7.6 ± 0.27 , 7.8 ± 0.52 and 7.9 ± 0.48 in the amblyopic, normal and fellow eyes respectively.

Amblyopic Eyes Versus Healthy Controls (Table 1)

In the amblyopia group the average age was 8.6 years (95% C.I. 7.2-9.9) with a male female ratio of 16:16. The normal group had an average age of 10.9 (95% C.I. 9.3-12.4) and a male female ratio of 7:5. Age distribution showed a significant difference between amblyopic and normal groups ($p=0.024$); no differences were found in the sex distribution. Twenty five eyes were right sided while seven were left sided in the amblyopia group; all eyes were right sided in the control group.

	Amblyopia (n = 32)	Normal (n = 24)	p value*
Age	8.6 (7.2 – 9.9)	10.9 (9.3-12.4)	0.024
Sex (M:F)	16:16	14:10	0.6
CDVA (median and range)	20/50 (20/30-20/200)	6/6 (all)	<0.001
Spherical Equivalent	-7.9 -to +10.1	-1.0 to +1.5	0.32
Arteriolar Saturation	84.5 (82.6-86.4)	83.2 (80.7-85.6)	0.39
Arteriolar diameter	125.7(121.5-130.0)	128.2 (124.4-132.0)	0.37
Venous Saturation	51.9 (50.4-53.4)	50.8 (48.2-53.4)	0.47
Venous Diameter	164.2 (157.8 – 170.5)	160.5 (154.6-166.4)	0.39
AVSD	32.7 (30.6-34.7)	32.4(29.9-34.8)	0.86

Table 1 - Demographics, oxygen saturation and diameter values of the amblyopia and normal groups *Independent t test for continuous variables; Chi-Square test for nominal data.

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The CDVA was lower in the amblyopia group with a median of 20/50 (range 20/200 –20/30) on Snellen's chart; all subjects in the normal group had a CDVA of 20/20 ($p<0.001$). The spherical equivalent in normals varied from -1.0 D to +1.75 D; in the amblyopia group it varied from (-7.8 D to + 10.1 D).

The average arteriolar saturation in the amblyopia and normal group were 84.5% and 83.2% ($p=0.393$). The venous saturations were respectively 51.9% and 50.8% ($p=0.465$) while the AVSD was 32.7% and 32.4% ($p=0.857$). None of the parameters showed a significant difference between the groups.

There were no significant correlations between the saturation and diameter parameters with the age, group, CDVA or spherical equivalent.

Amblyopic Eyes Versus Healthy Fellow Eyes (Table 2)

The average age of the amblyopic subjects that also had fellow eye measured ($N=13$) was 10.4 (95% C.I. 8.1-12.7). Their male female ratio was 6:7. Eight of the amblyopic eyes were right sided and five were left sided, being of course the reverse for the fellow eyes.

The CDVA was lower in the amblyopia eyes with a median of 20/50 (range 20/200 –20/30) on Snellen's chart. All fellow eyes had a CDVA of 20/20 ($p<0.001$). The spherical equivalent varied from +1D to +8D in amblyopic eyes and from -0.38 D to +6.25 D in fellow eyes ($p=0.003$).

Both arteriolar and venous saturation were significantly higher in the amblyopic eyes compared to the fellow eyes (85.4% and 80.8%, $p=0.013$, and 53.4% and 49.3%, $p=0.005$ respectively). The AVSD were 32.2% and 31.5% ($p=0.259$) respectively with no differences seen between the eyes.

	Amblyopia (n = 13)	Fellow (n = 13)	p value*
CDVA (median)	20/80 (range 6/9 – 6/60)	20/20 (all)	<0.001
Spherical Equivalent	+1 to +8	-0.38 - +6.25	0.003
Arteriolar Saturation	85.4 (83.0-87.8)	80.8 (78.6-82.9)	0.013
Arteriolar diameter	125.7(119.5-131.9)	126.7 (118.2-135.2)	0.75
Venous Saturation	53.4 (51.1-55.7)	49.3 (45.7-52.9)	0.005
Venous Diameter	166.2 (158.5-173.9)	156.7 (147.1-166.3)	0.20
AVSD	32.2 (29.0-35.5)	31.5(27.9-35.0)	0.26

Table 2 - Demographics, oxygen saturation and diameter values of the amblyopia and fellow groups. *Wilcoxon signed rank test

Healthy Fellow Versus Normal Eyes

We found no differences between the normal fellow eyes and eyes in the normal group, also after correcting for age.

DISCUSSION

This to the best of our knowledge is the first study of retinal oximetry in amblyopic eyes and one of the few studies (Liu 2017) to report oximetry values in subjects below the age of 18 years. We found no differences between the amblyopia group and the control group or the fellow eye and the control group, however the saturations in the amblyopia group were significantly higher than those in the fellow eyes.

Wiesel and Hubel in 1963 (Wiesel & Hubel 1963) put forth an interesting theory by studying cortical changes in amblyopic eyes of kittens. They said that binocularly driven cells no longer respond to stimuli from the deprived eye and undergo a change in function, becoming monocularly driven by stimuli from the sound eye. There is no change in the number of monocularly driven cells responding to stimuli from the deprived eye but the reduction in the total cell population driven by this eye provides

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electrophysiological evidence of cortical changes occurring in amblyopia. This can be a possible explanation for the significant difference seen between the amblyopia and the fellow group, however the limitation of the small sample size must be kept in mind before interpreting results.

Amblyopia is a condition where we find decreased visual acuity in a structurally normal globe. We had also studied the vascular diameters of the vessels that were used for oximetry. Comparison between the groups showed no differences. The comparable vascular diameters in the three groups conform with this theory. This data was not included in the results because the preferred way to study the vascular diameters would be to use the method described by Knudtson et al. (Knudtson 2003)

A decrease in thickness can alter the measured light intensities as seen in the study by Mohan et al. (Mohan 2016) and thus alter the final calculated optical density ratio (ODR). Also as postulated in the study a thinner RNFL may imply a smaller capillary free zone and thus higher measured saturations due to decreased oxygen consumption. It thus needs to be kept in mind that the above observations could be due to a change in functional metabolism or could be due to an optical artefact. As stated earlier in the introduction the literature on RNFL changes in amblyopia does not yield a conclusive trend hence it is difficult to hypothesise an explanation for our findings based on structural changes.

One limitation of our study was that we did not have corresponding OCT measurements to see if our findings were artefactual or true. In future we will follow up patients under treatment for amblyopia and see if oximetry values return to normal in eyes that respond and remain elevated in non-responsive eyes. A higher number of unaffected fellow eyes also would help us confirm the findings of a compensatory increase in function of fellow eyes.

Another limitation of the study was that the amblyopic group was significantly younger than the normal group. Probably, children in the amblyopia group presented earlier to the paediatric clinic due to decreased vision compared to otherwise normal children presenting for a general check-up. In our normative database (Mohan 2015) on adults of the same population age showed a positive correlation with measured saturations however in this study age did not show any correlation with the oxygen saturation values. The spherical equivalent was very variable ranging from -7.8 to +10.1 in the amblyopia group and this could possibly result in artefactual alterations however previous studies on normal adults (Jani 2014; Mohan 2015) show no significant association.

Conclusion

In conclusion we find higher measured saturations in amblyopic eyes versus the fellow eyes. This observation could be due to altered neuronal activity or could be due to a measurement artefact due to alterations in retinal reflectivity.

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CHAPTER 7

Oximetry in retinal dystrophies

IOVS 2015

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Chapter 7

ABSTRACT

Purpose

To study the oxygen saturation profiles in retinitis pigmentosa and macular dystrophies and compare it to age-matched normals

Methods

In a cross sectional prospective study, 62 subjects with retinitis pigmentosa (RP), 23 with macular dystrophies and 78 controls were enrolled and retinal oximetry was performed with the Oxymap T1 retinal oximeter (Oxymap ehf, Reykjavik, Iceland). The images were analyzed for oxygen saturation and diameter of retinal vessels.

Results

All parameters showed a significant difference between the three groups. Patients with RP showed significantly lower diameters ($98.4\mu\text{m}$ and $136.9\mu\text{m}$ arteriolar and venous) ($p < 0.001$), higher saturations (102.3% and 59.1%) ($p < 0.001$; 0.06) and higher arterio-venous saturation difference (AVSD) (43%) ($p < 0.001$) compared to the other two groups. Macular dystrophies showed higher global arteriolar values (96.7%) and AVSD (41.6%) but comparable venous values (54.9%) to the control group (90.6%, 57.4% and 33.3%).

Conclusions

Oximetry is sensitive in quantifying hemodynamic changes in retinal dystrophies. It is still unclear whether these hemodynamic changes are a cause or a result of the disease process.

INTRODUCTION

Retinitis pigmentosa (RP) encompasses a large group of hereditary diseases of the posterior segment of the eye characterized by degeneration, atrophy and finally loss of photoreceptors and retinal pigment epithelium (RPE), leading to progressive visual loss. (Konieczka 2012) The prevalence of RP is estimated to be 1 in 4000 individuals, with a total of about two million affected persons worldwide. (Bunker 1984; Chizzolini 2011) The prevalence has been reported to be higher in the Indian population. (Sen 2008)

Hereditary macular disorders are characterized by defects of the cone photoreceptors or retinal pigment epithelium underlying the macula, and include Stargardt disease, cone dystrophy, cone-rod dystrophy and other maculopathies. (Roosing 2014) Many genes affecting the photoreceptors have been implicated in the pathogenesis of these diseases. (Konieczka 2012; Roosing 2014) Although the exact mechanism of cell death in RP is not known, oxidative stress is known to play a major role. (Komeima 2007)

Disturbed ocular blood flow has been described as another potential causative factor in RP. (Konieczka 2012) This is an interesting hypothesis as reduced ocular blood flow can either occur as a primary event and cause ischemia and tissue loss, or occur as a physiological secondary response to reduced tissue and demand. Konieczka et al have suggested that reduction in ocular blood flow in RP patients could be a primary event. (Konieczka 2012)

An early ocular hemodynamic finding in patients with RP is increased arterio-venous (A-V) transit time and reduced blood flow velocity which have been observed before any clinically detectable ocular pathology. (Wolf 1991) Doppler imaging has demonstrated that peak systolic flow velocities are decreased in both ophthalmic arteries and posterior ciliary arteries. (Cellini 2010) Interestingly this decrease in blood flow velocity was not confined to the ocular circulation. Systemic findings like reduced flow in the cutaneous capillaries of the finger and longer recovery time after cold provocation has been observed in RP patients. (Cellini 2010)

There is evidence that in RP patients, ocular blood flow is reduced beyond what is attributable to reduction secondary to retinal atrophy. Konieczka et al hypothesize that the primary ocular blood flow is reduced in RP patients due to Peripheral vascular dysregulation syndrome (PVD). (Konieczka 2012) Cellini et al have further demonstrated a disturbance in peripheral blood flow in addition to that in the eye in RP patients. (Cellini 2010) Reduced blood flow in the retina and an increase in the AV transit time has been observed before the appearance of any ophthalmoscopic signs. (Wolf 1991) In summary, the observation of an association with PVD syndrome and

peripheral vascular abnormalities and the increased AV transit time occurring early in the disease argue strongly in favour of vascular disturbances being a primary event. (Konieczka 2012)

Apart from blood flow velocity and vascular diameters, it is possible to measure the saturation of oxygen in the hemoglobin contained in the ocular vessels. A photo-spectrometric device measures oxygen saturation in a non-invasive manner. (Hardarson 2006) An increase in venous saturations and a resultant decrease in AVSD in eyes with RP have been reported. (Eysteinnsson 2014; Turksever 2014; Ueda-Consolvo 2015)

A large majority of genes associated with cone dystrophies are yet to be discovered; this hints towards the existence of unknown cone-specific or cone-sensitive processes. (Roosing 2014) No established evidence exists to implicate ocular blood flow abnormalities in the pathogenesis of cone dystrophies. It would therefore be interesting to study the ocular blood flow and oxygen saturations in cone dystrophies.

We aimed to study and compare the oxygen saturation profiles and vascular diameters in RP and macular dystrophies and compare them to age- matched controls.

MATERIALS AND METHODS

One hundred and fourteen consecutive patients presenting to the Retina department at Narayana Nethralaya, Bangalore, diagnosed with retinitis pigmentosa (RP), cone dystrophy or Stargardt disease were enrolled in the study. The diagnosis of these conditions was made based on the presentation, clinical features and electrophysiological examination. A detailed history was taken for every patient and patients with any cardio-vascular disease, diabetes, hypertension or history of migraine or using any ocular or systemic medications were excluded. The study followed the tenets of Helsinki. The Ethics Committee and Institutional Review Board of Narayana Nethralaya approved the study. All patients gave a written consent for the study.

All patients underwent a complete ophthalmic examination. These included measurement of the best-corrected visual acuity (BCVA), anterior segment examination, measurement of the intraocular pressures and a fundus examination. All of them underwent fundus photography, spectral domain optical coherence tomography (SD-OCT) and full- field electroretinogram (ERG). Patients younger than 18 years of age, smokers, with a previous history of trauma, nystagmus and poor media clarity were excluded from the study.

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This paper summarises the oximetry findings and the SD-OCT and ERG findings were used only for confirmation of diagnosis. They have not been included as it would confound the reader with too much information

Image Acquisition

Following dilation with 1% tropicamide and 10% phenylephrine, all patients underwent dual wavelength photospectrometric Oximetry (Oxymap T1 retinal oximeter, Oxymap, Reykjavik, Iceland).

The initial images were obtained after the patients were allowed to rest for 5 minutes to eliminate exercise-induced fluctuations in readings. Resting blood pressure and pulse oximetry (Silicon Labs) readings were measured in all patients. None of the subjects had consumed caffeine within 2 hours of the examination. The aiming light was set at the lowest setting, flash intensity was 50W, small aperture and large pupil settings were applied to the Topcon TRC 50DX Fundus camera.

One experienced photographer obtained standardized images for all the subjects. We obtained 2 images per eye of 50° field that were disc centred in all subjects. (Fig 1) We ensured that all the images analyzed were in sharp focus. To achieve this, we chose the best quality image between the 2 eyes in bilateral cases, which not only ensured quality, but also ensured that we take only one reading per patient eliminating duplication of data. In unilateral cases or cases with only one available image, the best quality image was taken. If no image appeared satisfactory, or the eye had measurable vessels in only one quadrant, that patient was excluded from the study (n=29). A circle was drawn concentric to the optic disc leaving 50 pixels from the disc margin. A second concentric circle was drawn twice the diameter of the first one. Vessel segments were analysed between these 2 concentric circles to ensure that retinal eccentricity is uniform. Retinal eccentricity and cell density may have an impact on measured retinal saturations. Images were analyzed by choosing the thickest arteriole and venule representative of that quadrant.

Fig 1 – Shows images oximetry images with the pseudo-color maps for retinitis pigmentosa (a), macular dystrophy (b) and normal (c). Note the arteriolar attenuation and loss of analyzable vessels when threshold set to 8 pixels.

The oxymap algorithm for calculation of oxygen saturation compensates for vessel width. The compensation by the software is similar to that suggested by Geirsdottir et al (Geirsdottir 2012). Analyzable vessels were defined as vessels greater than 8 pixels ($74\mu\text{m}$) in width. (Geirsdottir 2012) This value was chosen as a cut-off because of the possibility of inaccurate results for very thin vessels. Global averages were obtained by simple averaging of the values of the 4 quadrants.

Statistical Analysis

Statistical analysis was done using IBM SPSS v22.0. All parameters were tested for normality using the Shapiro-Wilk Test. Parametric data was analyzed using the One-way ANOVA with the Turkey test for post-hoc analysis. Non-parametric data was compared using the Kruskal-Wallis test and the Mann-Whitney U test for the post-hoc analysis.

RESULTS

There were a total of 23 eyes with macular dystrophies, 62 eyes with retinitis pigmentosa and 78 controls.

Non-measurable Vessels

Analyzable vessels were present in all four quadrants in the macular and control group. The RP group had 14 eyes without measurable arterioles in both the superonasal and inferonasal quadrants, 15 eyes without infero-nasal arterioles only, 5 eyes without supero-nasal arterioles only, 1 eye without supero-nasal venule and 3 eyes without infero-nasal venules. In all those quadrants where the venules were not measurable, the corresponding arterioles were definitely not measurable, although the reverse was not true. In 32 eyes, the arterioles were not measurable but the venules were measurable. In all, 34 eyes/62 (54.8%) had non-measurable vessels in the nasal hemifield.

In our study, we found that the nasal vessels attenuated earlier compared to the temporal ones. In those eyes with not measurable temporal vessels, the nasal ones were also not measurable. This accounted for the 7 out of the 29 eyes that we had to exclude since both the temporal and nasal vessels were not measurable.

Demographics

The average age in the macular dystrophy group was 20.8 ± 11.5 , RP group was 30.4 ± 16.7 and the control group was 32.4 ± 9.4 . Age matched non-smoking controls were chosen according to the RP group, as the numbers were larger in that group. The age distribution in the macular group was significantly different from the other 2 groups. No significant differences were found in the sex distribution. None of the patients had any refractive error beyond ± 0.50 D. The intra-ocular pressures (IOP) for both the RP group (mean 14.5, range 10-18) and the macular dystrophy group (mean 14.4, range 9-18) were within normal ranges. The results have been summarized in Table 1.

Parameter (n=163)	Macular (n=23)	RP (n=62)	Controls (n=78)	Significance
Age	20.8 ± 11.5 (15.8-25.8)	30.4 ± 16.6 (26.2-34.7)	32.4 ± 9.4 (30.3-34.5)	$<0.001^a$
Sex (M: F)	16: 7	38: 24	36: 42	0.066 ^b

Table 1 - Demographics

- Kruskal-Wallis test to compare the age distribution of 3 groups. Mann-Whitney U post-hoc test - Macular vs RP, $p = 0.005$; Macular vs Controls, $p < 0.001$.
- Chi-Square test to compare difference in sex

Global Saturations And Diameters

The global saturations and diameters have been summarized in Table 2. All parameters showed a significant difference between the three groups. RP group showed significantly higher saturations, lower diameters, higher AVSD and lower A-V ratios compared to both the groups. Macular dystrophies showed higher global arteriolar values and AVSD but comparable venous values to the control group. Macular and RP groups also showed statistically significant differences in the global arteriolar and venous saturations and diameters.

Parameter	Macular Mean (95% CI) (n=23)	RP Mean (95% CI) (n=29)	Controls Mean (95% CI) (n=78)	Significance*
Arteriolar Saturation (%)	96.7 (92.3-101.9)	102.0 (98.5-105.6)	90.6 (89.2-91.9)	<0.001 ^a
Arteriolar Diameter (μm)	116.5 (110.1-122.7)	107.0 (99.0-115.0)	122.4 (120.1-124.6)	<0.002 ^b
Venous Saturation (%)	54.9 (51.9-58.0)	59.1 (56.4-61.8)	57.4 (56.0-58.8)	0.056
Venous Diameter (μm)	158.4 (149.8-167.1)	148.9 (141.0-156.8)	160.4 (157.4-163.4)	0.02 ^c
Arterio-Venous Difference (%)	41.6 (37.9-45.3)	43.0 (39.8-46.2)	33.3 (32.1-34.4)	<0.001 ^d
AV Ratio [†]	0.74 (0.70-0.78)	0.72 (0.68-0.76)	0.76 (0.74-0.78)	0.09

Table 2 - Summarising the global saturations and diameters.

*Kruskal-Wallis test used to obtain significance for non-parametric data

†AV Ratio implies the ratio of the diameters

Mann-Whitney U post-hoc analysis:-

- a. RP vs Controls $p < 0.001$; Macular vs Controls $p = 0.005$;
- b. RP vs Controls $p = 0.001$;
- c. RP vs Controls $p = 0.004$;
- d. RP vs Controls $p < 0.001$; Macular vs Controls $p < 0.001$;

Quadrant Saturations and Diameters

RP group had the highest saturations and lowest diameters in all the quadrants. The values have been summarized in Table 3

Quadrant Comparison

The groups showed significant differences for arteriolar and venous saturation and diameter ($p < 0.001$; < 0.001) for all the quadrants. Only the nasal venous saturations did not show a statistical difference.

Post hoc test with Mann-Whitney U mainly showed that the RP group significantly differed from controls in all the parameters except the nasal venous saturations.

Macular dystrophies differed from the controls mainly in the infero-temporal arteriolar saturations ($p < 0.001$), the global arteriolar saturation ($p = 0.005$) and the global AVSD ($p < 0.001$)

Macular dystrophies and RP group differed mainly in the superior hemifield values for arteriolar values, but the venous values showed differences in both hemifields.

Quadrant	Parameter (n)	Macular (n=23)	RP (n=62)	Controls (n=78)	Significance*
ST	Art Sat (%)	89.3	103.9	88.3	<0.001
	Art Dia (μm)	127.3	99.9	132.9	<0.001
	Ven Sat (%)	53.3	60.8	56.3	<0.001
	Ven Dia (μm)	170.7	151.8	170.8	<0.001
	AVSD (%)	36.1	43	32.1	<0.001
SN	Art Sat (%) (43)	99.4	106.2	94.5	<0.001
	Art Dia (μm) (43)	107.9	93.7	110.3	<0.001
	Ven Sat (%) (61)	57.7	61.0	60.6	0.306
	Ven Dia (μm) (61)	141.9	120.9	144.7	<0.001
	AVSD (%) (43)	42.0	44.9	33.8	<0.001
IN	Art Sat (%) (33)	99.9	102.5	93.3	<0.001
	Art Dia (μm) (33)	102.7	97.7	111.0	0.001
	Ven Sat (%) (59)	57.6	61.1	60.7	0.166
	Ven Dia (μm) (59)	139.0	114.2	144.4	<0.001
	AVSD (%) (33)	42.3	42.2	32.5	<0.001
IT	Art Sat (%)	97.8	102.2	86.5	<0.001
	Art Dia (μm)	127.2	106.9	134.7	<0.001
	Ven Sat (%)	51.1	56.9	51.9	<0.001
	Ven Dia (μm)	181.4	159.5	181.0	<0.001
	AVSD (%)	46.6	45.2	34.6	<0.001

Table 3 - Summarising the Quadrant wise arteriolar saturations and diameters for the 3 groups Number of quadrants analysed for each parameter are given in the header row. Where the number differs from the number in the header, it has been indicated in the parameter box.

*Kruskal-Wallis test used to obtain significance for non-parametric data

DISCUSSION

This study represents one of the largest description of oxygen saturation profiles in eyes with RP, the first description in macular dystrophies and its comparison with RP.

A decrease in vascular diameters, an increase in arteriolar (104.1%) and venous saturations (60.0%) and AVSD (44.1%) in the RP group in comparison to macular and control groups were observed in the study. Türksever et al (Türksever 2014), Eysteinnsson et al (Eysteinnsson 2014) and Ueda-consolvo et al (Ueda-Consolvo 2015) equivocally confirm the decrease in vascular diameter. Türksever et al (Türksever 2014) have reported an increase in arteriolar saturation with a mean of 99.3% whereas Eysteinnsson et al (Eysteinnsson 2014) have found no change in RP with a mean of 91.7%. Venous saturation was increased in all three studies (58.0%-66.8%). Our study was equivocal in the venous saturation increase, but found an opposite trend in the AVSD. Türksever et al (Türksever 2014) have mentioned that the AVSD correlated positively with the central macular thickness and have hence attributed its decrease to the progression of retinal atrophy. Eysteinnsson et al (Eysteinnsson 2014) on the other hand have stated that both AVSD and retinal blood flow decrease in RP, and coupled with the decrease in choroidal blood flow result in decreased oxygen delivery from the retinal circulation. Both the groups indicate that the effects are secondary to the degeneration rather than primary. The alterations observed in the saturations and diameters could be a primary and resulting in the dystrophy or be secondary to the disease process. We may hypothesize that findings that occur early in the disease are more likely primary and ones that are seen in advanced disease may most likely be an effect of the disease process.

Macular dystrophies differed from both RP and control group in the global arteriolar saturations, with the arteriolar saturations being highest in the RP group. Venous saturations differed from the RP group but not from controls.

Several theories have been postulated to explain the arteriolar attenuation seen in retinitis pigmentosa. One such hypothesis is that the photoreceptor atrophy and death causes less oxygen utilization that results in higher oxygen partial pressures in the inner retina. This would then result in a reflex constriction and reduction in ocular blood flow, thus indicating that the vascular changes are secondary to localized disease process. On the contrary, Konieczka et al (Konieczka 2012) have stated that there is a high prevalence of peripheral vascular dysregulation (PVD) syndrome in RP patients, which causes alteration early in the disease. Patients with PVD syndrome react differently to stimuli like coldness, physical or emotional stress. There is dysregulation of vessels with vasospasm. Hence a system-wide vascular spasm that also manifests in the eye could

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be one explanation whereas tissue loss (Konieczka 2012), atrophy and thus a decreased demand could be the other hypothesis. The above theories may probably explain why as many as 54.8% of eyes in the RP group lacked measurable vessels in the nasal hemifield. Expectedly there was no significant difference in the vascular diameters between the macular and control groups.

There exists a capillary free zone around larger vessels that derive oxygen directly from them. (Kur 2012) It has been shown that translocated cells of the retinal pigment epithelium can deposit a thick layer of extracellular matrix around retinal vessels. (Li 1995) This can effectively block oxygen diffusion out of the vessels and explain the high saturation seen in the arterioles but the unknown effect of these cells and the proximity of the measured segments to the optic disc throw enough doubt on this theory.

The thickness of RNFL is known to be decreased in RP. (Garcia-Martin 2012; Oishi 2013) In our own study (Mohan 2016) we found an inverse correlation between vascular saturation and perivascular retinal nerve fiber layer thickness in normative Asian-Indian eyes. This could explain our observation of increasing arteriolar and venous saturation.

In a normal eye, arteriolar saturations can be expected to increase when there is more demand and less oxygen tension in the inner retinal tissue. This phenomenon though will have to be accompanied by a corresponding increase in vascular diameters because that is the only established way the inner retina can increase oxygen delivery by physiological hyperemia. (Kur 2012) The arteriolar attenuation that we observed in the RP group makes this response unlikely.

An increase in arteriolar saturation can hypothetically cause a corresponding increase in venous saturation. The venous saturation can also increase due to less utilization secondary to tissue atrophy in RP. This possibly explains the increase in venous saturation seen in our patients and is similar to that noted in the other studies.

Oxygen utilization by tissue is the product of AVSD and blood flow. (Kur 2012) Blood flow is heavily dependent on the vascular diameters. It is known that the A-V transit time is increased and vascular diameters are decreased in RP, thus resulting in very low volume of blood flow. Hence for a given amount of oxygen extraction, the AVSD would increase if the blood flow decreases. This is accompanied by a decreased choroidal blood flow in RP. (Cellini 2010) We could possibly imply that even though tissue atrophy and death would cause lower oxygen demand, in view of the fall in blood flow, AVSD would have to increase to adequately meet the demands of the residual functioning retina. In addition, hypoxic tissue would extract more oxygen per unit volume of blood

(Eysteinnsson 2014). This could possibly explain the increased AVSD seen in the RP group in our study.

An alteration in oxygen saturation profiles was seen in all quadrants in the RP group but mainly in the infero-temporal quadrant in the macular group. The lower saturations in the infero-temporal venules in normative individuals has been attributed to its anatomic location in relationship to the optic disc. (Jani 2014) In macular dystrophies, it stands to reason that the macular blood supply is the most affected compared to those with RP, thus contributing to the altered oxygen saturation profiles that we noted in the infero-temporal quadrant.

Our study is limited by the relatively small sample size we had in the macular dystrophy group. Another limitation was that blood pressure was not measured, but it is assumed that there was no difference in blood pressure between those with retinal degeneration and age matched controls, given the relatively young age of the subjects included in the study. The current approach of our study excludes vessels narrower than 8 pixels or 74 microns, hence we cannot comment on vessels that were narrower than this cut off and therefore not measurable. A correlation of oxygen saturation profiles with RNFL thickness could throw further light on the reasons for our observations. A correlation with electro-retinographic findings can in the future help us obtain a better correlation between oximetry values and disease severity.

This study represents the largest study so far reported on retinal dystrophies and establishes the increase in arteriolar, venous saturations and AVSD increase. It is important to ascertain whether the hemodynamic observations are primary or secondary. If it is secondary it will only help us establish severity; if primary it have a multitude of therapeutic implications. It has also been shown that the central defects progress slower with nilvadipine (Nakazawa 2011) which is a calcium channel blocker (CCB). Magnesium, omega-3 fatty acids and fludrocortisone have all been hypothesized to help in the treatment of retinitis pigmentosa by altering the hemodynamic parameters. (Konieczka 2012) If these above methods prove to be effective, retinal oximetry can be used as a non-invasive tool to identify potential patients who might be benefitted and subsequently monitor response to treatment.

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CHAPTER 8

Oximetry in CRAO

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ABSTRACT

This study aims to retrospectively study the retinal vessel oxygen saturation in eyes with central retinal artery occlusion (CRAO).

The 11 consecutive patients included underwent retinal oximetry (Oxymap T1) in addition to a complete ophthalmic examination. Arteriolar and venous saturations and diameters were the main outcome measures.

The oxygen saturation was decreased compared to normals in both arterioles (83% vs 94%, $p=0.036$) and venules (47% vs 58%, $p=0.046$). The arterio-venous saturation difference (36% vs 36%) was not significantly different. An immediate increase in both arterial and venous saturations was seen in the 2 patients who underwent paracentesis. Visual acuity however had no correlation with the oximetry findings

In conclusion, oximetry in CRAO shows changes compared to age-matched controls but does not correlate with visual acuity outcomes. It can be used to understand physiological alterations in retinal perfusion in CRAO but it however does not provide reliable predictors for clinical outcomes.

INTRODUCTION

The retina is one of the most metabolically active tissues of the human body (Kur 2012). Therefore, an adequate supply of oxygen is extremely important for the proper functioning of the tissue.

Central retinal artery occlusion (CRAO) as the name suggests is blockage of blood flow through the central retinal artery. The incidence of acute CRAO at a tertiary referral centre is approximately 1 in 10,000 outpatient visits which may be lower in the general population at 0.85 cases per 100,000 per year (Rumelt 1999). The central retinal artery is the principal blood supply of the retina. It enters the eye through the optic disc and mainly supplies the inner retina through the superficial and deep capillary plexus. The involvement of inner retinal layers and relative sparing of the outer retinal layers in CRAO has been described already in 1971 (Hayreh 1971). Based on these findings, we would expect to see a drop in oxygen saturation levels in the entire inner retinal circulation in case of CRAO. Previous studies by Hardarson et al. (Hardarson 2013a; Hardarson 2013b) and Hammer et al. (Hammer 2009) have shown that oxygen saturations in CRAO are rather variable varying from a marked decrease to not very different from normal. However, these studies were limited by their small sample size (5 subjects).

Since determination of oxygen saturation is non-invasive and easily repeatable, it could serve as potential biomarker predicting visual outcomes in retinal vascular disorders.

In this paper we present retinal oximetry findings in a series of Asian-Indian patients who presented at our hospital and were clinically diagnosed to have CRAO.

METHODS

This prospective observational case series was approved by the institutional review board and was carried out following the tenets of declaration of Helsinki. An informed written consent was obtained from every patient.

Patients presenting to our hospital with history of sudden painless decrease in vision and diagnosed to have CRAO on clinical examination were included in the study. Typical clinical features include the presence of a cherry red spot, retinal whitening and box-carring of retinal vessels due to stasis of blood. Patients who underwent prior ocular treatment elsewhere in the form of ocular massage or paracentesis were excluded (pre-imaging exclusion). A detailed systemic evaluation including routine blood tests was performed by our internist; serum homocysteine and carotid doppler were done in select cases. These reports have not been included in the study due to the risk of adding too many parameters for a small sample size. All patients underwent complete ophthalmic evaluation including best corrected visual acuity (Snellen), intra-ocular pressure measurement (Goldmann tonometry), slit lamp evaluation and fundus examination. Retinal oximetry (Oxymap T1, Oxymap hf, Reykjavik, Iceland) was performed after pupil dilation with 1% tropicamide and 10% phenylephrine.

Each patient was allowed to rest for 5 minutes before the images were captured. This was done to eliminate exercise-induced fluctuations in readings. Resting blood pressure and pulse oximetry (Silicon Labs) readings were taken for all patients. None of the subjects had consumed caffeine within 2 hours of the examination.

The aiming light was set at the lowest; small aperture and large pupil settings were applied and flash intensity was set to 50Ws on the Topcon TRC 50DX fundus camera.

One experienced photographer obtained standardized images for all the subjects. Fifty-degree disc centred images in sharp focus were used for all subjects. Eyes with poor image quality (less than 6.0 on the automatic image quality analysis – Oxymap Analyzer 2.5.1) due media opacities or non-cooperation of patients were excluded from the study (post-imaging exclusion).

Anterior chamber paracentesis was performed in a minority of patients who consented to this procedure. Oximetry was done one hour before and 3 hours after paracentesis in 2 patients.

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Oxygen saturations and vessel diameters were determined separately for each quadrant and vessel type (arteriole, venule). Global values were obtained by simple averaging of the quadrant values. Calculations were done as described by the authors in a previous study (Mohan 2015).

Data obtained from involved eyes were compared with fellow eyes and also with eyes from age-matched normals, obtained from our normative database (Mohan 2015). After assessing for normality using the Shapiro-Wilk test, comparison between involved and fellow groups was done using the paired T-test. An independent T-test was used to compare involved and normal groups. For comparison between involved and fellow groups, only those involved eyes which had corresponding fellow eye measurements were included. All involved eyes were included for comparison between involved and normal groups.

RESULTS

A total of 20 eyes of 20 patients were eligible. 4 patients were excluded because of pre-imaging exclusion and 5 because of post-imaging exclusion. Hence 11 eyes of 11 patients were included and statistically analysed.

The average age was 54.3 (range 22-75) years and the male-female ratio was 7:4. The median BCVA was 20/1200 (range -perception of light to 20/60) on Snellen's acuity chart for distance. The average duration from onset of sudden painless decrease in vision to oximetry was 4.4 days (1-10 days). The demographics have been summarized in Table 1.

Oximetry in the fellow eye of 3 patients could not be assessed due to poor image quality.

There was no significant difference in the image quality between the three groups (6.9 in involved, 7.2 in normals [$p = 0.28$ vs involved] and 7.0 in fellow [$p = 0.6$ vs involved]). The global arteriolar (Fig. 1) and venous (Fig. 2) saturations in the involved group (respectively 83.0% and 47.1%) were lower as compared to the normal group (respectively 94.4% and 58.3%) (respectively $p=0.048$ and $p=0.058$). We found no differences between the affected and fellow eye; $p=0.16$ $p=0.093$ for arteriolar and venous saturation respectively. The arteriolar and venous diameters in the involved group were $120.1\mu\text{m}$ and $159.8\mu\text{m}$ respectively and were statistically non-significantly smaller as compared to the fellow and normal groups. All values have been summarized in Table 1. A comparison of the individual saturations of the involved and fellow eye, visual acuity at presentation and time to oximetry for each patient has been summarized in Table 2. There were no significant differences between the fellow eye and age matched normals.

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Fig 1 (left) & 2 (right) – Box plot demonstrating the variability in the global arteriolar and venous saturation distributions respectively among the patients in the 3 groups.

Parameter	Involved (I) (n=11)	Fellow (F) (n=8)	Normal (N) (n=12)	I vs F* p value	I vs N‡ p value
Age (years)	54.3 (44.8-63.8)	55.6 (48.4-62.8)	55.4 (52.1-58.7)	1.00	0.796
Sex (M:F)**	7:4	4:4	6:6	1.00	0.51
Arteriolar Saturation	83.0 (72.2-93.8)	95.0 (89.9-100.2)	94.4 (90.1-98.8)	0.158	0.036
Arteriolar Diameter	120.1 (110.2-131.5)	119.1 (104.0-134.3)	127.1 (116.3-137.9)	0.755	0.372
Venous Saturation	47.1 (36.1-58.1)	59.1 (52.3-66.0)	58.3 (53.4-63.1)	0.093	0.046
Venous Diameter	159.8 (146.1-173.6)	158.0 (145.7-170.3)	159.5 (152.1-166.9)	0.772	0.963
Arterio-venous saturation difference	36.0 (27.3-44.7)	35.6 (29.2-42.0)	36.4 (33.7-39.2)	0.878	0.917

Table 1 - This table summarises the demographics and the global saturations (%) and diameters (μm). The figures represent the mean values and the ones in brackets represent the 95% confidence interval of the mean.

*p values are calculated using the paired students t-test. (I vs F – Involved eyes (8) and their corresponding fellow eyes (8) are compared); ‡p values are calculated using the independent students t-test (I vs N – all involved eyes (11) are compared with age matched normals(12)). **For sex distribution, the chi-square test was used to calculate the p-value.

Fig 4 – Oximetry pseudocolor image showing pre and post paracentesis of CRA01 (a & b) & CRA07(c & d). Note the improvement in saturation (according to pseudocolor map)

Paracentesis was performed in 6 out of 11 patients and 2 of them underwent oximetry post paracentesis; the remaining 4 did not undergo post paracentesis imaging. These 2 patients (CRA01 and CRA07) showed an immediate improvement in arteriolar and venous saturation values post paracentesis. (Fig. 3 & 4) The visual acuity however improved marginally in CRA07.

There was no correlation between the oxygen saturation parameters and the duration since onset of symptoms.

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Fig 4 – Oximetry pseudocolor image showing pre and post paracentesis of CRA01 (a & b) & CRA07 (c & d). Note the improvement in saturation (according to pseudocolor map)

Patient ID	Age*	Sex†	Onset‡	BCVA	IA	IV	FA	FV	Systemic
CRA01	75	1	1	1/60	73	36	-	-	Old MI
CRA02	45	2	7	1/60	102	75	99	71	RA Factor +ve
CRA03	55	1	7	PL	68	28	-	-	Hypertension (HTN)
CRA04	46	1	5	6/60	93	59	98	53	Hyperhomocysteinemia and dyslipidemia
CRA05	54	1	2	1/60	92	61	95	62	Nil
CRA06	55	2	3	1/60	94	61	91	59	APLA syndrome
CRA07	70	2	10	6/18	77	22	83	45	Diabetes mellitus (DM) and arthritis
CRA08	63	1	3	2/60	87	38	95	64	DM, HTN
CRA09	61	2	3	1/60	81	40	95	65	Dyslipidemia
CRA10	51	1	2	1/60	47	41	104	54	Nil
CRA11	22	1	5	1/60	99	57	-	-	Nil

Table 2 - Summarising the individual saturations of the involved and fellow eye, visual acuity and time to oximetry for each patient.

*Age in years; †1=male, 2=female; ‡Days; IA: Involved eye arteriolar global; IV: Involved eye venular global; FA: Fellow eye arteriolar global; FV: Fellow eye venular global. PL: perception of light

DISCUSSION

Our study shows decreased arteriolar and venous saturations at presentation in eyes with CRAO compared to normal eyes. To the best of our knowledge, this study has the largest sample size till date and is also the first to demonstrate the effect of anterior chamber paracentesis on oxygen saturations.

Previous reports by Hardarson et al. (Hardarson 2013a; Hardarson 2013b) described 4 patients with variable measurements. Their study included 2 patients with oximetry one day after the insult, one patient 2 weeks later and the last one a month after the insult. Patients presenting early had lower saturations and the ones presenting late had saturations close to 100%. This finding is in line with our study that also shows lower saturations at early presentation (< 2 weeks). In another report by Hammer et al. (Hammer 2009), a mixed case series of central and branch arterial occlusions showed a reduction in arterial saturations that increased with 5 days of pentoxifylin therapy. An average arteriolar saturation of 91% was reported in the involved eyes. This study has a larger sample size as compared to the above two studies.

This study is limited by several factors. It is retrospective in nature, incomplete in not having contra-lateral eyes images of all subjects and has no follow up measurements. The patients presented at variable times after the insult. Convincing a patient for and performing an invasive investigation which is not necessarily going to change the treatment course but may predict prognosis is not possible in every patient. Fundus fluorescein angiography was not included as part of the protocol and hence perfusion by a cilioretinal artery was not documented.

In our study, patients were imaged at an average of 4.4 days following the occlusion. In many patients spontaneous re-canalisation after 72 hours is possible, however this period of ischemia has a high risk of apoptosis and cell death of inner retinal cells. Hence, we may observe patients with retinal saturations returning to normal but no improvement in visual recovery. Imaging as soon as possible after onset would have been ideal to draw a valuable inference. There was also no correlation observed between the oxygen saturation parameters and the duration of onset of symptoms.

We feel that the arteriolar saturation (83.0%) observed in our study is interesting as it is significantly lower than the normal group, but has not fallen to an absolute zero as can be expected in a global cut off of the blood supply. The reasons for this observation could be diffusion of oxygen from the still perfused choroid or recanalisation of retinal

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arterioles and restoration of part or whole of the blood supply. A transient nature of occlusion may also explain why there still exists measurable saturation in the vessel. We can also hypothesize that part of the retinal tissue would die, and this would result in decreased consumption. This may result in a new balance of decreased blood flow and decreased consumption.

The average arteriolar diameter in the involved group was lesser as compared to the normal group but the values were not statistically significant. The venous diameters were almost the same.

A capillary free zone (Kur 2012) surrounding the arterioles and venules is known to exist where oxygen exchange takes place. There is widespread edema of the inner retinal layers (Hayreh 1971) especially the retinal nerve fibre layer where this capillary free zone exists. We hypothesize that this edema could limit the exchange of oxygen and hence result in the retention of oxygen in the vessels despite drop in blood flow. The presence of retinal edema also could have played a role by loss of contrast. The algorithm for measurement of oxygen saturation considers the pixels neighboring the vessels, hence the alteration of contrast may also result in artefactual values.

Two of our patients who underwent paracentesis showed visible improvements in the pseudo colour maps with well-defined measurable vessels and an improvement in saturation values. This could be an important finding and may prove a predictor of the effectiveness of paracentesis in eyes with CRAO. In the above two patients both the arteriolar and venous saturations increased and the AVSD remained almost the same. If the diameters in both the eyes decrease after paracentesis, we can hypothesise that this could well be a measurement artefact, but this is unlikely as we saw an increase in diameters in one patient and a decrease in the other. The marginal improvement seen in vision could be incidental. Our interpretations and analysis though are based on static images and observing dynamic images indicative of blood flow should give us a better understanding.

We also used phenylephrine for dilatation in our study and are unaware if this affects retinal vessel oxygen saturation in any way. The other published normative databases by Jani PD et al, Geirsdottir et al and our own data have all used phenylephrine. A large percentage of eyes (45%) were excluded based on image quality, as a result there is no difference in image quality between the involved and other groups. This we feel has improved the reliability of our results.

In conclusion, this study describes the changes in retinal oximetry seen in eyes with CRAO and highlights the changes seen with anterior chamber paracentesis. There was importantly no correlation seen with visual acuity. In future, studies with a larger sample size that follow the patients over time at regular intervals and link the oximetry findings with functional outcomes can provide valuable information and may help to define the role of retinal oximetry in the management of central retinal arterial occlusion.

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CHAPTER 9

Oximetry in BRVO

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ABSTRACT

Purpose

To study the retinal oxygen saturation profiles in acute and chronic branch retinal vein occlusion (BRVO).

Methods

Eighteen consecutive patients, average age 54y (33-74) (M: F 1:1) with confirmed branch retinal vein occlusion on fundoscopy underwent oximetry. The patients were divided into two groups: acute (Group 1) and chronic (Group 2) based on history of duration of disease and clinical presentation. Peripapillary vessel segments were chosen and studied for oxygen saturation (%) and diameter(μm), both quadrant and global.

Results

There was no difference in the median corrected distance visual acuity (CDVA) between the 2 groups. Global arteriolar oxygen saturations ($p=0.01$) were higher in Group 1 (105.8%) than in Group 2 (99.8%). Global venous oxygen saturations (Group 1 62.7%; Group 2 60.1%) and arterio-venous oxygen saturation difference (AVSD) (Group 1 43.3% and Group 2 39.8%) showed no significant difference. On quadrant comparison, arteriolar oxygen saturations ($p=0.001$) and arterio-venous oxygen saturation difference (AVSD) ($p=0.038$) were significantly higher in Group 1 whereas the venous oxygen saturations were higher ($p=0.035$) and diameters were lower ($p=0.004$) in Group 2. The other eye showed significant higher values in the global arteriolar and AVSD as compared to normals. ($p<0.01$).

Conclusions

In the acute stage arteriolar and AVSD oxygen saturations were increased. An increase in venous oxygen saturation and a decrease in venous diameter accompanied by near normal arteriolar oxygen saturations occurred late in disease.

INTRODUCTION

Retinal vein occlusion (RVO) is a common, sight-threatening retinal vascular disorder. Branch retinal vein occlusion (BRVO) is an occlusion of either a major branch retinal vein draining one quadrant of the retina, a macular branch vein draining a portion of the macula, or a peripheral branch vein draining a portion of the retinal periphery, of which the prevalence ranges from 0.6% to 1.1%. (Ehlers & Fekrat 2011) Known risk factors for RVO are age, systemic vascular disorders such as diabetes mellitus, hypertension, ocular hypertension and glaucoma (Ehlers & Fekrat 2011) These systemic disorders may put the patient at an increased risk of BRVO in the fellow eye. A landmark study found that a BRVO in one eye portends a 10% risk of any RVO in the fellow eye within 3 years following the initial event. (Group 1986)

The common clinical presentation of acute BRVO is a segmental distribution of flame-like, dot blot haemorrhages, soft and hard exudates, signs of leakage and ischemia, and macular edema. At a later stage vessel changes like obliteration of veins are seen, these changes are represented as vascular sheathing and development of venous collaterals (Rehak & Rehak 2008) In addition, retinal neovascularization occurs in 36% of the eyes with an area of non-perfusion greater than 5 disc diameters. (Group 1986) Areas of non-perfusion leads to retinal hypoxia. In experimental BRVO, the hypoxic drive was shown to be present in the inner retina and low partial pressures (pO_2) in the pre-retinal vitreous. (Pournaras 1990) A greater area of ischemia leads to higher levels of vascular endothelial growth factor (VEGF) at the retina and vitreous cavity that increases the risk of macular edema and retinal neovascularization with vision loss as a consequence (Noma 2008; Rehak & Rehak 2008)

Measurement of retinal oxygenation in patients with BRVO may help to assess, in a non-invasive manner, the severity of the ischemia. It could in addition to other signs of ischemia predict prognosis which may enable a customized antiVEGF or retinal photocoagulation treatment. (Hardarson & Stefansson 2012) A recent study performed in ischemic and non-ischemic BRVO showed an increase in oxygen saturation mainly in the occluded and central arterioles; oxygen saturation changes in venules was not found to be significant. (Lin 2016)

The primary aim of the study was to evaluate the changes in vascular oxygen saturation in BRVO from acute to chronic in the different quadrants of the retina. We compared retinal vascular oximetry values with those in fellow eyes and eyes of age matched normal individuals. As a secondary aim we investigated the association between central foveal thickness (CFT) on spectral domain optical coherence tomography (SD-OCT) with oxygen saturation.

METHODS (FIG. 1)

Twenty-seven consecutive patients presenting to our retina outpatient department diagnosed with BRVO on fundoscopic evaluation were included in this cross-sectional observational study. Some of the patients came with complaints of vision loss, while in others the occlusion was observed incidentally during a routine check-up. Patients with a known history of ocular diseases such as uveitis, glaucoma or diabetic retinopathy, or a history of prior ocular surgery or trauma were excluded from the study.

Corrected distance visual acuity (CDVA) was recorded for all patients. All patients underwent a complete ophthalmic evaluation including slit lamp evaluation, Goldman applanation tonometry (GAT) and a dilated fundus evaluation. Patients with any abnormalities like significant cataracts, IOP higher than 21mm Hg on GAT or any retinopathy detected on fundus evaluation were excluded from the study.

Fig 1 – showing the color fundus images of acute and chronic group (a & b), the corresponding oximetry images with the pseudo-color map (c & d) and the corresponding macular SD-OCTs (e & f).

The patients were divided into 2 groups – Acute (group 1) and Chronic (group 2), based on clinical fundus evaluation. Patients were assigned to group 1 when fundus examination showed fresh haemorrhages with absence of collaterals. In the presence of venous collaterals, neovascularization or vascular sheathing patients were assigned to group 2 (Fig. 2). (Rehak & Rehak 2008) Age matched controls (group 3) were included from our normative database of 98 patients who were healthy normal controls who had come for a routine eye evaluation. They had no visual complaints. The eyes were perfectly normal with no detectable pathology except for spherical refractive error less than $\pm 6D$. They were normotensive nondiabetic patients.

After giving informed consent in writing, patients underwent retinal oximetry (Oxymap T1 retinal oximeter, Oxymap hf, Iceland). All patients were dilated with 1% tropicamide and 10% phenylephrine and were seated for 15 minutes before the test. Light and camera settings were standardized and kept constant for every patient. (50Ws flash intensity, small aperture setting). (Mohan 2015) Disc centered 50 degree images were taken and used for analysis.

Fig 2 – showing the flowchart of the patients depicting the different groups they land in and the tests performed

Images with hazy media, defocus or extensive haemorrhages extending within $50\mu\text{m}$ of the optic disc were excluded. The images which were hazy due to poor media clarity or defocus were excluded. Images which had extensive haemorrhages which left us with no measurable vessels in the quadrant were also excluded. Very few images had to be excluded in the control group as all the patients had clear media and no retinal pathology. Thus, only images due to defocus during image acquisition had to be excluded in the control group. Quadrant oxygen saturations were obtained by selecting the thickest arteriole and venule per quadrant which were then subsequently averaged to give us the global oxygen saturations. The length of the measured segment was $100\mu\text{m}$. We left $50\mu\text{m}$ from the edge of the optic disc and then traced the vessel. If an adequate length ($> 100\mu\text{m}$) could be obtained between the branch and $50\mu\text{m}$ from the optic disc we chose the segment, else we traced the vessel distal to the branching. Difference in the oxygen saturations of arterioles and venules in the respective quadrants gave us the arterio-venous oxygen saturation difference (AVSD) for the quadrant. CFT obtained from the thickness map of the SD-OCT macular scan (Spectralis, Heidelberg, Germany) was correlated with retinal vascular oxygen saturation and logMAR visual acuity for both the groups.

Both global and quadrant analysis were performed. In global analysis the global oxygen saturation values of three groups were compared. For quadrant analysis three quadrants were compared – the involved quadrant (I); opposite quadrant of the involved eye (O) and same quadrant of the age matched normal eye (NQ). (Yang 2017) Here, the opposite quadrant of the involved eye was defined as infero-temporal in cases of supero-temporal occlusions or vice-versa. Global oximetry values of the unaffected contralateral eyes when possible (subject to the patients granting consent and the imaged contralateral eyes having adequate image quality) were also compared to age matched controls.

Image quality measurements were also compared between the normal and contralateral groups, but not for the affected group as the haemorrhages may render the value (image quality) unreliable.

Statistical analysis was done using the IBM SPSS v22. All recorded values were tested for normality using the Shapiro-Wilk test. Normally distributed data was compared using the One-Way ANOVA with the Tukey post-hoc test. Pearson's test was used for correlations. Non-parametric data was compared using the Kruskal-Wallis test and the Mann-Whitney U test for the post hoc analysis. Spearman's tests were used for correlations. Chi-square test was used to compare nominal data between groups.

RESULTS

Of the 27 eyes imaged, 9 eyes were excluded due to poor image quality

Demographics

All subjects were of Asian Indian ethnicity. There were 18 eyes of 18 patients included in the study, 10 in group 1 and 8 in group 2. Group 3 contained 10 eyes of 10 different individuals whose right eye was included. There were no differences between the age and gender distributions of the 3 groups. Half of the patients affected with BRVO were hypertensives, which was significantly different from the control group ($p=0.036$). The median CDVA was 6/14 (range 6/9-3/60) in group 1 and 6/18 (range 6/6-6/60) in group 2, being significantly different from the control group 3 ($p<0.001$). Demographics are summarized in Table 1.

Parameters	Acute (n=10)	Chronic (n=8)	Controls (n=10)	p value
Age (years)	56.5 $\pm$ 8.4	50.4 $\pm$ 9.4	55.6 $\pm$ 4.1	0.21*
Sex (M: F)	6:4	3:5	6:4	0.56 [§]
CDVA	6/14 (6/9-3/60)	6/18 (6/6-6/60)	6/6 (6/6-6/6)	<0.001 [†]
Duration(weeks)	3.9 $\pm$ 2.0 (2.1-8.0)	59.1 $\pm$ 38.3 (13.1-108.7)	-	0.002 [‡]
CFT (μ m)	416.4 $\pm$ 196.0 (180 - 874)	232.4 $\pm$ 27.7 (208 - 275)	228.0 $\pm$ 14.1 (207 - 251)	0.032*
Location (ST:IT)	9:1	6:2	-	0.40 [§]
Hypertensives	5	4	0	0.036 [§]
Diabetics	2	2	0	0.32 [§]

Table 1 – Demographics of the 3 groups

M: F – Male: female ratio; CDVA – Corrected distance visual acuity on Snellen's chart; ST:IT – No of eyes having supero-temporal involvement vs infero-temporal. Numbers in brackets indicate range. CFT – Central foveal thickness.

*One-way ANOVA

§ Chi-square test

† Kruskal-Wallis test (post-hoc Mann-Whitney U test: Acute vs Controls, Chronic vs Controls $p<0.001$, Acute vs Chronic $p = 0.576$)

‡ Independent samples student's t-test for parametric data for comparison of means

Since retinopathy does affect the retinal oximetry, we specifically looked for it in the four patients with diabetes. They all had Type 2 diabetes of recent onset – less than 3 years - and showed no signs of retinopathy.

Global Comparison

Table 2 shows both global arteriolar and venous oxygen saturations as well as the global arterio-venous oxygen saturation in the three groups. The values were the highest values for group 1, although statistically significant differences were observed only for the arteriolar oxygen saturations ($p=0.01$). Post-hoc test revealed that group 1 differed significantly from group 3 ($p = 0.007$).

Parameters	Acute (1) (n=10) (95% CI)	Chronic (2) (n=8) (95% CI)	Normals (3) (n=10) (95% CI)	p value*
Global Arteriolar (%)	105.8 ± 9.3 (99.1 – 112.5)	99.8 ± 8.8 (92.4 – 107.2)	93.4 ± 6.6 (88.7 – 98.1)	0.01 [§]
Global Venous (%)	62.7 ± 5.1 (59.1 – 66.3)	60.1 ± 11.1 (50.9 – 69.4)	57.2 ± 7.9 (51.6 – 62.8)	0.334
Global AVSD (%)	43.3 ± 7.9 (37.6 – 49.0)	39.8 ± 9.5 (31.8 – 47.7)	36.5 ± 4.4 (33.4 – 39.7)	0.142

Table 2 – Global arteriolar, venous oxygen saturations and arterio-venous oxygen saturation difference (AVSD) in acute, chronic and controls

* Significance values obtained from One-way ANOVA

§ Post-hoc test (Tukey) – Acute vs Controls $p = 0.007$

Quadrant Comparison

Table 3 shows the results of the quadrant analysis in which three quadrants were compared - the involved quadrant (I); opposite quadrant of the involved eye (O) and same quadrant of the age matched normal eye (NQ). This comparison was done separately for group 1 & 2. Arteriolar oxygen saturations ($p=0.001$) and AVSD ($p=0.038$) were significantly increased in group 1 whereas the venous oxygen saturations were significantly increased ($p=0.035$) and venous diameters were decreased ($p=0.004$) in group 2.

Contralateral Eye

The contralateral eye of 12 patients showed arteriolar, venous and A-V difference oxygen saturations of 99 ± 6 , 59 ± 7 and 40 ± 5 respectively. The arteriolar and A-V difference oxygen saturations were significantly higher than normals ($p=0.007$ and 0.003). Image quality was comparable between the two groups ($7.5, 7.6$; $p=0.42$)

Central Foveal Thickness (cft)

The mean CFT (μm) was 416.4 ± 296.1 (ranging from 180-978 μm) in group 1 (10/10 eyes) and 232.4 ± 27.7 (range 208-275) in group 2 (5/10 eyes) ($p=0.05$). In group 1 CFT did not correlate with any oxygen saturation values. In group 2 CFT correlated with venous oxygen saturations (spearman's correlation, $r = 0.904$, $p=0.035$) and AVSD ($r = -0.933$, $p=0.021$)

DISCUSSION

To the best of our knowledge this is the first study on oxygen saturations in branch retinal vein occlusions where acute and chronic have been differentiated and studied. Our study shows a clear trend with raised global arteriolar, venous and A-V difference oxygen saturations in the acute and chronic stages compared to normals. In the quadrant analysis we found that in the acute stage mainly the arteriolar and arterio-venous difference oxygen saturations were higher whereas in the chronic stage the venous oxygen saturations were higher and diameters were significantly decreased compared to those in controls. The fellow eyes also showed an increase in arteriolar and arterio-venous difference oxygen saturations compared to normals.

We measured the thickest available arteriole and venule in each quadrant. However, it may not always be the thickest venule which would be affected in an occlusion. The thinking behind measuring the oxygen saturation in the thickest "available" vessels was that they would be representative of majority of the blood flow in that quadrant. Our global values hence could be indicative of the true metabolic response of the retina to the occlusion event and could reflect the metabolic derangements taking place.

We are now faced with two possible situations – the first one where the thickest "available" venule is the affected venule and the second one where a smaller or larger venule is the affected one. In the first situation, we would have no confusion with our method as we would be measuring the oxygen saturation in the affected vessel, however we may not be getting the true picture of the metabolism in that quadrant. In the second situation where the affected venule is a smaller or larger one, measuring the oxygen saturation in the largest available vessel would be most representative of majority of the blood flow of that quadrant and hence of the metabolism in that quadrant. We have no guaranteed way of determining which is the involved vessel without performing an angiogram. Being an invasive investigation, this was not performed in all patients. Choosing vessels based on a subjective feeling of which

vessel was involved can lead to bias and non-uniformity in results. We hence chose to have a uniform protocol for all eyes which was to measure the thickest available venule and arteriole in each quadrant.

Hardarson et al. (Hardarson & Stefansson 2012) reported that the oxygen saturations were variable and may reflect variable severity of disease, degree of occlusion, recanalization, collateral circulation, tissue atrophy, arteriovenous diffusion or vitreal transport of oxygen. Hypoxia was seen in some eyes but not in others.

Lin et al (Lin 2016) report an increase in only arteriolar oxygen saturations in ischemic BRVO; no changes were seen in non-ischemic BRVO. From the description of the fundoscopic findings in their study, most of the subjects appear to have an acute BRVO. These findings are very similar to what we have seen in our study as well.

The trend of raised global arteriolar, venous and arterio-venous difference oxygen saturations as observed in our study are contrary to what we would expect in the event of an occlusion. Yang et al found raised arteriolar and lower venous diameters in acute BRVO. (Yang 2017) Raised arteriolar global oxygen saturations seen in the acute stage probably reflect increased blood flow in response to ischemia, although an absence of any change in arteriolar diameters casts doubt on this theory. Interestingly Lin et al (Lin 2016) did find a decrease in arteriolar diameters and thus propose an increase in arteriolar blood flow as an explanation for the increased arteriolar oxygen saturation. Raised venous oxygen saturations can probably be explained by arterio-venous diffusion of oxygen. (Hardarson & Stefansson 2012) The increased metabolic derangements due to tissue damage may lead to higher than normal oxygen consumption leading to the observed higher arterio-venous oxygen saturation difference.

In the quadrant analysis the arteriolar and arterio-venous oxygen saturation difference was significantly raised in the acute stage whereas the venous oxygen saturations and diameters were significantly altered in the chronic stage. The initial vasoregulatory mechanisms may cause an increase in ocular blood flow leading to predominantly arteriolar changes as we observed in the acute group. Subsequent tissue and vascular re-modelling with formation of collaterals may result in the altered venous oxygen saturation and diameter values with near normal arteriolar parameters. The smaller venous diameters could also be a result of our measurement protocol where larger involved venules being unmeasurable, the smaller venules were measured.

In Table 3 we saw that the saturations in the involved quadrant were higher than the ones in the opposite quadrant. Most of the occlusions were in the supero-temporal

quadrant and thus were compared to the saturations in the infero-temporal quadrant. It is known that the infero-temporal saturations are generally the lowest physiologically.(Mohan 2015) Hence it raises doubt as to whether our findings were actually due to the occlusive pathology. It is important to note that the difference seen in our study is much larger than the physiological difference seen otherwise. Hence, we are led to believe that these alterations are largely pathological, although a part of the difference would be physiological. Designing the method of comparison was complicated and hence we used the method described in an earlier published report by Hardarson et al.(Hardarson & Stefansson 2012)

The significant differences that we observed in the global oxygen saturations of the unaffected fellow eye compared to normals is probably suggestive of a systemic disease causing an underlying sub clinical derangement of the retinal vascular physiology. This interesting finding warrants a larger sample size for confirmation. It could have profound implications on the screening and preventive care of BRVO patients.

Central foveal thickness (CFT) did not correlate with any of the parameters in the acute stage but in the chronic stage correlated positively and strongly with venous oxygen saturations and as a result negatively with arterio-venous oxygen saturation difference. This could mean that less the oxygen exchanged in the retina, more the ischemia and thus more the macular edema indicated by the higher CFT.

Our study was limited by our age matched controls having no history of systemic hypertension. Fifty percent ($n=9/18$) of our patients were hypertensives. We agree that this could be a confounding factor, however we also found a difference between the involved and the contralateral eye of the patients where hypertension would not be a confounding factor.

Another limitation of the study is the possible bias in selection of chronic cases with severe ischemia and hypoxia. Since history is not always reliable for BRVO, many do not notice any symptoms till they have significant macular edema, and a few others are diagnosed incidentally – hence exact duration of onset is unavailable and often unreliable – we have hence chosen to rely on clinical findings to classify the groups.

A larger sample size, serial follow ups, correlation of areas of non-perfusion on angiography with oximetry and studying the effects of treatment modalities like laser and anti-VEGF injections would serve to further strengthen our understanding of retinal venous occlusions

To our knowledge this is the first study to present data on central foveal thickness and the contralateral eye in BRVO patients. The data presented on the involved segments in the affected eye is more conclusive as compared to previously published literature showing a clear trend of increased oxygen saturations.

CONCLUSION

In summary, our study demonstrates that oximetry is sensitive in picking up changes in branch retinal vein occlusions, showing a trend of high oxygen saturations in venous occlusions. It also picks up subtle changes in the unaffected fellow eye of the patients, which if further validated can be used as a predictive marker for the development of BRVO

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CHAPTER 10

Oximetry in tubercular retinal vasculitis

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ABSTRACT

Aim

We aimed to study the retinal vascular oxygen saturation profiles in eyes with tubercular retinal vasculitis (TRV) and compared it with age-matched controls.

Methods

This is a cross sectional observational case series of 20 eyes of 15 patients diagnosed clinically with TRV. Apart from routine clinical examination, color fundus photography, optical coherence tomography, fundus fluorescein angiography and retinal oximetry (Oxymap T1, Oxymap hf, Reykjavik, Iceland) was performed for all the patients. The oximetry images were analyzed for oxygen saturation in arterioles and venules and arterio-venous oxygen saturation difference.

Results

Twenty eyes were included from 15 patients (5 bilateral, 10 unilateral). There were 42 eyes in the control group. The average arteriolar saturations were 91.1% and 90.2% ($p=0.77$); venous saturations were 53.9% and 57.2% ($p=0.16$) and the arterio-venous saturation difference (AVSD) was 37.2% and 33.2% ($p=0.004$) in the TRV and control groups respectively.

Conclusion

In conclusion this study shows that eyes with TRV have increased AVSD which can be an indirect indicator for altered retinal metabolism that results in increased oxygen consumption.

INTRODUCTION

Ocular tuberculosis (OTB) can present as anterior uveitis (TAU), intermediate uveitis (TIU), posterior uveitis (TPU), pan uveitis (TBP) or retinal vasculitis (TRV). (Gupta 2007; Agrawal 2019) Diagnosis of OTB presents a unique challenge, as the commonly used laboratory tests that also serve as the gold standard for confirming the diagnosis of tuberculosis, namely the demonstration of mycobacterium tuberculosis organisms by smear or culture in the sputum or the body fluids/ tissues, are seldom, if ever, positive from the ocular fluids.

The methods of determining disease activity in uveitic conditions so far have been predominantly subjective like grading of vitreous and anterior chamber inflammation by Standardisation of Uveitis Nomenclature (SUN) classification. (Jabs 2005) This can thus lead to intra and inter-observer variability. An objective method of determination of disease activity can add a new dimension to the management of this disease.

Oxygen is important for the normal physiology and metabolism in the body and in particular in the retina with its highest metabolic demand amongst all human tissues. (Kur 2012) Retinal vascular oxygen saturation can non-invasively be measured by a photo spectrometric method that uses a dual wavelength fundus photographic approach. (Hardarson 2006) Altered oxygen saturations can be a marker for disease activity. (Boeckert 2012; Hardarson 2013; Battu 2015)

A recent article by El-Asrar et al (Abu El-Asrar 2019) shows that retinal oxygen saturation is altered in uveitic conditions like VKH. They report an elevation in the oxygen saturation levels in both the arterioles and venules that return to normal upon resolution of the disease. It would be thus very interesting to see how the saturations are in eyes with TRV.

On literature review we did not find any study which has tried to study oxygen saturation of retinal vessels in TRV. In this study we aim to study the retinal vascular oxygen saturation profiles in cases of TRV and compare them with our age matched normative database. (Mohan 2015)

METHODS

Twenty-seven eyes of 22 consecutive patients presenting to the Ocular Immunology and Uveitis department at tertiary eye care center in Bangalore, India, diagnosed with TRV (Agrawal 2019) were included in this prospective cross sectional observational case study. The study followed the tenets of the Declaration of Helsinki and was approved by the ethics committee and Institutional Review Board. All patients gave a written informed consent for the study. Patients younger than 18 years of age, smokers, a history of ocular trauma or surgery or any other ocular disease were excluded from the study. Subjects with any systemic illness like hypertension or diabetes mellitus that could confound the readings were also excluded from the study. The patients were compared with age matched controls, chosen from our normative database who had a corrected distance visual acuity (CDVA) of 20/20 on Snellen's chart for distance and had no ocular or systemic pathology.

All patients underwent a complete ophthalmic examination. This included measurement of CDVA, anterior segment examination using slit lamp bio microscopy, measurement of the intraocular pressures with Goldmann applanation tonometer and a fundus examination with indirect ophthalmoscopy with scleral indentation. Fundus photography and Spectral Domain Optical Coherence Tomography (SD-OCT) was also performed. Mantoux test and Chest X-ray were done for all the patients; HR CT Chest, QuantiFERON TB Gold test, Aqueous Tap for PCR and endoscopic ultrasound bronchoscopy (EBUS) was done for selected patients. The ocular inflammation was considered to be tubercular in origin based on positive immunological tests (such as Mantoux/interferon gamma release assay) and/or radiological tests (such as chest computerized tomography indicative of old/healed tuberculosis) and not necessarily a confirmed test such as histopathology or polymerase chain reaction. Isolated retinal vasculitis (either peri phlebitis and/or arteritis) with/ without occlusive disease was labelled as TRV. (Agrawal 2019)

Following dilation with 1% tropicamide and 10% phenylephrine, all patients underwent dual wavelength photo spectrometric Oximetry (Oxymap T1 retinal oximeter, Oxymap, Reykjavik, Iceland). The initial images were obtained after the patients were allowed to rest for 5 minutes to eliminate exercise-induced fluctuations in readings. Resting blood pressure was measured in all patients to confirm that they were not hypertensive. None of the subjects had consumed caffeine within 2 hours of the examination. The aiming light was set at the lowest setting, flash intensity was 50W, small aperture and large pupil settings were used, and the images were obtained by one experienced

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photographer. Two images which were 50 degree and disc centered were obtained per eye. (See Fig 1)

We used the Oxymap Analyzer version 2.5.1. Eyes with automatic image quality less than 7.5 were excluded. Segments were manually selected according to the method from our previous study. (Mohan 2015) The vessels were at least 8 pixels wide ($72\text{ }\mu\text{m}$), at least 100 pixels long, were minimum 50 pixels from the optic disc margin and at least 100 pixels from the edge of the image. In cases vessel branchings were less than 150 pixels from the optic disc, segments after the branching were taken.

Statistical analysis was done using IBM SPSS v24.0 for Mac. All parameters were tested for normality using the Shapiro-Wilk Test. Comparison of means for parametric data was carried out using the independent t-test. Chi-square test was used for nominal data. The level of significance was $p < 0.05$. In order to account for subjects with both eyes included a mixed model analysis was performed with laterality and subject ID as covariables.

Fig 1 – a-Mid phase fundus fluorescein angiography image of the right eye showing macular hyperfluorescence due to active choroiditis and hyperfluorescent staining of the scar margins temporally; b & c-Corresponding 570 and 600nm monochromatic images taken on the Oxymap from which the retinal vessel oxygen saturations are calculated; d-570 nm image with pseudo color oximetry map overlay. Note the near normal to high saturation in the arterioles with the lower than normal saturations in the venules which leads to the higher arterio-venous saturation difference. e-Mid phase fundus fluorescein angiography image of the left eye showing the hyperfluorescent staining of the scar margins with interspersed hyperfluorescent areas of

blocked fluorescence due to pigmentation. f & g- Corresponding 570 and 600nm monochromatic images taken on the Oxymap from which the retinal vessel oxygen saturations are calculated; h- 570 nm image with pseudo color oximetry map overlay. Note the near normal saturation in the arterioles with the lower than normal saturations in the venules which leads to the higher arterio-venous saturation difference.

RESULTS

From a total of 27 eyes (22 patients), 20 eyes were included from 15 patients (5 bilateral, 10 unilateral). Seven eyes were excluded due to inadequate image quality due to defocus or media haze. There were 42 eyes in the control group (all unilateral). Demographics and clinical features have been summarized in Table 1. The average age in the TRV group was 33.9 ± 12.6 years (95% CI 28.0 - 39.8) and 36.0 ± 13.7 years (95% CI 31.7 - 40.3) in the control group ($p=0.55$). The male female ratio was 13:7 and 16:26 in the two groups respectively ($p=0.043$).

Parameter	TRV (n=20) (95% CI)	Controls (n=42) (95% CI)	Significance*
Age (years)	33.9 (28.0-39.8)	36.0 (31.7-40.3)	0.545
Sex (Male:Female)	13:7	16:26	0.043 [#]
Arteriolar Saturation (%)	91.1 (85.5-96.7)	90.2 (88.3-92.2)	0.770
Arteriolar Diameter (μm)	121.8 (114.4-129.2)	123.70 (119.8-127.6)	0.646
Arterio-Venous Difference (%)	37.2 (34.4-40.0)	33.2 (31.8-34.6)	0.004
Venous Saturation (%)	53.9 (49.5-58.2)	57.2 (55.3-59.1)	0.161
Venous Diameter (μm)	164.2 (156.4-172.1)	160.1 (156.4-163.9)	0.333

Table 1 - Summarising the demographics and global saturations and diameters.

*Independent t-test for comparison of means

[#]Chi-square test for Nominal data

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Clinically all patients had some vitritis and a component of retinal vasculitis confirmed on FFA.

Retinal Vascular Oxygen Saturations have been summarized in Table 1. Mean arteriolar saturation was 91.1% (95% CI 85.5 - 96.7) in the TRV group and 90.2% (95% CI 88.3 - 92.2) in the control group ($p=0.77$). Mean venous saturations were 53.9% (95% CI 49.5 - 58.2) and 57.2% (95% CI 55.3 - 59.1) ($p=0.16$) and the arterio-venous saturation differences (AVSD) were 37.2% (95% CI 34.4 - 40.0) and 33.2% (95% CI 31.8 - 34.6) ($p=0.004$) in the two groups respectively.

AVSD in males of $36.8 \pm 4.9\%$ was higher than $32.4 \pm 4.8\%$ in females ($p=0.001$) in the TRV group. On multivariate analysis with AVSD as the dependent variable and group and sex as the independent variables the group effect remained (intercept $\beta = 45.2$, $p<0.001$; group $\beta = 3.0$, $p=0.024$ and sex $\beta = 3.7$, $p=0.004$).

The results of the above multivariate analysis persisted in the mixed model analysis with the dependent variable as AVSD, independent variables as group and sex and covariables as laterality and subject ID the group effect remained (intercept $\beta = 31.9$, $p<0.001$; group $\beta = 3.1$, $p=0.032$ and sex $\beta = 3.5$, $p=0.007$, covariance subject ID = 5.3 and laterality = 16.0 for the right eye and 19.2 for the left eye)

To avoid bias due to paired sample two separate analysis were conducted – one including only the right eyes of the bilaterally affected and the second including only the left eyes. Both analyses yielded the same result. With including only, the right eyes ($n=15$) the arteriolar saturation was 92.6% ($p=0.326$), venous saturation was 55.9% ($p=0.574$) and AVSD was 36.7% ($p=0.014$). With including only, the left eyes the respective values were 93.6% ($p=0.165$), 55.8% ($p=0.531$) and 37.8% ($p=0.003$)

DISCUSSION

To the best of our knowledge this is first study to describe retinal vascular oxygen saturations in eyes with tubercular retinal vasculitis. AVSD was the only significant difference between the two groups; arteriolar and venous saturations and diameters were not significantly different.

In our study, the retinal vascular oxygen saturations were comparable in the arterioles but were lower in the venules resulting in a higher AVSD in the TRV group. It is seen in Table 1 that apart from AVSD, there was a significant difference in the sex distribution as

well. Due to concern that the difference in AVSD between groups could be due to sex differences we corrected for sex using a multivariate analysis. The significant difference in AVSD between the cases and controls persisted. This means that the oxygen extraction was higher in the eyes with inflammation as compared to the controls which stands to reason as inflammation can result in higher metabolism and thus higher oxygen consumption. Using a similar principle of spectroscopy ten patients with gingival inflammation were studied by Hanioka *et al.* (Hanioka 1991) They report that the decreased oxygen saturation during the inflammatory period returned to normal on resolution of the disease.

The arteriolar saturation is slightly higher although not significantly in the TRV eyes as compared to the controls. Arteriolar saturation can be affected by lung function, however with compromised lung function in patients with components of pulmonary tuberculosis we would expect a lower saturation.

Eyes with inflammation can have media haze that can artefactually alter the measured saturation values. Hence eyes with significant media haze were excluded and only eyes with an image analysis score of 7.5 and above were included to ensure the saturation values were not artefactually altered.

Retinal vasculitis can alter the background reflectance due to the presence of perivascular inflammatory material and loss of transparency of the vessel wall and surrounding retina. This can in turn alter the measured saturation values. The retinal vasculitis in our cases involved both the arterioles and venules and hence this effect would then be seen on both, however there was no significant difference between the disease and control group for either arterioles or venules. We had also studied the vascular diameters of the vessels that were used for oximetry. Comparison between the groups showed no differences. The data on retinal vascular diameters was not included in the results because the preferred way to study the vascular diameters would be to use the method described by Knudtson *et al.* (Knudtson 2003) It must also be kept in mind that the degree of retinal vasculitis can be different between the different TRV subjects. It will be difficult to quantify exactly how much occlusion exists in each subject however this factor must be kept in mind while interpreting the results. The arteriolar saturations being relatively close to normal and the absence of any significant difference in the arteriolar and venous diameters could mean that the values obtained from the venules are reliable.

With inflammation the vascular calibre and permeability can be affected. This can in turn affect the blood flow velocity. A recent study by Jeppesen *et al* (Jeppesen & Bek

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2019) shows that blood flow velocity has an inverse relationship with retinal saturation. This effect must be kept in mind while interpreting the results.

There were 5 subjects with both eyes included and the other 10 with only one eye. Since oxygen concentration depends not only on local factors but also on systemic factors, eyes from the same subject cannot be considered as independent. Hence, we performed a mixed model analysis with the subject ID as an error and found that the group effect remained. We also excluded one eye for the subjects with bilateral involvement and found that the difference in AVSD persisted.

Our study is limited by the small sample size and as a result it is difficult to derive any reliable conclusions. The lack of a significant difference can be due to the smaller sample size and hence a larger sample set needs to be accommodated in future studies. However, this is a pilot study and the first of its kind in ocular tuberculosis. Since retinal vasculitis can be a potential confounder a study could be planned that has the presence of vasculitis as an exclusion criterion.

In conclusion this study shows that eyes with TRV have increased AVSD which can be an indirect indicator for altered retinal metabolism that results in increased oxygen consumption.

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CHAPTER 11

Miscellaneous Observations

Chapter 11

DIABETIC RETINOPATHY AND ISCHEMIC DISEASE

Presented at multiple state conferences and as a postgraduate thesis for Diplomate of National Board 2017

We wanted to further study how ischemia affects the retinal vascular oxygen saturations. We thus compared the retinal vascular oxygen saturations in subjects with diabetic retinopathy to the previous groups of vascular occlusions. We found that the arteriolar saturation was lowest in the CRAO group at 83%, statistically significantly below that of 94% in the normal group ($p < 0.001$). The rest of the groups had arteriolar saturations above normal with Mild NPDR at 97.8% ($p = 0.47$), Moderate NPDR at 99.3% ($p = 0.03$), Severe NPDR at 102.2% ($p < 0.001$) and BRVO at 101.4% ($p = 0.035$). The venous saturation was lowest in the CRAO group at 47.1%, significantly below that of 58.9% in the normal group ($p = 0.005$). Only severe NPDR at 66.4% was significantly above normal ($p = 0.023$) while the rest of the groups (Mild NPDR at 59.0%, Moderate NPDR at 63.7% and BRVO at 60.2%) we found no differences with the normal group. We found no difference in AVSD between the groups.

Fig 1 – Oxymap images with the pseudocolor maps superimposed on the 570nm monochromatic image. A – Normal, B- Chronic BRVO, C- Acute CRAO, D- Mild NPDR, E- Moderate NPDR and F- Severe NPDR

For the above, there were a total of 143 eyes of which there were 32 (32 subjects) in the normal group, 19 (11 subjects) in Mild NPDR, 40 (24 subjects) in moderate NPDR, 29 (18 subjects) in severe NPDR, 12 (12 subjects) in chronic BRVO and 11 (11 subjects) in acute CRAO. The average age of 48.5 ± 6.6 was significantly lower in the control group as compared to other groups. There were more females than males (12:20) in the control group; the opposite trend was seen in the other groups. There was no significant correlation of age or sex with any of the variables.

Thus, we saw that eyes with CRAO had the lowest arteriolar and venous saturations. Arteriolar saturations in other groups were above normal. The rest of the groups had comparable venous saturations to normal with only Severe NPDR significantly above normal. The oxygen extraction indicated by AVSD was not different between the groups. The increase in saturation with increasing severity of disease agrees with previous reports. (Boeckeaert 2012; Hardarson & Stefansson 2012; Khoobehe 2013; Jorgensen 2014; Torp 2018) While the findings in CRAO seem logical the arteriolar changes in BRVO and DR are not fully explained physiologically and hence could be optical artifacts. While all the retinal vascular conditions are characterized by hypoxia, we noticed that only eyes with CRAO had measured oxygen saturations below normal values. CRAO was also the only acute condition, while the others are chronic.

OPTIC DISC EDEMA

Presented at NANOS 2015 and ARVO 2016

Next, we performed retinal oximetry in eyes with optic disc edema. We hypothesized that optic disc edema can have a unique effect on the retinal circulation. Twenty consecutive eyes diagnosed to have disc edema secondary to various pathologies diagnosed on basis of clinical examination were included. Arteriolar diameters were reduced, and venous diameters were increased in the affected eyes as compared to the values of our normative database (Chapter 2). Arteriolar saturations were increased, venous saturations were decreased and arterio-venous saturation difference (AVSD) was increased as compared to the age matched normals. A significant positive correlation was noted between arteriolar saturation and mean deviation on standard automated perimetry.

We hypothesized that there could be a possible compartment like syndrome (Kerr 2009) that causes a compression of vessels leading to two events; an increase in arterio-venous transit time and decreased vascular diameters. This would give more time for oxygen extraction resulting in decreased venous saturations and increased AVSD.

Fig 2 – Left – retinal oximetry image of the left eye with optic disc edema at presentation where the visual acuity was 6/60, arteriolar saturation was 111% and diameter was 102 μ m, venous saturation was 70% and the diameter was 193 μ m with an AVSD of 41%. Center – Greyscale representation of the subject's perimetry showing an inferior field defect with an altered mean deviation and visual field index. Right – follow up of the same eye with resolution of the optic disc edema, arteriolar saturations remained at 112%, diameters increased to 107 μ m, venous saturations increased to 78% with diameters decreasing to 144 μ m, the AVSD normalised to 34%.

GLAUCOMA

Presented at EGS 2015, multiple Indian state conferences and Dutch Ophthalmic Society 2017

Next we performed retinal oximetry in eyes with glaucoma. 53 eyes were analyzed and had an average age of 64.9 ± 8.8 years. Arteriolar saturation was $103.2 \pm 12.9\%$, venous saturation was $59.4 \pm 9.2\%$ and AVSD was $44.1 \pm 13.1\%$. Average RNFL thickness was $56.5 \pm 14.7 \mu\text{m}$, MD was -13.8 ± 9.0 and VFI was 59.1 ± 29.2 . Arteriolar saturations correlated significantly with MD ($r = -0.34$; $p = 0.014$).

Previously higher saturations in venules and no effect in arterioles have been reported.(Olafsdottir 2014) These alterations have been reported to be correlating with functional visual field changes.(Shimazaki 2016) It has also been reported that the changes are seen irrespective of RNFL thickness changes.(Hammer 2016) However, in our cohort we found that both the arteriolar and venous saturations were elevated thus having a resultant increase in AVSD. As tissue loss would lead to less demand and extraction and thus a narrower AVSD, our findings of increased AVSD seem counter-intuitive.

PARKINSON'S DISEASE

Presented at Euretina 2018

Parkinson's disease is a progressive neurodegenerative disorder. It affects people in the elderly age group with a prevalence of seven to 10 million individuals worldwide.(Beitz 2014) The retina being a neuroectodermal tissue, bears embryological and anatomical similarities to the vasculature of the brain.(London 2013) The retina is also the only tissue in the body where non-invasive visualization of vasculature is possible. The possibility of predicting cerebral pathology with respect to alterations in the vascular functioning of the retina can thus be explored.(Cheung 2014; Einarsdottir 2016)

We performed a cross sectional observational study of 22 eyes of 11 MRI confirmed patients with Parkinson's Disease. The average age of the patients was 56.2 years (95% CI – 53.4-59.0), age of onset of disease was 50.8 years (95% CI – 47.7-53.9), duration of disease was 5.1 years (95% CI – 3.7-6.5), UPDRS III score was 27.9 (95% CI – 25.1-30.7), H&Y score was 2.2 (95% CI – 2.1-2.4) and Levodopa equivalent dosage per day was 626(95% CI - 532.8-719.2). The pRNFL thickness was 103.8 μm (95% CI – 100.3-107.3) and macular thickness was 274.1 μm (95% CI – 263.8-284.4). The arterial saturation was 97.5% (95% CI – 94.3-100.9), venous saturation 60.1% (95% CI – 58.2-61.9), AVSD was 37.5% (95% CI – 34.4-40.6), arteriolar diameter was 113.3 μm (95% CI – 107.9-118.7), venous diameter 152.4 μm (95% CI – 147.7-157.1). The H&Y score correlated negatively with the age of onset ($r=-0.459$, $p=0.048$), positively with the UPDRS score ($r=0.623$, $p=0.002$), and Levodopa dosage ($r=0.671$, $p=0.001$). The arteriolar saturation correlated inversely with Levodopa dosage ($r=0.554$, $p=0.011$) while positively with AVSD ($r=0.840$, $p<0.001$). The RNFL thickness did not show any significant associations with any of the other parameters.

We concluded that retinal metabolic parameters like arteriolar saturation and AVSD showed a significant correlation with Levodopa dosage. This may imply that retinal oximetry changes occur in association with underlying central nervous system derangements.

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CHAPTER 12

Pitfalls in Oximetry And Mathematical Modelling

Chapter 12

PITFALLS IN OXIMETRY

Values above 100%

A number of studies (Hardarson & Stefansson 2012; Battu 2015) show saturations above 100% which do not make sense physiologically. Hardarson et al. (Hardarson 2013) state that these values are measured oxygen saturations and hence are relative and not absolute. Although as such they may be used for a comparison of means between two groups or can be used to follow up the same individual, they cannot be used on a single individual at a single point of time to obtain reliable values. (Fig. 1)

Fig 1 – The solid red lines show that the saturations have reached the ceiling of the pseudo color map. Obtained saturations are in excess of 100%. This is an image of a subject with severe non proliferative diabetic retinopathy.

Variability within the eye

The central retinal arteriole brings in oxygenated blood from the heart and divides into a superior and inferior branch which further divides into nasal and temporal branches. It would stand to reason that the saturation in these 4 branches which are measured within 1000 μm of the optic disc should be more or less equal as the blood in them was mixed just a second ago. It must be noted that no significant oxygen exchange can have taken place yet as it is still contained in large arterioles. Observing the oxy-hemoglobin dissociation curve between saturations of 90-100% reveals that a corresponding change of 70-100% would be needed in the PO_2 – implying that for each percent change in saturation the PO_2 needs to change by 3% - a large variation that cannot be physiologically explained. (Fig 2)

Fig 2 – Depicting the variability within the eye and the variability due to the angle of gaze. Upper left is an optic disc centered image (note the difference in color in arterioles between nasal and temporal halves; nasal ones being more solid red) and the upper right is an inferior temporal quadrant image of the same eye. The white box indicates the same section of retina imaged and magnified below to show the difference due to angle of gaze. Also note the rattlesnaking in the images.

Variability due to angle of gaze

It has been shown that vessel location within an oximetry image, depending on the gaze of the subject, has a significant effect on measured oxygen saturation in arterioles and venules. (Geirsdottir 2012) It is not possible to have the exact same angle of gaze every time for a given vessel segment. This will give rise to an artefactual inter and intra subject variability. Also, if it was only the background pigmentation that was different between individuals and races which could influence measured saturations then the angle of gaze should not affect it significantly. However, because the angle of gaze does affect the measured saturation values it leads us to look more closely at the retinal micro-anatomy. The anatomical considerations will be discussed in the next section. (Fig. 2)

Rattlesnaking Artefacts

A common artefact in oximetry is 'rattlesnaking', (Fig. 2) where estimated oxygen saturation spuriously varies along the length of a blood vessel, causing a stripey pattern to appear when visualized as a colour-coded map. This can arise due to a number of reasons, including variations in RBC concentration, optical scattering, background pigmentation, and vessel-fitting errors. (MacKenzie & Harvey 2018)

ANATOMICAL CONSIDERATIONS

Effect of RNFL Thickness

It is known that the vessel diameter has an apparent inverse relationship with the measured saturation values. (Beach 1999; Hammer 2008) Hence the Oxymap uses an inbuilt vessel width correction. However, despite this correction the inter arterial variation still exists. Mohan et al. (Mohan 2016) reported an inverse relationship between perivascular RNFL thickness and calculated oxygen saturation and showed that this relationship was stronger than the previously established relationship between diameter and oxygen saturation. Interestingly the thicker RNFL affected the I_o (reflected light intensity on the side of the vessel) values at 570 and 600nm which may partly explain the changes in the ODRs.

The I_o taken on the side of the vessel is supposed to be representative of every factor except for the vessel itself. However, it must be noted that the RNFL being much thicker

here as compared to on the vessel, it offers a very different reflectance pattern. This may in part explain the findings of higher ODRs with higher perivascular RNFL thickness in the study by Mohan et al. (Mohan 2016)

Effect of Media Opacities and Contrast

It is known that changes in the ocular media like cataract or vitreous haze can decrease contrast and artefactually lower the measured saturation values. (Patel 2013) It thus stands to reason that any unaccounted reflection or absorbance of light before it reached the blood column alters the measured saturation. On observing the OCT, it is interesting to note that a few vessels lie at the surface while a few lie much deeper in the RNFL layer. This variable presence of RNFL tissue above the vessel (pre-RNFL), though small can theoretically alter measured saturation values. (Fig. 1)

Fig 1 – The image of the right shows a well-focused image; the one of the right shows a defocused image of the same eye. Note the differences in saturation.

Effect of the central light reflex

On observing the intensity profiles of the vessel and its background we notice a dip in intensity in the region of the vessel. However, an interesting rise in intensity is noted at the very center of the vessel. This led us to believe that there is a significant component of vessel wall reflection that must be considered. The vessel wall too like the pre-RNFL interacts with light before it interacts with the blood column and thus can alter measured saturations, especially when it is thicker like with aging and arteriosclerosis. In the Oxymap the points on the vessel itself are chosen so as to avoid the central light reflex. (Hardarson 2013)

Effect of Background Reflectivity

It has been known that there exists a certain reflectivity of the photoreceptor outer segments, RPE and Bruch's layers which can have an impact on measured light intensities. The photoreceptor density is maximum centrally and decreases with eccentricity (Steinberg 1973) and aging. The RPE residual body content increases with aging. The Bruch's membrane also is known to become thicker, more elastic and calcified with aging or diseases like ARMD. (Ramrattan 1994; Okubo 1999) Changes occurring in BM with age include increased calcification of elastic fibers, increased cross-linkage of collagen fibers and increased turnover of glycosaminoglycans. In addition, advanced glycation end products (AGEs) and fat accumulate in BM. (Booi 2010) The magnitude of impact of this photoreceptor-RPE-Bruch's reflection (PRB) must be studied and taken into account if significant. (Fig. 2)

Fig 2 – Image of an eye with retinitis pigmentosa showing a number of spurious signals (in purple) due to changes in background pigmentation.

MATHEMATICAL MODELLING

Need for a mathematical model

To re-iterate, we saw that eyes with aging, retinal dystrophies and glaucoma, venous occlusions, increasing diabetic retinopathy severity and eyes with optic disc edema were associated with increased saturations. Increased metabolic demand or decreased RNFL thickness were the main hypothesis. In eyes with minimal structural change like amblyopia or Parkinson's too there were changes in retinal oximetry that correlated with the functional changes. Hence the changes seen are a combination of true metabolic alterations and artefactual alterations due to structural and optical derangements. After the initial description of a simple model of measurements at two wavelengths (Hickam 1959) there have been attempts to compensate for the variable fundus pigmentation (Beach 1999) however the contribution of the retinal nerve fiber layer (RNFL) and vessel wall to the optical properties of the fundus was not taken into account.

Lemaitre et al. (Lemaitre & Ramella-Roman 2009) in 2009 proposed a dynamic eye phantom that took into account the optical properties RPE, choroid and sclera. Chen et al. (Chen 2019) a decade later recently published a fundus simulating phantom for calibration of retinal oximetry devices based on the original work of Lemaitre et al. Their phantom consists of a transparent top layer, a middle vascular layer and lower base layer. However these optical properties can not only differ from race to race or individual to individual, they can also differ vessel to vessel within the same eye.

The observations of this thesis highlight the need to separate the effect of true metabolic alterations from optical artefacts on the measured oxygen saturation values. None of the published literature has described a comprehensive model that considers the dynamic nature of RNFL, pre-RNFL, vessel wall reflection, thickness and saturation of the blood column, photoreceptor, Bruch's and chorio-scleral reflection.

Developing a comprehensive model may further help us use the knowledge in multispectral imaging oximetry which is an optical technique that utilises the oxygen saturation dependent absorption spectra of haemoglobin within blood to quantify the oxygen saturation with high spatial and temporal resolution. (MacKenzie & Harvey 2018) This would be dealt with in the next chapter.

Methods

We describe five models below which incorporate different optical effects of the retinal micro-anatomy. In the last model we use in vivo data of 25 healthy individuals. These individuals had no ocular or systemic abnormalities and had presented to our center for a routine eye evaluation. Ocular examination was normal with a corrected distance visual acuity of 20/20 on Snellen's chart. Imaging was done as described in Chapter 2.

The software version was Oxymap 2.5.2 which has an automatic image quality analysis for which a cut off of 7.5 was used. The four thickest arterioles and venules were chosen with segments between 100 to 150 pixels in length. Vessel point analysis data was obtained which gives apart from the saturation and width, the I (on the vessel) and I_0 (on the side of the vessel) values for the two wavelengths. These values were then averaged to obtain one value for each parameter for each eye for arterioles and venules.

Results

Common equations for all models

$$ODR = OD_{600}/OD_{570} \quad (1)$$

$$OD_{600} = \log_{10}(I_{0\ 600}/I_{600}) \quad (2)$$

$$OD_{570} = \log_{10}(I_{0\ 570}/I_{570}) \quad (3)$$

$$Transmission_{\lambda} = 10^{- (Optical\ Density_{\lambda} * Width)} \quad (4)$$

$$Optical\ Density_{\lambda} = Sat/100 * HbO_{2\lambda} + (1-Sat/100) * Hb_{\lambda} \quad (5)$$

HbO₂ was taken as 0.01062 at 570 nm and 0.00043 at 600nm. Hb was taken as 0.01048 and 0.00306 respectively. Vessel width was assumed to be 125 μ m.

Model 1

This is a simple model with retinal (B) and choroidal (C) reflectance and an overlying vessel. This has no RNFL or pre-RNFL. This model was described to prove the linear inverse relationship between the ODR and the calculated saturation. (Fig.3)

Fig 3 – Schematic representation of Model 1

Equations

$$I_{0\ 570} = B + 0.01C = E \quad (6)$$

$$I_{0\ 600} = B + C = F \quad (7)$$

$$I_{570} = \text{Transmission}_{570} * E \quad (8)$$

$$I_{600} = \text{Transmission}_{600} * F \quad (9)$$

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We found that the ODR had an inverse linear correlation with saturations as seen in Fig. 4

Fig 4 – Results of Model 1

Model 2

This model builds on Model 1 by adding RNFL on the side of the vessel. We calculated how it can impact saturations separately for arterioles (92%) and venules (57%). (Fig. 5)

Fig 5 – Schematic representation of Model 2

$$I_{0\ 570} = \text{RNFL} + E \quad (10)$$

$$I_{0\ 600} = \text{RNFL} + F \quad (11)$$

I_{570} & I_{600} as per equation 8 & 9 in Model 1. RNFL is the thickness in microns.

We saw that with saturations kept constant for both arterioles and venules at 92% and 57% respectively, the calculated ODR increased with increasing RNFL thickness. (Fig. 6)

Fig 6 – Results of Model 2

Model 3

This is Model 1 with RNFL above the vessel and the vessel wall reflectance (pre-reflectance) and how it can separately affect arteriolar and venous saturations. (Fig. 7)

Fig 7 – Schematic representation of Model 3

Equations

$I_{0\ 570}$ & $I_{0\ 600}$ as per equations 6 & 7

$$I_{570} = \text{Pre-reflectance} + \text{Transmission}_{570} * E \quad (12)$$

$$I_{600} = \text{Pre-reflectance} + \text{Transmission}_{600} * F \quad (13)$$

We saw that for the arterioles the calculated ODR decreased and became negative as the pre reflectance increased. The venous ODR initially saw an increase which was then followed by a decrease. (Fig. 8) Hence at higher saturations the ODRs decrease and even become negative, at lower saturations they initially increase and then start to decrease. It is worth mentioning here that there exists an inverse relationship between ODR and saturations. Also negative ODRs will give saturations in excess of 100%.

Fig 8 – Results of Model 3

Model 4

This is a combination of Model 2 & 3 with both RNFL and pre-reflectance incorporated at fixed values against changing saturations. (Fig. 9)

Fig 9 – Schematic representation of Model 4

Equations used – 10, 11, 12 & 13.

We saw that at lower saturations the estimated ODRs were much higher, this effect reduced at higher saturations. (Fig. 10)

Fig 10 – Results of Model 4

Model 5

In this model we use the in vivo data and try to apply Model 4 to it. There were a number of assumptions made for this model fit.

1. The arteriolar saturation for all subjects was 92%
2. B and C would be the same for arterioles and venules when averaged across four vessels across four different quadrants. Hence the value obtained of B and C from the arteriolar values can be substituted for venules.
3. Equations

Using Equations 6 & 7 we can obtain

$$C = (F-E)/0.99 \quad (14)$$

Further using equations 6,7, 12 and 13 we can obtain

$$B = (I_{600} - I_{570} - (Transmission_{600} - 0.01 * Transmission_{570}) * C) / (Transmission_{600} - Transmission_{570}) \quad (15)$$

Where assuming the saturation to be 92% and using the measured vessel width from the software, $\text{Transmission}_{600} - \text{Transmission}_{570}$ could be calculated for arterioles using equations 4 & 5

Now that we have B and C from arterioles we tested these on the $I_{0.570}$ and $I_{0.600}$ obtained from venules with equations 10 & 11 to see if the returned RNFL values were similar. There was no observed statistically significant difference. ($p=0.995$)

Now that the values of retinal (B) and choroidal (C) reflectance obtained from arterioles was applied to the reflectance values obtained from venules and validated we proceeded to determine the corrected saturation. Venous $\text{Transmission}_{570}$ was determined using equation 4 & 5 and the measured vessel width from the software. The saturation here did not matter as this is a saturation insensitive wavelength. However as a standardisation the following equation was used

$$\text{Venous Transmission}_{570} = 10^{-(0.01055 * \text{Vessel Width in } \mu\text{m})} \quad (16)$$

Then the venous $\text{Transmission}_{600}$ was determined with the following equation

$$\text{Venous Transmission}_{600} = (I_{600} - I_{570} - \text{Venous Transmission}_{570} * E) / F \quad (17)$$

Finally the saturation was obtained from the Venous $\text{Transmission}_{600}$ and the vessel width as under

$$= ((-\log_{10} \text{Venous Transmission}_{600}) / \text{Width} - 0.00306) * 100 / -0.00263 \quad (18)$$

This corrected venous saturation was very variable at $56 \pm 21\%$.

The venous saturation values were also obtained by calculating ODR from the venous light intensities

$$\text{Saturation} = 124 - 128 * \text{ODR} \quad (19)$$

The obtained saturations were $53 \pm 9\%$ which were far less variable.

Discussion

We had hypothesized that optical artefacts would exist that alter the measured saturation values. We proved this with Model 1 that showed that if the retina is made up of only a vessel that lies over a layer of retina and choroid then the measured ODR would be inversely related to the saturation. This is the concept used in all the currently available algorithms which fail to account for changes in RNFL thickness surrounding the vessel.

We further strengthened our concept with Model 2, 3 and 4 go on to show how RNFL and pre reflectance change the measured ODR and lead to incorrect calculated saturations. Model 3 shows us how negative ODRs can be obtained due to increased pre-reflectance and gives us an insight into the optical factors in play when saturations go above 100%. Hence in theory we can see how Model 1 will lead to incorrect measured saturations in vivo if the effects of Model 4 are not considered.

Model 5 then attempts to validate Model 4 with measured in vivo data to obtain less variable venous saturations and helps us ascertain the relative contributions of the different components. The results of this attempt however are surprising and disappointing as it turned out to have more variations than if Model 1 alone was used as is being used currently. The reason for the above could be that Model 5 wrongly estimates certain artefacts and overcorrects for them increasing the errors in the measured saturations.

Hence through our models, while we expose theoretical flaws in the current method of oxygen saturation estimation, we failed to provide a better method to estimate the same. Further work on this model would be essential to obtain more reliable values.

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CHAPTER 13

General Discussion

Chapter 13

There are several important points that emerge from the work of this thesis. Firstly, we established the normative database for Indian eyes and presented an automated objective method to select the vascular segments and analyse the global arteriolar and venous saturations. We also demonstrated possible artefacts in the same eyes due to RNFL thickness and angle of gaze. Secondly, we performed retinal oximetry in a diverse set of disease states to understand how oxygen saturation can change in disease. Lastly, we present a mathematical modelling that may help explain several observations in normal and disease states and help overcome artefacts.

PART 1 – IN NORMAL EYES

Our normative database had an age range of 18-63 years the results of which was comparable to previous studies.(Geirsdottir 2012; Jani 2014) The average arteriolar saturation was $90.3\% \pm 6.6\%$ and the venous saturation was $56.9\% \pm 6.3\%$. The average arterio-venous saturation difference (AVSD) was $33.2\% \pm 5.2\%$. Age, vessel diameter and ocular perfusion pressure were the significant factors that influenced the saturation. Arteriolar and venous saturations increased with age while the AVSD did not change significantly.

The current practice to calculate oxygen saturation from the fundus image, however, requires considerable manual effort. To start with, one must manually outline the OD and segment a ring-shaped region of interest that is concentric with the OD. Subsequently, the arterioles and venules must be identified, and the oxygen saturation level and arterio-venous saturation difference (AVSD) must be estimated. This procedure is not only tedious but also introduces subjectivity. The automatic method introduced by us from a clinical perspective is important because it shows that the algorithm could be used in place of manual measurements. Apart from being automatic and time saving, it eliminates inter-observer variability and thus lays the foundation for standardising oximetry measurements. It also demonstrates for the first time the existence of bimodal peaks in the oxygen saturation distribution and with this it incorporates the advantage of being immune to extreme values.

Next, we noticed a significant negative linear relationship between oxygen saturation values and the measured RNFL thickness. The background for this study includes anecdotal observations of saturations going above 100% in many cases and that nasal values were higher than the temporal ones in normal subjects. This not being a theoretical possibility raised a strong suspicion of an artefact. While this variation by

location may be possible in venules that contain blood after their journey through the retina, it is not physiologically explained for arterioles wherein, the contained blood that just seconds before would have all been present mixed in the central retinal arteriole.

This relationship between perivascular RNFL could be physiological or artefactual. If physiological, it would explain and form the basis of altered oxygen saturation in a multitude of diseases. If artefactual, it would necessitate the incorporation of a correction factor. Our observations have also helped to explain the findings in several subsequent studies. It must however be kept in mind that the perivascular RNFL measurements have been made at a single point on the vessel and might not be representative of the entire length of the vessel selected.

In our normative database, we chose the thickest arteriole and venule in each quadrant to represent the saturation of that quadrant. We also wanted to see how this value changes if we image only the quadrant in question and average the values over three vessels each for arterioles and venules. We observed a decreased intra-eye variation when individual quadrants were observed. The most plausible explanation was from the studies by Knighton *et al.* (Knighton 1992; Knighton & Qian 2000) where the RNFL has been termed as “ribbons” and they have reported that the temporal “ribbons” were brighter than the nasal ones. It must be noted that the structure of the RNFL again forms the basis for the explanations of these observations as well.

Our observations of the relationship of perivascular RNFL and the quadrant imaging prove a strong case for optical artefacts related to changes in RNFL thickness. This must be kept in mind while interpreting and understanding the results of the next section.

PART 2 - OXIMETRY IN DISEASE

In amblyopia, we observed higher mean saturations than in fellow eyes. In Parkinson's disease, there was no comparative group, however we observed that the arteriolar saturations correlated negatively with Levodopa usage. There are several studies that have evaluated the microstructural changes in amblyopia and Parkinson's disease, however there are only few that demonstrate functional changes in these diseases where the retina appears grossly normal clinically. Functional problems perceived by the patient include a gross decrease in acuity in amblyopic eyes, while only minor changes like visual hallucinations or photopsias are perceived in Parkinson's disease. When we consider the results of both these studies together, we may conclude that as a group there is a deviation from normal, however as individual eyes we fail to see the

same. Additionally, the amblyopia study also provides a small dataset for normal values in a paediatric age group.

Next, we studied the saturations in inherited retinal dystrophies. We noticed that the saturations were increased as compared to normals in dystrophies like retinitis pigmentosa that involved the peripheral retina and in Stargardt's disease that involved the macula. The saturations were more elevated in retinitis pigmentosa as compared to the macular dystrophy group and the normals. We concluded that these observations could be a biomarker for haemodynamic changes that take place in these disease states. However, an optical effect due to RNFL thinning and due to an alteration of background reflectance cannot be ruled out.

Next, we aimed to study how the oximetry changes with a retinal vascular disease. In arteriolar occlusions, we saw that the saturations were decreased in both the arterioles and venules, in microvascular disease like NPDR we observed that the arteriolar saturations were increased as compared to normal in moderate and severe NPDR; the venous saturations were only increased in severe NPDR. Moving on to venous occlusions we observed that in the acute stage arteriolar saturations and AVSD was increased while an increase in venous oxygen saturation and a decrease in venous diameter accompanied by near normal arteriolar oxygen saturations occurred late in disease. While it is intuitive that the saturations in arterioles and venules are decreased in acute arterial occlusions and venous saturations are increased in chronic vein occlusions, it is surprising to see that in acute vein occlusions, we observed an increase primarily in the arteriolar saturations. We can have tissue edema in both acute arteriolar and venous occlusions, however in venous occlusions, there would be intra-retinal haemorrhages which can influence the measured saturations. In the chronic stage, there would be tissue atrophy and vascular remodelling and thus there would be metabolic and optical changes in the retinal tissue. It can be argued that tissue atrophy would mean less oxygen demand and thus higher venous saturations as observed in the chronic subject's group. We saw that eyes with CRAO had the lowest arteriolar and venous saturations. Arteriolar saturations in other groups were above normal. The rest of the groups had comparable venous saturations to normal with only Severe NPDR significantly above normal. The oxygen extraction indicated by AVSD was not different between the groups.

Next, we studied how the saturations are affected in vascular inflammations. We chose a group of eyes with tubercular retinal vasculitis and found that the most noticeable change was in the AVSD; the arteriolar and venous saturations were not statistically

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different from normals. This could mean that in such retinal inflammatory states, the retinal metabolism is heightened and thus there is a higher demand for oxygen which is evidenced by the increase in the saturation difference.

In optic nerve head edema hypothesized that there could be a possible compartment like syndrome that causes a compression of vessels leading to two events; an increase in arterio-venous transit time and decreased vascular diameters. This would give more time for oxygen extraction resulting in decreased venous saturations and increased AVSD. In glaucoma we found that both the arteriolar and venous saturations were elevated thus having a resultant increase in AVSD. As tissue loss would lead to less demand and extraction and thus a narrower AVSD, our findings of increased AVSD seem counter intuitive.

We thus saw that significant and intriguing changes occur in disease states that are distinctly different from normal subjects. What we also note is that there is a trend which can be appreciated across different types of disease that include ischemic, inflammatory, degenerative, and developmental pathologies. Firstly, we see that in amblyopia, Parkinson's disease, retinal dystrophies, NPDR, BRVO, vasculitis and glaucoma the arteriolar saturations are increased. This increase in arteriolar saturations seems alarming as not only do they increase, they exceed 100%. The only place in the body where saturations can increase is in the lungs. The rest of the tissues extract oxygen. It is thus counter intuitive as to why we see an increase in any of the disease states.

Secondly, we see an increase in AVSD. It can be argued that this is entirely explained by the earlier observation of arteriolar saturation increase, however we have also seen a decrease in venous saturation in a significant subset. This finding is also unexplained in glaucoma and retinitis pigmentosa where we know that the amount of retinal tissue decreases. Hence it may be fair to conclude that there is indeed heightened metabolism in the retina due to certain disease states leading to increased oxygen extraction. Despite considering all the possible optical artefacts this single parameter appears like a genuine observation, and even if this finding is an optical artefact, it can be used as a biomarker for disease as an optical change could reflect an underlying disease state.

Lastly, we also know that most of the earlier mentioned diseases would have some change in the optical characteristics of the retina like RNFL thinning, vascular or perivascular edema, altered background reflectance or media opacities. It is thus imperative that these are explored in detail.

PART 3 - THE ARTEFACTS AND MATHEMATICAL MODELLING

In parts 1 & 2 we discussed the possibility of various optical artefacts in healthy as well as diseased eyes. In chapter 11, we have summarised the main observed artefacts like saturations exceeding 100%, intra eye variations, variations with the angle of gaze and rattle snaking artefacts. We also speak about the anatomical factors that can be responsible like the RNFL thickness, vessel wall thickness and background pigmentation. We discussed interesting phenomena like large intra-eye variations unexplained physiologically in normal subjects, oximetry measurements that inversely with the RNFL thickness, vary with the angle of gaze and are different in disease states as compared to normal, and saturations exceeding 100% which cannot be explained by the pathology of the disease, hence raise a strong suspicion that these observations could be artefactual.

A comprehensive discussion has been presented in chapter 5 about why the findings should be artefactual. Briefly, the results of the oxy-hemoglobin saturation curve support the theory that such large intra-eye variations seen in optic disc centered imaging especially with arterioles must be artefactual. Since the blood comes from the central retinal arteriole and divides into the four major vessels the saturation in the four major vessels should be nearly the same. Each percent difference in saturation requires a change of more than 3 mm Hg in PO_2 ; this difference in PO_2 is unexplained physiologically especially in normal eyes.

Since the algorithm takes into consideration neighbouring pixels, the state of the retina on the side of the vessel also becomes crucial. In diseases like acute BRVO or CRVO with extensive haemorrhages it can become impossible to perform oximetry. In CRAO, extensive retinal edema can also impact measured saturations. Retinitis pigmentosa can cause non-uniform pigmentary changes thus altering measured saturations. Media opacities like vitritis can also render several eyes not amenable to oximetry.

We then present 5 different mathematical models which have many interesting arguments. Model 1 draws entirely from the equations in literature based on optical density. We prove that this would be linearly related to oxygen saturation, only if there was no tissue above and on the side of the vessel. This, however, is not true in vivo. Models 2, 3 and 4 incorporate RNFL on the side of, above and both side and above the vessel respectively and show how the linear relationship would change if the optical effects of these tissues are considered. It also makes a strong point as to why the linear relationship which currently forms the core principle of retinal oximetry is flawed. Subsequently, Model 5 was performed exclusively on major arterioles expected to have

more or less the same saturation within a given eye. We expected Model 5 to build on these calculations and apply them to in vivo data to obtain a corrective factor. At this, however we failed as even by considering the optical effects of Model 2,3 and 4 we failed to reduce the intra-eye variations. We concluded that there were possibly too many unknowns and more equations would be needed to correct the present flaws in the algorithm. More equations would mean more wavelengths and thus we feel that multispectral imaging may be better than dual wavelength imaging.

One major limitation is that we have neither ventured into in depth pathological reasoning of the oximetry findings, nor have we validated the clinical studies against the pitfalls and mathematical modelling. While we raised several questions and suggested possible mathematical modelling, we did not have sufficient data to discuss the findings in various diseases with the help of the different mathematical models.

Firstly, due to the knowledge that a substantial part of the findings could be artefactual, there is a considerable doubt that such alterations are not due to the disease process. Hence, proposing elaborate pathological theories to explain them would be inappropriate. Secondly, we would have preferred to use our proposed mathematical models on the results of the various clinical studies to make them more reliable, however, our models have used OCT measurements of RNFL thickness, vascular diameters and choroidal thickness which is unavailable to us in the studies on disease states. During the planning of this project, there was an underestimation of the impact the artefacts could have, largely due to their absence in published literature at that time. As a result, OCT and other measurements needed for mathematical modelling were not considered in the study designs. During the course of the study, we learnt that they indeed do play a significant role and must be given weightage while interpreting observations or for future development.

PART 4 - FUTURE DIRECTIONS

Our experience with retinal oximetry taught us that presently oximetry has questionable value in evaluating a single patient at a single visit. Several patients of a disease when compared to an equally large cohort of normals may show a significant difference, however readings of a single individual do not give any reliable insights. Alternatively, if a single patient must be imaged, there needs to be follow up and multiple observations. The multiple readings can then be compared as the optical artefacts unique to that eye can hypothetically cancel out leaving us with true changes that occur over the measured

follow up period. Hence our first recommendation is that future studies with the present technology should be longitudinal and not cross sectional.

We have focused extensively on the pitfalls and attempted mathematical modelling. One pitfall that is unacceptable is that the nasal arterioles have higher saturations than the temporal ones. We tried unsuccessfully to calculate a correction factor for each eye by assuming that the four major arterioles should have the same saturation. Our second recommendation is that research should be focused on obtaining similar saturations in the four major arterioles. There may be multiple ways to achieve this. Two suggestions are multimodal imaging and multispectral imaging.

We noticed that with just four equations (two for each wavelength), we had too many unknowns to solve the problem. Thus, we felt the need for multispectral oximetry which would give us more equations to work with. Multispectral imaging oximetry is an optical technique that utilizes the oxygen saturation dependent absorption spectra of hemoglobin within blood to quantify the oxygen saturation with high spatial and temporal resolution.(MacKenzie & Harvey 2018) and may be the next logical step to iron out the theoretical flaws in dual wavelength retinal oximetry.

The second option is multimodal anatomical imaging. Our models have used OCT data extensively and hence would immensely benefit from OCT data at each of the points considered for automatic retinal oximetry. With image registration, this can be achieved and can possibly enable us to correct for changes in perivascular or pre-vascular RNFL, diameter and vessel wall thickness, choroidal and RPE, Bruch's factors for each of the points.

Retinal oximetry thus gives the percent change in saturation; however, it gives us no information about the absolute amount of oxygen extracted. Knowledge of the volume of blood flowing coupled with the saturation change can tell us exactly how much oxygen has been extracted. Our last recommendation is that we find a way to study the actual amount of oxygen extracted per unit time.

In conclusion, in future we should try to plan studies with a more longitudinal study design, use either multispectral or multimodal imaging with mathematical modelling to iron out the pitfalls and lastly, use flow volume data to calculate the amount of oxygen extracted per unit time

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CHAPTER 14

Summary

Chapter 14

In this thesis, we aimed to study how the saturations are in normal subjects and how they change with disease. We also aimed to study how the possible optical artefacts are in various diseases and how they affect the measured saturation values. Chapter 1 Chapter 1 includes a general introduction about retinal oximetry, specifically about its history, principles, and commercially available machines. We provided the normative database (Chapter 2) for an Indian population which is comparable to previous studies. We reported that age, vessel diameter and ocular perfusion pressure were the significant factors that influenced the saturation. Arteriolar and venous saturations increased with age while the arterio-venous saturation difference (AVSD) did not change significantly. While the above requires manual segment selection, we also developed an ImageJ plugin that can automatically provide the oximetry results which exhibited a high consistency across the dataset indicating that the automated technique (Chapter 3) is an accurate alternative to the manual procedure.

We were the first to report an inverse relationship between oximetry values and perivascular RNFL thickness and to speculate that this relationship could be physiological or artefactual. (Chapter 4) The results of this study enabled us to expect increased saturation in areas of thinner RNFL or vice-versa with the current methodology of retinal vessel oximetry as obtained by dual wavelength photo-spectrometric fundus imaging. We also observed that the direction of gaze has a significant impact on the measured saturations which is more apparent temporally than nasally. (Chapter 5)

We performed retinal oximetry in several disease states. In amblyopic eyes we observed higher mean oxygen saturations than the fellow eyes. (Chapter 6) In retinal dystrophies, oximetry was sensitive in quantifying hemodynamic changes, however it was unclear whether these hemodynamic changes were a cause or a result of the disease process. (Chapter 7) Oximetry in CRAO showed changes compared to age-matched controls but did not correlate with visual acuity outcomes. (Chapter 8) In BRVO, the acute stage arteriolar and AVSD oxygen saturations were increased. (Chapter 9) An increase in venous oxygen saturation and a decrease in venous diameter accompanied by near normal arteriolar oxygen saturations occurred late in disease. We saw that eyes with severe NPDR has saturations significantly above normal. (Chapter 11) In inflammatory states like tubercular retinal vasculitis, we observed an AVSD which can be an indirect indicator for altered retinal metabolism that results in increased oxygen consumption. (Chapter 10) In optic nerve head edema we hypothesized that there could be a possible compartment like syndrome that causes a compression of vessels that explained the increase in arterio-venous transit time and decreased vascular diameters.

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(Chapter 11) In glaucoma we found that both the arteriolar and venous saturations were elevated thus having a resultant increase in AVSD. (Chapter 11) In Parkinson's disease we concluded that retinal metabolic parameters like arteriolar saturation and AVSD showed a significant correlation with Levodopa dosage. (Chapter 11) This may imply that retinal oximetry changes occur in association with underlying central nervous system derangements.

We then described the major problems faced in retinal oximetry namely saturations exceeding 100%, variability within the eye, variability due to the angle of gaze and rattle snaking artefacts. (Chapter 12) We subsequently explored anatomical factors like the effect of RNFL thickness, of the central light reflex and of the background reflectance that can be responsible for the artefacts. This helped us propose 5 different mathematical models. While we exposed theoretical flaws in the current method of oxygen saturation estimation, we failed to provide a better method to estimate the same.(Chapter 12)

In conclusion, this thesis shows that dual wavelength retinal oximetry can be sensitive to detect changes in disease states, however also exposes significant artefacts prompting us to be cautious while interpreting oximetry results and urges us to advance further either by developing better mathematical models or using multiple wavelengths to possibly overcome the theoretical limitations of dual wavelength retinal oximetry.

CHAPTER 15

Impact Paragraph

Chapter 15

Oxygen is one of the most essential elements, if not the most essential for our lives. We can survive without water and food for days, however, surviving for more than two minutes without oxygen is not possible. Hence it stands to reason that alterations in oxygen saturation would have far reaching effects on tissue function. If we can reliably detect this alteration, we can be certain of some tissue dysfunction. Furthermore, this can not only be used to diagnose disease, but also to monitor response to treatment. If deranged saturations show a trend towards normal, we can safely assume that the tissue function would also return to normal.

Pulse oximetry is measured on the fingers using the principle of photo spectrometry. A point worth considering is that while the fingers are covered with skin and nails which can potentially mask true variation, the retina is easily visible with transparent tissue anterior to it. Thus, it stands to reason that the measurements obtained from the retina can be a more accurate representation of true physiological values. It must be emphasised that the saturation values thus obtained will not only be suggestive of the changes occurring locally in the retina but can also give us information about systemic alterations.

The arguments presented above make a strong case for retinal oximetry, however, this investigation is not yet a part of mainstream clinical investigations. With the advent of multimodal imaging, importance is given to obtaining a comprehensive picture comprising of anatomical and functional information. Retinal oximetry if added to this ensemble can provide crucial information about the metabolic functional giving us a more complete understanding.

So why is retinal oximetry still not mainstream, despite more than 50 years of research? One of the main reasons for this is the presence of unresolved artefacts that render the obtained values less precise and accurate. Through our mathematical modelling we exposed the fact that there were too many variables that were assumed to be constant among individuals. We showed that not only do they have inter-subject variation, but they also have intra-eye variation between different vascular segments. Thus, more wavelengths and better algorithms would be needed to reliably calculate oxygen saturation.

Disease is a dynamic process and taking a static snapshot may not always tell us the complete picture. Oxygen saturation gives the percentage of haemoglobin that is saturated with oxygen; however, it does not tell us what quantity of oxygen has been delivered to the retina. For this information on flow is critical. Machines like the retinal function imager have tried to address this issue by measuring the velocity of blood flow

and the diameter of the vessels. The diameter and velocity will tell us the volume of blood that courses through the retina per unit time and the saturation can tell us what amount of oxygen is being carried per unit volume. When these parameters are combined, we can estimate the amount of oxygen delivered and consumed per unit time. Understanding this parameter can have far reaching implications and can remarkably alter our understanding of disease. Additionally, retinal vascular imaging encompassing anatomical factors like vessel width and fractals and functional parameters like flow and oxygen saturation can be used as important biomarkers for the function of a number of organs ranging from the brain to the heart to the lungs.

While with any technology, we should proceed with caution, we should also be excited about its prospects and the positive impact it could have. Let us imagine the potential of autofocussing retinal cameras with automatic imaging, segmentation, and selection. We can potentially have a fully automatic system in the future that can give the vascular saturations and even a grading of the level of oxygenation to the retina. These can then be set up as kiosks in malls and other public spaces allowing for a rapid screening of the patient's retina. For a retina clinic, this can be additionally combined with dynamic flow volume information. This would form an important aspect of any multimodal imaging protocol and pave the way for a complete anatomical and functional understanding of the patient's retinal status.

CHAPTER 16

Acknowledgements

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Acknowledgements

It is said that success is not what you do for yourself, but what others do for you. I understood the true meaning of this statement during my PhD. Numerous individuals have positively helped me throughout this journey. This journey started off at a meeting in Hyderabad where my mentor Dr. Rashmin Gandhi introduced me to my co-supervisor Dr. Rohit Shetty. It was through their vision that I embarked on this journey under the guidance of Dr. Shetty, who has always gone the extra mile whenever needed to ensure that I was in the right direction and provided me with more resources than one can ask for. He ensured that I understood the field of science completely by encouraging and guiding me to present at national and international conferences. I am certain that this degree would fill him with as much pride as it fills me for it was as much his struggle as it was mine, it was as much his journey as it was mine.

The journey taught me so much through all its ups and downs. There were times when I would feel despondent and Dr. Gandhi would always be there to encourage me and give me the necessary strength. I experienced a fair share of personal issues during the journey and my wife Shruti was there for me unconditionally. Each article when accepted would have her more excited than me and this is the single most important factor that made the journey enjoyable and memorable. Her painting is also the front cover of this thesis.

I met Dr. Tos for the first time during his India visit. We discussed the subject in detail, and he rekindled the curiosity and inquisitiveness in me. He urged me to question everything that I see, and it would be fair to say that the overall direction of my research work had changed for the better after his inputs. For almost three years we have had regular Skype calls every week and he has patiently spent time guiding and teaching me. Over the years our rapport has grown and the weekly interactions with him will leave a lacuna difficult to fill.

After I registered for the PhD, I was introduced to my supervisor, Prof. Dr. Webers. I will never forget one email interaction where Prof. Webers felt I was too much in a hurry and as a result producing half-baked work. He chided me as to how I should give everything a second thought and not be in a hurry to complete the PhD track. He taught me that quality is of utmost importance and things done in a hurry can adversely compromise it. I thank him for the opportunity to spend time in Maastricht twice, spanning a total of 5 weeks. During this time, he gave instilled confidence in my work and assured me that if we focus on quality, one day success would come. Dr. Tos went the extra mile to make things memorable for me, right from taking me on a guided tour of Maastricht, to treating me to a variety of local cheese, to a home cooked meal in his backyard that ended with a fun filled visit to one of the underground caves. He asked me

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to write my name on the cave wall and told me that now that its written, it would stay there forever. This was symbolic of how the interactions that I have had with Maastricht University, the city, and my supervisors in particular, will remain forever etched in my memory.

My father was one of my main inspirations to complete this thesis. He taught me the importance of completing academic achievements on time and whenever I would feel distraught, I would be reminded of his words and move on. This journey also showed me a few qualities I never knew I had. I always felt I was one to give up easily, however I am surprised at how giving up was never an option I considered, a quality I feel I have inherited from my mother. It is said that a friend is someone who understands your past, believes in your future, and accepts you just the way you are. I have been fortunate to have one such friend in Suyog. There have been countless occasions where in the past where he has been one of the pillars of my strength and his emotional support has played a significant part in the completion of this thesis. His unconditional confidence is a weapon I have often borrowed and one that I will continue to in the future.

Walking on a different path has unique and unprecedented challenges and through these adversities, often Dr. Anand Vinekar, Dr. Padmamalini Mahendradas and Dr. Chaitra Jayadev have been like guardian angels for me. Their genuine concern and well wishes can never be thanked enough.

While writing this acknowledgement I am choked with mixed feelings. At one end, I am filled with humility at the realisation that this journey required the support of so many well wishers and at the other end I am filled with pride that I had their support, which cannot be quantified, however if it ever can be, I am sure it will be statistically significant!

CHAPTER 17

Curriculum Vitae

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Dr. Ashwin Mohan

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PREVIOUS WORK

- ◆ Vitreo-retinal surgeon and Research Lead, Chaithanya Eye Hospital and Research Institute, Trivandrum – July 2019 – September 2020
- ◆ Vitreo-retinal surgeon, Suseela Nethralaya, Kurnool – June 2018 – March 2019
- ◆ Junior Consultant – Vitreo-retina – Narayana Nethralaya, Bangalore – July 2016 – March 2018

EDUCATION

- ◆ PhD Scholar – Maastricht University, Netherlands (Defense April 2021)
- ◆ Fellowship in Vitreo-retinal surgery - Narayana Nethralaya, Bangalore - 2014-2016
- ◆ Ophthalmology Residency - Medical Research Foundation, Chennai - Diplomate of National Board - 2010-2013
- ◆ Observerships - Emergency Medicine and General Surgery - SUNY Downstate, Brooklyn 2008
- ◆ Medical School - Lokmanya Tilak Municipal Medical College, Sion, Mumbai - 2002 - 2008
- ◆ College - D.G.Ruparel, Matunga, Mumbai 2000-2002
- ◆ School - Maneckji Cooper Education Trust - 1989-2000

RESEARCH EXPERIENCE IN OPHTHALMOLOGY/MEDICINE

1. Actively involved in imaging based clinical research in retina. Well versed with retinal oximetry, retinal functional imaging and OCT angiography.
2. Reviewer for Acta Ophthalmologica, Indian Journal of Ophthalmology, Ocular Immunology and Inflammation and Scientific Reports.
3. Project Associate at HTIC - IIT-M Research Park - 2013 - worked as an ophthalmology expert for a machine based automatic diabetic fundus image grading system.
4. Worked on Sankara Nethralya's Clinical Decision Support System - 2013.
5. Short term Research studentship - Indian Council for Medical Research - 2005 - Neurosurgery.

NOTABLE AWARDS

1. Gold medal in Neuro-ophthalmology in Sankara Nethralaya (2012)
2. IJO Honour Award for Peer Review (2019)

NOTABLE CONFERENCE PRESENTATIONS

3. ARVO 2016 - Seattle - SIG - Pitfalls in retinal oximetry and introduction to the Retinal Function Imager
4. ARVO 2016 - Seattle - Poster - Optic disc centered imaging versus quadrant imaging in retinal oximetry
5. ARVO 2015 - Denver - Poster - Oxygen saturation profiles in Asian Indian eyes with central retinal artery occlusion (CRAO)
6. NANOS 2015 - San Diego - Poster - Retinal Oximetry (Oxygen Saturation) and peripapillary vascular diameters in normal eyes and non-arteritic ischemic optic neuropathy (NAION)

PEER-REVIEWED PUBMED INDEXED PUBLICATIONS

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