Retinal Oximetry: The Indian Experience in Healthy and Diseased Eyes

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ABSTRACT
To study the alterations in retinal oxygen saturations in healthy and diseased eyes. Patients presenting to our hospital underwent an additional non-invasive procedure to measure oxygen saturation in their retinal vessels. After dilatation, oximetry was done using the Oxymap T1 retinal oximeter (Oxymap hf, Reykjavik, Iceland). Normal patients and patients with arteriolar and venous occlusions, retinal dystrophies and glaucoma, were evaluated. Arteriolar, venous and arterio-venous saturation difference (AVSD) values were determined for each of the groups. In the normal subjects \((n = 98)\), the average arteriolar saturation was 90.3 ± 6.5, and the venous saturation was 56.9 ± 6.3. The average AVSD was 33.4 ± 5.0. In arterial occlusions \((n = 10)\), we have seen an initial fall in arteriolar (85.8%) and venous (49.7%) saturations in the acute stage in eyes with central retinal artery occlusion with subsequent increase in saturations. In venous occlusions \((n = 18)\), there was an initial increase in all global saturation parameters in the acute stage (arteriolar: 105.8%, venous: 62.7%, AVSD: 43.3%), followed by a gradual decrease in saturations in the chronic stage (arteriolar: 99.8%, venous: 60.1%, AVSD: 39.8%). Eyes with retinitis pigmentosa \((n = 62)\) showed higher saturations (104.15%) and higher AVSD (44.15%) compared to macular dystrophies \((n = 23)\) (96.7% and 41.61%) and normal controls (90.6% and 33.3%). Macular dystrophies showed higher global arteriolar values and AVSD but comparable venous values to the control group. In glaucoma \((n = 44)\), we have seen raised arteriolar and AVSD values. Oximetry is sensitive in picking up changes in diseased eyes that are distinct from normal values. In the future, it may prove to be useful in pre-clinical screening studies and in therapeutic decision making.

KEY WORDS: Oximetry, Retinal circulation, Saturation

INTRODUCTION
Retinal oximetry was first described by Hickam in 1959.[1] This is a technique to quantify the percentage of hemoglobin in the blood that is saturated with oxygen. The retina is one of the most metabolically active tissues of the body. Any change in oxygen saturation could potentially disturb the normal functioning of the retina. In disease states, the oxygen saturation measurements may thus be altered and can serve as a potential biomarker.

Owing to the transparency of the cornea and the lens, the retina is the only place in the body where we can directly visualize the blood vessels. Retinal oximetry may thus not only help us in observing vascular changes due to local pathology but may also serve as a window to system-wide vascular disturbances.

Retinal oximetry is a non-invasive tool with an excellent safety profile that can be used as a rapid diagnostic adjunct to clinical examination. In this article, we aim to describe an overview of oximetry and parameters in normal subjects, outcomes in retinal vein and arterial occlusions, dystrophies such as retinitis pigmentosa and Stargardt’s disease and other diseases with a known vascular derangement like glaucoma.

RELEVANT VASCULAR DYNAMICS
In normal persons, the blood leaving the left ventricle is fully saturated, which may subsequently desaturate during the passage to the end organ because of diffusion of oxygen into the surrounding tissue. Exchange of oxygen within the optic nerve head where the central retinal artery and vein lie in close proximity may also play a role. The amount of residual arterial oxygen after an exchange in the
retinal microcirculation will increase when the blood flow is increased, or the oxygen extraction in the retinal tissue is reduced or vice versa and is reflected in the venous saturation.²

**PRINCIPLE**

Oxy and de-oxy hemoglobin behave differently to different wavelengths of light. At particular wavelengths, they have the same absorption of light and these wavelengths are called isosbestic; the wavelengths at which they behave differently are called non-isosbestic. This is the basic principle on which retinal oximetry measurement is based. It is thus a photo-spectrometric method of measurement.

Light intensities are measured at points on the blood vessel (I) and also the neighboring points falling on the background retina (I₀). The log of the ratio of the above two light intensities gives the optical density at that point at a particular wavelength (Equation 1). Ratio of the optic densities at a non-isosbestic and an isosbestic wavelength give the optic density ratio (Equation 2). The optical density ratio (ODR) is linearly related to the oxygen saturation (Equation 3).

**Equations**

1. \( \text{OD}_x = \log_{10}(I_0/I) \)
2. \( \text{ODR} = \frac{\text{OD}_{\text{non-isosbestic}}}{\text{OD}_{\text{isosbestic}}} \)
3. \( \text{Saturation} = a + b \times \text{ODR} \)

Since the first description by Hickam where the optic disc was used for \( I_0 \), retinal oximetry has evolved manifold.³ From a tedious manual procedure of measuring each point, it has now become an easy to use the commercially available automated system.

There have been different wavelengths of light used for isosbestic and non-isosbestic measurements like 570 and 600 nm or 586 and 605 nm. Measurement techniques where algorithms incorporate more than 2 wavelengths are called hyperspectral imaging. There are a few commercially available oximetry machines. The authors’ experience is based on the Oxymap T1 retinal oximeter (Oxymap hf, Reykjavik, Iceland) mounted on a Topcon fundus camera TRC 50DX.

**IMAGE ACQUISITION**

Retinal oximetry relies on reflected light from the fundus and hence the room lighting conditions and the machine parameters like aperture and flash can play a pivotal role in the final measured values. It is important to standardize parameters such as room lighting, flash settings, and aperture for every patient. We follow the practice of keeping the ambient light to a minimum for all our oximetry measurements. Exercise, caffeine intake and tobacco can influence the saturation values and hence it is important to keep the patient seated for 10 min and ensure no caffeine or tobacco intake close to the test procedure. We have ensured caffeine or tobacco intake within 2 h as an exclusion criteria for all our test subjects.

**SEGMENT SELECTION AND ANALYSIS**

The Oxymap gives a pseudocolor map (Figure 1) that shows the oxygen saturations measured at each of the points automatically chosen by the software. Geirsdottir et al. have stated important guidelines for segment selection.⁴

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.⁴ Our experience and other normative articles are based on optic disc centered images.⁴,⁵

One important limitation to keep in mind is that the algorithms work well as long as the background pigmentation is uniform. So, in conditions where...
the neighboring pixels are affected like colobomas, retinal pigment epithelial hyperplasias, or retinal to sub-retinal hemorrhages, we can expect values which are not very reliable. Hence, it is important to avoid the area 50 µm concentric to the optic disc, vascular segments with non-uniform background pigmentation and vessel crossings while choosing our vessel segments.

Vessels are chosen (Figure 2) and labeled per quadrant to give quadrant values for arteriolar, venous and arterio-venous saturation difference (AVSD) and averaged for global values.

**Variability**

The values obtained show physiological variability within and between clinical sessions. This we feel implies that oxygen saturation is a rapidly changing dynamic parameter that is dictated by a number of local and systemic factors. Authors have suggested averaging values across 100-150 µm to get reliable results.[6]

**Normal eyes**

We studied 98 eyes in the age range of 18-63 years (Figure 3). The global average arteriolar saturation ranged from 90.3% to 92.2%, and the venous saturation ranged from 55.3% to 56.9% in our study and other normative studies.[4,5] The average AVSD was around 33.2%. The inferotemporal quadrant had the lowest saturations. The average global arteriolar diameters were 121 µm, and the venous saturations were 160 µm.

Interestingly, the average difference between the pulse oximetry values and the retinal oximetry values were 7%. We hypothesize that since the eye has transparent media and lacks the masking effect of skin and nails, we may be directly observing the true physiological variability seen in humans. This makes retinal oximetry a challenging and intriguing field of vascular imaging.

We have seen an increase in arteriolar and venous saturations with age. The AVSD did not change significantly with age. Ocular perfusion pressure which was seen to increase with age also correlated positively with the increase in arteriolar and venous saturations.

The increase in retinal saturations with age could be attributed mainly to the decrease in retinal nerve fiber layer (RNFL) tissue and increase in ocular perfusion pressure. We know that the total oxygen extraction with age would decrease owing to the decrease in total tissue. This is accompanied by a corresponding decrease in ocular blood flow volume due to smaller diameter vessels secondary to arteriolosclerosis. In our experience, we found the AVDS to be unchanged with age and was the least variable of the parameters. Hence, we feel that healthy eyes are efficient at regulating and maintaining the AVDS. This could prove to be a very important parameter and be a sensitive indicator of any derangement in ocular perfusion.

We also analyzed images focused at the periphery and found that larger arterioles had the highest saturations.

![Figure 2: Method of segment selection](image)

![Figure 3: Summary of the arteriolar, venous and arterio-venous saturation differences in our study patients](image)
which decreased in smaller arterioles followed by the smaller venules and was the least in the larger venules.

**Arterial occlusions**

Published literature states that the oximetry findings in central retinal artery occlusion (CRAO) are variable.\(^7\) In another report by Hammer *et al.*, a mixed case series of central and branch arterial occlusions showed a reduction in arterial saturations that later increased with 5 days of pentoxifilin therapy.\(^8\) In our own experience, we studied 10 eyes and had seen an initial fall in arteriolar (85.8%) and venous (49.7%) saturations in the acute stage in eyes with CRAO with a subsequent increase in saturations. On follow-up, they showed a gradual increase in saturations with time. We have seen in 2 of our cases that the saturation and ocular perfusion dramatically improve within minutes after anterior chamber paracentesis (Figure 4).

Our results showed the saturations did not fall to an absolute zero in the event of an occlusion indicating that there is some residual perfusion present even in the setting of acute severe visual loss and that improvement in oxygen saturations is associated with an improvement in visual acuity.

We can expect that in the obstruction of the main blood supply to the inner retina there would be very low saturations a few hours after the insult as the residual blood in the arterioles would quickly lose the oxygen to the surrounding tissues. We know that there exists a capillary-free zone surrounding the arterioles and venules where oxygen exchange takes place.\(^9\) We also know that there is widespread edema of the inner retinal layers, especially the RNFL where this capillary-free zone exists.\(^10\) We hypothesize that this edema could limit the exchange of oxygen and hence results in the retention of oxygen in the vessels in spite of drop in blood flow. Decreased blood flow with impaired exchange due to mass inner retinal edema seems to be the main hypothesis in explaining our results.

**Vein occlusions**

Branch retinal vein occlusions (BRVO) have been reported to show very variable saturations attributable to the degree of occlusion and stage of resolution.\(^11\)

Since the occluded segment is obscured by hemorrhages, it is not technically possible to measure the ODR. We have hence measured the segment proximal to the occlusion. We hypothesize that the proximal segment carries the blood that has coursed through the occlusion and hence may possibly give us valuable insights into the disease pathology and metabolic derangements. We found in our study of 18 patients that there was an initial increase in all global saturation parameters in the acute stage (arteriolar: 105.8%, venous: 62.7%, AVSD: 43.3%), followed by a gradual decrease in saturations in the chronic stage (arteriolar: 99.8%, venous: 60.1%, AVSD: 39.8%) (Figure 5). The age-matched normal controls had lower saturations (arteriolar: 93.4%, venous: 57.2%, AVSD: 36.5%) Venous saturation and diameter changes occurred late in disease. We hypothesized that the induced ischemia would produce an increase in the local mediators thus resulting in increased blood flow and increased arteriolar saturations. When we examined the involved quadrant, we found that the arteriolar and venous saturations were high in the acute stage, with the arteriolar saturations returning to normal in the chronic stage while the venous saturations remained high. The decrease in arteriolar saturations could be indicative of the demand returning to baseline. Tissue atrophy and less oxygen extraction could be the cause for the high venous saturations observed in the chronic stages. A predictable decrease in diameters can be explained by the tissue atrophy and also the formation of collaterals that would divert the blood through

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*Figure 4: Images on the left show the oximetry at presentation and the images on the right show the oximetry images after paracentesis*
other channels. The other eye showed significant differences as compared to normal indicating that there are subclinical changes, which may help us in the screening of patients identifying the “at risk” patients. Central vein occlusions are more complex to study. In oximetry, the peripapillary and perivascular retina plays a crucial role in the reflection of light and hence measurement. Hemorrhages on both sides of the vessel will alter the value of $I_o$ and render the measured values inaccurate. The occlusions in BRVO occur close to an arterio-venous crossing allowing us enough measurable vessels between the disc and the occlusion. In CRVO, the occlusion in within the optic disc, thus leaving very little or in some cases no measurable vessels. This is the biggest technical challenge faced with oximetry in CRVO. Hardarson has reported that lower venous saturations than the fellow eye in cases of CRVO.\[3\]

**Retinal dystrophies**

In retinitis pigmentosa, the photoreceptor atrophy and death causes less oxygen utilization that result in higher oxygen partial pressures in the inner retina. This results 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. have stated that there is a high prevalence of peripheral vascular dysregulation syndrome in RP patients, which causes alteration early in disease and implying that vascular changes can be primary and be causative.\[12\] This difference of opinion makes vascular imaging in retinitis pigmentosa very interesting and may have clinical implications when understood better.

We saw that in our study of 85 patients (Figure 6), ones with RP ($n = 62$) showed significantly lower diameters (98.4 µm), higher saturations (104.15%) and higher AVSD (44.15%) compared to macular dystrophies ($n = 23$) (116.5 µm, 96.7% and 41.61%), and normal controls (122.4 µm, 90.6% and 33.3%). Macular dystrophies showed higher global arteriolar values and AVSD but comparable venous values to the control group.

Previous studies have also reported similar findings. Türksever et al., and Eysteinsson et al., equivocally confirm the decrease in vascular diameter.\[13,14\] Türksever et al. have reported increase in arteriolar saturation with a mean of 99.3%, whereas Eysteinsson et al., have found no change in RP with a mean of 91.7%. Venous saturation was increased, and AVSD was decreased in both the studies (58.0%-66.8%).

It has been shown that translocated cells of the retinal pigment epithelium can deposit a thick layer of extracellular matrix around retinal vessels.\[15\] This can
effectively block oxygen diffusion out of the vessels and explain the high saturation seen in the arterioles.

The thickness of RNFL is known to be decreased in RP.\textsuperscript{[16,17]} In our own study (unpublished), we found inverse correlation between vascular saturation and perivascular RNFL thickness in normative Asian-Indian eyes. This could explain our observation of increasing arteriolar and venous saturation. 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.

**Glaucoma**

Published reports in glaucoma state that there is no change in the arteriolar saturations, an increase in venous saturation, and a decrease in AVSD.\textsuperscript{[18,19]} We have seen an increase mainly in arteriolar and AVSD in 44 eyes that we have studied.

The hypothesis by other authors is that with tissue loss, there would be less utilization and hence higher venous saturations and lower AVSD. We have seen an opposite trend which is intriguing. Interestingly, one common event in physiological aging, retinitis pigmentosa and glaucoma is a loss of retinal tissue and RNFL. We have noted the same trend in saturations in these diseases - An increase in measured saturation values. In normal retinas though we saw that the AVSD remained unchanged, but pathological conditions like RP and glaucoma saw an increase in the AVSD as well. We have also noted a negative correlation with mean deviation on visual fields and retinal saturations - As the mean deviation values get more negative, we see higher vascular saturations.

**DIABETES, HYPERTENSION, AND AGE-RELATED MACULAR DEGENERATION**

Reports on diabetes reveal a trend of increasing arteriolar and venous saturations with disease.\textsuperscript{[20]} Higher venous saturations were seen with increasing severity of disease in non-proliferative diabetic retinopathy (NPDR), and higher arteriolar and venous saturations were seen in the proliferative disease.\textsuperscript{[2]}

The higher venous saturations in NPDR have been explained by less extraction of oxygen implying that the microcirculatory disturbance becomes more widespread as the disease progresses.

We performed a comparison between patients with a history of well-controlled hypertension and no visible retinopathy and normal controls. We found no statistically significant difference. Increased blood pressure should increase the ocular perfusion pressure and cause the blood to course faster in the eye. Vessel wall changes with hypertension also might change the way light interacts and thus alter the measured saturation. It would be interesting to see if oximetry can reveal markers of hypertensive disease.

Age-related macular degeneration is characterized by lower extraction of oxygen.\textsuperscript{[21]} It is interesting to note that a disease localized to the macula and characterized by a primary derangement in the choroidal circulation can have a significant effect on the global average retinal saturations.

**FUTURE DIRECTIONS**

In the future, correlation of oximetry with areas of capillary non-perfusion on fundus fluorescein angiography would be a significant step ahead. Non-invasive non-contact methods like these that can aid in decision-making would be a huge benefit to the patients and the treating doctors. Correlation of oxygen saturation levels with VEGF levels would help us better understand the alterations in saturations with ischemia. This can then be used even in therapeutic decisions as to a choice between anti-VEGF injections or intravitreal steroids. The procedure for analysis and segment selection involves a lot of manual input and calculation. Automation of this procedure and summarizing the general state of retinal oxygenation by a number or in the form of a percentage would greatly help in cursory evaluation and screening.

**REFERENCES**


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