The Relationship Between Retinal Vessel Oxygenation and Spatial Distribution of Retinal Nonperfusion in Retinal Vascular Diseases

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Purpose. We study the relationship between retinal vessel oxygenation and the spatial distribution of retinal nonperfusion using ultrawide field angiography in eyes with retinal vascular diseases.

Methods. This prospective single center study recruited 57 eligible eyes from 44 patients with retinal vascular diseases. Retinal oximetry measurements were obtained using the Oxymap T1 device to determine the arteriovenous (AV) difference. Retinal nonperfusion was measured from ultrawide field angiography images taken with the Optos 200TX system and superimposing the images with the concentric rings template to determine the area and distribution of retinal nonperfusion.

Results. Seven (12.3%) eyes had a diagnosis of a branch or hemiretinal vein occlusion, 24 (42.1%) with central retinal vein occlusion and 26 (45.6%) with diabetic retinopathy (11 [19.3%] nonproliferative and 15 [26.3%] proliferative diabetic retinopathy). The correlation between the total area of retinal nonperfusion with the AV difference controlling for age was not statistically significant ($R = -0.103$, $P = 0.449$). However, when analyzing the correlation of AV difference with the area of retinal nonperfusion in the posterior pole controlling for age and peripheral nonperfusion, this was significant ($R = -0.295$, $P = 0.029$). This was not significant for the area of retinal nonperfusion in the periphery while controlling for posterior pole nonperfusion and age ($R = 0.124$, $P = 0.368$).

Conclusions. Retinal nonperfusion has a negative correlation with AV difference measured on retinal oximetry. This correlation is significant in the posterior pole, but not in the peripheral retina.

Keywords: oxygenation, oximetry, retinal nonperfusion, diabetic retinopathy, retinal vein occlusion

The public health burden of visual impairment due to retinal vascular diseases is significant. The most common retinal vascular diseases are diabetic retinopathy and retinal vein occlusion. The majority of sight-threatening complications of retinal vascular diseases are the sequelae of retinal capillary nonperfusion.

The retina is unique in its oxygenation because retinal vessels must provide and extract oxygen without structurally obscuring the visual axis. This requires efficient oxygen delivery given the limited vasculature permitted. Retinal nonperfusion in retinal vascular diseases can result in ischemia. The retina has the highest oxygen consumption of any tissue of the body and most oxygen is consumed by the retinal photoreceptors.[1,2] The photoreceptor has limited mitochondrial reserve capacity increasing its vulnerability to metabolic changes.[3] In the diseased state, because of the retina's unique demand for oxygen, even subtle decreases in oxygen availability can result in a hypoxic state.[1,4]

Studies on retinal oxygenation are essential in further understanding the relationship of retinal oxygenation and ischemia in retinal vascular disorders. Early retinal oxygenation studies involved invasive tests and were restricted to studies in animal models. Human studies require noninvasive methods to evaluate retinal oxygenation. A recent method for measuring retinal vessel oxygenation has been developed by Hardarson et al.[5] in Reykjavic, Iceland. It is composed of a fundus camera and beam splitter. A software (Oxymap analyzer) then is used to analyze the data obtained. The validity of retinal oximetry measurements has been studied with reports of excellent reliability and reproducibility.[6,7]

Current literature on the use of oximetry in retinal diseases is expanding. Several studies have evaluated retinal vessel oxygenation in retinal vein occlusion, diabetic retinopathy, retinitis pigmentosa, and neovascular age-related macular degeneration.[8–11] Early studies evaluated retinal oximetry measurements in different retinal vascular diseases and revealed varying results.[8,12–14] The concept of arteriovenous difference then was used to reveal oxygen consumption and several studies evaluated this parameter in varying severity of diabetic retinopathy with inconsistent results, but a general trend toward reduction in AV difference with increasing severity of diabetic retinopathy.[15–17] A similar finding is observed in retinal

vein occlusion.[18–20] The variability in area of retinal nonperfusion is evident in these retinal vascular diseases and, thus, more recent studies have focused on the relationship of retinal nonperfusion in these conditions with the AV difference with more promising results.[15,21] However, these studies were done on small sample sizes; the largest subgroup was 17 for diabetic retinopathy and 23 for retinal vein occlusion, and again with varying results. Secondly, the metabolic demand is not equally distributed within the retina and, therefore, the location of ischemia will likely have an effect on oxygen consumption.[22] Finally, age may be a confounding variable as well and controlling for this variable was scarcely performed in these previous studies.

We prospectively assessed the correlation between the spatial location of retinal nonperfusion measured using the validated concentric rings method with the AV difference as measured on retinal oximetry in a sufficiently powered study.

Methods

This prospective cross-sectional study was done from December 30, 2014 to August 24, 2016 at the National Institute for Health Research Moorfields Biomedical Research Centre and the Institute of Ophthalmology, University College London, United Kingdom. The study was granted approval by the Wales Research Ethics Committee 6 (14/WA/1224). The study was implemented in accordance with Good Clinical Practice and the Declaration of Helsinki.

Inclusion and Exclusion Criteria

Eligible patients 18 years or older with a diagnosis of any retinal vascular diseases who had the capacity to provide informed consent were included. Sufficient media clarity and pupillary dilatation for adequate fundus photographs were required. Participants must have undergone fluorescein angiography using the Optos ultrawide field system (Optos, Dunfermline, Scotland) within 6 months or were due to undergo one in a month. Patients with poor quality images on angiography or retinal oximetry were excluded. Previous retinal laser treatment and other retinal diseases also were excluded.

Investigative Procedures

Oximetry. Retinal oximetry was performed using the retinal oximeter (Oxymap T1 device connected to Topcon TRC50-DX fundus camera; Oxymap ehf., Reykjavik, Iceland). It consisted of a fundus camera with an attached beam splitter as well as a digital camera. The Oxymap T1 acquires two images at two different wavelengths of the same area (586 nm, which is insensitive to oxygen, and 605 nm, which is oxygen-sensitive). The ratio of these optical densities (absorbance) is approximately linearly related to hemoglobin oxygen saturation[12,23,24] The oximeter is calibrated to yield relative oxygen saturation values.[5] The two spectral images are processed by the Oxymap analyzer software to give predicted oxygen saturation values.

Optic disc centered images were captured through dilated pupils and the images were 1200 × 1600 pixels and covered a 50° field of central retina. The images were captured for both eyes, but only the affected eye(s) was used in this analysis. Images were analyzed using the Oxymap Analyzer software (Oxymap ehf.). A minimum vessel width of eight pixels was set and vessels less than eight pixels were automatically excluded from the analysis. Oximetry measurements were performed using previously reported methods whereby the intragrader intraclass correlation coefficient was reported to be 0.89 to 0.99.[25] In brief, an initial central circle was used to delineate the optic disc. Two additional measurement circles are made, one three times and the following circle 1.5 times the diameter of the central circle. The area between these two additional circles centered on the optic disc was analyzed. Areas where vessel detection would prove inaccurate (branching, overlapping, background hemorrhage, or intersecting), and segments of vessels less than 50 pixels long were excluded. Vessels to be measured were selected manually. The retinal oximetry data streams were extracted from the source data file. Data collected from the Oxymap software included mean arteriolar oxygen saturations (SaO2), mean venular oxygen saturations (SvO2), and mean arterial vessel, and mean venular diameter. The difference in arterial and venular oxygenation was determined and referred to as the AV difference.

Fluorescein Angiography. The ultrawide field angiography images were obtained using the Optos 200TX ultrawide field system observing a standard protocol after an intravenous bolus infusion of 5 mL 20% fluorescein sodium. The protocol consisted of acquiring images in transit phase, arteriovenous phases, and late frames. A single investigator (LN) identified the best macula centered fluorescein angiography (FA) image in the arteriovenous phase from the FA series of each eligible eye.

Image Processing

A correction factor was automatically applied for flattening of the three-dimensional (3D) image to a two-dimensional image via the Optos V2 Vantage Pro software. The concentric rings template then was added to each image according to previously described methods.[26] This validated method incorporates a macular ring with a radius of 2.5 disc diameters (DD) and five additional concentric rings (rings 1–5), each with a 2.5 DD increment in radius. Each of the six rings (Rings M and 1–5) was divided into 12 segments. Each segment was graded as upgradeable, not perfused, or perfused if 50% or more of the segment was involved. In addition to quantifying nonperfusion, the concentric rings method allowed documentation of location of nonperfusion. The area of each cell in each concentric ring was modified based on the enlargement factor identified using 3D printed model eyes. The modified area of each segment based on the corresponding enlargement factor in each ring was used.[27,28] The montaged image, using a steering protocol to obtain three images (superior, central, inferior), introduced projection distortions that as yet had not been studied or validated as the location of the same area of the retina is distorted differently in different images given the different angular location. Therefore, only the central image was used in this study as the projection distortion in this has been studied previously and we have made the appropriate corrections.

Image Analysis

Two graders who are retinal specialists (LN and CVA) measured the area of retinal nonperfusion using the concentric rings method on a Samsung LED 46-inch 1080p television screen. The area measured was averaged between the two graders. The average values for each ring (Rings M, and 1–4) were determined. Ring 5 was excluded from the analysis due to the high number of ungradable segments reported previously. A single investigator (LN) reviewed ultrawide field autofluorescence images taken with the Optos 200TX system of eyes in the cohort where available to assess for loss of autofluorescence which may be suggestive of photoreceptor loss.

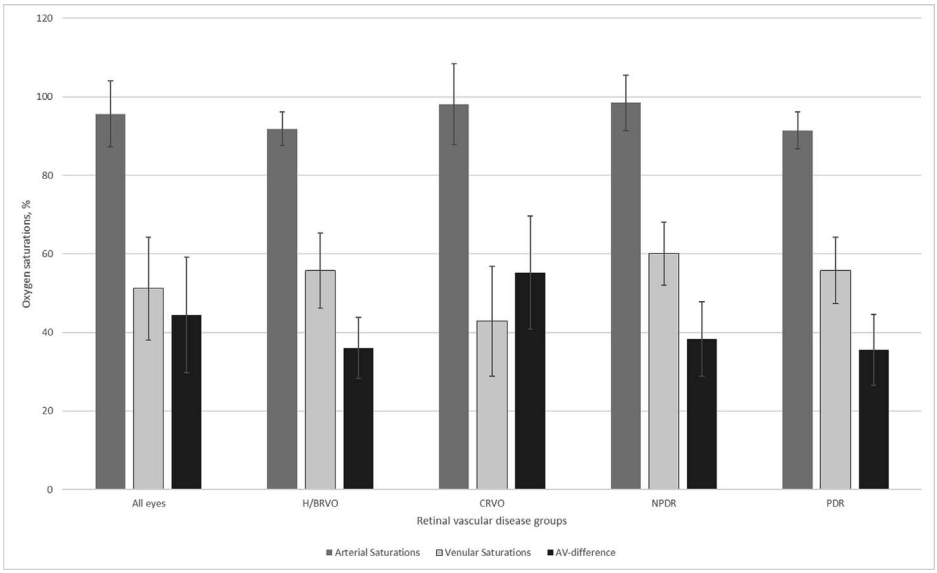

Figure. The arteriolar and venular oxygen saturations and AV differences in oxygen saturations in retinal vascular diseases.

Statistical Analysis

The sample size was calculated based on the primary objective. To our knowledge, no previous studies exist comparing this specifically at the start of the study. Observing previous reports on oximetry readings in retinal vein occlusion and proliferative diabetic retinopathy, a medium effect size was expected.[8,21,29] Therefore, with a probability level of 5% and to achieve 80% power for correlation analysis, a sample size of 54 was required. The association between AV difference and area of retinal nonperfusion was measured using a partial correlation controlling for age. The association between posterior nonperfusion and AV difference was controlled for age and peripheral nonperfusion, and the associated between peripheral nonperfusion and AV difference was controlled for age and posterior nonperfusion. Analysis was performed using SPSS version 22.0 (IBM Corp., Armonk, New York, USA).

Results

A total of 57 eyes from 44 patients (mean age, 58.2 ± 20.2 years; range, 25–94) fulfilled the eligibility criteria and were analyzed. Seven (12.3%) eyes had branch or hemiretinal vein occlusion, 24 (42.1%) central retinal vein occlusion, and 26 (45.6%) diabetic retinopathy (11 [19.3%] nonproliferative and 15 [26.3%] proliferative).

Mean area of retinal nonperfusion in the whole cohort measured 98.7 ± 77.4 DA. Mean arterial oxygen saturations were 95.64% ± 8.37% and venular saturations were 51.19% ± 13.13%. Mean AV difference calculated was 44.46% ± 14.65%. The correlation between the total area of retinal nonperfusion with AV difference controlling for age was not statistically significant ($R = -0.103$, $P = 0.449$). However, when analyzing the correlation of AV difference with the area of retinal nonperfusion in the posterior pole (Rings M and 1) while controlling for age and peripheral nonperfusion, the correlation was significant ($R = -0.295$, $P = 0.029$). The correlation was not significant for the AV difference and peripheral nonperfusion while controlling for age and posterior nonperfusion ($R = 0.124$, $P = 0.368$).

The arteriolar and venular saturations and AV difference for branch or hemiretinal vein occlusions, central retinal vein occlusions, and nonproliferative and proliferative diabetic retinopathy are presented in the Figure. The correlation between AV difference and area of retinal nonperfusion for the total cohort and individual diseases also are presented in the Table. All 32 eyes that had autofluorescence imaging (32/32) had no abnormal loss of autofluorescence.

Discussion

In this prospective study, we found a negative correlation between the AV difference in oxygen saturations and the area of retinal nonperfusion in eyes with retinal vascular diseases. Globally, statistical significance was not achieved, but when studied within regions, the AV difference was correlated significantly with area of nonperfusion in the posterior pole. The association was not significant for the periphery. This finding can be explained by the original hypothesis that the larger the area of retinal nonperfusion, the lesser the amount of viable retinal tissue and so the oxygen consumption is reduced. This reduced use leads to reduce oxygen extraction and a reduced AV difference. Interestingly, this association is significant only in the posterior retina and not in the periphery. This may explain the varied results seen in previous reports and, to an extent, the global effect of retinal nonperfusion with AV difference in our cohort. This negative correlation between AV difference and posterior nonperfusion corroborates previous findings of the significance of posterior nonperfusion in retinal diseases, such as central retinal vein occlusion.[28] The importance of posterior ischemia and the metabolic demands of the retina has not been described as the noninvasive

Table. The Correlation of Area of Retinal Nonperfusion With AV Differences in Retinal Vascular Diseases While Controlling for Age

Disease Category	Number of Eyes	Mean Area of Retinal Nonperfusion, DA	*R*	*P* Value
All eyes	57	98.7 ± 77.4	−0.103	0.449
Retinal vein occlusion	31	58.7 ± 52.2	0.149	0.433
Diabetic retinopathy	26	124.3 ± 58.9	−0.053	0.801

assessment of metabolic demands of the retina was not easily tested previously. With retinal oximetry, we have now identified a relationship between these variables. The results suggested the significant oxygen demands of the posterior pole and nonperfusion in this region results in reduced oxygen extraction from the retinal circulation. This may be a contributory factor for the development of neovascularization which has been shown to be related to posterior pole nonperfusion and oximetry measurements of new vessels that have near arterial oxygen saturation levels.[28,30,31] In a clinical setting, using the AV difference measured with a noninvasive tool can aid the clinician in identifying an ischemic or nonischemic retina should angiography not be possible or available.

The retinal thickness decreases significantly toward the periphery.[32] The oxygen distribution curve has been reported to have two peaks with increased oxygen tension in the inner retina mainly at the superficial retina, which then decreases to a minimum at approximately 150 μm depth and increases toward the Bruch's membrane, depth 400 to 450 μm, in animal models.[1,33] Toward the periphery, the vascular density also is reduced from 10.1% at the macula to 1.3% at the far periphery.[34] The oxygen distribution curve of an induced artery occlusion and in an avascular retina in animal models are similar with reduced inner retinal oxygenation and peak levels toward Bruch's membrane.[1] The oxygen demand of the inner retina toward the peripheral retina is likely to be much less compared to the posterior pole. Therefore, the significance of the relation of posterior pole nonperfusion and the AV difference in our study may be explained by the higher retinal volume in the posterior pole having a larger effect on retinal oxygenation as opposed to the periphery. The clinical manifestations of posterior pole nonperfusion also have been reviewed in its significance in neovascularization in central retinal vein occlusion.[28]

The significant association with nonperfusion in the posterior pole and poorer correlation with the periphery also interestingly mirrors the photoreceptor density map.[22,35] The concentric rings template, when superimposed onto a rod photoreceptor density map, reveals that rings M and 1 also correspond to the zones of the retina with the highest rod photoreceptor density.[22] It is well established that retinal photoreceptors are highly metabolically active cells.[1] The rod photoreceptors also are known to have a higher oxygen demand.[36,37] Therefore, the observation that the posterior pole (Rings M and 1) corresponds to the zones with highest rod photoreceptor density could be related to the high oxygen consumption of the rod photoreceptor in the retina. However, we acknowledge that in this study, OCT imaging over the peripheral retina was not performed to ascertain the presence of the ellipsoid layer over the mid-peripheral and peripheral retina. Instead, we assessed the ultrawide field autofluorescence image where available to ensure no loss of autofluorescence suggesting loss of photoreceptor function was present.

The strengths of this study lies in the prospective nature of the data collection, area of retinal nonperfusion that is corrected for projection artefacts, measurement of area of nonperfusion and retinal vessel oxygenation using a validated method, and analysis that is controlled for age. Furthermore, to our knowledge, no report has been published studying the significance of the regional effects of nonperfusion on retinal oxygenation. Also to our knowledge, this is the largest single cohort series assessing the correlation between retinal nonperfusion with AV difference. We acknowledge several limitations, such as the fact that we included diabetic retinopathy and retinal vein occlusion in our cohort. However, the study specifically evaluated retinal nonperfusion from a cross-sectional approach. The Oxymap system also had its limitation in only being able to assess the global retinal oxygenation by measuring the retinal vessels in the peripapillary area. Therefore, regional oximetry measurement is not possible or have been validated using the current system. Furthermore, previous validation studies were performed mainly in healthy eyes.[25,38] Therefore, retinal oximetry measurement in eyes with severe retinal vascular diseases despite multiple previous publications has not specifically been validated.

In conclusion, retinal nonperfusion has a negative correlation with AV difference measured on retinal oximetry. This correlation is significant in the posterior pole, but not in the peripheral retina.

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