Oxygenation in Glaucoma

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HÁSKÓLI ÍSLANDS

Súrefnismettun í gláku

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Ritgerð til meistaragráðu í líf- og læknavísindum

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Ágrip

Markmið:

Deilt hefur verið um orsakir gláku í 150 ár. Margt bendir til þess að blóðflæði í augum glákusjúklinga sé minnkað eða því illa stjórnað og getur það hugsanlega leitt til súrefnisskorts. Markmið verkefnisins er því að mæla súrefnismettun sjónhimnuæða í glákusjúklingum og kanna hvort tengsl séu á milli súrefnismettunar og sjónsviðsskemmda í gláku.

Efni og aðferðir:

Súrefnismettun í sjónhimnuæðum sjúklinga með gleiðhornsgláku með og án flögnunarheilkennis (e. pseudoexfoliation) var mæld í fyrsta og annars stigs sjónhimnuæðum með súrefnismæli (Oxymap retinal oximeter) og borin saman við sjónsvið fengin úr Octopus 123 sjónsviðsmæli. Meðalsjónsviðsskemmd (e. mean visual field defect, MD) var metin með tilliti til súrefnismettunar í samsvarandi sjónhimnuæðum (n=45). Súrefnismettun sjónhimnuæða var einnig metin á milli ósamhverfra glákuskemmdra svæða í sama auga (n=13). Framkvæmd voru Pearson's r fylgnipróf og stúdents t-próf hvað varðar tölfræðilega marktækni.

Niðurstaða:

Jákvæð fylgni var á milli súrefnismettunar bláæða og meðalsjónsviðsskemmdar (r=0.36, p=0.015). Sömuleiðis minnkaði munur í súrefnismettun milli slagæðlinga og bláæðlinga marktækt með aukinni meðalsjónsviðsskemmd (r=-0.42, p=0.00037). Engin fylgni var milli súrefnismettunar í slagæðlingum og meðalsjónsviðsskemmdar (r=-0.12, p=0.43).

Hjá sjúklingum með ósamhverfar glákuskemmdir innan sjónsviðs í sama auga mældist lægri súrefnismettun hjá slagæðlingum (98±5%) sem tilheyrðu svæði með dýpri glákuskemmd samanborið við heilbrigðara svæði (102±6%, p=0.04). Munur í súrefnismettun milli slagæðlinga og bláæðlinga var einnig lægri á glákuskemmdum svæðum (30±10%) samanborið við heilbrigðari svæði (37±10%, p=0.04). Enginn marktækur munur fannst á súrefnismettun bláæðlinga (p=0.3).

Umræða:

Svæði með dýpri glákuskemmd hafa minni mun í súrefnismettum milli slagæðlinga og bláæðlinga og hugsanlega minni súrefnisflutning til vefsins. Þetta kann að stafa af minni súrefnisnotkunar vegna rýrnunar sjónhimnu og glákuskemmda. Niðurstöður okkar benda til truflunar á súrefnisefnaskiptum í sjónhimnu glákusjúklinga og kalla á frekari rannsóknir á súrefnis-efnaskiptum sjónhimnu í glákusjúklingum til þess að auka skilning á lífeðlisfræði gláku og hlutverki súrefnis í þróun sjúkdómsins.

Abstract

Purpose:

For 150 years there has been a debate about what causes glaucoma. There is considerable evidence to support the idea that there is decreased or poorly controlled ocular blood flow in glaucomatous eyes, which could possibly cause glaucoma. The purpose of the study is to measure retinal vessel oxygen saturation in glaucoma patients and determine whether there is a correlation between retinal oxygen saturation and glaucomatous visual field defects.

Methods:

Retinal vessel oxygen saturation in patients with open-angle glaucoma with and without pseudoexfoliation was measured in first and second degree retinal vessels with a spectrophotometric retinal oximeter (Oxymap retinal oximeter) and evaluated with visual fields attained from Octopus 123. The mean visual field defect was evaluated in relation to oxygen saturation of hemoglobin in corresponding retinal blood vessels (n=45). Retinal vessel oxygen saturation between areas with asymmetrical visual field defects within the same eye was also compared (n=13). Statistical analysis was performed with Pearson's r Correlation and Student´s t-test.

Results:

Oxygen saturation in retinal venules was positively correlated with visual field mean defect (r=0.36, p=0.015) A statistically significant correlation was observed between arteriovenous difference in oxygen saturation (r=-0.42, p=0.00037) and visual field mean defect . No correlation was found in retinal arterioles (r=-0.12, p=0.43).

In patients with asymmetrical visual field defects within the same eye, the mean arteriolar oxygen saturation was lower (98±5%) in areas corresponding to a deeper visual field defect compared to healthier areas (102±6%, p=0.04). The arteriovenous difference was also lower in areas corresponding to deeper visual field defect (30±10%) compared to healthier areas (37±10%, p=0.04). The venules showed no statistically significant difference (p=0.3).

Conclusion:

Deeper glaucomatous visual field defects are associated with decreased arteriovenous difference in retinal oxygen saturation and possibly a decreased oxygen delivery to the retinal tissues—perhaps resulting from less oxygen consumption because of degeneration of the retina and glaucomatous damage. The data suggest disturbed oxygen metabolism in the glaucomatous retina indicating the need of further study on oxygen metabolism in glaucoma for a better understanding of the physiology and the role of oxygen in the pathogenesis of the disease.

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Abbrevations

IOP = intra-ocular pressure
mmHg = millimeter mercury
MOAP = blood pressure in the mean ophthalmic artery
F = blood flow
R = resistance
r = radius
l = length
η = viscosity
cmH_2O = centimeter water
NO = nitric oxide
LOXL-1 = lysyl oxidase-like 1
n = number
HIF-1α = hypoxia-inducible factor 1alpha
$SatO_2$ = oxygen saturation
HbO_2 = oxygenated hemoglobin
Hb = deoxygenated hemoglobin
OD = optical density
I_0 = intensity of incident light
I = intensity of transmitted light
L = liter
mmol = millimole
cm = centimeter
nm = nanometer
ODR = optical density ratio
dB = decibel
SP = systolic blood pressure
DP = diastolic blood pressure
MAP = mean arterial pressure
CI = confidence interval
r = coefficient of correlation
AV = arteriovenous
PEX = pseudoexfoliation syndrome
SD = standard deviation

1 Introduction

1.1 Structure of the eye

The wall of the eye consists of three layers or tunics; the outer fibrous tunic, the intermediate vascular tunic and the inner neural tunic. The fibrous tunic is composed of the cornea, the conjunctiva and the sclera. It provides mechanical support and protection for the eye. It also serves as an attachment for the extrinsic eye muscles. The vascular tunic (or the uvea) is made of the iris, the ciliary body and the choroid. The choroid supplies oxygen for parts of the inner tunic (the retina). The iris regulates the amount of light that enters the eye by means of dilation and contraction.[1, 2] The ciliary body can be divided into a few layers, including the ciliary muscle, the ciliary processes and the ciliary epithelium. It participates in the production of aqueous humor which is secreted across the epithelium from the ciliary processes to the posterior chamber of the eye. The aqueous humor is a clear fluid that provides structural integrity and nourishment for the eye. It flows from the ciliary body through the pupil into the anterior chamber, exiting the eye via two pathways; the uveoscleral outflow or through the trabecular meshwork-Schlemm's canal-venous system.[3] The canal is a passageway that encircles the anterior chamber angle. It can be divided into three major layers, the uveal, the corneoscleral and the juxtacanalicular layer. It serves as a check valve whereby the aqueous humor flows down a hydrostatic-pressure difference between the intra-ocular and episcleral venous pressures. Any event resulting in the elevation of episcleral venous pressure will result in the elevation of intra-ocular pressure (IOP, measured in mmHg)—allowing aqueous humor outflow to the venous system of the sclera. If the IOP is lower than the episcleral pressure, the trabecular meshwork may collapse or blood may reflux into the Schlemm's canal.[3, 4] The rate of removal of aqueous humor normally keeps pace with the rate of generation of aqueous humor at the ciliary processes.[1]

The ciliary muscle can facilitate aqueous drainage through the trabecular meshwork by contraction. The ciliary muscle also plays part in accommodation by influencing the shape of the crystalline lens.[5] Lying in the posterior of the cornea, the crystalline lens is held in place within the lens capsule by the zonular fibers, which are connected to the ciliary body. As mentioned previously, the ciliary muscle controls accommodation by changing the curvature of

the lens and thus, contributing to the main function of the lens which is to focus the visual image on the photoreceptors. The transparency of the lens is dependent on a precise combination of structural and biochemical characteristics. Deviations from the balance of these precise combinations result in loss of transparency and the formation of a cataract—where senile cataracts are the most common form of cataracts. Furthermore, with time, the lens can also turn yellowish and eventually begin to lose its own transparency.[1]

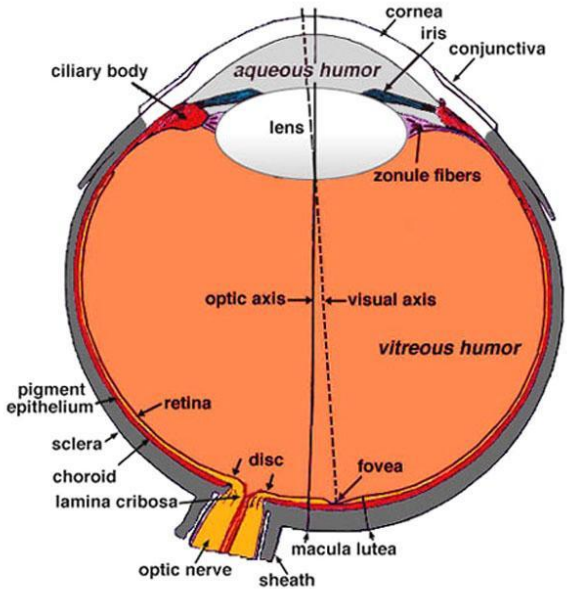

Figure 1. Sagittal horizontal section of the eye. Link to figure: http://webvision.med.utah.edu/imageswv/draweye.jpeg

The innermost tunic (the retina) is the neural layer of the eye. It will be described in detail below (chapter 1.1.1). Light enters through the cornea and passes through the aqueous humour into the anterior chamber, crystalline lens and a transparent gelatinous mass called the vitreous humour. From there it passes into the vitreous cavity and onto the retina where it is transformed in to electrical signals by the photoreceptors. [1, 2]

1.1.1 The retina

The retina is the innermost layer of the eye—located just inside the choroid. These two layers are separated by Bruch's membrane (figure 1). The retina covers inside the back of the eye and ends by the ciliary epithelium. It consists of different layers as described in table one. As a part of the central nervous system, the retina is mostly made of different types of neurons and glial cells. There are five major classes of neurons (photoreceptors, horizontal cells, bipolar cells, amacrine cells and ganglion cells) in the mammalian retina.

They can be divided into ~60 individual types.[6] The signal route from photoreceptors towards the ganglion cells is quite complex and involves different types of cells where the neural signals produced by the photoreceptors are processed.[7]

Photoreceptors (rods and cones) detect light and transform it into electrical signals. Rods have a low threshold for detecting light and function best in dim light conditions. Cones, on the other hand, are less sensitive to light and operate best under daylight conditions. They provide the color in our vision and add to a high visual acuity. Maximal density of cones is within the fovea, which is a part of the macula. Photoreceptors release the neurotransmitter glutamate for signaling.

Bipolar cells exist between photoreceptors and ganglion cells. There are approximately nine types of bipolar cells that synapse cones but only one that synapse rods. Bipolar cells are glutamatergic neurons, categorized into either ON or OFF cells depending on their response to the glutamate released by the photoreceptors.[7]

A numerically small proportion of the retina's interneurons, are horizontal cells. They make a feedback system for the photoreceptors by enhancing contrast between adjacent light and dark region. In other words, they adjust the whole system's response to the overall level of illumination by measuring illumination across a broad region and subtract it from a signal transmitted to the inner retina about a local image. Horizontal cells are thought to be inhibitory.[8]

Amacrine cells are inhibiting interneurons that synapse with ganglion cells, seemingly to shape and control ganglion cell response. It is believed that they account for correlated firing amongst ganglion cells. Most amacrine cells are GABAergic but the rest is glycinergic.[7]

Table 1. Layers of the retina.[1, 2, 7]

The layers are in direct order, with the outermost layer on top of the table.

Retinal layers	**Some main functions of the retinal layers**
Pigment epithelium	Outermost layer, next to the choroid. Absorbs light, reducing light scatter. Participates in the process of reconversing a metabolized photopigment by transporting it from the photoreceptors to choroid.
Photoreceptor layer	Photoreceptors; rods and cones which detect light and alternate it into electrical signals.
External limiting membrane	Müller cells and photoreceptors connections.
Outer nuclear layer	Photoreceptor nuclei
Outer plexiform layer	Where photoreceptors synapse with bipolar cells and horizontal cells.
Inner nuclear layer	Cell bodies and nuclei of number of cell types; bipolar cells, horizontal cells, amacrine cells and Müller cells.
Inner plexiform layer	Where bipolar and amacrine cells synapse with ganglion cells.
Ganglion cell layer	Formed by ganglion cells which transmit visual information to the brain.
Optic fibre layer	The axons of ganglion cells, covers the innermost part of the retina, avoiding the macula but entering the optic nerve.
Inner limiting membrane	Innermost layer of the retina. Formed by end feet of the Müller cells.

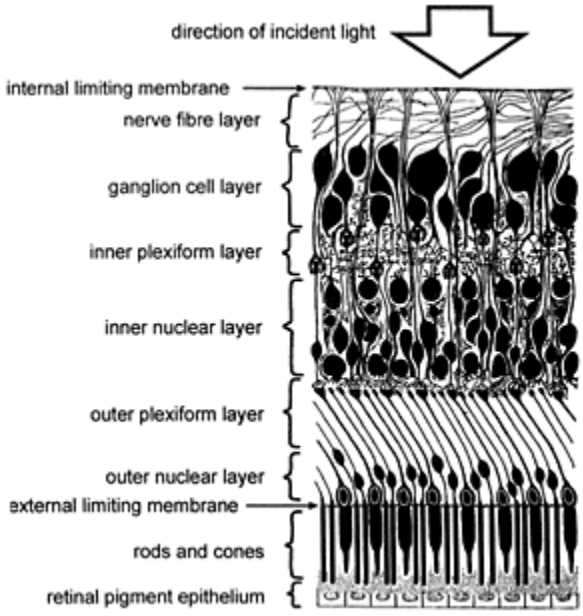

Figure 2. Layers of the retina, as described in table one. Link to figure: http://www.answersingenesis. org/tj/v13/i1/retina.asp (modified from Brash J.C. [ed.], 1951. Cunningham's Textbook of Anatomy, p. 1169)

Retinal ganglion cells transmit visual information to the brain. These cells differ in size and response to visual stimulation. There are only a few types of ganglion cells where most are generally glutamatergic. The most common ones are the midget cells which have a one-to-one connection ratio to midget bipolar cells, carrying information from a single cone.[8, 9] The ganglion cell's axons cover the retina and converge on the optic disc where they turn to penetrate the sclera and proceed from that point as the optic nerve to the brain.[1]

The central retinal artery and the central retinal vein (see below), pass through the center of the optic nerve and emerge on the surface of the optic nerve head. There are no photoreceptors on the optic nerve head. The anterior portion of the optic nerve can be divided into four regions; the superficial nerve fiber layer, the prelaminar cribrosa, the laminar cribrosa and the retrolaminar cribrosa.[10]

Glial cells perform specialized functions in support of neurons where nearly every aspect of the development, homeostasis and function of the visual system involves a neuron-glia partnership. The major glial cell type in the retina and optic nerve head are astrocytes which exhibit significant homeostatic interactions with the retinal ganglion cells and axons and provide energy support. Müller cells play an important role in controlling the extracellular environment, maintaining the extracellular glutamate and ion balance. Glial cells also sheathe the blood vessels in the retina and provide signals that have an impact on the development and maintenance of the blood-retinal barrier.[11]

1.2 Vasculature structure of the retina and the optic nerve head

Blood supply to the eye is provided by the ophthalmic artery. Branching from the internal carotid, (see figure 4A) it supplies the eye via the branches of the central retinal artery and posterior ciliary trunks. On average, there are 2-3 ciliary trunks that supply the medial and lateral long posterior ciliary arteries. Each posterior ciliary artery then divides into approximately 10-20 short posterior ciliary arteries just before or even after penetrating the sclera (see figure 4A and 4B). The central retinal artery branches usually directly off the ophthalmic artery, entering the optic nerve approximately 10 mm behind the globe.[10]

1.2.1 The retinal circulation

As mentioned previously, the outermost layer of the retina; photoreceptor layer, gets its metabolic substrates and oxygen delivered from the choroidal system. The retinal vessels provide oxygen and a metabolic substrate for the inner layers of the retina. The central retinal artery divides into arterioles outward from the optic disc, each generally supplying

one quadrant of the retina. A cilioretinal artery hooks around the temporal margin of the optic disk in approximately 25% of human eyes and together with the temporal arterioles, supplies the macular region.[12] The larger vessels lie in the innermost part of the retina, close to the internal limiting membrane. At the arterio-venous crossing sites, deeper vessels lay as inset at the outer plexiform or outer nuclear layer. The venous system is similar to the arteriolar arrangement where the central retinal vein drains venous blood from the eye through the optic nerve.[11, 13]

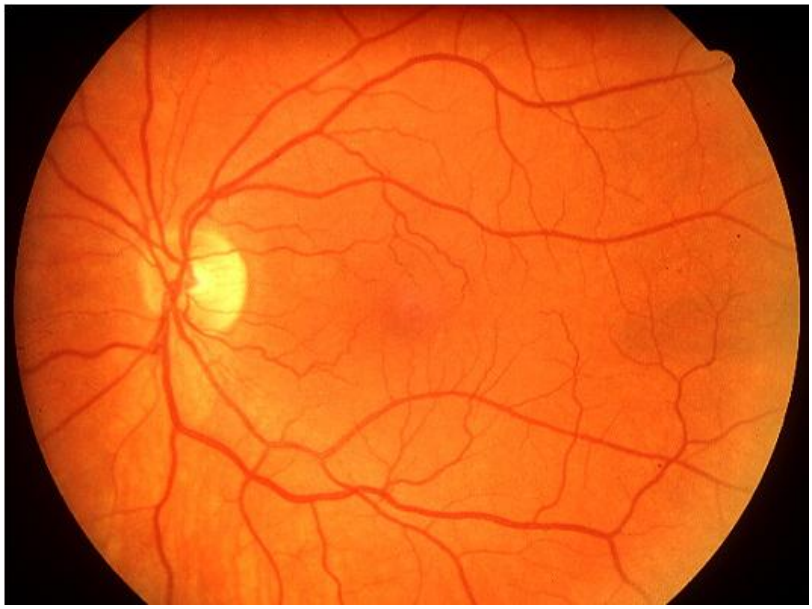

Figure 3. A healthy retina. The retinal vessels leave and enter the eye through the optic nerve head. Link to figure: http://webeye.ophth.uiowa.edu/dept/service/photo/cfundus.htm

1.2.2 The optic nerve and optic nerve head

The blood supply and vasculature of the optic nerve head is more complex than that of the retina. The main source of blood supply to the optic nerve head is the circulation of posterior ciliary arteries. The most anterior portion of the optic nerve head—the surface nerve fiber layer—is typically supplied by the retinal arterioles. In some cases its temporal region may instead be supplied by the posterior ciliary arteries from the deeper prelaminar region. The prelaminar cribrosa region is supplied by the peripapillary choroid which gives out branches to the corresponding part of the prelaminar cribrosa region. The lamina cribrosa region is supplied by the centripetal branches from the short posterior ciliary arteries, either directly or indirectly from the circle of Zinn-Haller, when that is present. The retrolaminar cribrosa region may have a dual source of blood supply. The peripheral centripetal vascular supply is always present and formed by the pial vascular plexus. The major source supply is the recurrent pial branches

arising from the circle of Zinn-Haller or the short posterior ciliary arteries in that area and the peripapillary choroid. Pial branches from the central retinal artery may also contribute. The other source is the axial centrifugal vascular supply which is inconsistent. The source of supply is derived from branches of the central retinal artery, if it gives out any branches.[14]

A. **B.**

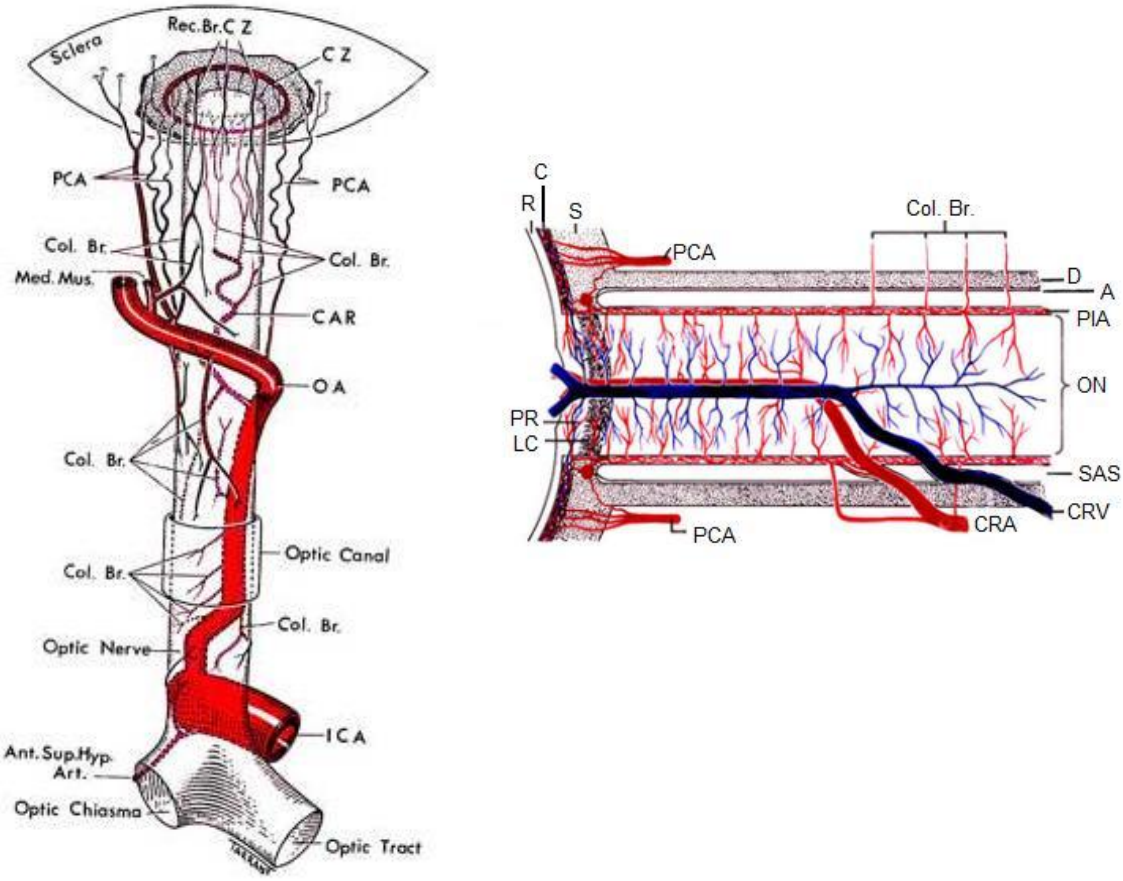

Figure 4. The anatomy of the optic nerve and optic-nerve head vasculature.

A. Diagrammatic representation of blood supply of the various parts of the optic nerve. Reproduced from Hayreh.[15]

Link to figure: http://webeye.ophth.uiowa.edu/dept/aion/images/fig24.htm

B. Arterial supply of the optic nerve. Reproduced from Hayreh.[15]

Link to figure: http://webeye.ophth.uiowa.edu/dept/aion/images/fig1.jpg

Ant.Sup.Hyp. Art. = anterior superior hypophyseal artery; C = choroid; CRA = central retinal artery; Col. Br. = Collateral branches; CZ = circle of Zinn; ICA = internal carotid artery; Med. Mus. = medial muscular artery; OA = ophthalmic artery; ON = optic nerve; PCA = posterior ciliary artery; R = retina; Rec. Br. CZ = recurrent pial branches from peripapillary choroid/CZ; S = sclera

1.3 Vascular physiology of the eye

The rate of retinal blood flow is dependent on interaction of various factors which determine the perfusion pressure and the vascular resistance generated by the retinal vessels and the blood viscosity.[11] Perfusion pressure drives blood through the retina and is the difference between mean blood pressure in the ophthalmic artery (MOAP) and the central retinal vein. Because the venous pressure is almost equal to the IOP, perfusion pressure is defined as MOAP-IOP.[11] However, direct intravascular pressure measurements show that venous pressure is somewhat higher than the IOP. Therefore, perfusion pressure as defined here must be regarded as an estimation of the actual perfusion pressure.[11, 16] If the IOP increases, perfusion pressure decreases. Also, if systemic blood pressure is raised, perfusion pressure decreases.

Ocular blood flow can be described with following equation:

$$F = \frac{(MOAP - IOP)}{R} \qquad (1)$$

where F = blood flow and R = resistance. In the retinal vasculature, the resistance of a blood vessel depends primarily on the diameter of the vessel. Ocular blood flow also follows the Hagen-Poiseuille's equation.

$$F = \frac{(MOAP - IOP)\pi r^4}{8\eta l} \qquad (2)$$

where F = blood flow, r = radius of a vessel, l = lenght of a vessel, η = viscosity of blood. The ocular blood flow is controlled by the MOAP, IOP and the vascular resistance. There is evidence that autoregulation controls blood flow both in the optic nerve head and the retina by alterations in the tone of the blood vessels and therefore, the vascular resistance. [17] Autoregulation of blood flow can be described as the intrinsic tendency of an organ to keep its blood flow constant despite changes in perfusion pressure. [17, 18] If there is a moderate increase in IOP, the oxygen tension in the optic nerve head and the retina remain for the most part unaffected because of the autoregulation.[19] The arterioles dilate and decrease their resistance to meet the decreased perfusion pressure of the eye. However, autoregulation breaks down when the perfusion pressure goes below or above a critical range (see figure 5). [17]

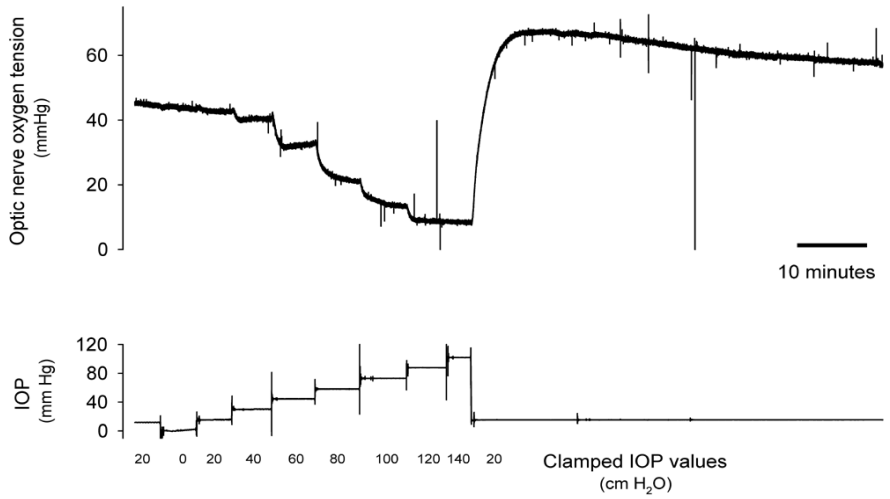

Figure 5. Optic nerve oxygen tension during increased levels of IOP.

When IOP is increased to above 40 mmHg, optic nerve oxygen tension starts to fall, probably because of an overwhelmed autoregulation. When IOP is reduced, oxygen tension levels reach above the previous baseline in a post-ischemic hyperemic response. Clamped IOP values are shown in cmH_2O. Actual measured IOP values are shown in mmHg. The figure is from la Cour et al.[20]

Autoregulation is also defined as the capability of an organ to control its blood supply in accordance with its metabolic needs (figure 6).[11] This implies that blood flow is actively regulated by metabolism through the action of local factors that change the tone of the retinal resistance vessel. These factors are released by the vascular endothelium and/or neural tissue surrounding the vessels. They can either be vessel tone relaxing like nitric oxide (NO) or vessel contracting like endothelium-1. In humans, a decrease in oxygen pressure (hypoxia), induces a vasodilation of the retinal arterioles.[11] In anesthetized cat retinal vessels, endothelium derived NO was shown to be a vasodilating mediator in the response of retinal blood flow to hypoxia.[21]

The retinal circulation is independent of the influence of autonomic nerve terminals.[11, 22]

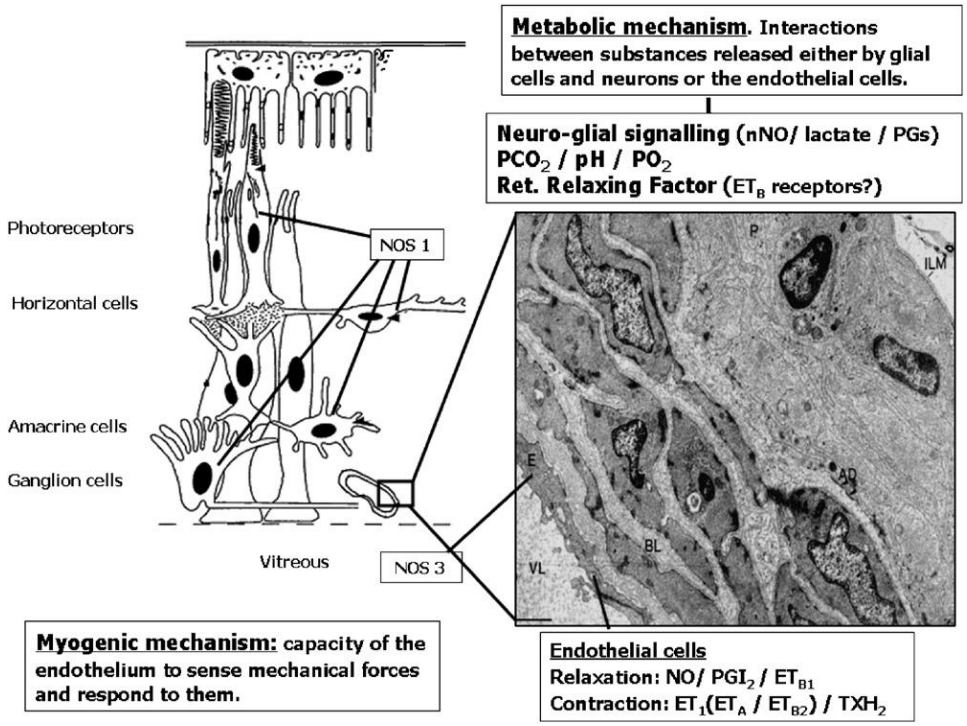

Figure 6. Myogenic and metabolic pathways in retinal blood flow regulation.

NO, ET and prostaglandins and its receptors are constitutively released by endothelial and neural cells and act on smooth cells and pericytes.

NOS = nitric oxide synthase, PGs = prostaglandins, PCO2 = carbondioxide pressure , pH = acid level, PO2=oxygen pressure , NO = nitricoxide, ET = endothelins, TXH2 = dihydrogenthromboxin .

The figure is from Pournaras et al.[11]

Alterations of ocular perfusion could cause ischemia in the tissues in the optic nerve head and the retina because of a restriction in blood supply. In ischemia, the tissue is deprived of adequate oxygen supply and hypoxia results. It has been proposed that ischemia might play a role in some ocular diseases such as glaucoma.[23]

1.4 Glaucoma

Glaucoma is a term used for group of diseases that share an optic neuropathy associated with ganglion cell death and visual field loss[4, 24] where elevated intraocular pressure (IOP) is one of the primary risk factors.[4] It is the second leading cause of blindness globally, considered to affect about 60 million people in the year 2010, increasing to around 80 million by 2020.[25] In Iceland, the prevalence of open-angle glaucoma in the age group 50 years or older is 4% and the prevalence is age related. [26]

In general, glaucoma is classified as open-angle or closed-angle glaucoma and as primary or secondary glaucoma. In open-angle glaucoma there is no mechanical blockage of the trabecular meshwork as in angle-closure glaucoma. Open-angle glaucoma is primary

when there is no anatomically identifiable underlying cause of outflow interruptions. On the other hand, when an abnormality is identified and its supposed role in the pathogenesis of the disease can be recognized, glaucoma is classified as secondary.[4] In this thesis, the focus will be on open-angle glaucoma with and without pseudoexfoliation.

Open-angle glaucoma without pseudoexfoliation syndrome is primary glaucoma. It is the most common form of glaucomatous loss of optic nerve fibers[25]. It often has a hereditary predisposition and is generally a bilateral disease. Its severity may be detected in the appearance of the optic disk or the nerve fiber layer and/or the presence of characteristic defects in the visual field.[4, 27] Open-angle glaucoma with pseudoexfoliation syndrome is secondary glaucoma. In some prevalence surveys, pseudexfoliation glaucoma is not classified as secondary glaucoma but as a variant of open-ange glaucoma. This view remains to be fully vindicated.[26, 28] Pseudoexfoliation glaucoma is characterized clinically by the presence of whitish, fibrillar material in the anterior segment of the eye. The exact nature of exfoliation material and the pathogenesis of the disease continue to be unknown.[4] However, Thorleifsson et al. identified two risk variants in the lysyl oxidase-like 1 (*LOXL1*) gene associated with pseudoexfoliation glaucoma.[29] The results have largely been replicated worldwide. The product of *LOXL1* is a protein responsible for the synthesis and maintenance of elastin fibers, lending a strong support for the role of dysfunctional elastogenesis in pseudoexfoliation syndrome and pseudoexfoliation glaucoma.[30, 31]

According to the American Academy of Ophthalmology, the main risk factor for open-angle glaucoma is raised IOP. Other risk factors are advanced age, a positive family history, ethnicity and decreased corneal thickness. Some of the main symptoms of open-angle glaucoma can be generalized enlargement of the cup in the optic nerve head (figure 7B), nerve fiber layer (splinter) hemorrhage by the optic nerve head, abnormal visual field and in many cases, raised IOP. [4] The reason for the increasing size of the cup in glaucoma is because of the loss of ganglion cell axons.

Pooled data from large epidemiologic studies indicate that the mean IOP is 16 mmHg. No clear line exists between safe and unsafe IOP but the value >21mmHg has been used in the past to separate normal and abnormal pressures and to define which patients require ocular hypertension therapy.[4] IOP is the only treatable symptom with use of medication and/or surgery or surgeries where the goal is to lower the IOP.

A. **B.**

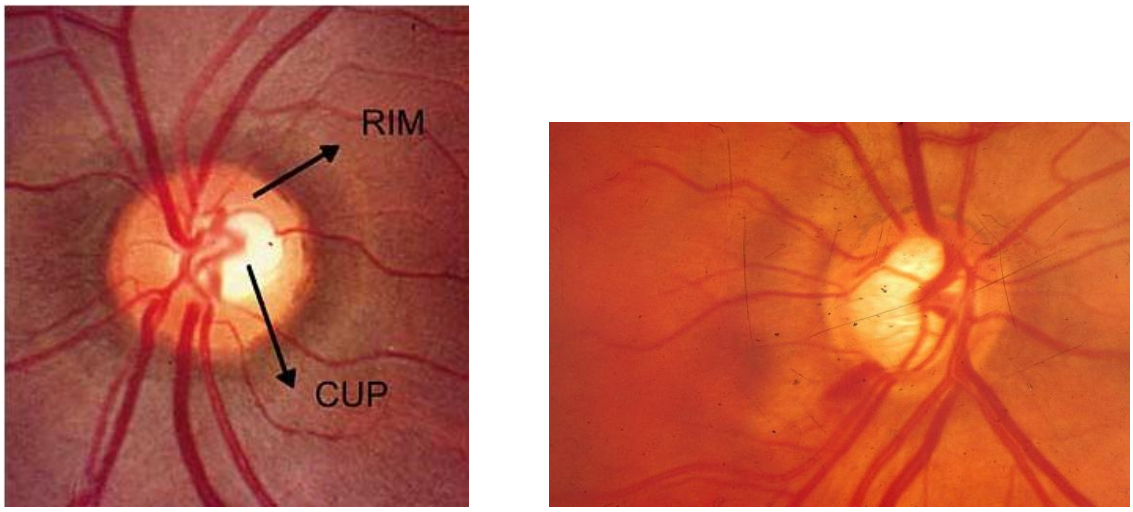

Figure 7. Optic nerve head.

A. Healthy optic nerve head. Arrows point from the outer rim of the optic nerve head and the cup. The cup is the point of entry for retinal vessels, from the optic nerve to the retina.
B. Optic nerve head with glaucoma excavation (cupping) and hemorrhage.
Link to figure A:
http://journals.prous.com/journals/dot/20023808/html/dt380563/images/Weinreb_f2.jpg
Figure B is supplied by Maria Soffia Gottfredsdottir, MD.

The exact cause of open-angle glaucoma still remains unknown. Two conflicting and unresolved theories were proposed to explain the pathogenesis of the disease 150 years ago, one mechanical and the other vascular.[32] The former points to mechanical consequences of elevated IOP as a direct cause with displacement of the lamina cribrosa leading to pinching of the ganglion cells axons.[33, 34] Also, optic nerve head astrocytes may respond to changes in IOP, altering their physiological relationship with axon bundles, vasculature and connective tissues, leading to axonal loss and tissue remodeling.[35] While elevated IOP is the major risk factor for open-angle glaucoma, a high percentage of individuals with elevated IOP do not develop glaucoma.[36] Further, in some studies such as the Reykjavik eye study[26], amongst others[37, 38], IOP has been eliminated from the definition of glaucoma because of the big overlap of IOP in open-angle glaucoma and non open-angle glaucoma eyes.[39] The vascular theory postulates insufficient or poorly regulated blood supply to the retina and the optic nerve head, leading to ischemia, hypoxia and resultant tissue damage.

1.5 Blood flow and glaucoma

There is growing evidence that blood flow in glaucomatous eyes is reduced or its regulation disturbed compared to normal, non-glaucomatous, eyes.[40] Blood flow deficiencies have been reported in the choroidal[41, 42] and retrobulbar [42-46] circulations in glaucoma patients.[47] Also, for

instance, Michelson et al. showed that eyes with primary open-angle glaucoma have significantly reduced blood flow of the juxtapapillary retina and neuroretinal rim compared to controls.[48] Also, Plange et al. have shown that in asymmetric glaucoma defined by visual field defect, blood velocity in the central retinal artery and ophthalmic artery is reduced in the more glaucomatous affected eye compared to the less affected eye. Their patients (n=25) underwent color Doppler imaging of their retrobulbar vessels and visual field testing. [49] Sato et al. have shown that neuroretinal rim blood flow is reduced in areas corresponding to scotoma within asymmetric normal tension glaucoma eyes. Their method was based on scanning laser Doppler flow meter using the Heidelberg retina flow meter, which gives blood flow values obtained from the surface nerve fiber layer of the optic nerve head, supplied by the retinal circulation and its deeper layer, supplied by the posterior ciliary arteries. They conclude that impaired circulation is a causative factor in the pathogenesis of the disease.[50]

Systemic and localized vascular abnormalities have been linked to open-angle glaucoma.[51-54] Reduced ocular perfusion pressure has been linked to both the prevalence [55-57] and incidence of glaucoma [58], indicating strongly that vascular factors may play a role in glaucoma. Flammer et al. theorized that vascular autoregulation is impaired in glaucoma resulting in impaired blood flow and energy metabolism.[40, 59] Glaucoma patients often have increased endothelin-1 plasma levels which is a vasoconstrictor and may be an indirect sign of altered blood flow.[60] Oku et al. were able to create chronic optic nerve head ischemia by repeated intravitreal injections of endothelin-1.[61]

It is unknown whether diminished blood flow is a primary cause of glaucomatous atrophy of ganglion cells and the optic nerve, or secondary to the atrophy, where autoregulation reduces blood flow in response to decreased metabolic demand and oxygen consumption in an atrophic tissue.[43, 44] It is not known whether glaucomatous eyes have a primary metabolic impairment from ischemia in which case tissue hypoxia should be present; or physiologically reduced blood flow secondary to tissue atrophy, in which case tissue oxygen levels should be normal. While tissue oxygen levels cannot currently be measured in human glaucoma patients, retinal vessel oxygen saturation provides data on retinal oxygen metabolism and possible hypoxia.

The functional loss of vision in glaucoma is caused by death of retinal ganglion nerve cells and their axons. This is at least partly due to cell apoptosis.[62] It is also known from experimental animal studies and clinical studies in humans that ischemia can lead to ganglion cell death and optic nerve head atrophy.[23]

The relationship between blood pressure and IOP may be important in glaucoma. Systemic hypotension, especially while sleeping, has been suggested as a possible cause of decreased optic nerve perfusion resulting in glaucomatous damage.[63] It has been suggested that IOP fluctuation is a stronger risk factor than a stable increase in IOP.[59]

1.6 Retinal oxygenation and glaucoma

Non-invasive measurements of retinal oxygen saturation in humans with glaucoma are relatively new. Earlier attempts to measure oxygen saturation were limited to invasive methods in animals. Blumenröder et al. measured decreased intravascular oxygen pressure of the retinal vessels and the optic nerve head in pigs with raised IOP.[64] la Cour et al. measured decreased oxygen tension with raised IOP in the optic nerve head in pigs (see figure 5, page 21).[20] Novack et al. measured cytochrome a,a3 levels and showed that optic nerve head oxidative metabolic response is dependent on perfusion pressure in cats (see figure 8).[65] Cytochrome a,a3 is a terminal member of the respiratory chain. It is very sensitive to small changes in oxygen availability and can therefore be used as an indicator of the metabolic state of the tissue.[66]

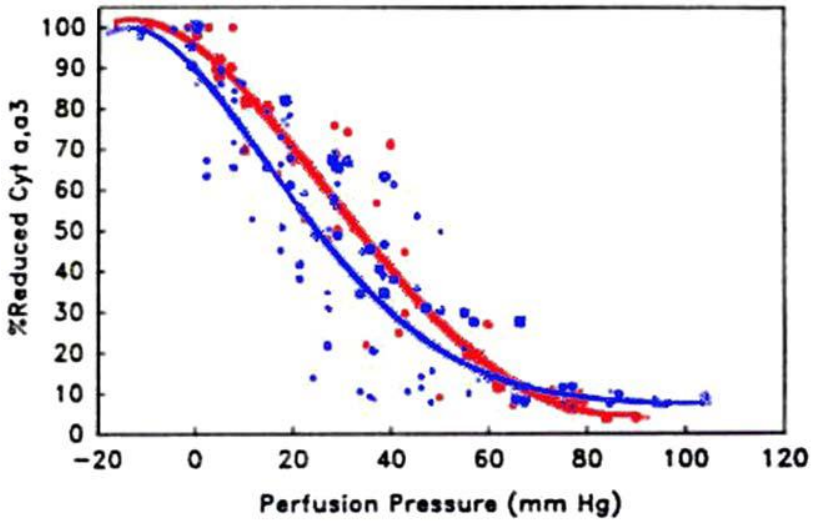

Figure 8. Magnitude of cytochrome a,a3 reduction as a function of the ocular perfusion in cats.

Blue line indicates measurements where the perfusion pressure was controlled by varying IOP. Red line indicates measurements where the perfusion pressure was controlled by means of intravenous sodium nitroprusside while constant IOP was maintained.

The figure is from Novack et al.[65]

On the other hand, Shonat et al. did not observe major changes in oxygen saturation of the retina or the optic nerve head when acute, moderate increase in IOP was applied to cats.[19] Khoobehi et al. performed non-invasive hyperspectral imaging on monkeys to measure oxygen saturation. When the IOP was raised, optic nerve oxygenation was

decreased.[67] In general, most of these studies show that when perfusion pressure declines and autoregulation of blood flow is overwhelmed, optic nerve oxygenation decreases.

More recently, Michelson and Scibor used spectrometric imaging to measure oxygen saturation in glaucoma. They found decreased retinal arteriolar oxygen saturation in people with normal-tension glaucoma compared to normal human eyes only when it was correlated with the rim area of the optic nerve head but not with the mean defect of the visual field. These changes were not seen in high-tension glaucoma. When all examined eyes (normal-tension plus high-tension glaucoma) were correlated with the rim area, oxygen saturation decreased significantly in retinal arterioles with decreasing size of the rim area. They also observed significant correlation between the size of the rim area and arteriovenous difference over all examined eyes. Their data suggested a trend towards increased values of oxygen saturation in venules in high-tension glaucoma compared to normal eyes, even though it was not significant. The site of their measurements was only at the superior temporal retina in one arteriole and one venule. [68] On the other hand, Ito et al. detected some differences in oxygen saturation in the retinal tissue in both normal-tension and high-tension glaucoma patients compared to normal controls. Their technique is based on a Fourier transform-based spectral retinal imaging system. Seven points in the retina were analyzed, five juxtapapillary points avoiding the visible vessels, one arteriole and one venule. There was some diversity in their results, for example; correlation was found between mean deviation and oxygen saturation of the inferotemporal point in high tension glaucoma but not in the low tension glaucoma group.[69] Our group (Hardarson et al.) found a 2% increase in oxygen saturation values in arterioles after glaucoma surgery, but no difference in venules and no difference between oxygen saturation values in arterioles and venules (arteriovenous difference).[70]

These direct measurements on oxygenation indicate that retinal oxygenation is altered in glaucoma. The role of oxygenation in glaucoma has also been suggested by a report that hyperbaric oxygen treatment has positive effects on glaucomatous visual field.[71] Furthermore, Tezel and Wax found that immunostaining for hypoxia-inducible factor 1α (HIF-1α) is increased both in the retina and the optic nerve head of human donor glaucomatous eyes compared with control eyes. They also examined the relationship between retinal regions exhibiting increased immunostaining for HIF-1α and the locations of visual field defects. This demonstrated that the increased immunostaining for HIF-1α was most prominent in retinal regions corresponding to visual field defects. HIF is the

primary hypoxic signaling protein in cells for regulating angiogenesis, indicating a presence of tissue hypoxia.[72]

1.7 Oximetry

The measurement of oxygen saturation in the retinal vasculature is recognized as oximetry. The basic concept of oximetry is relatively simple where the difference in color of oxygen-rich and oxygen-poor blood is used to calculate oxygen saturation.

Oxygen saturation, $SatO_2$, is the percentage of hemoglobin bound to oxygen:

$$SatO_2 = \frac{[HbO_2]}{[Hb]+[HbO_2]} * 100\% \qquad (3)$$

where $[HbO_2]$ is oxygenated hemoglobin and [Hb] deoxygenated hemoglobin.

A common, non-invasive method to measure oxygen saturation is a pulse-oximeter. It is widely used in hospitals where a probe transilluminates a thin part of the body such as a fingertip or an earlobe. The pulse-oximeter is limited only to measure oxygen saturation in arteries. The method of pulse-oximetry, where light intensity is measured from behind the tissue, cannot be used the same way in tissues such as the retina, where it is difficult to measure light intensity behind it. Similar concept is introduced for oxygen saturation measurements in the retinal vessels. As in pulse-oximetry, it is based on the Beer-Lambert law[73]

$$OD = \log\left(\frac{I_0}{I}\right) \qquad (4)$$

where OD is the optical density, I_0 is the intensity of incident light and I is the intensity of transmitted light. Because, as stated before, it is impractical to use transmittance in retinal vessels to determine oxygen saturation, reflection must be used. So I_0 becomes light reflected just outside a vessel and I is the light reflected inside a vessel.[73]

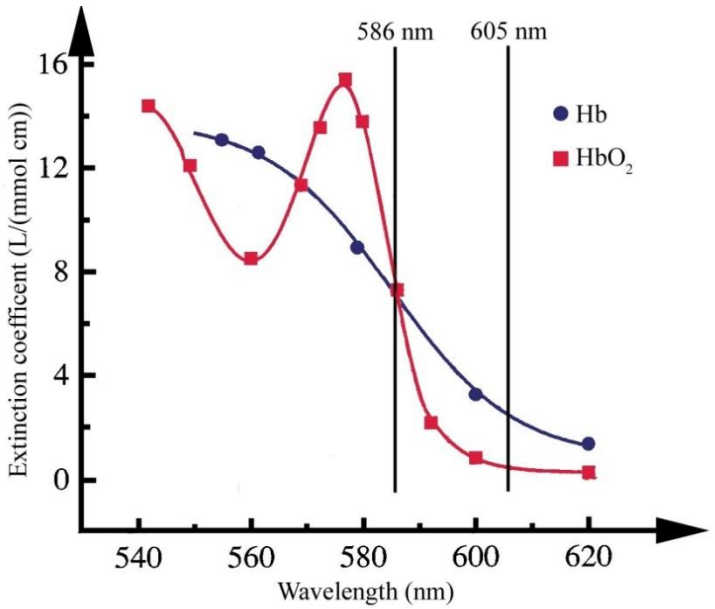

Figure 9. Light absorbance at different wavelength for oxygenated hemoglobin (HbO_2) and deoxygenated hemoglobin (Hb).

OD equals extinction coefficient (L/(mmol cm)). The two wavelengths, 586 nm and 605 nm, are the ones used in the study.
The figure is from Sveinn Hakon Hardarson, reproduced from van Assendelft.[74]

Optical density ratio (ODR) is the ratio of two different wavelengths:

$$ODR = \frac{OD_X}{OD_Y} \qquad (5)$$

where OD_X is the optical density at wavelength X and OD_Y is the optical density at wavelength Y where wavelength X is non-isosbestic (sensitive to oxygen saturation) and wavelength Y is isosbestic (non-sensitive to oxygen saturation). The ratio of OD at two different wavelengths has a linear and approximately inverse relationship with oxygen saturation:

$$SO_2 = a + k \cdot \left(\frac{OD_X}{OD_Y}\right) = a + k \cdot ODR \qquad (6)$$

where SO_2 is the percentage of oxygen bound hemoglobin, a and k are constants and ODR is oxygen density ratio.

In this research, the wavelengths used are at 586 nm (isosbestic) and 605 (non-isosbestic) (figure 9).

Several research groups have contributed to the development of techniques to measure retinal oxygenation.[67, 73, 75-81] These measurements are based on color changes in blood depending on hemoglobin oxygen saturation levels. The retinal oximeter used in this study

(figure 11, page 36) is based on this technique. The reliability of the oximeter has been tested by Hardarson et al. whereby healthy individuals underwent pure oxygen breathing whilst being measured by the oximeter. Increase in oxygen saturation was detected both in arterioles and venules during hyperoxia. Therefore, the retinal oximeter is sensitive to changes in oxygen saturation and reliable.[81]

The most reliable measurements are made on the larger retinal vessels. Non-invasive measurements of tissue oxygen saturation are hampered by the fact that the signal (light attenuation) from the retinal capillaries is small and needs to be separated from the choroidal background.

1.8 Perimetry

Perimetry, the evaluation of the visual field and functional damage, is an important diagnostic test in ophthalmology for managing glaucoma. Standard visual field testing involves measuring contrast sensitivity or the ability of an observer to distinguish a target from a background. It is common to perform a standard achromatic perimetry which is a visual field testing with a white target against a more dimly illuminated white background (‘white-on-white’). Currently, there are two main types of standard achromatic perimetry used in clinical practice, kinetic and static perimetry.[82] In this thesis, the focus will be on automated static perimetry.

In static perimetry, stimuli of the same size and different intensity are randomly presented in predetermined locations of the visual field and patients' responses are registered. Visual field indices help to assess the visual field. Some of the main indices are the mean sensitivity and mean defect index. The mean sensitivity index is the average of all measured values of differential light sensitivity in dB. The mean defect index is the average of all local defects.[83]

A. **B.**

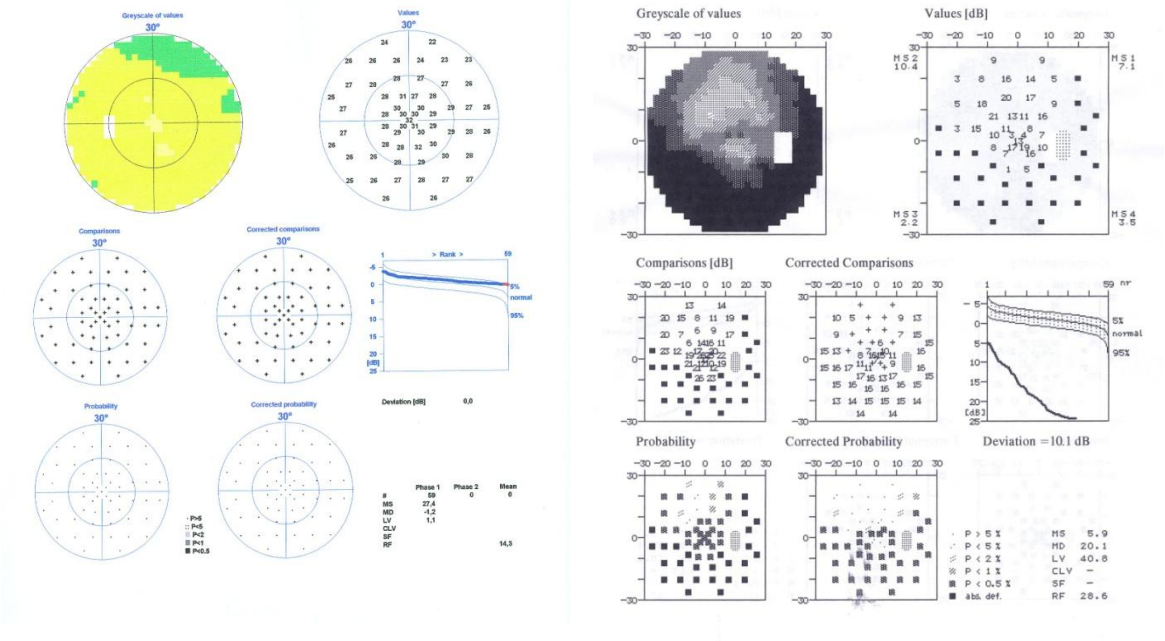

Figure 10. Visual field printouts.

A. Visual field from a healthy individual. B. Visual field from an individual with glaucoma. The patient has an inferior visual field defect (scotoma). The printouts are from the same Octopus 123 visual field analyzer.

Figures are supplied by Maria Soffia Gottfredsdottir MD.

Patient‘s understanding of a test and cooperation are critical for reliability of perimetric test results. Therefore, algorithms intended to measure patients' reliability are included in most computerized perimetric programs. These algorithms measure frequencies of false-positive, false- negative responses and fixation losses. There is also the reliability factor which is a sum value that is calculated from the positive and the negative answers. The reliability factor should not exceed 15%.[83] If a patient gives a positive response to an invisible stimulus, a false-positive answer occurs. On the other hand, if a patient does not response to a stimulus that should be visible, a false-negative answer occurs.[84] Higher rates of false-negative have been reported amongst glaucoma patients compared to healthy individuals.[85-87] This implies that high frequencies of false-negative are associated with glaucoma rather than the patient reliability.[84, 85, 88] Katz and Sommer found that most test results are unreliable on a single measure due to the risk of fixation loss. In their research, patients with high false-positive rates tended to have high rates of fixation loss.[85]

2 Aims

Non-invasive spectrophotometric retinal oximetry [81, 89, 90] was used to measure oxygen saturation in retinal arterioles and venules in patients with open-angle glaucoma, with and without pseudoexfoliation syndrome.

The overall aim was to detect whether oxygen might play a role in glaucoma.

The specific aims were to test the following hypotheses:

1. Retinal vessel oxygen saturation levels correspond to visual field mean defect in eyes with open angle glaucoma.
2. Retinal vessel oxygen saturation corresponds to the depth of visual field defect in eyes with asymmetric visual field defect.

3 Methods

The study was approved by the National Bioethics Committee of Iceland and The Icelandic Data Protection Authority and adhered to the tenets of the Declaration of Helsinki. All individuals signed an informed consent.

3.1 Patients

Eligible individuals were screened by an ophthalmologist in a glaucoma clinic (Augnlæknar Reykjavíkur, Hamrahlíð 17) and qualified open-angle glaucoma patients were invited to participate in the study. The inclusion criteria were open-angle glaucoma with or without pseudoexfoliation syndrome [30, 91, 92] and no other ocular diseases. All individuals were 40 years of age or older. Individuals receiving anti-hypertensive medication for elevated systemic blood pressure were not excluded. Individuals with cataract were not excluded. Individuals with other systemic diseases, such as diabetes, were also excluded from the study.

Pseudoexfoliation syndrome was diagnosed based on morphologic alterations of the anterior lens capsule.[30, 91] The characteristic appearance of pseudoexfoliation material was diagnosed when a peripheral granular zone, with or without a central zone, was seen. The pupil was dilated before lens changes were diagnosed. Pupils were dilated by an ophthalmologist with 1% tropicamide (Mydriacyl®, S.A. Alcon-Couvreur N.V., Puurs, Belgium). When needed, it was supplemented with 10% phenylephrine hydrochloride (AK-Dilate, Akorn Inc., Buffalo Grove, IL., USA) and 0.5% proparacaine hydrochloride (Alcaine®, S.A., Alcon-Couvreur N.V., Puurs, Belgium).

3.2 Oximetry

Non-invasive spectrophotometric oximetry was performed on the same day as ophthalmologist evaluations. The oximeter is based on a fundus camera with an attached beam splitter and a digital camera linked to a computer.

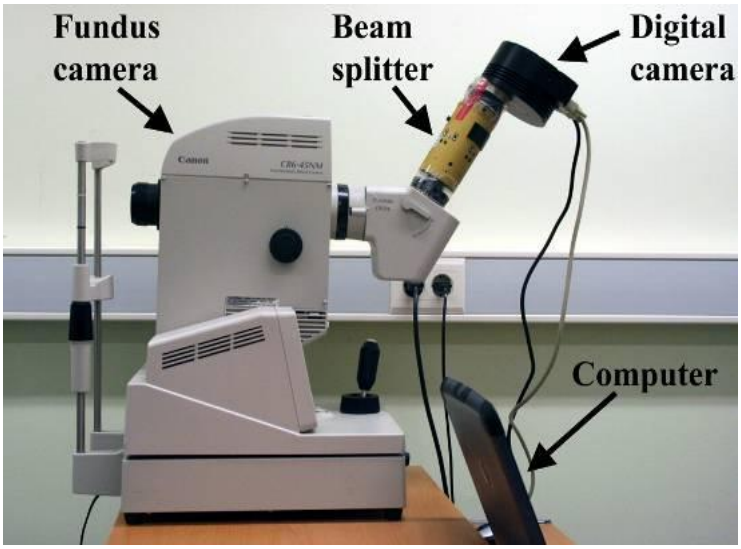

Figure 11. The retinal oximeter used in the research.

It is composed of a fundus camera, beam splitter and digital camera which is linked to a computer.

The instrument delivers four images at four different wavelengths, 542nm, 558nm, 586 nm and 605 nm. Only two wavelengths were used for oxygen saturation measurements in this research, 605nm which is non-isosbestic and 586nm which is isosbestic (see figure 9, page 29).

A. B.

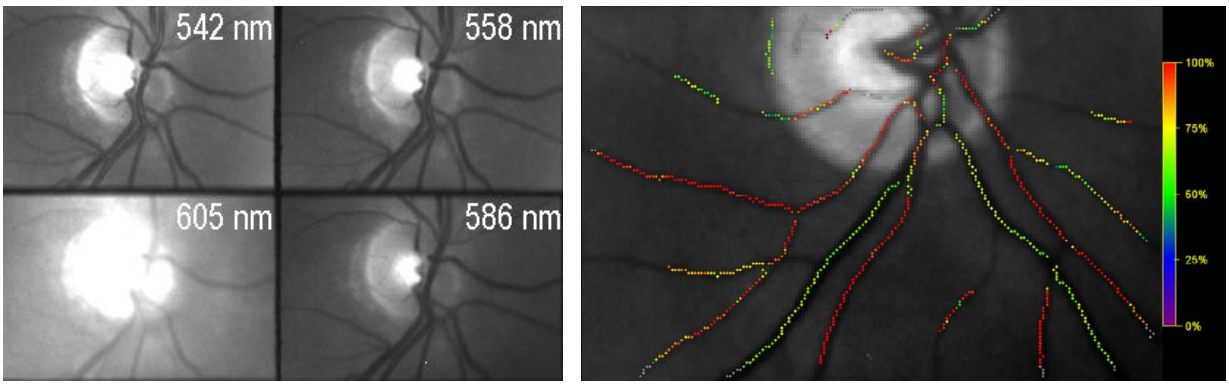

Figure 12. An output from the oximeter.

A. A typical output from the oximeter showing images from all wavelengths. B. A pseudocolor map after the oxygen saturation has been measured by the software used with the oximeter. In this photo, arterioles are denoted as red vessels and venules are denoted as green vessels.

A software algorithm calculates optical density (OD) of retinal vessels from the two acquired images according to equation 3. The ratio of the OD at 605nm and the OD at 586nm is inversely related to hemoglobin oxygen saturation. Measurements were made in first and second degree retinal arterioles and venules.

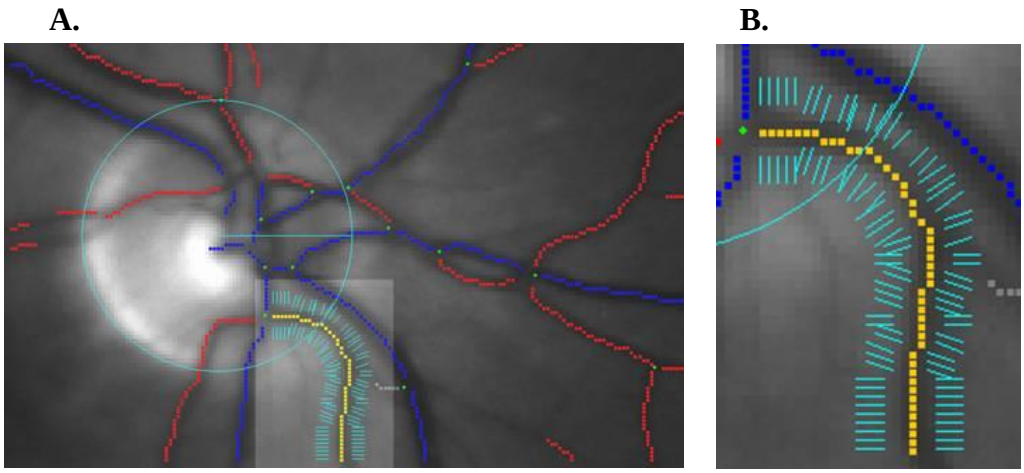

Figure 13. Measurement points.

A. The software automatically locates vessels on the fundus image. Measurements points inside and outside the vessels are also automatically selected. Venules are denoted as blue vessels, arterioles are denoted as red vessels. B. The yellow dots denote selected vessel (measurement of I). The light blue lines are the dots outside the vessel which give an averaged number of I_0. The photos were acquired from an older version of the software.

For the subject being measured, the experience is identical to traditional fundus photography. The analysis is made with specialized software which identifies retinal arterioles and venules and chooses measurement points automatically for calculation of oxygen saturation, see figure 13.

3.3 Perimetry

All visual field testing was performed using an Octopus 123 (Interzeag AG, Schlieren, Switzerland), program G1.

The Octopus 123 uses a full-size Goldman spherical cupola, which covers the entire 90° visual field area. As it is very rare in glaucoma that changes in the differential light sensitivity commence only in the periphery without any sign of loss in the central field, the visual field examination were focused on the central 30° area.

3.4 Other measurements

IOP was measured using Goldmann applanation tonometry mounted on a Haag-Streit slit lamp (Haag-Streit BQ 900, Haag-Streit International, Köniz, Switzerland) on the same day as the oximetry was performed.

Systolic and diastolic blood pressure (SP and DP, respectively) were measured using an automatic sphygmomanometer (Omron HEM-705CP, Omron, Kyoto, Japan). Mean arterial pressure (MAP) was calculated as follows; MAP=2/3 DP + 1/3 SP. Mean ocular perfusion pressure was calculated as 2/3 MAP-IOP.

To evaluate whether systemic oxygen saturation was normal, finger oximetry was performed using an Ohmeda Biox 3700 pulse oximeter (Ohmeda, Boulder, CO, USA) with the probe placed on the index finger of the right hand, before the fundus oximetry.

Statistical analysis was performed with Prism, version 5.01 (GraphPad Software Inc., LaJolla, CA, USA). Pearson´s correlation was used to detect correlation between mean defect and oxygen saturation levels. Student's t-test was used to compare retinal oxygen saturation in asymmetric glaucomatous visual field within-eye.

3.4.1 Correlation of retinal oxygen saturation and visual field mean defect

45 individuals were enrolled into this part of the study. The right eye in each patient was used for the study except in the case of low image quality from the oximeter in which the left eye was measured. Additional details of patients in the study are shown in table 2.

For 33 participants, the visual field tests were performed on the same day as the oximetry. For 12 individuals, the perimetry was not performed on the same day as the oximetry, but no more than five months before the oximetry. The reliability factor did not exceed 15%[83], where the frequency of false-positive answers in a visual field were the limiting factor. Since the false-negative answers represent the status of the eye rather than patient status[84, 88], they did not count as an exclusion factor.

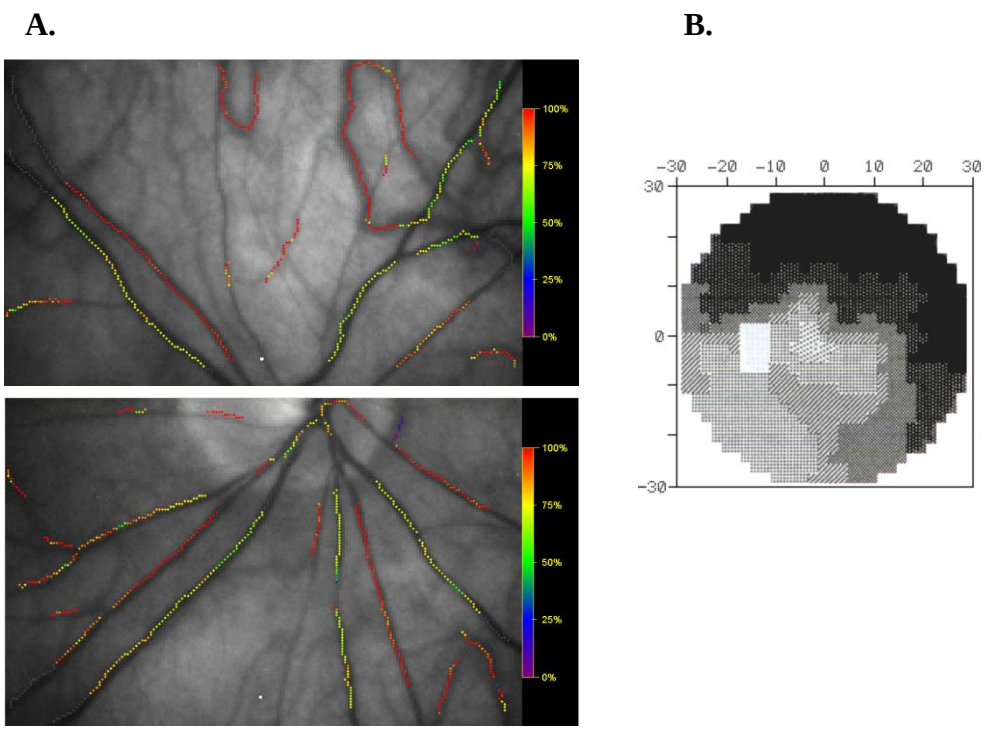

Figure 14. All data is from the same eye. A. A typical pseudocolor maps used in the study. B. Visual field that was matched with the pseudocolor maps.

3.4.2 Retinal oxygen saturation difference between asymmetric glaucomatous visual field within-eye

For the study of visual field asymmetry within-eye, 13 individuals met the criteria which, for this part of the study, also included an asymmetric visual field defect within an eye. However, here, the reliability factor did not count as an exclusion factor. Eight individuals from part one of the study; correlation of retinal oxygen saturation and visual field mean defect, were also included in this part of the study.

The visual field asymmetry was evaluated visually from the Octopus printout (see figure 16) and also by the mean sensitivity for each hemisphere. The mean sensitivity was acquired from the visual field exam where it had been calculated for each quadrant of the visual field. It was then averaged for the inferior part and the superior part of the visual field. The more damaged areas had an average mean sensitivity of 6±4dB where the less damaged areas had an average mean sensitivity of 16±6dB. Figure 15 shows the areas that were photographed and measured in each of the patients. Further clinical details for the patient group are in table 4.

A. **B.**

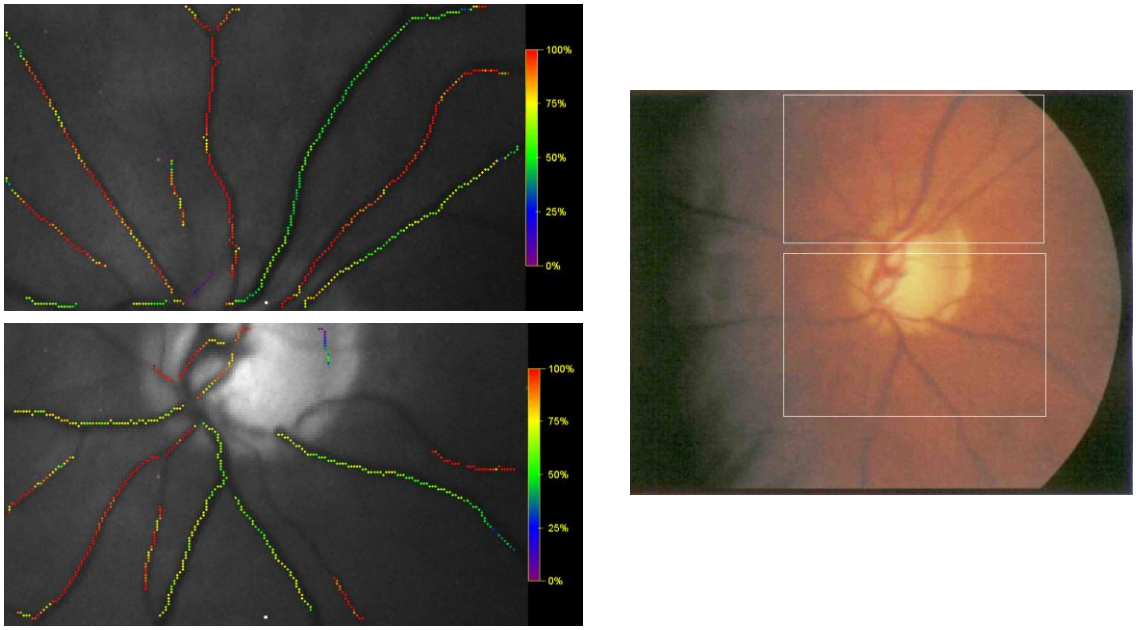

Figure 15. All photos are from the same eye. A. Pseudocolor maps of a fundus showing oxygen saturation in retinal vessels. B. Fundus image.

Frames indicate area of oximetry measurements. The photo has been rotated so the areas inside the frames resemble the pseudocolor maps (A).

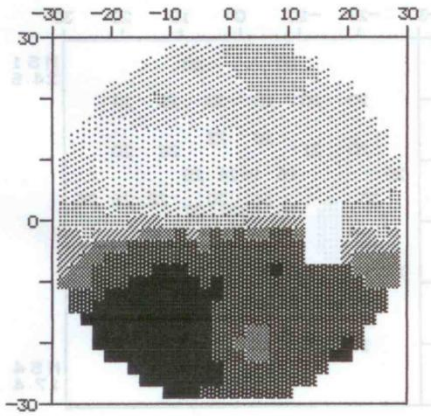

Figure 16. The visual field is from the same eye as in figure 15. It shows inferior scotoma and a healthier superior area.

4 Results

4.1 Correlation of retinal oxygen saturation and visual field mean defect

In open-angle glaucoma patients (n=45), there was a statistically significant negative correlation between arteriovenous difference in oxygen saturation and visual field mean defect levels (r= -0.42, p=0.0037, Pearson´s r, figure 17). The slope of the regression line for arteriovenous difference was -0.33 %/dB with 95% confidence interval (CI) ranging from -0.55 to -0.11. Statistically significant correlation was found between oxygen saturation in retinal venules and visual field mean defect levels (r=0.36, p=0.015, figure 17), where the venous oxygen saturation increased with worsening visual field. The slope of the regression line for venules was 0.28 %/dB with 95% CI from 0.057 to 0.49 (figure 17). No significant correlation was found between saturation in retinal arterioles and visual field mean defect (r=-0.12, p=0.43, figure 17). The slope of the regression line for arterioles was -0.056 %/dB with 95% CI from -0.20 to 0.088 (figure 17).

For OAG patients without pseuodoexfoliation syndrome (n=31), there was a negative statistically significant correlation between arteriovenous difference and visual field mean defect (r=-0.55, p=0.0013, figure 18). The slope of the regression line for arterioveonus difference was -0.36 %/dB with 95% CI from -0.57 to -0.15 (figure 18). Positive statistical correlation was found between oxygen saturation in venules and visual field mean defect (r=0.43, p=0.015, figure 18) with the slope of the regression line at 0.28 %/dB where 95% CI ranged from 0.058 to 0.50 (figure 18). In retinal arterioles, there was no significant correlation with visual field mean defect (r=-0.14, p=0.38, figure 18). The slope of the regression line for arterioles was -0.082 %/dB with 95% CI ranging from -0.27 to 0.11 (figure 18).

For the group of open-angle glaucoma patients with pseudoexfoliation syndrome (n=14) there was no significant correlation between arteriovenous difference and visual field levels (r=-0.082, p=0.78, figure 19). The slope of the regression line for arteriovenous difference was -0.08 %/dB with 95% CI ranging from -0.73 to 0.56 (figure 19). There was no correlation detected between oxygen saturation levels in venules and visual field levels (r=0.15, p=0.60, figure 19) where the slope of the regression line was 0.15 %/dB with 95% CI from -0.45 to 0.75 (figure 19). No significant correlation was found between oxygen

levels of arterioles and visual field levels (r=0.22, p=0.44, figure 19). For arterioles, the slope of the regression line was 0.065 %/dB with 95% CI from -0.11 to 0.24 (figure 19).

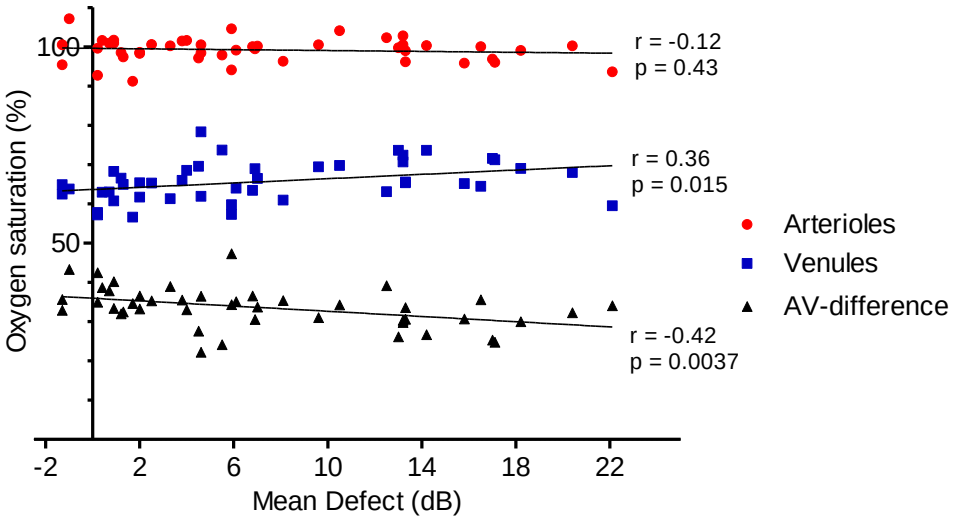

Figure 17. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in eyes with open-angle glaucoma, with or without pseudoexfoliation syndrome.

The slope of the regression line for arteriovenous difference was -0.33 %/dB, for venules it was 0.28 %/dB and -0.056 %/dB for arterioles. n=45, r=coefficient of correlation.

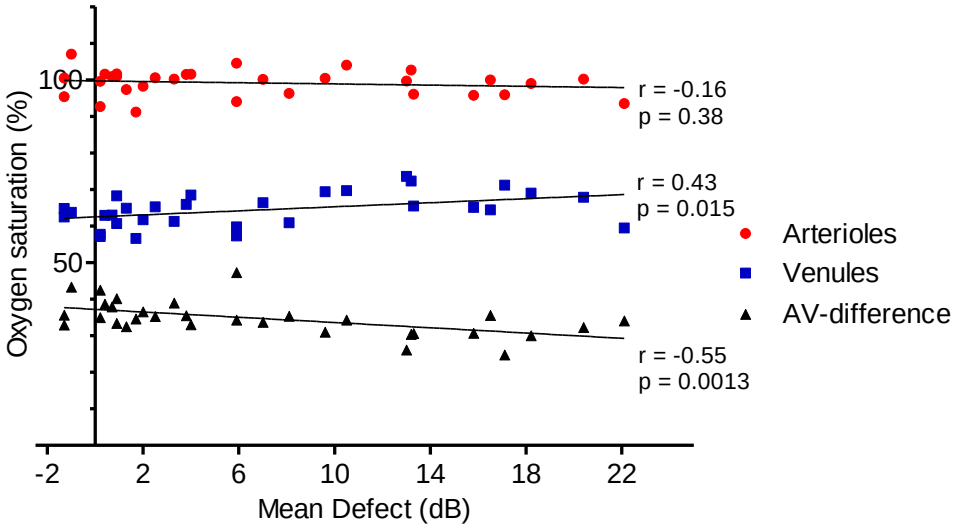

Figure 18. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in patients with open angle glaucoma without pseudoexfoliation syndrome.

The slope of the regression line for arteriovenous difference was -0.36 %/dB, for venules it was 0.28 %/dB and -0.082 %/dB for arterioles. n=31, r=coefficient of correlation.

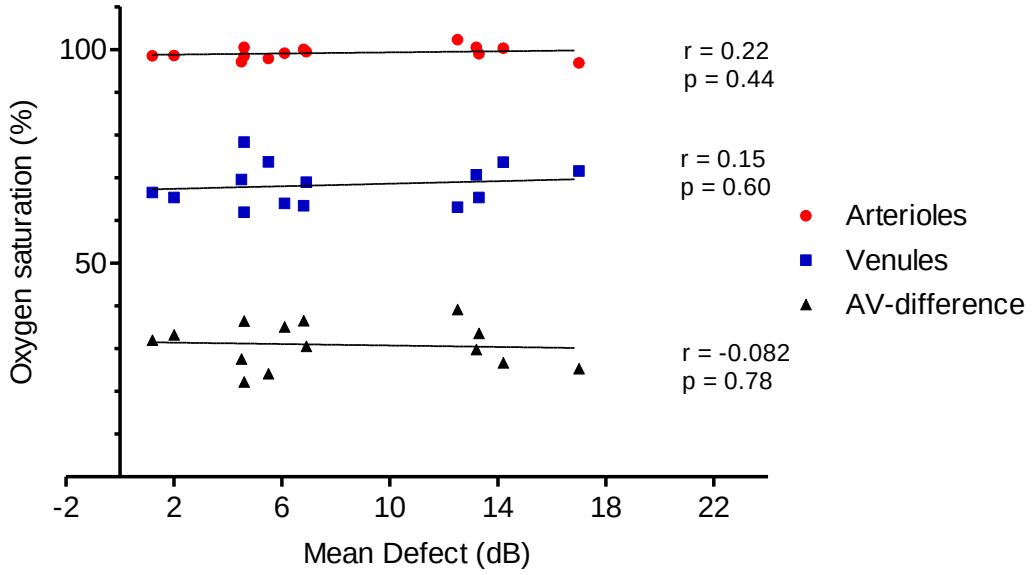

Figure 19. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in patients with open angle glaucoma with pseudoexfoliation syndrome.

The slope of the regression line for arteriovenous difference was -0.08 %/dB, for venules it was 0.15 %/dB and 0.065 %/dB for arterioles. n=14, r=coefficient of correlation.

Table 2. Clinical data for the entire study group. SD=standard deviation

Age (mean ± SD, year)	69 ± 13
Gender	27 females, 18 males
Number with pseudoexfoliation	14
Number without pseudoexfoliation	31
Intraocular pressure (mean ± SD, mmHg)	17 ± 4
Perfusion pressure (mean ± SD, mmHg)	50 ± 11[a]
Number using drugs for high blood pressure	19
Number using drugs for glaucoma	32
Trabeculectomy	8
Shunt (Ahmed tube)	2
Systolic blood pressure (mean ± SD, mmHg)	136 ± 23[a]
Diastolic blood pressure (mean ± SD, mmHg)	82 ± 13[a]

[a]Blood pressure measurements from three individuals were unavailable.

Table 3. Mean oxygen saturation (%) in 1st and 2nd degree retinal vessels in all glaucoma patients, glaucoma patients without pseudoexfoliation syndrome and glaucoma patients with pseudoexfoliation syndrome.

Data are presented as mean ± standard deviation. PEX=pseudoexfoliation syndrome, AV=arteriovenous.

	All (n=45)	No PEX (n=31)	PEX (n=14)
Arterioles	99 ± 3%	99 ± 4%	99 ± 1%
Venules	66 ± 5%	64 ± 5%	68 ± 5%
AV- difference	33 ± 5%	35 ± 5%	31 ± 5%

4.2 Retinal oxygen saturation difference between asymmetric glaucomatous visual field within-eye

In patients with asymmetrical visual field defects within the same eye, the mean arteriolar oxygen saturation was lower (98±5%) in areas corresponding to a deeper visual field defect compared to healthier areas (102±6%, p=0.04, table 5, figure 20A). The arteriovenous difference was also lower in areas corresponding to deeper visual field defect (30±10%) compared to healthier areas (37±10%, p=0.04, table 5, figure 20C). The venules showed no statistically significant difference with oxygen saturation levels at 68 ± 7% in areas corresponding to deeper visual field defect and 65 ± 9 % in healthier areas (p=0.3, table 5, figure 20B).

Table 4. Clinical data for the study group with asymmetrical visual fields.

SD=standard deviation

Age (mean ± SD, year)	77 ± 11
Gender	9 females, 4 males
Number with pseudoexfoliation	3
Number without pseudoexfoliation	10
Intraocular pressure (mean ± SD, mmHg)	16 ± 5
Perfusion Pressure (mean ± SD, mmHg)	52 ± 9
Number using drugs for high blood pressure	7[a]
Number using drugs for glaucoma	10
Trabeculectomy	4
Shunt (Ahmed tube)	1
Systolic blood pressure (mean ± SD, mmHg)	145 ± 30
Diastolic blood pressure (mean ± SD, mmHg)	82 ± 13

[a]Information from three people were not listed

Table 5. %Oxygen saturation for the study group with asymmetrical visual fields, n=13. Mean ± SD.

	Arterioles	Venules	AV difference
Scotoma	98 ± 5*	68 ± 7	30 ± 10*
No scotoma	102 ± 6*	65 ± 9	37 ± 10*

* Paired t-test, p=0.04

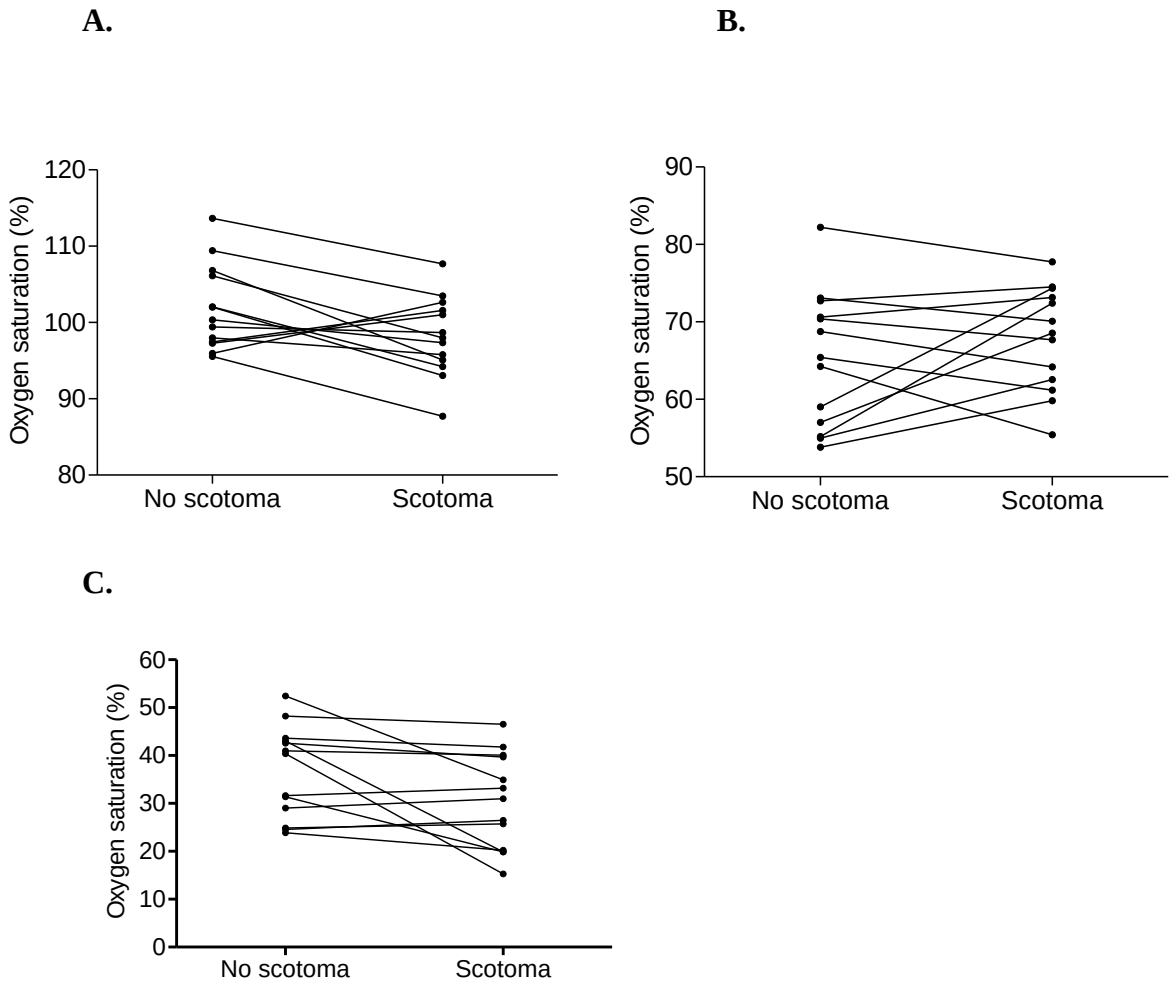

Figure 20. Oxygen saturation (%) in measured eyes for the study group with asymmetrical visual fields (n=13).

The lines connect measurement points in the same eye for oxygen saturation values in A. Arterioles. B. Venules. C. Arterio-venous difference.

5 Discussion

Oxygen saturation in retinal vessels in patients with open-angle glaucoma with and without pseudoexfoliation syndrome was studied. The results indicate that oxygen saturation is affected in glaucoma. Oxygen delivery is decreased in retinal tissue with worse glaucoma.

5.1 Correlation of retinal oxygen saturation and visual field mean defect

The results from the group as a whole show a correlation between arteriovenous difference and mean defect in the visual field. The arteriovenous difference in oxygen saturation decreases with worsening visual field (figure 17). Oxygen saturation in venules increases with a worsening visual field (figure 17).

In the context of open-angle glaucoma pathophysiology, arteriovenous difference decreased with worse glaucoma. Lower arteriovenous difference along with decreased blood flow in glaucoma, seen in earlier studies[40, 42-50], suggest less oxygen delivery by the retina with worsening glaucoma.

In primary tissue atrophy, the oxygen consumption of the tissue would be decreased. This reduces the relative proportion of oxygen extracted from the blood and although this is met by autoregulation, vasoconstriction and lower blood flow, it would be expected that the oxygen saturation of the venules would be stable or slightly increased in primary atrophy. Our data show an increase in venous oxygen saturation which may be secondary to tissue atrophy.

In primary ischemia, where the blood flow is insufficient for the oxygen demand of the tissue, the tissue would extract an increased proportion of the oxygen content from the blood causing the oxygen saturation levels in the venules to fall. This is not what was observed in our study. Oxygen saturation in the venules increased with worse visual field and this speaks against primary ischemia and tissue hypoxia.

However, the data do not completely exclude ischemia and hypoxia from the pathogenesis of glaucoma. Ischemia and hypoxia may be intermittent. It may be present when the glaucoma treatment is poor, when IOP spikes or blood pressure drops, for example at night.[93, 94] Our patients were under active glaucoma treatment and control at the

time of the study, the intraocular pressure was 17 mmHg on average, and this may prevent retinal hypoxia. Oximetry measurements in progressive glaucoma patients with poorly controlled IOP and low perfusion pressure are needed to further study the role of ischemia/hypoxia in the pathophysiology of glaucoma.

Michelson and Scibor[68] measured oxygen saturation in patients with high- or normal-tension glaucoma. For all examined eyes, they found that arteriovenous difference in saturation decreased with decreased area of the optic nerve head. This is in agreement with our results, i.e. arteriovenous difference in saturation decreases with advancing of glaucoma, measured either by mean defect or rim area.

The oximetry data gives an objective measurement that corresponds to the psychophysical data provided by perimetry. With further development and testing, this may support visual field testing in glaucoma or even prove to be a more reliable and objective measure of the degree of glaucomatous atrophy. An objective measurement has obvious benefits over the subjective visual field and, in addition, oximetry provides a numerical measurement.

When oxygen saturation data from the glaucoma patients without pseudoexfoliation syndrome was correlated with the mean visual field defect, the results were similar to those of the whole group. Venous saturation increased and the arteriovenous difference decreased with deeper visual field defect. As before, no correlation was seen between arterioles and visual field mean defect (figure 18). On the other hand, no statistically significant correlation was found between oxygen saturation levels and visual field mean defect in eyes with pseudoexfoliation glaucoma (figure 19), but this group was relatively small. A larger group of patients with pseudoexfoliation syndrome needs to be studied to confirm that oxygen metabolism is truly different in glaucoma patients with and without pseudoexfoliation syndrome.[95, 96]

The study is not without limitations. Some patients were on glaucoma drugs whilst others were not and some had undergone glaucoma surgery (table 2). A report by Hardarson et al.,[89] indicate that glaucoma surgery could affect our results, but not dramatically. Hardarson et al found a 2% increase in oxygen saturation in arterioles after surgery, but no statistically significant difference in venules and arteriovenous difference. The mean oxygen saturation values obtained in this study (table 3) compare well to

previous measurements by our group[81, 89, 90] and values obtained by other research groups with different oximeters.[68, 97]

5.2 Retinal oxygen saturation difference between asymmetric glaucomatous visual field within-eye

Comparisons were made in retinal vessel oxygen saturation corresponding to regions with asymmetric glaucomatous visual field defect within an eye. It was observed that AV-difference is lower in areas corresponding to glaucomatous visual field defect (table 5). The mean difference in the arteriovenous difference between the two areas was 30% in oxygen saturation which implies that, if we also look at blood flow data from other researchers, that oxygen uptake is less in more damaged areas in the visual field. That corresponds with the results from the first part of the study.

Because the comparison was made within an eye, variations between individuals, such as blood pressure differences, were avoided. However, the limitations to part one of the study (page 48) do, of course, also apply to this part of the study. Other factors are also included as limitations for this part; reliability factor in visual field testing (chapter 1.8) was not considered as an elimination factor because of the low number of patients. The data is preliminary, further studies are needed with higher number of patients.

6 Conclusion

Worsening visual field is related to an increase in oxygen saturation in veins and decreased arteriovenous difference. The same general finding is seen when oxygen saturation and visual field are correlated in eyes with asymmetrical glaucomatous visual field defects. This is consistent with and may result from tissue atrophy in glaucoma. On the whole, oxygen saturation in retinal blood vessels seems to be affected in glaucoma.

Our preliminary data do not show hypoxia in glaucomatous eyes, at least in our group of patients. Further clinical studies are needed to confirm these findings and to advance the use of oximetry in glaucoma research and treatment. This technology allows us to test the 150 years old ischemic theory and its role in the pathophysiology of glaucoma.

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Appendix

Article submitted for publication:

Retinal Oximetry in Open-Angle Glaucoma

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Abstract

PURPOSE: To test whether glaucomatous visual field defects are associated with alterations in retinal vascular oxygen saturation in patients with open-angle glaucoma.
DESIGN: Prospective, non-randomized clinical trial.
METHODS: Forty-five patients with open-angle glaucoma with and without pseudoexfoliation syndrome underwent spectrophotometric retinal oximetry and Octopus 123 visual field evaluation. Pearson's correlation was used to test whether the mean visual field defect was correlated with oxygen saturation of hemoglobin in corresponding retinal blood vessels.
RESULTS: Deeper visual field defects were correlated with smaller arteriovenous difference in oxygen saturation ($r=-0.42$, $p=0.0037$, $n=45$). Oxygen saturation in retinal venules increased significantly as the visual field mean defect deepened ($r=0.36$, $p=0.015$), whereas saturation in retinal arterioles remained constant ($r=-0.12$, $p=0.43$).
CONCLUSION: Deeper glaucomatous visual field defects are associated with decreased arteriovenous difference in retinal oxygen saturation, possibly indicating decreased oxygen delivery to the retina. These data suggest a change in oxygen metabolism in the glaucomatous retina, possibly related to tissue atrophy. They may also provide an objective measurement of the severity of glaucomatous atrophy.

Introduction

The exact cause of open-angle glaucoma (OAG) remains unknown. Glaucoma is considered to be an optic neuropathy associated with retinal ganglion cell death and visual field loss.[1] Two conflicting and unresolved theories were proposed to explain the pathogenesis of the disease 150 years ago, one mechanical and the other vascular.[2] The former points to mechanical consequences of elevated intra-ocular pressure (IOP) as a direct cause. The vascular theory postulates insufficient or poorly regulated blood supply to the retina and optic nerve head, leading to ischemia, hypoxia and resultant tissue damage. While often described seperately, reduced blood flow within eye tissues may also be the result of elevated IOP through a reduction in ocular perfusion pressure.

There is growing evidence that blood flow in glaucomatous eyes is reduced or its regulation disturbed compared to normal, non-glaucomatous eyes.[3] Blood flow deficiencies have been reported in the choroidal[4, 5] and retrobulbar[5-9] circulations in glaucoma patients.[10, 11] For instance, Plange et al. have shown that blood velocity in the central retinal artery is reduced in the more affected eye in asymmetric glaucoma.[12] Sato et al. have shown that neuroretinal rim blood flow is reduced in areas corresponding to scotoma within asymmetric normal tension glaucoma eyes.[13] Systemic and localized vascular abnormalities have been linked to OAG.[14-17] Reduced ocular perfusion pressure has been linked to both the prevalence[18-20] and incidence of glaucoma[21], indicating strongly that vascular factors

may play a role in glaucoma. Flammer et al. theorized that vascular autoregulation is impaired in glaucoma, resulting in impaired blood flow and energy metabolism.[3, 22]

It is unknown whether diminished blood flow is a primary cause of glaucomatous atrophy of ganglion cells and the optic nerve, or secondary to the atrophy, where autoregulation reduces blood flow in response to decreased metabolic demand and oxygen consumption in an atrophic tissue. Do glaucomatous eyes have a primary metabolic impairment from ischemia in which case tissue hypoxia should be present; or physiologically reduced blood flow secondary to tissue atrophy, in which case tissue oxygen levels should be normal? While tissue oxygen levels cannot currently be measured in human glaucoma patients, retinal vessel oxygen saturation provides data on retinal oxygen metabolism and possible hypoxia.

In the current investigation we use non-invasive spectrophotometric retinal oximetry[23-25] to measure oxygen saturation in retinal arterioles and venules and correlate with visual field defects in patients with OAG with and without pseudoexfoliation syndrome.

Methods

Eligible individuals were screened in the glaucoma clinic and qualified OAG patients were invited to participate in the study. The inclusion criteria were OAG with or without pseudoexfoliation syndrome[26-28] and no other ocular diseases. All individuals were 40 years of age or older. Individuals receiving anti-hypertensive medication for elevated systemic blood pressure were not excluded. Individuals with cataract were not excluded. Individuals with other systemic diseases, such as diabetes, were excluded from the study. In all, 45 individuals were enrolled into the study. The right eye in each patient was used for study except in the case of low image quality from the oximeter in which the left eye was measured. Additional details of patients in the study are shown in table 1.

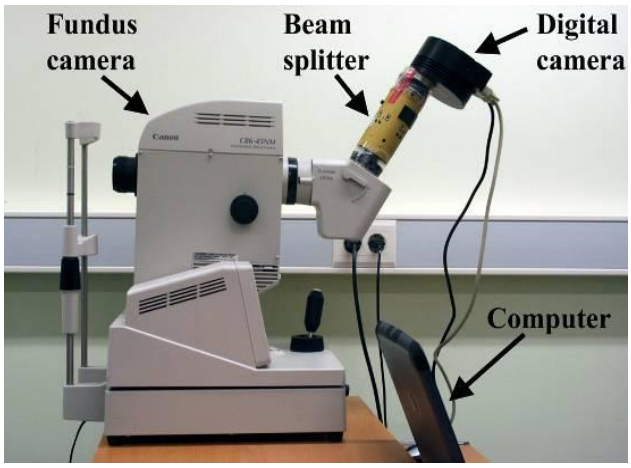

Figure 1. The oximeter used in the research.

Non-invasive spectrophotometric oximetry was performed on the same day as ophthalmologist evaluations. The oximeter is based on a fundus camera (Canon CR6-45NM, Canon Inc., Tokyo, Japan) with an attached beam splitter (Multispec Patho-Imager, Optical Insights, Tucson, AZ.) and a digital camera (SBIG ST-7E, Santa Barbara Instrument Group, Santa Barbara, CA) (figure 1). The instrument (described in detail elsewhere [23]) delivers two images at two different wavelengths, 605nm which is sensitive to oxygen saturation and 586nm which is not sensitive to oxygen saturation. A software algorithm calculates optical density (OD) of retinal vessels from the two acquired images according to the equation $OD=\log (I_o/I)$ where I_o is light reflected by the background and I is the light reflected from the vessel. The ratio of the OD at 605nm and the OD at 586nm is inversely related to hemoglobin oxygen saturation. Measurements were made in first and second degree retinal arterioles and venules (figure 2). Pupils were dilated with 1% tropicamide (Mydriacyl®, S.A. Alcon-Couvreur N.V., Puurs, Belgium). When needed, it was supplemented with 10% phenylephrine hydrochloride (AK-Dilate, Akorn Inc., Buffalo Grove, IL., USA) and 0.5% proparacaine hydrochloride (Alcaine®, S.A., Alcon-Couvreur N.V., Puurs, Belgium).

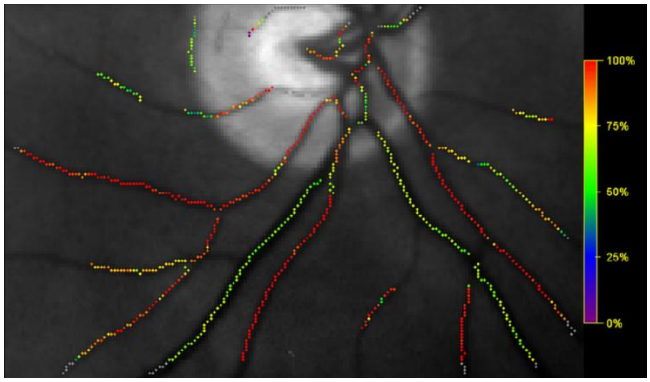

Figure 2. A pseudocolor fundus map from the oximeter.

All visual field testing was performed using an Octopus 123 (Interzeag AG, Schlieren, Switzerland), program G1. For 32 participants, the visual field tests were performed on the same day as the oximetry. For 12 individuals, the perimetry was not performed on the same day as the oximetry, but no more than five months before the oximetry. The frequency of false-positive answers in a visual field was limited to< 15%. Since the false-negative answers represent the status of the eye rather than patient status[29, 30], they did not count as an exclusion factor.

IOP was measured using Goldmann applanation tonometry mounted on a Haag-Streit slit lamp (Haag-Streit BQ 900, Haag-Streit International, Köniz, Switzerland) on the same day as the oximetry was performed. Pseudoexfoliation syndrome was diagnosed based on morphologic alterations of the anterior lens capsule.[26, 27] The characteristic appearance of pseudoexfoliation material was diagnosed when a peripheral granular zone, with or without a central zone, was seen. The pupil was dilated before lens changes were diagnosed.

Systolic and diastolic blood pressure (SP and DP, respectively) were measured using an automatic sphygmomanometer (Omron HEM-705CP, Omron, Kyoto, Japan). Mean arterial pressure (MAP) was calculated as follows; MAP=2/3 DP + 1/3 SP. Mean ocular perfusion pressure was calculated as 2/3 MAP-IOP. Finger oximetry was performed using an Ohmeda Biox 3700 pulse oximeter (Ohmeda, Boulder, CO, USA) with the probe placed on the index finger of the right hand.

Statistical analysis was performed with Prism, version 5.01 (GraphPad Software Inc., LaJolla, CA, USA). Pearson´s correlation was used to detect correlation between mean defect and oxygen saturation levels.

Results

In OAG patients (n=45), there is a statistically significant negative correlation between arteriovenous difference in oxygen saturation and visual field mean defect levels (r= -0.42, p=0.0037, Pearson´s r, figure 3). The slope of the regression line for arteriovenous difference was -0.33 %/dB with 95% confidency interval (CI) ranging from -0.55 to -0.11. Statistically significant correlation was found between oxygen saturation in retinal venules and visual field mean defect levels (r=0.36, p=0.015, figure 3), where the venous oxygen saturation increased with worsening visual field. The slope of the regression line for venules was 0.28 %/dB with 95% CI from 0.057 to 0.49 (figure 3). No correlation was found between saturation in retinal arterioles and visual field mean defect (r=-0.12, p=0.43, figure 3). The slope of the regression line for arterioles was -0.056 %/dB with 95% CI from -0.20 to 0.088 (figure 3).

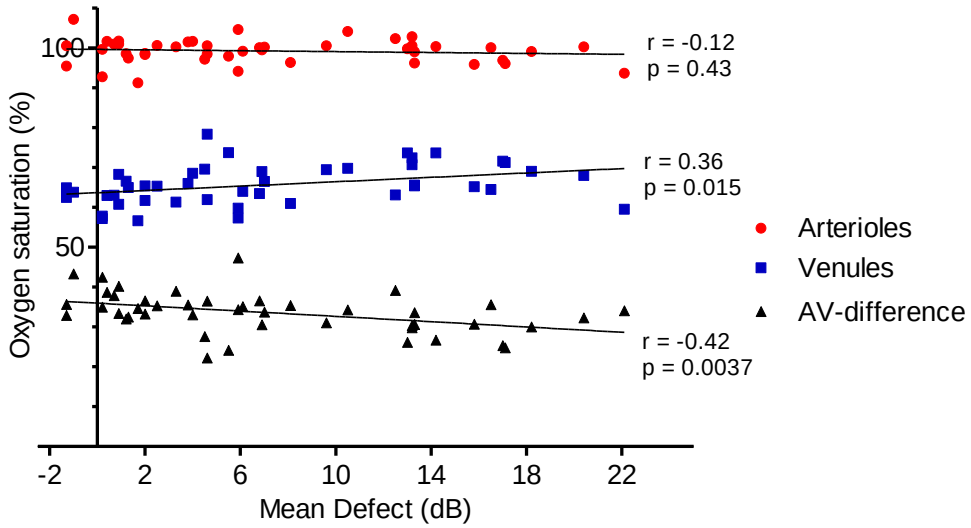

Figure 3. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in eyes with open-angle glaucoma, with or without pseudoexfoliation syndrome. The slope of the regression line for arteriovenous difference was -0.33 %/dB, for venules it was 0.28 %/dB and -0.056 %/dB for arter ioles. n=45, r=coefficient of correlation.

For OAG patients without pseudoexfoliation syndrome (n=31), there was a negative statistically significant correlation between arteriovenous difference and visual field mean defect (r=-0.55, p=0.0013, figure 4). The slope of the regression line for arterioveonus difference was -0.36 %/dB with 95% CI from -0.57 to -0.15 (figure 4). Positive statistical correlation was found between oxygen saturation in venules and visual field mean defect (r=0.43, p=0.015, figure 4) with the slope of the regression line at 0.28 %/dB where 95% CI

ranged from 0.058 to 0.50 (figure 4). In retinal arterioles, there was no correlation with visual field mean defect (r=-0.14, p=0.38, figure 4). The slope of the regression line for arterioles was -0.082 %/dB with 95% CI ranging from -0.27 to 0.11 (figure 4).

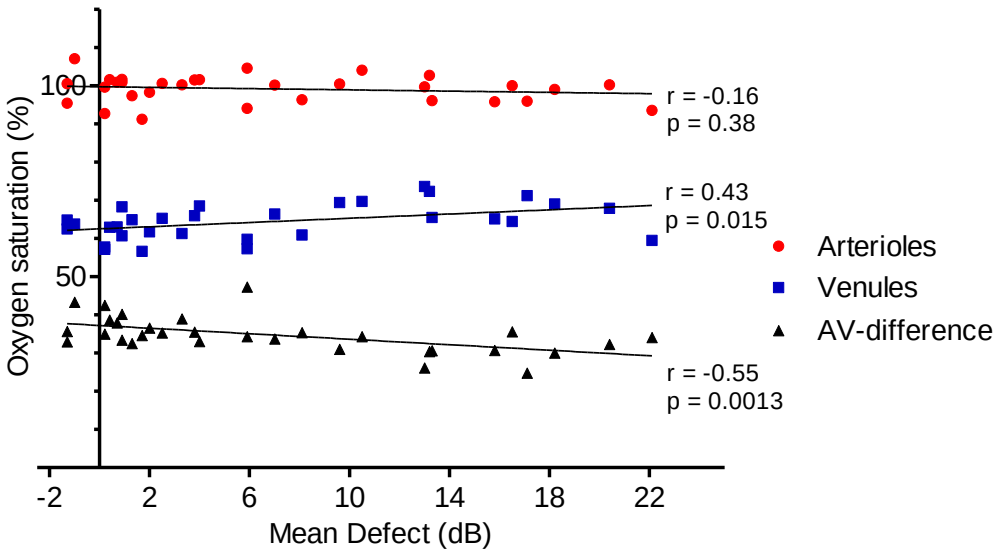

Figure 4. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in patients with open angle glaucoma without pseudoexfoliation syndrome. The slope of the regression line for arteriovenous difference was -0.36 %/dB, for venules it was 0.28 %/dB and -0.082 %/dB for arterioles. n=31, r=coefficient of correlation.

For the group of OAG patients with pseudoexfoliation syndrome (n=14) there was no correlation between arteriovenous difference and visual field levels (r=-0.082, p=0.78, figure 5). The slope of the regression line for arteriovenous difference was -0.08 %/dB with 95% CI ranging from -0.73 to 0.56 (figure 5). There was no correlation detected between oxygen saturation levels in venules and visual field levels (r=0.15, p=0.60, figure 5) where the slope of the regression line was 0.15 %/dB with 95% CI from -0.45 to 0.75 (figure 5). No correlation was found between oxygen levels of arterioles and visual field levels (r=0.22, p=0.44, figure 5). For arterioles, the slope of the regression line was 0.065 %/dB with 95% CI from -0.11 to 0.24 (figure 5).

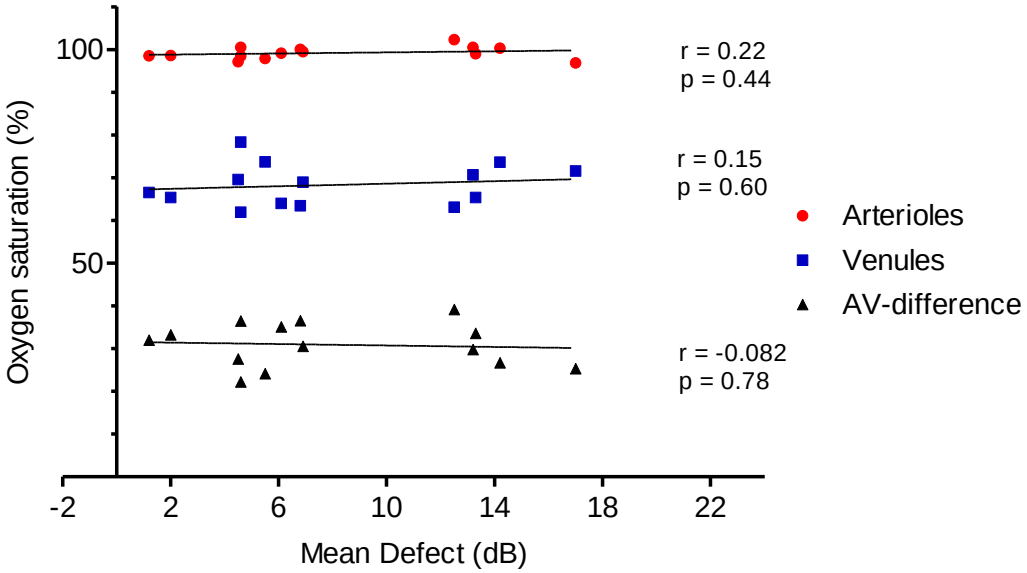

Figure 5. Correlation of visual field mean defect with oxygen saturation in arterioles (red), venules (blue) and arteriovenous difference (black) in patients with open angle glaucoma with pseudoexfoliation syndrome. The slope of the regression line for arteriovenous difference was -0.08 %/dB, for venules it was 0.15 %/dB and 0.065 %/dB for arterioles. n=14, r=coefficient of correlation.

Table 2. Clinical data for the study group. SD=standard deviation

Age (mean ± SD, year)	69 ± 13
Gender	27 females, 18 males
Number with pseudoexfoliation	14
Number without pseudoexfoliation	31
Intraocular pressure (mean ± SD, mmHg)	17 ± 4
Perfusion Pressure (mean ± SD, mmHg)	67 ± 10[a]
Number using drugs for high blood pressure	19
Number using drugs for glaucoma	32
Trabeculectomy	8
Shunt (Ahmed tube)	2
Systolic bloodpressure (mean ± SD, mmHg)	135 ± 23[a]
Diastolic bloodpressure (mean ± SD, mmHg)	82 ± 13[a]

SD=standard deviation
[a]Blood pressure measurements from three individuals were unavailable.

Table 2. Mean Oxygen Saturation (%) in 1st and 2nd Degree Retinal Vessels in all Glaucoma Patients, Glaucoma Patients without Pseudoexfoliation Syndrome and Glaucoma Patients with Pseudoexfoliation Syndrome [a].

	All (n=45)	No PEX (n=31)	PEX (n=14)
Arterioles	99 ± 3%	99 ± 4%	99 ± 1%
Venules	66 ± 5%	64 ± 5%	68 ± 5%
AV- difference[b]	33 ± 5%	35 ± 5%	31 ± 5%

PEX=pseudoexfoliation syndrome, AV=arteriovenous

[a] Data are presented as mean ± standard deviation.

[b] AV-difference denotes the difference in oxygen saturation between arterioles and venules.

Discussion

We studied oxygen saturation in retinal vessels in patients with OAG with and without pseudoexfoliation syndrome. The results from the group as a whole show a correlation between arteriovenous difference and mean defect in the visual field. The arteriovenous difference in oxygen saturation decreases with worsening visual field (figure 3). Oxygen saturation in venules increases with a worsening visual field (figure 3). There is no correlation between oxygen saturation values in arterioles and visual field levels (figure 3).

In the context of OAG pathophysiology, arteriovenous difference decreased with worsening glaucoma. Lower arteriovenous difference along with decreased blood flow in glaucoma, seen in earlier studies[3, 5-13], suggests less oxygen uptake by the retina with worsening glaucoma. In primary ischemia, where the blood flow would be insufficient for the oxygen demand of the tissue, the tissue should extract an increased proportion of the oxygen content from the blood and the oxygen saturation levels in venules would fall. This is not what we observed in this study. Oxygen saturation in venules increased with worse visual field.

Conversely, in primary tissue atrophy, the oxygen consumption of the tissue would be decreased. This reduces the relative proportion of oxygen extracted from the blood and although this is met by autoregulation, vasoconstriction and lower blood flow, we would expect oxygen saturation of the venules to be stable or slightly increased in primary atrophy. Our data supports this concept as the change in oxygen saturation and blood flow appears to be secondary to tissue atrophy. However, the data do not exclude ischemia and hypoxia from the pathogenesis of glaucoma. Ischemia and hypoxia may be intermittent. It

may be present when the glaucoma treatment is poor, when IOP spikes or blood pressure drops, for example at night.[31, 32] Our patients were under active glaucoma treatment and control at the time of the study, the intraocular pressure was 17 mmHg on average, and this may prevent retinal hypoxia. Oximetry measurements in progressive glaucoma patients whose IOP is poorly controlled and possibly with low perfusion pressure are needed to elicit additional information on the role of ischemia/hypoxia in the pathophysiology of glaucoma.

Michelson and Scibor[33] measured oxygen saturation in patients with high or normal tension glaucoma. They found that arteriovenous difference in saturation decreased with decreased area of the optic nerve head. This is in agreement with our results, i.e. arteriovenous difference in saturation decreases with advancing of glaucoma, measured either by mean defect or rim area.

The oximetry data gives an objective measurement that corresponds to the psychophysical data provided by perimetry. With further development and testing, this may support visual field testing in glaucoma or even prove to be a more reliable measure of the degree of glaucomatous atrophy. An objective measurement has obvious benefits over the subjective visual field and, in addition, oximetry provides a numerical measurement.

When oxygen saturation data from the glaucoma patients without pseudoexfoliation syndrome was correlated with the mean visual field defect, the results were similar to those of the whole group. Venous saturation increased and the arteriovenous difference decreased with deeper visual field defect. As before, no correlation was found between arterioles and visual field mean defect (figure 4). On the other hand, no statistically significant correlation was found between oxygen saturation levels and visual field mean defect in eyes with pseudoexfoliation glaucoma (figure 5), but this group was relatively small. A larger group of patients with pseudoexfoliation syndrome needs to be studied to confirm that oxygen metabolism is truly different in glaucoma patients with and without pseudoexfoliation syndrome.[34, 35]

Our study represents an initial clinical study and is not without limitations. Some patients were on glaucoma drugs whilst others were not and some had undergone glaucoma surgery (table 1). Previous results from Hardarson et al.,[24] however, indicate that glaucoma surgery would not dramatically affect our results. This prior study found a 2% increase in oxygen saturation in arterioles after surgery, but no difference in venules and arteriovenous

difference. The mean oxygen saturation values obtained in this study (table 2) compare well to previous measurements by our group[23-25] and values obtained by other research groups with different oximeters.[33, 36] It would also be ideal if we could have included direct measures of retinal blood flow in addition to oximetry values. Assessing the diurnal fluctuations in all of the aforementioned parameters may also provide a more complete understanding of retinal function and metabolism.

In summary, we found correlation between worsening visual field and increase in oxygen saturation in veins as well as the arteriovenous difference. This is consistent with and may result from tissue atrophy in glaucoma. There may be a metabolic difference between glaucoma patients with and without pseudoexfoliation syndrome. Further clinical studies are needed to confirm and further expand the use of oximetry in glaucoma to enhancing our understanding of pathophysiology and hopefully clinical evaluation of glaucoma pathology.

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B. Financial Disclosures: OBO, employee of Oxymap ehf; SHH, employee and stockholder of Oxymap; MSG, none; AH, advisor for Oxymap; ES stockholder of Oxymap.

C. Contribution of Authors: Design of the study (OBO, SHH, MSG, ES); conduct of the study (ES); collection and management of the data (OBO, SHH, MSG); preparation of manuscript (OBO, ES); review and approval of manuscript (OBO, SHH, MSG, AH, ES).

D. Conformity with Author Information: The study was approved by the National Bioethics Committee of Iceland and The Icelandic Data Protection Authority and adhered to the tenets of the Declaration of Helsinki. All individuals signed an informed consent.

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