Abstract

**Purpose:** To evaluate retinal blood flow changes in glaucomatous eyes after intraocular pressure (IOP) lowering and to determine whether a correlation exists between retinal capillary blood flow and IOP changes using optical coherence tomography angiography (OCTA)

**Materials and methods:** OCTA images were obtained from glaucomatous eyes for the macula (n=24) and optic disc (n=21) in a prospective, cross-sectional observational study. Microvascular changes were analysed at two retinal layers: deep vascular plexus (DVP) and the superficial vascular plexus (SVP) as well as choriocapillaries (CC) were compared pre and six weeks post intraocular pressure (IOP) lowering treatment. Blood flow changes were analysed with ImageJ software and statistical analysis was performed via Stata with a \( p \) value of < 0.05 considered as statistically significant.

**Results:** Retinal blood flow across the macula showed no statistical significance at: SVP (z-score = -0.887), DVP (z-score = -1.093) and CC (z-score = -1.423). Further, no statistically significant difference in blood flow was observed across the disc: SVP (z-score = -0.918), DVP (z-score = -1.057) and CC (z-score = -0.295). While IOP reduction was achieved (z-score = -5.049), visual acuity deteriorated (z-score= 1.686)

**Conclusion:** No statistical difference was found in the data for all layers for both the areas surrounding the disc and macula at 6 weeks. OCTA can detect microstructural defects and future longitudinal studies may provide further insight into effects of IOP lowering treatment on retinal blood flow in glaucoma patients in longer term.

**Keywords:** Glaucoma, Intraocular pressure (IOP), Optical coherence tomography angiography (OCTA), Macula, Optic disc, Retinal nerve fibre layer, Visual acuity

Introduction

Glaucoma is a prevalent eye disease and is one of the leading causes of irreversible blindness and induced vision loss worldwide [1]. Currently, the global prevalence of glaucoma worldwide estimates that it affects 3.54% of the population aged between 40 and 80 years of age [2]. In 2013, approximately 64.3 million people worldwide between 40 and 80 years of age had glaucoma, with worrying estimates of this figure increasing to 76.0 million and 111.8 million in 2020 and 2040, respectively [2]. Currently, the single most validated treatment for glaucoma is intraocular pressure (IOP) lowering therapy [3,4]. However, despite achieving adequate IOP control visual acuity loss continues to progress in many patients [3]. Glaucoma is a condition that affects the optic nerve and associated structures. Diagnosis is based on progressive visual field loss and typical optic nerve head changes, dependent or independent of IOP [3]. Glaucoma, a multifactorial disease, is reported as being linked to genetics, age, gender, ethnicity, diabetes
mellitus, hypotension, sleep apnoea, migraine and primary vasospasm [5-7]. While elevation in IOP is an important finding in glaucoma patients it is not the only attributable cause of optic nerve damage and retinal ganglion cell (RGC) death [8].

Glaucoma is a neurodegenerative disease, yet to date there is limited understanding of how vasculature changes in glaucoma. A growing body of evidence suggests that vascular factors, such as a reduction of retinal blood flow and perfusion pressures, play a critical role in the development of glaucoma [9-11]. An explanation for this could be an impaired neurovascular unit and endothelial cell dysfunction, where a reduction in retinal capillaries showed a strong correlation in reduction of macula thickness [12]. Some prospective studies have demonstrated that in primary open-angle glaucoma (POAG) arterial blood flow is diminished in the ophthalmic, retinal, choroidal, and retrobulbar circulations [10,11,13]. The importance of understanding the mechanisms regulating the neurovascular system and the retinal haemodynamics in glaucoma may provide new insight into new strategies to prevent and treat glaucoma [14]. A recent study by Wu et al detected a reduction in macular vessel density and capillary perfusion using OCTA in individuals with primary open angle glaucoma (POAG) suggesting that OCTA may play a future role in facilitating diagnosis and early treatment of glaucoma [15].

Recently, a newly developed Optic Coherence Tomography Angiography (OCTA) has demonstrated the ability to quantify retinal and optic nerve head blood flow rapidly and accurately [9-11,13,15]. It is a non-invasive technique that does not require the injection of any exogenous dye or contrast agent and yet provides near-automatic quantification of blood perfusion. OCTA uses sequential, high speed OCT B-scans taken at precisely the same cross-section to provide a highly detailed map of retinal blood flow [9,11,16]. OCTA makes it possible for clinicians to visualise and assess the superficial and deep retinal capillary networks as well as choriocapillaris [9,11,16]. However, at present there is limited literature regarding the interpretation of OCTA in retinal diseases and even fewer in glaucoma. It is well demonstrated in the literature that the primary insult resulting in optic neuropathy in glaucoma is due to retinal ganglion cell loss, however a growing body of evidence is correlating retinal thinning of the inner macula with functional visual loss [9,16,17]. Therefore, in this study we wished to explore both the optic nerve head and macular microvasculature in determining the effects of IOP lowering treatment on retinal blood flow and visual function.

The aim of this study is:

1) To investigate for changes of retinal blood flow in glaucoma patients after treatment using OCTA.
2) To correlate the defect of retinal capillaries with intraocular pressure changes and RNFL in glaucoma patients pre and post treatment using OCTA.

Methods

This is a prospective, cross-sectional study that uses non-invasive OCTA to assess whether glaucoma treatment changes the perfusion of both the optic nerve head and macula by assessing blood flow pre and post treatment using OCT angiography and to determine whether the microvascular changes correlate with retinal ganglion cell loss.

This study was conducted at the Royal Victorian Eye and Ear Hospital, Melbourne, Australia. The study protocol and ethics approval for this study were approved by the Human Research Ethics Committee at the Royal Victorian Eye and Ear Hospital in Melbourne, Victoria (Protocol no. 17/1359H). This study adhered to the declaration of Helsinki as outlined in the National Health and Medical Research Council. Written informed consent was obtained from all of the patients involved in this study.

A total 24 participants were enrolled into this study of which 24 eyes for the macula and 21 for the disc layer were imaged using the Spectralis OCT Angiography Module (Heidelberg Engineering). Each patient underwent basic visual examination parameter testing prior to imaging including: best correct visual acuity and intra-ocular pressure measurement with Goldmann applanation tonometry. Data including age, gender and systemic blood pressure, were collected from participants medical records. The study involved one single OCTA imaging of the participants at the end of their routine clinic visit. OCT is already a routine imaging for the care of glaucoma patients and OCTA is an additional image for research purposes. All eyes were scanned using the Spectralis OCT Angiography Module (Heidelberg Engineering).

Only the eye being treated for glaucoma was additionally scanned using the OCTA. Four volumetric raster scans, including two horizontal priority (x-fast) and two vertical priority (y-fast) scans were obtained consecutively over 3 x 3 mm area for both optic disc and macular regions.
The two layers analysed at both the optic nerve head and the macula were the choriocapillaries (CC), deep vascular plexus (DVP) and superficial vascular plexus (SVP). Using a two-sample t-test the data pre and post treatment were analysed to determine if results differed significantly ($p<0.05$).

In order to quantify optic disc and macular circulation, en face retinal angiograms from OCTA acquired images were outputted and the vessel density at the disc and macula were processed with Image J software. Image J is a well-known, Java-based image-processing program, designed for scientific multidimensional imaging, developed at the National Institute of Health and the Laboratory for Optical and Computational Instrumentation.

The inclusion criteria included: (1) any patients 18 years or above or mature minor; (2) best corrected visual acuity of greater than 6/60; (3) patients requiring treatment to manage their glaucoma (including both medical (such as drops) or surgical treatment (such as laser or surgery); (4) pre-existing glaucoma or glaucoma suspects with either evidence of neural rim loss at the optic disc and/or visual field defects on automated visual field testing; (5) the intra-ocular pressure was higher than 20mmHg and below 40mmHg.

The exclusion criteria included: (1) any ocular comorbidity contributing to visual fields loss (including dense cataracts, uveitis, ocular trauma, non-glaucomatous optic neuropathy or any retinal pathology); (2) best corrected visual acuity worse than 6/60; (3) significantly poor mobility restricting ability to be examined using OCTA; (4) vulnerable individuals or those lacking capacity; (5) glaucoma patients with pressures above 20mmHg who weren’t being treated.

The Spectralis OCT Angiography provides a non-invasive visualisation of the choroidal and retinal vasculature based on low-coherence interferometry. High-resolution OCTA images were acquired using the Spectralis OCT Angiography Module with a lateral resolution of 5.7 μm/ pix. Fine capillary networks were visualised in greater detail through the precision of the TruTrack Active Eye Tracking. The highly detailed axial resolution of 3.9 μm/ pixel allowed for precise segmentation of all four histologically-validated retinal vascular plexuses. Within the superficial and deep vascularplexuses, custom slabs provided a more comprehensive clinical evaluation. An additional feature of the Spectralis OCT Angiography is the projection artifact removal (PAR) tool which removes artifacts from the OCTA images through utilization of information from the superficial vascular plexus. This enabled a more precise visualization of retinal vascular pathology and structure.

No internal algorithm was available using the Spectralis OCT. Instead, in order to quantify retinal circulation at the disc and macula, en face retinal angiograms from the OCTA acquired images were processed and vessel density was calculated using an external program called Image J. Each image was opened in Image J and the type was adjusted to 32bit to allow for the most precise detail acquisition. Each of the three layers at the disc and macula had a standardised colour threshold set by image J program which was used to analyse all the images in that particular retinal layer and location.

For analysis descriptive statistics were used to calculate mean and median difference of retinal nerve fibre layer (RNFL) vessel density acquired through Image J to calculate standard deviations. Two-sample t-tests via the statistical program Stata was used to calculate the difference in retinal blood flow of glaucomatous eyes pre and post treatment and a p-value was determined on the mean difference of flow pre and post treatment. A two-sample difference t-test was performed to analyse the data. A p value of $< 0.05$ or a confidence interval not containing zero was interpreted as a significant result. Further, the Mann-Whitney-Wilcoxon test, a well-validated test used to analyse data which does not follow the normal distribution, was used where the two-sample t-test would not be reliable. Linear regression analysis was used to investigate whether the measurement of retinal vessel blood flow was affected by intraocular pressure.

**Results**

**Demographics**

A total of 24 eyes were analysed for the macula area with 50% of eyes belonging to males and 50% to female participants. A smaller group of 21 eyes were analysed for the disc area except for the disc choriocapillaries were there were only 20. For the disc cohort 52.4% of the eyes belonged to male participants and 47.6% belonged to female participants. Unfortunately, ten participants or 12 eyes were lost to follow up. The average age of the cohort in this study was 63.13 years old with a 95% confidence interval (CI) of (54.34, 71.91). The method of IOP lowering used on participants in this study included use of topical and oral medication in 50%, laser in 16.7%, incisional surgery in 16.7% or a combination of methods in 16.7%.
Retinal blood flow at the macula

Table 1 summarises the raw pixel data for the retinal layers pre and post treatment. Table 2 is a summary of the analysis of the raw data in table 1 providing the two-sample t-test along with the 95% Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the Macular retinal layers. A two-sample difference t-test for the macula SVP layer showed no significant difference between the pre and post treatment with a p value of 0.4441 and a 95% CI of -6.93 to 3.09. At the macula DVP layer the two-sample difference t-test showed no significant difference between the pre and post treatment with a p value of 0.3690 and a 95% CI of -7.78 to 2.95. Further, analysis of the macula CC layer using a two-sample difference t-test showed no significant difference between the pre and post treatment with a p value of 0.1902 and a 95% CI of -13.40 to 2.74. On further analyses using the Mann-Whitney-Wilcoxon test, to cater for a lack of a normal distribution of the data, the z-score for the macula SVP, DVP and CC was -0.887, -1.093 and -1.423, respectively. This validates the result of the two-sample t-test with results showing no statistical significance between pre and post treatment capillary density at the macula.

Retinal blood flow at the optic disc

Table 3 summarises the two-sample t-test along with the 95% Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the optic disc retinal layers. A two-sample difference t-test for the optic disc SVP layer showed no significant difference between the pre and post treatment with a p value of 0.3590 and a 95% CI of -7.04 to 2.61. At the optic disc layer the two-sample difference t-test showed no significant difference between the pre and post treatment with a p value of 0.3933 and a 95% CI of -4.05 to 1.63. Further, analysis of the optic disc CC layer using a two-sample difference t-test showed no significant difference between the pre and post treatment with a p value of 0.7112 and a 95% CI of -6.16 to 4.25. On additional analyses using the Mann-Whitney-Wilcoxon test, to cater for a lack of a normal distribution of the data, the z-score for the optic disc SVP, DVP and CC was -0.918, -1.057 and -0.295, respectively. This validates the result of the two-sample t-test with results showing no statistical significance between pre and post treatment capillary density at the optic disc. Table 4 summarises the mean capillary density with 95% confidence interval pre and post IOP lowering treatment that shows no statistical significance pre and post treatment. However, whilst no statistical significance was found the mean capillary density was lower post treatment for all retinal layers.

Intraocular pressure and visual acuity changes pre and post treatment

Table 5 summarises the raw values of the intraocular pressure (IOP) and visual acuity pre and post treatment. An analysis of the raw values is provided in table 6, which summarises the two-sample t-test along with the 95%, Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the IOP and visual acuity (VA) pre and post treatment. Interestingly, despite no statistically significant findings in retinal capillary density across all retinal layers at the optic disc and macula, a two-sample difference t-test of IOP pre and post treatment showed statistical significance with a p value of 0.000 and a 95% CI of -16.70 to -8.89. This was further validated on the Mann-Whitney-Wilcoxon test finding a statistically significant Z-score of -5.049. Despite, statistically significant improvement in IOP pre and post treatment, visual acuity showed a statistically significant reduction post treatment with a Mann-Whitney-Wilcoxon Z-score 1.686. A Mann-Whitney-Wilcoxon test was performed on VA as the data showed skew meaning the two-sample difference t-test for VA changes pre and post treatment was not reliable.

Discussion

In recent times, a number of innovative technologies have been designed for the assessment of ocular blood flow, blood vessel diameter and levels of oxygen saturation, such as bidirectional laser Doppler velocimetry, retinal vessel analyser, colour Doppler imaging and video angiography [18]. However, none of these advanced techniques provide accurate measurement and direct visualization of retinal capillaries [18]. OCTA offers novel and imperative information of retinal haemodynamics using a non-invasive, detailed and fast acquisition of images [9,11,16]. This study looked at changes of retinal blood flow in glaucoma patients after treatment using OCTA and whether any correlation exists with intraocular pressure changes and retinal capillaries in the RNFL in glaucoma patient’s pre and post treatment. This study hoped to provide novel information on the effects of current IOP lowering therapies on retinal blood flow and visual function, however a lack of a statistically significant association was found. Future, prospective studies with a larger number of patients and longer follow up periods will be required to further validate the conclusion.

We have demonstrated that no statistical significance exists in retinal blood flow pre and post IOP lowering treatment in the retinal layers surrounding both the optic disc and macula. Despite no statistical significance found
in capillary density pre and post IOP lowering treatment, the IOP decrease of the cohort was statistically significant. However, we found a statistically significant deterioration in visual acuity pre and post treatment. Figure 1 and 2 below demonstrate the OCTA images pre and post IOP lowering treatment at both the optic disc and macula for the SVP, DVP and CC for the same patient.

**Figure 1**: Demonstrates the OCTA images pre and post IOP lowering treatment at the macula for the SVP, DVP and CC for the same patient.

(A SVP Pre-treatment, B SVP Post Treatment, C DVP Pre-Treatment, D DVP Post Treatment, E CC Pre-Treatment, F CC Post Treatment)

**Figure 2**: Demonstrates the OCTA images pre and post IOP lowering treatment at the optic disc for the SVP, DVP and CC for the same patient.

(A SVP Pre-treatment, B SVP Post Treatment, C DVP Pre-Treatment, D DVP Post Treatment, E CC Pre-Treatment, F CC Post Treatment)

IOP plays a vital part in damage to retinal ganglion cells in glaucoma, but more recently has been shown to play a crucial role of blood flow insufficiency in the development and progression of glaucoma [13]. This is strengthened through the association found between normal tension glaucoma and POAG with nocturnal hypotension, vasospasm, peripheral vascular abnormalities and migraines [19,20]. Latest imaging by magnetic resonance imaging provides additional proof of cerebral small-
Table 1: Summary of the raw pixel data for the retina layers pre and post treatment.

| Eye | Pre Disc DVP | Post Disc DVP | Pre Disc SVP | Post Disc SVP | Pre Disc CC | Post Disc CC | Pre Mac DVP | Post Mac DVP | Pre Mac SVP | Post Mac SVP | Pre Mac CC | Post Mac CC |
|-----|--------------|---------------|--------------|---------------|-------------|--------------|-------------|--------------|-------------|--------------|-------------|--------------|
| 1   | 10.24        | 5.06          | 28.61        | 18.66         | 18.58       | 10.34        | 23.16       | 8.25         | 32.11       | 16.67       | 43.95       | 17.94       |
| 2   | 20.25        | 21.69         | 38.2         | 37.71         | 24.72       | 22.25        | 35.57       | 36.99        | 43.78       | 48.4         | 52.71       | 47.2         |
| 3   | 17.57        | 16.78         | 14.43        | 14.17         | 18.38       | 16.41        | 23.34       | 26.59        | 24.6        | 30.57        | 24.44       | 16.84       |
| 4   | 19.34        | 16.31         | 32.98        | 29.8          | 17.8        | 14.81        | 24.12       | 23.54        | 32.47       | 33.61        | 18.06       | 20.23       |
| 5   | 15.88        | 14.44         | 26.74        | 23.73         | 19.7        | 12.01        | 15.64       | 21.71        | 25.64       | 29.4         | 20.28       | 19.33       |
| 6   | 14.99        | 17.5          | 22.75        | 20.57         | 18.92       | 25.63        | 14.01       | 16.85        | 23.55       | 23.63        | 11.52       | 17.91       |
| 7   | 9.79         | 9.95          | 8.03         | 8.24          | 3.37        | 4.06         | 4.56        | 3.19         | 18.99       | 14.27        | 10.75       | 6.04        |
| 8   | 18.48        | 14.8          | 28.85        | 20.81         | 30.29       | 22.54        | 30.25       | 27.71        | 31.12       | 26.92        | 50.07       | 42.34       |
| 9   | 16.73        | 10.12         | 14.37        | 14.36         | 14.11       | 6.52         | 18.89       | 15.37        | 15.94       | 15.93        | 29.83       | 23.1        |
| 10  | 15.12        | 16.34         | 24.75        | 25.68         | *           | *           | 18.24       | 13.84        | 31.97       | 27.1         | 21.74       | 18.14       |
| 11  | 11.72        | 12.92         | 21.32        | 21.25         | 13.92       | 14.34        | 12.96       | 13.69        | 21.18       | 20.73        | 16.14       | 18.81       |
| 12  | 8.71         | 10.49         | 10.44        | 10.77         | 8.46        | 21.25        | 4.09        | 3.94         | 5.87        | 4.82         | 11.69       | 14.33       |
| 13  | 4.59         | 7.69          | 10.22        | 15.09         | 5.78        | 11.97        | 16.21       | 15.79        | 19.84       | 21.26        | 11.76       | 12.26       |
| 14  | 15.04        | 14.76         | 20.38        | 21.07         | 18.84       | 18.99        | 31.67       | 31.15        | 20.21       | 21.14        | 40.64       | 40.87       |
| 15  | 10.88        | 10.19         | 11.12        | 6.07          | 5.79        | 5.55         | 21.87       | 28.8         | 21.57       | 24.57        | 34.25       | 50.59       |
| 16  | 8.26         | 7.09          | 17.6         | 16.5          | 6.48        | 4.4          | 18.66       | 16.94        | 25.76       | 26.04        | 24.65       | 18.91       |
| 17  | 18.29        | 17.05         | 16.92        | 18.15         | 29.22       | 32.02        | 33.2        | 29.81        | 20.55       | 19.54        | 55.02       | 34.52       |
| 18  | 8.29         | 3.09          | 20.38        | 10.65         | 6.81        | 3.4          | 23.5        | 11           | 31.18       | 19.83        | 26.9        | 15.68       |
| 19  | 16.34        | 12.01         | 27.64        | 21.53         | 14.61       | 11.85        | 22.25       | 20.9         | 27.43       | 24.71        | 38.04       | 34.37       |
| 20  | 16.45        | 13.96         | 27.88        | 24.44         | 10.14       | 8.14         | 25.71       | 17.17        | 33.43       | 29.61        | 27.2        | 10.41       |
| 21  | 8.83         | 8.1           | 23.59        | 21.43         | 3.97        | 4.23         | 17.72       | 8.09         | 38.39       | 27.18        | 16.75       | 8.54        |
| 22  |              |               |              |               |             |             | 16.18       | 6.14         | 17.85       | 10.9         | 21.61       | 2.99        |
| 23  |              |               |              |               |             |             | 6.57        | 3.67         | 15.34       | 14.07        | 11.76       | 4.06        |
| 24  |              |               |              |               |             |             | 6.77        | 6.01         | 15.26       | 17.04        | 9.36        | 5.83        |

Table 2: Two sample t-test along with the 95% Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the macular retinal layers.

| Retinal layer | p value | 95% Confidence Interval | Z-score |
|---------------|---------|-------------------------|---------|
| Macular SVP   | 0.44    | (-6.93, 3.09)           | -0.89   |
| Macular DVP   | 0.37    | (-7.78, 2.95)           | -1.09   |
| Macular CC    | 0.19    | (-13.40, 2.74)          | -1.42   |

Table 3: Two sample t-test along with the 95% Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the optic disc retinal layers.

| Retinal layer | p value | 95% Confidence Interval | Z-score |
|---------------|---------|-------------------------|---------|
| Disc SVP      | 0.36    | (-7.04, 2.61)           | -0.92   |
| Disc DVP      | 0.39    | (-4.05, 1.63)           | -1.06   |
| Disc CC       | 0.71    | (-6.16, 4.25)           | -0.3    |
**Table 4:** Mean capillary density with 95% confidence interval pre and post IOP lowering treatment.

| Retinal Layer | Mean Pre Treatment Capillary density and 95% CI | Mean Post Treatment Capillary density and 95% CI |
|---------------|-----------------------------------------------|-----------------------------------------------|
| Disc DVP      | 13.61 (11.58, 15.64)                          | 12.40 (10.28, 14.51)                          |
| Disc SVP      | 21.30 (17.60, 24.99)                          | 19.08 (15.74, 22.42)                          |
| Disc CC       | 14.50 (10.71, 18.28)                          | 13.54 (9.71, 17.36)                           |
| Mac DVP       | 19.38 (15.71, 23.05)                          | 16.96 (12.88, 21.08)                          |
| Mac SVP       | 24.75 (21.16, 28.34)                          | 22.83 (19.14, 26.52)                          |
| Mac CC        | 26.21 (20.27, 32.15)                          | 20.89 (15.10, 26.67)                          |

**Table 5:** Summarises the raw values of intraocular pressure (IOP) and visual acuity pre and post treatment.

| Eye | Pre Treatment IOP | Post Treatment IOP | Pre Treatment Visual Acuity | Post Treatment Visual Acuity |
|-----|-------------------|--------------------|-----------------------------|------------------------------|
| 1   | 42                | 17                 | 0.6                         | 1.3                          |
| 2   | 44                | 16                 | 0                           | -0.1                         |
| 3   | 22                | 10                 | 0                           | 0                            |
| 4   | 22                | 14                 | -0.1                        | -0.1                         |
| 5   | 22                | 8                  | -0.1                        | 0                            |
| 6   | 33                | 18                 | 0.2                         | 0.2                          |
| 7   | 26                | 9                  | 0.3                         | 0.6                          |
| 8   | 33                | 18                 | 0                           | 0.5                          |
| 9   | 34                | 7                  | 0                           | 0.1                          |
| 10  | 25                | 16                 | 0.2                         | 2                            |
| 11  | 31                | 16                 | 0.2                         | 0.2                          |
| 12  | 27                | 4                  | 0.5                         | 0.6                          |
| 13  | 29                | 7                  | 0.4                         | 0.3                          |
| 14  | 24                | 24                 | 0.2                         | 0.6                          |
| 15  | 25                | 8                  | 0.2                         | 0.8                          |
| 16  | 13                | 14                 | 0.5                         | 0.6                          |
| 17  | 10                | 14                 | 1.3                         | 1.3                          |
| 18  | 24                | 18                 | 0.3                         | 0.3                          |
| 19  | 24                | 24                 | 0                           | 0.1                          |
| 20  | 28                | 16                 | 0                           | -0.1                         |
| 21  | 20                | 21                 | -0.1                        | 0                            |
| 22  | 25                | 9                  | 0.3                         | 0.8                          |
| 23  | 27                | 10                 | 0                           | 0.6                          |
| 24  | 35                | 20                 | 0.4                         | 0.3                          |

**Table 6:** Two-sample t-test along with the 95% Confidence Interval and the Mann-Whitney-Wilcoxon Z-score for the IOP and VA pre and post treatment.

| Variable | p value | 95% Confidence Interval | Z-score |
|----------|---------|-------------------------|---------|
| IOP      | 0       | (-16.70, -8.89)         | -5.05   |
| VA       | 0.06    | (-0.01, 0.48)           | 1.69    |
vessel infarcts and ischaemic changes in individuals with normal tension glaucoma when compared to aged matched controls. While in our study the reduction in capillary density observed across the macula and optic disc was not statistically significant other studies have described a reduction of peripapillary vessel density in glaucomatous eyes [15,16,21,22].

An explanation of these results could be due to the fact that no internal algorithm exists on the Heidelberg Engineering OCTA to analyse the blood flow unlike those used in previous studies where internal algorithms would have allowed for more accurate and specific calculations of retinal blood flow [15]. Further, Image J softwares pixel threshold may in fact limit the use of external software to analyse and calculate percentage of retinal vessel density. A recent study by Wu that looked at retinal blood flow in primary open angle glaucoma patients and found a statistical significance between retinal blood flow between normal and glaucoma patients acquired the use of an internal algorithm in the OCTA machine to calculate retinal blood flow [15].

Reduced blood perfusion in glaucomatous eyes as a result of retinal ganglion cell loss versus being a causative factor has long been a topic of discussion [23,24]. Table 4 summarises the mean capillary density with 95% confidence interval pre and post IOP lowering treatment. While the cause of visual loss and the process of retinal cell death is not the principal focus of this study and could be attributed to coexisting ocular pathology, interestingly, while not statistically significant retinal vessel density seemed to have decreased post treatment in all of the retinal layers surrounding the optic disc and macula. This is in contrast to most understanding of IOP lowering treatment which suggests a reduction of IOP is aimed at improving retinal blood flow and perfusion to the optic nerve head and macula in glaucoma patients.

Conclusion

No significant difference was found in the data for all layers of both the areas surrounding the disc and macula at 6 weeks post glaucoma treatment. Further studies of OCTA exploring blood flow to the retina in larger cohorts are required to assess whether glaucoma treatment changes the perfusion of both the optic nerve head and macula and to determine whether the micro vascular changes correlate with retinal ganglion cell loss.

Limitations of this study include a smaller sample size and only one follow up post treatment, which resulted in a lack of normalization of the data. While acquiring the OCTA images the requirement of maintaining a fixed gaze at a certain angle by participants provided further challenges for the elderly and those with poor visual acuities resulting in suboptimal image quality. Future studies, which follow up patients for longer periods of time, use internal software to calculate percentage of retinal blood flow across the different retinal layers may provide further insight into the use of OCTA in diagnosis and detection of glaucomatous changes prior to visual acuity and visual field changes. It may further provide insight into the long-term effects of IOP lowering therapy in glaucoma patients on retinal vessel density.

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