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PERIPAPILLARY VESSEL DENSITY IN NORMAL POPULATION AGED 50-60 YEARS IN HEALTHY INDIAN EYES

Ophthalmology

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ABSTRACT

The aim of the study was to establish the normative data of peripapillary vessel density in the population aged 50-60 years in South India. The study design is a prospective analysis. Sixty normal subjects aged 50 and 60 years, who presented to the out patient department from April 2021 to April 2022 were considered for the study.All patients underwent vision, refraction, detailed slit- lamp examination with applanation tonometry, gonioscopy, dilated fundus examination with stereophotography of optic disc and red- free retinal nerve fiber layer (RNFL) photography, Humphrey SITA visual field test using the program 30-2 and swept source optical coherence tomography (OCT) to prove they have normal ocular findings.They were then subjected to optical coherence tomography angiography (OCTA) to study the peripapillary vessel density and blood supply to optic nerve. The Pearson correlation between axial length and RNFL thickness was found to be significant. In our study, we studied the normative data of 60 subjects where the mean of RPC inside was 52.29 and standard deviation was 0.680. RPC whole mean was 52.18 +/- 0.663. The mean of superior peripapillary vessel density was 52.94 +/- 0.689. Inferior peripapillary mean was 54.76 +/- 0.55. Nasal ppv mean was 54.50 +/- 0.4625. Temporal ppv mean was 72.59 +/- 0.85887. Axial length mean was 24.29 +/- 0.14. The study focused on peripapillary vessel density in the South Indian population aged 50-60 years. The above-mentioned normative data can be used to detect retinal vascular pathology such as diabetic retinopathy, CRVO and glaucoma at an early stage.

KEYWORDS

INTRODUCTION

Glaucomatous optic neuropathy (GON), characterized by loss of retinal ganglion cell (RGC), is the leading cause of irreversible blindness worldwide[1]. Apart from raised intraocular pressure (IOP), there is much evidence suggesting that vascular insufficiency in the optic nerve head (ONH) can contribute to the development of GON[2,3]. Some studies even showed that vascular abnormalities arise earlier than retinal nerve fiber layer (RNFL) thinning and visual field defect[4,5].Therefore, evaluation of blood perfusion at the site of optic disc might be of significance in the screening of subjects with risk of developing glaucoma, and could be a potential diagnostic predictor for glaucoma in future.

There are four vascular networks in the retina.[6] Among them, the nerve fiber layer plexus (NFLP, previously called radial peripapillary capillaries) is most studied for its unique 'radial' distribution, which makes it more vulnerable and sensitive to ischemia than other retinal capillaries in the reticular formation[7,8]. NFLP, from internal limiting membrane (ILM) to RNFL, is restricted to the microvasculature within the RNFL and is responsible for the nutritional supply of RGC axons.[9,10] Therefore, evaluation of blood perfusion at NFLP is important both academically and clinically.

Optical coherence tomography angiography (OCTA) is a time-efficient imaging and non-invasive technique developed on the basis of OCT. OCTA reads the movement of red blood cells by measuring the changes from repeated OCT scans and generates three-dimensional (3D) angiographic information, allowing clinicians to investigate in - depth the morphology, as well as their interaction with the retinal layers. In addition, OCTA is able to synthesise qualitative images into quantitatively comparable values, such as vessel density (VD), using its own software. These advantages allow OCTA to be used in population-based fundus vascular studies quantitatively.

The peripapillary area comprises the region around the optic nerve head (ONH), which is critical for maintaining normal vision. By assessing VD in this region, OCTA provides valuable insights into the vascular health of the optic nerve and surrounding tissues. Changes in peripapillary vessel density (PPVD) can indicate early signs of various ocular and systemic diseases, making it an essential tool for improving public health outcomes.

Early detection of ocular diseases: PPVD measurements can help identify early signs of eye conditions such as glaucoma, optic neuropathy, and diabetic retinopathy. Detecting these diseases at an early stage allows for timely intervention, preventing irreversible vision loss and improving long-term outcomes.

Here are a few reasons why peripapillary vessel density measured by OCTA is of great public health importance:

Monitoring disease progression: OCTA enables longitudinal monitoring of peripapillary vessel density over time. This longitudinal data can aid in understanding disease progression and in evaluating the effectiveness of interventions and treatments. It helps clinicians make informed decisions and provide personalized care to patients.

Peripapillary vessel density measured by OCTA holds significant implications for public health. OCTA is a non-invasive imaging technique that allows for high-resolution visualization of the microvasculature in the optic nerve head and the surrounding peripapillary region.

Population-based Screening: Incorporating peripapillary vessel density measurements into population-based screening programs can help identify individuals at risk of ocular and systemic diseases. Early detection and intervention can prevent disease progression, reduce healthcare costs, and improve overall public health outcomes.

Research And Public Health Policy: Data on peripapillary vessel density obtained through OCTA can contribute to research studies and inform public health policies. By analyzing population-level trends and associations, researchers can gain insights into disease prevalence, risk factors, and potential interventions, ultimately leading to improved preventive strategies and healthcare guidelines.

Systemic Disease Associations: Changes in peripapillary vessel density have been linked to various systemic conditions, including hypertension, diabetes, and cardiovascular diseases. Monitoring peripapillary vessel density can provide valuable information about a patient's overall vascular health, allowing for early detection and management of systemic diseases that may have ocular manifestations Currently, OCTA has been used in the assessment of ONH perfusion[11,12]. However, existing studies are all based in hospitals with potential selection bias and with limited sample size. Therefore, we conducted this population-based study in subjects without optic nerve or retinal diseases among the Indian population, aiming to provide a normative reference for the clinicians to quantitatively grade the OCTA data of ONH and peripapillary VD at NFLP slab, as well as explore the ocular and systemic associations of NFLP VD. If we could derive a population map of ONH and peripapillary NFLP VD, it might make OCTA a more popular diagnostic tool for early glaucoma, like RNFL measurement.

Sample Size

Sample Size Formula:

$$n = \frac{\left(Z_{1-\beta} + Z_{1-\frac{\alpha}{2}}\right)^2}{\left(\frac{r^2}{1-r^2}\right)}$$

Where,

r : Correlation coefficient.

$Z_{1-\alpha/2}$: Desired confidence level

1-β : Power

Sample Size Calculation:

Regression methods - Correlation coefficient	
Correlation coefficient	-0.264
Power (1- beta) %	80
Alpha error (%)	5
1 or 2 sided	2
Required sample size	**62**

Sample size was estimated by using nMaster software Version 2.0 by applying following details in the above formula. Based on the study "José Ignacio Fernández-Vigo, Bachar Kudsieh, Hang Shi, Lucía De-Pablo Gómez-de-Liaño, Irene Serrano-Garcia, José María Ruiz-Moreno, José María Martínez-de-la-Casa, Julián García-Feijóo & José Ángel Fernández-Vigo (2020): Normative database of peripapillary vessel density measured by optical coherence tomography angiography and correlation study, Current Eye Research, DOI: 10.1080/02713683.2020.1744164" Negative correlation was detected between SCP VD and age (R=-0.264; P<0.001)). Based on the above parameter with an alpha of 0.05 (2 sided) and **power of 80%** the estimated sample size using the sample size formula for Regression methods - Correlation coefficient **62 was arrived**.

Statistical Analysis Plan

The collected data will entered in the Microsoft Excel 2016 and will be analysed with IBM SPSS Statistics for Windows, Version 29.0.(Armonk, NY: IBM Corp). To describe about the data descriptive statistics frequency analysis, percentage analysis will be used for categorical variables and for continuous variables the mean and S.D will be used.

To find the significant difference between the bivariate samples in Independent groups the Unpaired sample t-test or Mann-Whitney U test will be used as on the normality of the data.

To predict the relationship and influencing factors between the variables the Regression analysis will be used.

To find the relationship between the variables the correlation will be used. To find the association of significance in categorical data the Chi-Square test or Fisher's exact test will be used.

Any other necessary test found appropriate will be dealt at the time of analysis, based on the data distribution. In all the above statistical tools the probability value .05 will be considered as significant level.

Definitions

Frequency Analysis

The study of quantitatively describing the characteristics of a set of data is called descriptive statistics. Frequency Analysis is a part of descriptive statistics. In statistics, frequency is the number of times an event occurs. Frequency Analysis is an important area that deals with the number of occurrences (frequency) and analyzes measures of central tendency, dispersion, percentiles, etc.

Mean:

The mean is defined as the sum of the observations and dividing them by the number of terms in the set.

Standard Deviation:

The Standard Deviation is a measure of how spreads out numbers are.

Independent Samples t-Test & Mann-Whitney U Test

The independent samples t-test & Mann-Whitney U test were used when two separate sets of independent and identically distributed samples are obtained, one from each of the two populations being compared with respect to normal and skewed data respectively.

Correlation Coefficient

It is a number between -1 and 1 which measures the degree to which two variables are linearly related. If there is perfect linear relationship with positive slope between the two variables, we have a correlation coefficient of 1; if there is positive correlation, whenever one variable has a high (low) value, so does the other. If there is a perfect linear relationship with negative slope between the two variables, we have a correlation coefficient of -1; if there is negative correlation, whenever one variable has a high (low) value, the other has a low (high) value. A correlation coefficient of 0 shows that there is no linear relationship between the variables.

Chi-Square Test

The chi-square (I) test is used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories.

Fisher's Exact Test

To find the significance in categorical data if the expected cell frequency is less than the count 5 in 2×2 tables then the Fisher's Exact will used.

METHODS

In our study prospective analysis of 60 normal subjects aged between 50-60 years who presented to ophthalmology outpatient department of Uma Eye Clinic from April 2021 to April 2022 was done under the following criteria

The Inclusion Criteria For Control Subjects Were As Follows: (1) no evidence of glaucoma and normal retina; (2) normal intraocular pressure (<21 mmHg); (3) gonioscopy showing open angle; (4) normal appearing ONH and RNFL

Exclusion Criteria Were As Follows: (1) best-corrected visual acuity (BCVA) less than 20/40, (2) gonioscopy showing narrow angle, (3) retinal and neurologic diseases leading to abnormal visual field test, (4) history of any recent intraocular surgery, (5) VF mean deviation (MD) < -15.0 decibels (dB),(6) refractive error >± 6.0 diopters (D).

All subjects underwent detailed ophthalmologic examinations, including BCVA, refractive error measurement by manual refraction, slit-lamp examination, IOP measurement with Goldmann applanation tonometry, gonioscopy examination for assessing angles, dilated fundus examination with stereophotography of the optic disc and red-free RNFL photography, Humphrey SITA VF tests using central 30–2 Humphrey Field Analyzer (Zeiss 24 visual field analyzer), and spectral-domain OCTA (Optovue RT Avanti).

OCTA scan taken using RTVue XR Avanti by Optovue. After complete dilatation of pupils, OCTA scans were taken with the patient focussing on the given target for fixation. The optic disc was centered and after a clear image was obtained with good signal strength, the trigger was clicked to obtain serial optic disc, RNFL and GCC scans. The scans were considered for evaluation if the quality of the scan was at least 7/10 of signa; strength.

OCT-A (Optovue RT Avanti) uses 840 nm light source and provides a noninvasive OCT-based method to visualize the retinal vascular structures. The A-scan rate is 70,000 scans per second and the bandwidth is 50 nm. Each of the optic disc cubes contain 304 clusters of two repeat B-scans that has 304 A-scans each. Split-spectrum amplitude-decorrelation angiography (SSADA) algorithm was used to improve the signal-to-noise ratio as it helps in splitting the spectrum to give multiple repeat OCT frames from the two original repeat OCT frames[13].

OCT-A can characterize vascular information at various retinal layers as a VD (%) map. Vessel density is the proportion of measured area occupied by flowing blood vessels (pixels) having decorrelation values acquired with the SSADA algorithm above the threshold level.

The prototype modality of imaging is the RTVue XR Avanti by Optovue. The image acquisition ranges to 70,000 A-Scans per second with a tissue resolution of 5 mm axially and a beam width of 15 mm.

(1) The algorithm followed for the detection of blood flow within the vascular columns is the comparison between serial consecutive B-scans using the property of contrast of motion. This is the Split Spectrum Amplitude Decorrelation Angiography (SSADA)[2,14–16].

The mobility of erythrocytes and moving blood within the vascular columns result in a variation of reflectivity over successive scans, resulting in a high decorrelation between the various time frames and images obtained. The elimination of the sequential decorrelated frames of images and immobile outliers from the frame provides better resolution and image clarity, as the tissue motion artifacts are eliminated.

In addition to this, the spectrum of the source of illumination is divided into four components, in an attempt to eliminate the noise present in the image and the decorrelation step is assessed individually by each of the components respectively.

This SSADA algorithm results in lesser noise and high transverse resolution.

(2) The highly organized retina is a structure that has got segmental vascular supply and retinal segmentation for various layers (Figure1). The complex data analysis performed on the images acquired processes the vessel density and index of flow separately for four layers of the retinal choroidal complex. These four areas of in- depth scanning include:

a) The vasculature in the ganglion cell layer, which comprises of the superficial plexus
b) The network of blood vessels in between the outer boundary of the inner plexiform layer and the central zone of the outer plexiform layer.
c) The layer of photoreceptors which is relatively avascular
d) A 30 micron off shoot for the vasculature of the choroid

Along with this, the installed software processing provides detailed information regarding the indices of perfusion in the following areas.

I. The central 3mm of the parafoveal zone
II. The surrounding 3– 6mm of the perifoveal zone
III. The foveal avascular zone is automatically calculated by an exclusion analysis from the measurements of perfusion index

Although the OCTA is a reliable tool for both imaging and diagnosis of various retinal pathologies, only little is known about the normative database for different age groups.

However, Matsunaga et al demonstrated that the OCTA provides detailed high-resolution images (Figure 2) obtained from healthy individuals comparable with the routine fundus fluorescein angiography techniques.Comparison between FFA and OCTA is given in Table 1

In this study, VD in peripapillary RNFL was analyzed for images with a 4.5 x 4.5 mm field of view on the optic disc. Using the standard OCT-A software (version 2015.1.0.90), in the RPC layer, VD within the RNFL was measured as a programmed setting from the internal limiting membrane to posterior boundary of RNFL. For nine areas measurements were obtained (Fig 1). Inside the disc, VD was measured for the region inside the optic disc boundary. Whole peripapillary VD was calculated for the region of 750 μm-wide elliptical annulus extending from the optic disc boundary, while the whole en face VD was measured for the entire 4.5 x 4.5 mm image. Sectoral division of the peripapillary region including nasal, inferonasal, superonasal , temporal, inferotemporal and superotemporal area was performed using Garway-Heath regionalization [39]. The VD at the whole peripapillary region, superotemporal region (without RNFL defect), and inferotemporal region (with RNFL defect), was used to compare VD at areas with or without RNFL defect.

Poor quality images with the following characteristics were excluded from analysis. (1) signal strength index <48, (2) local weak signal, (3) irregular vessel pattern or disc boundary showing residual motion artifacts (4) poor clarity or (5) RNFL segmentation errors. For accuracy, the location of the disc margin was reviewed and adjusted manually if necessary.

The Spearman Rank Correlation Coefficient was used to assess the correlation between RNFL segments and the relevant segments of the VD.

The degree of correlation is expressed as a number between - 1 and + 1. A negative number denotes that the two variables do not have a linear relationship. A positive number indicates that the variables are connected in a linear fashion. The greater the association, the closer the value is to +1. The lower the connection, the closer the value is to - 1.

The Mann-Whitney U test was used to compare the two groups for each quadrant of the ONH in terms of RNFL and OCT angio vessel density.

Statistical analysis was performed using SPSS statistics V.26.0 (IBM SPSS). The data were presented in the form of mean, SD, 95% CI for mean, minimum and maximum. Missing values were excluded from data analysis. Linear regression analysis was performed to determine the associations between the NFLP VD and ocular and systemic parameters. The ocular and systemic parameters with a p value less than 0.05 in the univariate analysis were enrolled into the multivariate regression analysis. A two-tailed p value less than 0.05 was considered as statistically significant.

Ethical Issues

Since it is an observational study , Ethics approval was not required.

RESULTS

Various studies have analyzed PPVD in patients with glaucoma and normal eyes. One such study is Peripapillary Vessel Density In Unilateral Preperimetric Glaucoma(PPG).The mean RNFL and GCC average thickness in microns differed significantly ($p<0.001$) between preperimetric glaucoma (PPG) (82.4±7.1, 81.4±5.9), fellow eyes (F) (91.0±7.1, 88.5±3.8) and healthy eyes (H) (103.5±6.0, 99.3±5.7). Pre perimetric glaucoma compared with Fellow eyes showed significantly ($p<0.001$) lower mean PPVD (43.8%±3.0% versus 47.8%±3.2%) and wiVD (45.9%±3.5% versus 50.1%±3.9%). Mean PPVD (49.7%±2.4%) and wiVD (52.6%±3.0%) in Healthy eyes were not significantly higher than in Fellow eyes.

DISCUSSION

In our study we studied the normative data of 60 subjects where the mean of RPC inside was 52.29 and standard deviation was 0.680. RPC whole mean was 52.18 +/- 0.663.The mean of superior PPVD was 52.94 +/- 0.689. Inferior peripapillary mean was 54.76 +/- 0.55. Nasal ppv mean was 54.50 +/-0.4625. Temporal ppv mean was 72.59 +/- 0.85887. Axial length mean is 24.29 +/- 0.14.(Table :2, Graph :1, Graph :2).The non parametric tests with normative data and the statistical confidence interval values in our study supports the same (Table 3,Table 4).Graph 3 shows the gender comparison of RNFL thickness and axial length, with not much variation among the male and female subjects.

The pathogenesis of glaucoma is known to include ganglion cell loss and accompanying nerve fiber layer thinning. Although frequent visual field testing enables us to monitor glaucoma development, it is patient-specific. The loss of ganglion cells and the RNFL occurs before scotomas are seen in glaucoma. OCT helps us to see these changes in the retina and diagnose them early. By analyzing the vascular density in these segments with OCT-angiography, we may draw a connection between the loss of the nerve fiber layer and vascular alterations.

This study shows that glaucoma patients have a considerable drop in vascular density. Surprisingly, the loss in vascular density is especially noticeable in women.

It is quite clear that the role of vascular changes that occur in the pathophysiology of glaucoma cannot be ignored. Angio analysis of the optic nerve disc could act as a surrogate marker in the diagnosis and prognosis of the disease. The question of whether the nerve fiber layer loss or vascular damage occurs first is still unclear and requires further investigation.

There are a few limitations that are worth mentioning. The investigation requires minimal cooperation from the patient in that the subject would be required to fixate for an optimal scan to be taken.

Patients with very poor vision might be unable to do so. Media opacities such as hard cataract might make it difficult to obtain a clear picture for analysis. High myopia also poses a challenge in obtaining an image. Pre-existing retinopathy could be a confounding factor while trying to correlate the vascular changes with glaucoma.

This study focused on VD in the RNFL. The approach aimed to reduce potential projection artefacts and should facilitate segmentation of retinal layers in the ONH area. Other studies analyzed vessel density in the whole retina between internal limiting membrane and Bruch's membrane opening. Aging is known to result in a physiological loss in morphometric parameters such as bruchs membrane opening - minimum rim width(BMO-MRW), bruchs membrane opening - minimum rim area (BMO-MRA) and RNFL thickness. In our study group the mixed regression models showed an impact of aging on vascular density of the ONH (Graph:1, Graph: 2). In many patients' glaucoma severity is closely linked to aging, as structure and function deteriorates over the course of the disease. Therefore normative VD data on healthy subjects in different age groups will be helpful to better understand the impact of aging.

CONCLUSION

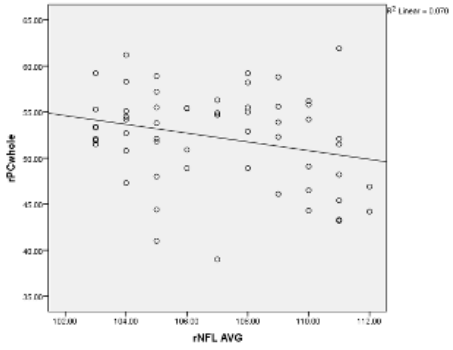
In conclusion, peripapillary vessel density measured by OCTA plays a crucial role in promoting public health. Its ability to detect early signs of ocular and systemic diseases, monitor disease progression, and contribute to population-based screening and research makes it a valuable tool in promoting eye health and overall well-being.The above mentioned normative data in the South Indian population aged 50-60 years will be useful to compare and evaluate patients for other diseases as well.

Table 1: Comparison between Fundus Fluorescein Angiography and OCT Angiography

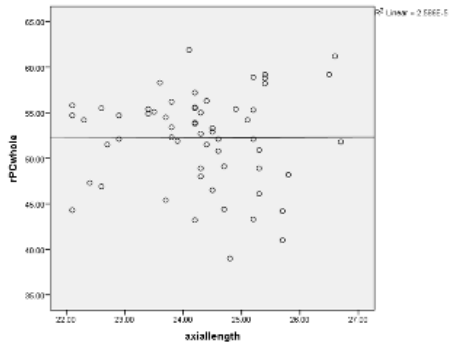
	FUNDUS FLUORESCEIN ANGIOGRAPHY	OCT ANGIOGRAPHY
Duration	5mins to 30 mins	Less than 5 mins
Invasiveness	Invasive technique with usage of fluorescein dye injections	Non invasive
Field of view	Wide field visualization	Restricted to 12mm x 12mm
Quality of image	Lower resolution 2 dimensional imaging technique	Higher resolution 3 dimensional imaging technique
Depth of imaging	Images only the superficial retina clearly	Clearly demarcates the superficial, deep, outer retinal layers and the choroid
Information on blood flow	Providesinformation on dynamic blood flow	Provides information on static blood flow
Motion artifacts	Less common, not affected by motion	More common, more affected by motion
Adverse Effects	Nausea, vomiting and anaphylactic reactions can occur	No known adverse reactions

Table 2 showing Pearson Correlation of Average RNFL and Axial Length with RPC whole

				rPCwhole
rNFL AVG	Pearson Correlation			-.264*
	Sig. (2-tailed)			.044
	N			59
		95% Confidence Interval	Lower	-.487
			Upper	-.012
Axiallength	Pearson Correlation			.005
	Sig. (2-tailed)			.970
	N			59
		95% Confidence Interval	Lower	-.270
			Upper	.280



Graph 1 showing linear regression of RPC whole and Average RNFL



Graph 2 showing linear regression between RPC whole and Axial length

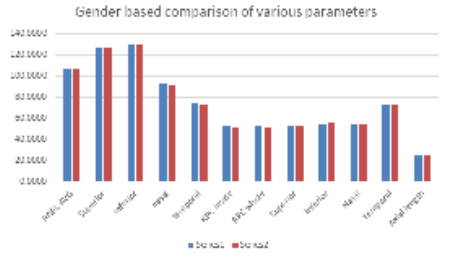
Table 3 showing nonparametric test and outcomes for the data.

	RNFL AVG	Superior	Inferior	nasal	temporal	RPC inside	RPC whole	Superior	Inferior	Nasal	Temporal	Axial length
Mann-Whitney U	375.500	404.000	414.500	379.500	350.500	374.000	344.500	365.500	359.000	382.000	412.500	403.000
Wilcoxon W	700.500	999.000	739.500	704.500	675.500	699.000	669.500	690.500	954.000	977.000	737.500	998.000
Z	-0.764	-0.323	-0.162	-0.701	-1.147	-0.783	-1.235	-0.925	-1.025	-0.672	-0.193	-0.338
Asymp. Sig. (2-tailed)	0.445	0.746	0.871	0.483	0.251	0.434	0.217	0.355	0.305	0.501	0.847	0.735

Table 4: Table showing confidence interval for average Retinal nerve fiber layer thickness and axial length

		Statistic	Std. ErrorStd. Error
RNFL AVG	Mean	107.1356	0.38640
	95% Confidence Interval for Mean	Lower Bound	106.3621
		Upper Bound	107.9091
Superior	Mean	126.7797	0.65053
	95% Confidence Interval for Mean	Lower Bound	125.4775
Inferior	Mean	130.1186	0.54225
	95% Confidence Interval for Mean	Lower Bound	129.0332
		Upper Bound	131.2041
Nasal	Mean	91.7966	0.44563
	95% Confidence Interval for Mean	Lower Bound	90.9046
		Upper Bound	92.6886
Temporal	Mean	74.0508	0.54458
	95% Confidence Interval for Mean	Lower Bound	72.9608
		Upper Bound	75.1409
RPC inside	Mean	52.2915	0.68041
	95% Confidence Interval for Mean	Lower Bound	50.9295
		Upper Bound	53.6535
RPC whole	Mean	52.1847	0.66336
	95% Confidence Interval for Mean	Lower Bound	50.8569
		Upper Bound	53.5126
	Kurtosis	-0.145	0.613
Superior	Mean	52.9492	0.68950
	95% Confidence Interval for Mean	Lower Bound	51.5690
		Upper Bound	54.3293
Inferior	Mean	54.7627	0.55726
	95% Confidence Interval for Mean	Lower Bound	53.6472
		Upper Bound	55.8782

	Kurtosis	3.805	0.613
Nasal	Mean	54.5085	0.46526
	95% Confidence Interval for Mean	Lower Bound	53.5772
		Upper Bound	55.4398
Temporal	Mean	72.5932	0.85887
	95% Confidence Interval for Mean	Lower Bound	70.8740
		Upper Bound	74.3124
Axial length	Mean	24.2949	0.14574
	95% Confidence Interval for Mean	Lower Bound	24.0032
		Upper Bound	24.5867



Graph 3 showing gender-based comparison.

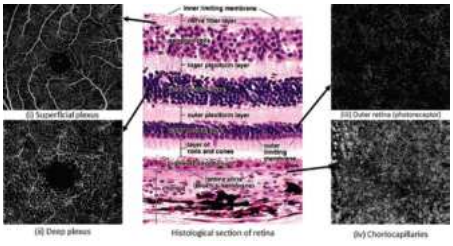


Figure 1: The location of different en-face zones in relation to histology of the human retina. The four en-face zones include (i) the superficial plexus, the capillary network in ganglion cell layer and nerve fiber layer (ii) the deep plexus, a network of capillaries in the inner plexiform layer with offshoot of 55 micron (iii) the outer retina (photoreceptors) and (iv) the choriocapillaris (choroid) with offshoot of 30 microns.

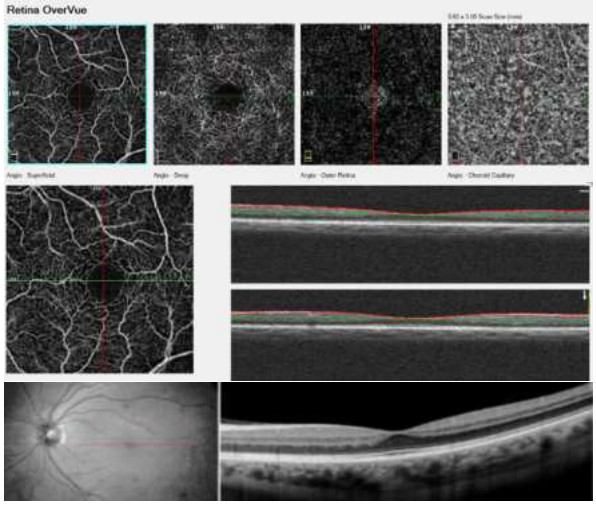


Figure 2 OCT Angiogram Fields of View and Segmentation Layers on Angiovue.

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