DOI:10.14744/bej.2020.75437
Beyoglu Eye J 2021; 6(1): 37-42

Original Article

Normative Data of Superficial Retinal Vascular Plexus and the Relationship to Retinal Layers

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Abstract

Objectives: The aim of this study was to evaluate the relationship between optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA) parameters in a healthy population and to detect any changes that occur with age.

Methods: A total of 100 healthy participants were included in this prospective, observational, and comparative study. The participants were categorized in 4 groups according to age: Group 1: 21-30 years, Group 2: 31-40 years, Group 3: 41-50 years, Group 4: 51-60 years of age. Mean macular thickness, retinal nerve fiber layer (RNFL), ganglion cell inner plexiform layer (GC-IPL), and the choroidal thickness (ChT), vessel density (VD), perfusion density (PD), foveal avascular zone (FAZ), and parapapillary perfusion density parameters were recorded and analyzed.

Results: In comparisons between groups, no significant difference in OCTA parameters was observed. There were inverse correlations between the outer VD, PD, and intraocular pressure (IOP) (r= -0.307, p=0.006 and r= -0.284, p=0.011, respectively). The correlation between parapapillary perfusion density and IOP was close to being significant (r= -0.213, p=0.059). There were significant relationships between OCTA parameters and macular, RNFL, and GC-IPL thickness. No significant relationship between ChT and OCTA parameters was seen.

Conclusion: The size and characteristics of superficial VD, PD, parapapillary perfusion density, and FAZ were determined in a population with standardized demographic and ocular clinical features, and the relationship between these parameters and retinal layers was established.

Keywords: Healthy population, optical coherence tomography, optical coherence tomography angiography, superficial vascular plexus

Introduction

Optical coherence tomography (OCT) angiography (OCTA) can generate, rapid, non-invasive, high-contrast, and well-defined images of the vascular layers of the retina as well as the radial peripapillary capillary network (1). OCTA has the advantage of also providing very similar images of the inner plexus of the retinal vessels, which can be seen in the early phases of fluorescein angiography (FA) without the use of dye (2). OCTA has even been reported to detect pathology not seen with FA (3). OCTA has been shown to be a valuable imaging method to evaluate common ophthalmological diseases, such as non-neovascular

How to cite this article: Isik MU, Akay F, Akmaz B, Guven YZ, Isik IG. Normative Data of Superficial Retinal Vascular Plexus and the Relationship to Retinal Layers. Beyoglu Eye J 2021; 6(1): 37-42.

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Submitted Date: May 19, 2020 **Accepted Date:** November 19, 2020 **Available Online Date:** February 15, 2021

and neovascular age-related macular degeneration, diabetic retinopathy, retinal vascular occlusion, central serous chorioretinopathy, and glaucoma (3,4). In addition to significant changes, it is also useful to detect previously unknown subclinical effects of ocular and systemic diseases on the retina and optic nerve vasculature (5-7).

The association between OCTA parameters and ocular and systemic features has been examined previously. It has been reported that there is a relationship between foveal avascular zone (FAZ) values and vascular indices, and age, gender, axial length (AL), spherical equivalent (SE), body mass index (BMI), and central macular thickness (CMT) in a healthy population (8-11). It has also been noted that OCTA parameters vary according physiological conditions, such as pregnancy (12). In the majority of OCTA studies of healthy subjects, AL and BMI differences of the participants were not considered (13-18). A relationship between FAZ area and CMT has been also demonstrated (16, 19). However, to our knowledge, there is no study that comprehensively evaluates the relationship between OCT parameters (RNFL, GC-IPL, CMT, retinal nerve fiber layer [RNFL], ganglion cell-inner plexiform layer [GC-IPL], CMT, and choroidal thickness [ChT]) and OCTA (vessel density [VD], perfusion density [PD], parapapillary PD, FAZ area, perimeter, and circularity index).

This study had 2 objectives: First, to determine and compare normal values of parameters obtained with OCTA according to age, and second, to evaluate any relationship between OCT and OCTA.

Methods

In this prospective, observational, and comparative study, 100 healthy participants (mean age: 39.5±11.2 years) were included. All of the participants were informed about the study procedure, and written consent was provided. The research observed the tenets of the Helsinki Declaration and was approved by the institutional ethics committee (ID: 40, 2020).

All of the participants underwent a detailed ophthalmological examination performed by a single ophthalmologist (BA). Individuals with an AL of <21 mm or >24 mm, significant refractive errors (>3 D SE refraction), intraocular pressure ≥21 mmHg, retinal vascular disease, uveitis, glaucoma, pseudoexfoliation, ocular surgery, or presence of any macular degeneration were excluded from the analysis. None of the participants had any systemic disease or used topical/systemic drugs.

After pupil dilatation, macular angiography was performed for a 6x6 mm area using a Zeiss Cirrus 5000 system (Carl Zeiss Meditec AG, Jena, Germany). A speed of 68.000 A-scans per second and 840 nm wavelength were used for the examinations. The images of the retinal capillary plexus were analyzed using Cirrus OCTA software (AngioPlex, version 10.0; Carl Zeiss Meditec AG, Jena, Germany). The macular 6x6 mm area was divided into sectors similar to the circles of the Early Treatment Diabetic Retinopathy Study subfields. Mean macular thickness (MMT), RNFL, GC-IPL, and ChT, VD, PD, FAZ, and parapapillary PD parameters were recorded.

Macular thickness, RNFL, and GC-IPL values obtained from the scan were calculated automatically by the device. The macular thickness was evaluated as CMT and MMT. RNFL was assessed in 4 quadrants (temporal, inferior, nasal, and superior) and determining the average. GC-IPL was divided into 6 regions (inferior, superior, inferotemporal, inferonasal, superotemporal, and superonasal) and the average was calculated. For the ChT values, 3 lines were drawn at 1000 μm intervals at nasal and temporal positions, centered on the subfoveal sclerochoroidal junction. VD and PD values were evaluated in central (1 mm), inner, outer, and full (6 mm) groups. FAZ parameters were assessed in 3 groups: area, perimeter, circularity index.

Statistical Analysis

The statistical analysis was done in 2 stages. Four age groups of 25 individuals were formed: Group 1: 21-30 years, Group 2: 31-40 years, Group 3: 41-50 years, and Group 4: 51-60 years of age. In the first stage, normal OCTA values were determined and comparisons were made between the groups. In the second stage, all of the participants were evaluated and relationships between ocular and systemic features were examined.

IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) was used to perform the analyses. Descriptive statistics were expressed as mean±SD for variables with normal distribution, median (interquartile range) for those with non-normal distributions, and the number of cases and percentage was used for nominal variables. The Kolmogorov-Smirnov distribution test was used to assess normal distribution. The Pearson chi-square test and Fisher's exact test were used for comparisons of descriptive statistics, as well as qualitative data. Mann-Whitney U test was used for comparisons of non-normally distributed quantitative data between 2 groups, and Student's t test was used for normally distributed data. The Kruskal-Wallis test was performed to compare more than 2 groups of non-normally distributed quantitative data, and the Mann-Whitney U test was applied to analyze the difference. For comparisons between more than 2 groups of normally distributed quantitative data, analysis of variance was performed and the significance of difference was examined with a post hoc Tukey test. The relationship between parameters was investigated with Pearson's correlation test (r value) for normally distributed data, and Spearman's correlation test (rho value) was used for nonparametric data.

The results were calculated with a 95% confidence interval and p<0.05 level of significance.

Results

No significant difference was seen in a comparison of the OCT and OCTA parameters between the right and left eyes of all of the participants (p>0.05 for all comparisons). Only the right eye was used for comparisons and correlation analysis. There were 9 males/16 females in Group 1, 10 males/15 females in Group 2, 9 males/16 females in Group 3, and 11 males/14 females in Group 4 (p=0.927). Comparisons between the groups revealed no significant difference in OCTA parameters (Table 1).

Assessment of the entire cohort yielded no difference in OCTA parameters in terms of gender (p>0.05 for all). When the relationship between OCT and OCTA parameters and patient age, BMI, and IOP values was evaluated, inverse correlations were seen between CMT, MMT, and ChT and age (p<0.001 for all). There was also an inverse correlation between BMI and MMT (rho= -0.361; p=0.001). When the relationship between OCT and OCTA parameters and IOP values was examined, there were inverse correlations between outer VD, PD, and IOP (r=-0.307; p=0.006 and r=-0.284; p=0.011, respectively). The correlation between parapapillary PD and IOP was close to being significant (r= -0.213; p=0.059).

Significant interactions between macular thicknesses and OCTA parameters are shown in Table 2. In addition, there was an almost significant correlation between central PD and CMT (rho=0.214; p=0.057). Significant interactions between OCTA parameters and RNFL thickness are shown in Table 3 and GC-IPL thickness can be seen in Table 4. No significant correlation was observed between superonasal, inferonasal, superior, and inferior GC-IPL quadrants and OCTA parameters (p>0.05 for all). In addition, there was no significant relationship seen between ChT and OCTA (p>0.05 for all).

Table 2. Significant correlations between macular thicknesses and OCTA parameters

	CMT		MMT	
	rho	p	rho	p
Central VD	0.223	0.047	0.284	0.011
Central PD	NS		0.286	0.010
FAZ area			-0.306	0.006
FAZ perimeter			-0.299	0.007

CMT: Central macular thickness; FAZ: Foveal avascular zone; MMT: Mean macular thickness; NS: Not significant; OCTA: Optical coherence tomography angiography; PD: Perfusion density; VD: Vessel density; rho: correlation coefficient.

Table 1. Comparison of OCTA parameters according to age group

	Group 1 (n=25)	Group 2 (n=25)	Group 3 (n=25)	Group 4 (n=25)	Total (n=100)	p*
			Mean±SD			
VD						
Central	10.7±2.4	10.0±3.0	10.0±2.5	10.3±2.6	10.3±2.5	0.812
Inner	18.6±1.0	18.7±0.9	18.8±0.8	18.7±0.8	18.7±0.9	0.872
Outer	18.8±0.8	18.9±0.6	19.0±0.6	19.0±0.4	18.9±0.6	0.564
Full	18.5±0.8	18.6±0.7	18.7±0.6	18.7±0.5	18.6±0.7	0.710
PD						
Central	24.2±5.6	22.9±6.9	22.9±5.9	23.2±6.2	23.4±6.0	0.872
Inner	44.6±2.3	45.0±2.3	44.9±2.1	44.8±2.0	44.8±2.1	0.935
Outer	46.8±2.0	46.8±1.6	47.4±1.5	47.1±0.9	47.0±1.6	0.536
Full	45.6±1.9	45.7±1.7	46.1±1.4	45.9±1.1	45.9±1.6	0.721
FAZ						
Area	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.473
Perimeter	2.0±0.4	2.2±0.4	2.2±0.4	2.2±0.5	2.2±0.4	0.484
Circularity Index	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.7±0.1	0.445
Parapapillary	45.1±1.2	45.7±1.0	45.6±1.4	45.1±1.1	45.3±1.2	0.284
Perfusion density						

FAZ: Foveal avascular zone; OCTA: Optical coherence tomography angiography; PD: Perfusion density; VD: Vessel density; * p value for comparisons between groups 1, 2, 3, and 4.

Table 3. Significant correlations between RNFL quadrants and OCTA parameters

	Retinal nerve fiber layer									
	Average		Superior		Temporal		Inferior		Nasal	
	r	p	r	p	r	p	r	p	r	p
VD										
Inner	0.248	0.027	0.362	0.001	NS		NS		NS	
Outer	0.318	0.004	0.299	0.007			0.291	0.009		
Full	0.301	0.007	0.332	0.003			0.244	0.030		
PD										
Inner	0.276	0.014	0.383	<0.001			NS			
Outer	0.261	0.020	0.258	0.021			0.242	0.032		
Full	0.295	0.008	0.332	0.003			0.236	0.036		
Parapapillary perfusion density	0.535	<0.001	0.426	<0.001	0.252	0.025	0.373	0.001	0.397	<0.001

NS: Not significant; OCTA: Optical coherence tomography angiography; PD: Perfusion density; RNFL: Retinal nerve fiber layer; VD: Vessel density; *r: correlation coefficient.

Table 4. Significant correlations between GC-IPL quadrants and OCTA parameters

	Ganglion cell-inner plexiform layer							
	Average		Superotemporal		Inferotemporal		Minimum	
	r	p	r	p	r	p	r	p
VD								
Outer	0.306	0.006	NS		0.308	0.005	0.321	0.032
Full	0.275	0.014			0.235	0.036	0.321	0.031
PD								
Outer	0.378	0.001	0.251	0.025	0.323	0.003	0.326	0.035
Full	0.359	0.001	0.235	0.036	0.274	0.014	0.348	0.019
Parapapillary perfusion density	0.244	0.031	0.346	0.002	0.382	0.001	NS	

GC-IPL: Ganglion cell inner plexiform layer; NS: Not significant; OCTA: Optical coherence tomography angiography; PD: Perfusion density; VD: Vessel density.; *r: correlation coefficient.

Discussion

Microvascular changes in diabetic retinopathy, retinal venous or arterial occlusion, choroidal neovascularization, and glaucoma have been already demonstrated with OCTA (18). In addition to microvascular changes in apparent retinopathy, subclinical retinal microvascular changes in systemic and ocular diseases and their relationship to retinal thickness have been shown (5,20). The relationship between FAZ area and vascular parameters and CMT has also been investigated in healthy subjects (8,14-18). However, although relationships between OCT and OCTA parameters have been examined, especially in studies investigating subclinical changes, relationships between OCTA parameters and sublayers of the retina in a healthy population have not yet been assessed. Therefore, it is uncertain whether the relationships are due to subclinical changes or whether they are already present in healthy individuals.

Consistent with our findings, previous comparisons of right and left eye OCTA parameters revealed no significant difference (11,13,15,17). When comparing VD values between

genders, as in our study, it has generally been reported that VD values were similar, (13,14,17) while in some research, the VD values were higher in males (21,22). In contrast to our results, some publications demonstrated a negative correlation between VD values and age (11,13,22,17,18,21). The lack of participants over the age of 60 in the present study may have produced this difference. The results of research comparing FAZ area between genders have also varied. In some studies, the FAZ area was found to be wider in females, (8,23) while we and other authors observed no difference between genders (13,16,17). Some studies have reported that the FAZ area expanded with age, (13,21) yet in others, no significant relationship to age was seen, as in our study (8,16,23). Also consistent with our results, an evaluation of peripapillary VD did not find a correlation with age (24).

The relationship between VD and CMT has also been examined. Falavarjani et al. (14) reported a moderate relationship (r=0.58) and our research determined a weak relationship (r=0.284). A moderate-to-strong correlation between the FAZ area and central retinal thickness has been reported in previous studies (r= -0.47 to -0.712) (8,14-16,19). Although our results were consistent with those of previous studies, the relationships between mean retinal thicknesses and OCTA parameters were weak (r= -0.306). In their study of glaucoma eyes and normal eyes, Jia et al. (25) reported no association between disc flow index and RNFL in either group. Richter et al. (20) observed a relationship between macular OCTA parameters and GCIPL thickness in eyes with glaucoma. These differences between studies may be due to different AL, SE, and BMI values or the use of different OCTA devices and software.

The inner retinal layers receive nutritional and oxygen supplementation through the branches of the central retinal artery. Larger vessels are located in the nerve fiber layer. They lengthen and eventually split into the two-layer capillary plexus. The superficial plexus provides oxygen and nutrients to the nerve fiber layer, ganglion cell layer, and the inner plexiform layer, while the deep layer supports the inner nuclear layer and the outer plexiform layer (26). The RNFL consists of retinal ganglion cell (RGC) axons and the GC-IPL consists of RGC dendrites. Since there is a direct relationship between the RGC number and RGC layer thickness, a change in GC-IPL thickness reflects the actual change in the number of ganglion cells. RGCs receive the vast majority of the oxygen supply from the superficial retinal capillary plexus (27). We believe this is the histological explanation of the relationship we found between GC-IPL and VD, PD, and parapapillary PD. However, we believe that some variables in retinal circulation physiology that we could not explain likely also affect this relationship, due to the weakness of some of the relationships we observed. Since lower and upper branch vessel diameters are higher than those of the nasal quadrant, it can be assumed that lower and upper blood flow is higher. This assumption has been proposed to explain the presence of higher venous oxygen saturation in the nasal quadrant (28). Apart from the relationship between parapapillary PD and RNFL quadrants, the relationship between superior and inferior RNFL and VD and PD values in the current study can be explained with this approach.

In this study, we included participants with AL, BMI, and SE parameters that were within the limits considered normal. Therefore, we believe that our results reflect standardized data. However, the study has some limitations. The elderly population was not included, the deep retinal plexus and choriocapillaris could be evaluated, and the relatively small sample size are some examples of elements that limit interpretation of the results. The relationship between retinal vascular indices and retinal layers will be better understood by evaluating all retinal and choroidal vascular structures in larger groups and in a broader age range.

Disclosures

Ethics Committee Approval: Committee of İzmir Katip Çelebi University, ID: 40, 2020.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (MUI, FA, YZG); preparation and review of the study (MUI, FA, YZG); data collection (FA, YZG, BA); and statistical analysis (MUI, IGI, FA).

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