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Repeatability Assessment of Optical Coherence Tomography Angiography in the Overall and Different Age Groups of the Turkish Population

Türk Popülasyonunun Genel ve Farklı Yaş Gruplarında Optik Koherens Tomografi Anjiyografinin Tekrarlanabilirlik Değerlendirmesi

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ABSTRACT

Background: To measure the repeatability of foveal avascular zone (FAZ) area, vessel density (VD), and flow area using AngioVue Analytics optical coherence tomography angiography (OCTA) in the overall and different age groups of the Turkish population.

Materials and Methods: This study included 100 eyes of 100 healthy subjects (54 females; mean age: 43.2±15.2 years). They were divided into five age groups- 20-29, 30-39, 40-49, 50-59, and >60 years- with 20 subjects each. The optical disk region and macula were imaged thrice using a 4.5x4.5 mm and 3x3 mm scan, respectively. Intraclass correlation coefficient (ICC) and coefficient of variation (CV) analyses were used to evaluate repeatability.

Results: CV and ICC for the FAZ area were 2.96% and 0.990, respectively. ICC values of entire image VD of superficial capillary plexus (SCP) and deep capillary plexus (DCP) were 0.828 and 0.842, respectively. CV and ICV for peripapillary VD were 2.32% and 0.990, respectively. ICC was 0.865 for the choriocapillaris flow area. CV values of different age groups showed no statistical differences, except for the FAZ area.

Conclusion: OCTA measurements of the FAZ area, VDs of the SCP, DCP, and optic nerve, and the choriocapillaris flow area showed relatively good overall repeatability. Repeatability results of different age groups should guide the optimal use of OCTA.

Keywords: Flow area, foveal avascular zone, OCTA, repeatability, vessel density

ÖZ

Amaç: AngioVue Analytics optik koherens tomografi anjiyografi (OKTA) kullanarak foveal avasküler bölge (FAB) alanı, damar yoğunluğu (DY) ve akış alanının tekrarlanabilirliğini Türk popülasyonunun genel ve farklı yaş gruplarında ölçmek.

Gereç ve Yöntemler: Bu çalışmaya 100 sağlıklı kişinin (54 kadın; ortalama yaş: 43,2±15,2 yıl) 100 gözü dahil edildi. Her biri 20 denek olmak üzere beş yaş grubuna (20–29, 30–39, 40–49, 50-59 ve >60 yaş) ayrıldılar. Optik disk bölgesi ve maküla sırasıyla 4,5x4,5 mm ve 3x3 mm tarama kullanılarak üç kez görüntülendi. Tekrarlanabilirliği değerlendirmek için sınıf içi korelasyon katsayısı (SKK) ve varyasyon katsayısı (VK) analizleri kullanıldı.

Bulgular: FAZ alanı için VK ve SKK sırasıyla %2,96 ve 0,990 idi. Yüzeyel kapiller pleksus (YKP) ve derin kapiller pleksus (DKP) tüm görüntü DY'sinin SKK değerleri sırasıyla 0,828 ve 0,842 idi. Peripapiller DY için VK ve SKK sırasıyla %2,32 ve 0,990 idi. Koryokapillaris akış alanı için ICC 0,865 idi. Farklı yaş gruplarının VK değerleri, FAZ alanı dışında istatistiksel olarak farklılık göstermedi.

Sonuç: FAZ bölgesinin OKTA ölçümleri; YKP, DKP ve optik sinirin DY'leri; ve koryokapillaris akış alanı genel olarak nispeten iyi tekrarlanabilirlik gösterdi. Farklı yaş gruplarının tekrarlanabilirlik sonuçları, OKTA'nın optimal kullanımına rehberlik etmelidir.

Anahtar Kelimeler: Akım alanı, foveol avosküler bölge, OKTA, tekrarlanabilirlik, damar yoğunluğu

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Introduction

Imaging of the vascular structure of the retina is necessary for the diagnosis, follow-up, and treatment of most ocular diseases. To date, retinal vasculature has been studied using various instruments and methods, such as histological techniques, fundus fluorescent angiography (FA), and laser ophthalmoscopy (1,2). However, these techniques have limited use in clinical practice and research because they are non-quantitative methods. FA is the gold standard method for evaluating retinal vascular diseases. However, it requires a dye injection to observe the retinal microvasculature, making it invasive and requiring a certain amount of time.

Optical coherence tomography angiography (OCTA) was first reported by Makita et al. (3) using Doppler OCT (3). OCTA is a highly up-to-date, non-invasive, and non-contact imaging method that allows detailed imaging of the retinal capillary network (4). This method relies on motion contrast to distinguish structures with blood flow from those without blood flow. The AngioVue Imaging System (RTVue XR Avanti; Optovue, Inc., Fremont, CA) uses its automated algorithm, split-spectrum amplitude decorrelation angiography (SSADA). This algorithm enables simultaneous three-dimensional structural visualization of the retina and the creation of en face maps of blood flow (5). It is widely used in the evaluation of retinal diseases and glaucoma. OCTA also enables quantitative measurements of the retinal microvasculature (6,7). Many studies have reported the repeatability of OCTA measurements in the eyes of healthy individuals; however, the repeatability in the normal eye across different age groups has not been sufficiently studied (6,8-12).

Repeatability is important for assessing the change in normal and the diagnosis of diseased eyes and their change over time. The repeatability of microvasculature measurements at the same retinal region of the same patient is critical during the follow-up and treatment periods. Many previous studies have evaluated OCTA in terms of repeatability in a specific retinal vascular structure region. In the present study, repeatability was evaluated comprehensively in terms of the foveal avascular zone (FAZ) area, peripapillary area, choriocapillaris flow area, and vascular density (VD) of the superficial capillary plexus (SCP) and deep capillary plexus (DCP).

In this study, we aimed to evaluate the repeatability success of OCTA measurements and obtain OCTA data across different age groups to provide an additional contribution to database creation for a Turkish population.

Materials and Methods

This retrospective interventional comparative study was approved by the Local Ethics Committee (2023.06.236) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before registration.

Subjects with healthy eyes without any ocular diseases were included in this study and divided into five age groups-20-29, 30-39, 40-49, 50-59, and >60 years old-with 20 subjects each. Patients with any ocular diseases causing corneal opacities, cataract, vitreous opacities, retinal diseases, glaucoma, or optic nerve pathologies were excluded. In addition, smokers, pregnant women, patients with a history of any ocular surgeries, axial length (AL) of <21 mm_or >24 mm and with spherical refraction of more than ±3.0 D, cylinder refraction of more than ±2.0 D, and best-corrected visual acuity (BCVA) of less than 20/25 were excluded from this study. All subjects who met the inclusion criteria underwent a complete ophthalmologic examination (external ocular examination, auto-refractometry, BCVA, slit-lamp and fundoscopy examination, Goldmann applanation tonometry, and AL measurement using an AL-Scan (Nidek Co., Gamagori, Japan).

OCTA Imaging Protocol

OCTA images were acquired through previously dilated pupils by the same experienced technician, and similar mesopic conditions were adopted throughout the study. OCTA imaging was performed using an SSADA algorithm to detect flow area and vascular density. The SSADA algorithm has been described in detail in previous studies (7,13). Before imaging, each subject's right pupil was dilated with a combination of tropicamide (0.5%) twice and phenylephrine (2.5%) once, 5 min apart. Study participants underwent imaging consisting of the macula (3 mm x3 mm) and optic disk (4.5 mm x4.5 mm) with the eye tracking system activated. Imaging was repeated three times for only the right eye with 5 min between scanning sessions. Manual adjustments were not conducted because it was planned to analyze the repeatability of the FAZ area, VD, and flow area measured using AngioVue software automatically. Imaging with a signal strength <70 were considered poor quality and excluded, and repeated acquisitions were performed until the signal strength index was ⩾70.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows version 26.0 (SPSS, Inc., Chicago, IL, USA). The coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated to analyze the repeatability of the OCTA measurements in healthy subjects.

Repeatability was examined by CV, coefficient of variation, and ICC. CV was accepted as 20% or less. Comparisons of numerical variables in independent groups were made using the Kruskal-Wallis test. Subgroup analyses were performed using the Mann-Whitney U test and interpreted with Bonferroni correction. The statistical alpha significance level was set as $p<0.05$.

Results

A total of 100 eyes of 100 healthy subjects [54 females (54%) and 46 males (46%)] were included in this study. The mean age was 43.2±15.2 years. The mean intraocular pressure was 15.5±3.0 mmHg, and the mean AL was 23.4±1.0 mm.

The mean FAZ area was 0.28±0.10 mm^2; further, the CV and ICC values for the FAZ area were 2.96% and 0.990, respectively. Table 1 shows the repeatability assessment of the FAZ area for the overall age groups. When different age groups were evaluated separately, the CV value was below 10% in all age groups (Table 2). Specifically, the CV values for the 20-29, 30-39, 40-49, 50-59, and >60 years age groups were 1.52%, 1.41%, 4.38%, 4.16%, and 3.61%, respectively; this indicates that the measurement repeatability between the groups differed significantly ($p<0.05$). Further, as shown in Table 3, the corresponding ICC values were 0.994, 0.998, 0.987, 0.981, and 0.975 for the age groups, respectively; this indicates that the ICC values evaluated separately for all groups showed excellent reliability.

The ICC and CV values were assessed for VDs of five subregions of the SCP and DCP: whole area, superior hemi, inferior hemi, fovea, and parafovea. The whole image VDs showed good reliability for both SCP and DCP. The VDs of the SCP and DCP for different age groups showed CV values below 10% for all age groups (Table 2). CV values did not show significant differences between the different age groups ($p>0.05$, for all). The ICC values showed moderate or better reliability for all age groups (Table 3).

ICC and CV values were assessed for VDs of three subregions of the optic disk: entire image, inside disk and peripapillary. The CV and ICC values for the peripapillary were 2.32% and 0.740, respectively. The VD of the optic disk entire image showed moderate reliability overall. VDs of the optic disk for different age groups showed CV values below 10% for all age groups (Table 2). The ICC values showed moderate or better reliability for all age groups (Table 3).

The mean choriocapillaris flow area for all volunteers was 2.08±0.12 mm^2. The ICC value of the choriocapillaris flow area showed good overall reliability. The ICC values of

Table 1. CV and ICC values of OCTA parameters for overall age groups

		Mean ± SD	CV (%)	ICC
FAZ		0.28±0.10	**2.96**	**0.990**
Vessel density of the SCP	Whole image	47.2±3.5	**2.69**	**0.828**
	Superior hemi	47.1±3.7	**2.73**	**0.837**
	Inferior hemi	47.3±3.4	**2.86**	**0.794**
	Fovea	17.3±5.7	**6.43**	**0.954**
	Parafovea	50.2±3.8	**2.62**	**0.842**
Vessel density of the DCP	Whole image	52.0±3.7	**2.19**	**0.876**
	Superior hemi	52.0±4.2	**2.96**	**0.658**
	Inferior hemi	52.0±3.7	**3.04**	**0.510**
	Fovea	33.6±6.8	**2.97**	**0.972**
	Parafovea	54.0±4.4	**3.32**	**0.562**
Vessel density of the optical disk	Whole image	50.0±2.1	**2.02**	**0.736**
	Inside disk	49.7±4.9	**4.66**	**0.745**
	Peripapillary	52.7±2.5	**2.32**	**0.740**
	Superior	53.1±2.4	**2.53**	**0.670**
	Inferior	52.3±3.0	**2.48**	**0.793**
Flow area	Choriocapillaries	2.08±0.12	**1.86**	**0.865**

CV: Coefficient of variation, ICC: Intraclass correlation coefficient, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus, SD: Standard deviation
Interpretation for ICC agreement measures: 0.50, poor; between 0.50 and 0.75, moderate; between 0.75 and 0.9, good; greater than 0.9, excellent reliability

Table 2. Average and CV values of the OCTA parameters for different age groups

		20-29 years		30-39 years		40-49 years		50-59 years		≥ 60 years		
		Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	Mean ± SD	CV (%)	p
FAZ		0.27±0.08	1.52	0.33±0.13	1.41	0.26±0.11	4.38	0.27±0.08	4.16	0.29±0.10	3.61	0.012[a*]
Vessel density of the SCP	Whole image	48.7±2.9	2.58	47.6±3.7	3.05	47.2±2.2	2.58	47.8±3.5	2.34	44.7±3.8	2.84	0.931[a]
	Superior hemi	48.7±3.0	2.60	47.7±3.6	3.15	47.1±2.3	2.42	47.6±3.4	2.42	44.3±4.4	2.99	0.436[a]
	Inferior hemi	48.7±3.0	2.66	47.5±3.9	3.06	47.4±2.2	2.88	47.9±3.7	2.55	45.1±3.3	3.09	0.956[a]
	Fovea	19.1±5.4	5.21	15.1±6.6	7.44	19.0±5.5	5.23	18.6±4.2	5.46	15.0±5.4	8.63	0.369[a]
	Parafovea	51.8±3.0	2.56	50.9±4.2	2.83	50.0±2.4	2.76	50.8±3.8	2.14	47.7±4.4	2.71	0.854[a]
Vessel density of the DCP	Whole image	53.5±4.1	2.02	52.5±3.5	2.32	53.4±2.5	2.05	51.4±4.5	2.40	49.5±2.0	2.18	0.866[a]
	Superior hemi	53.6±4.2	2.11	52.3±3.6	2.43	52.8±5.0	5.38	51.7±4.7	2.48	49.3±2.4	2.26	0.894[a]
	Inferior hemi	53.4±3.9	2.34	52.7±3.6	2.23	53.3±2.5	2.27	50.2±5.0	6.36	49.9±2.1	2.61	0.980[a]
	Fovea	36.0±5.0	2.37	31.7±8.3	2.65	35.1±7.2	2.59	35.3±5.2	3.29	30.5±5.8	4.03	0.208[a]
	Parafovea	55.6±4.0	1.72	54.2±5.2	5.07	55.4±2.2	2.18	53.6±4.7	2.03	50.9±4.3	5.28	0.525[a]
Vessel density of the optical disk	Whole image	49.8±1.6	2.30	50.0±2.8	2.20	50.8±1.8	1.35	49.8±2.2	2.20	49.4±2.0	2.09	0.088[a]
	Inside disc	51.3±4.2	5.30	50.6±5.6	5.12	49.8±5.3	4.08	48.7±3.9	4.41	48.1±4.7	4.35	0.751[a]
	Peripapillary	52.5±1.9	2.62	52.9±2.9	2.57	53.3±2.4	1.71	52.6±2.7	2.49	52.2±2.7	2.25	0.089[a]
Flow	Choriocapillaries	2.20±0.11	1.57	2.10±0.11	1.83	2.05±0.08	1.92	2.04±0.11	1.71	2.04±0.13	2.25	0.764[a]

[a]One-way ANOVA with *post-hoc test (Tukey-Kramer test)
In the post-hoc test, while there was no difference between other age groups in terms of FAZ, the 30-39-year age group was statistically different from the others (p<0.001).
CV: Coefficient of variation, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus

the choriocapillaris flow area for the different age groups were all below 10%. As shown in Table 2, the CV values for the 20-29, 30-39, 40-49, 50-59, and >60 years age groups were 1.57%, 1.83%, 1.92%, 1.71%, and 2.25%, respectively; there was no statistically significant difference between the different age groups (p>0.05). The ICC values showed good reliability for all age groups (Table 3).

Discussion

The accuracy of repeatability measurement is important when considering the implementation of OCTA in clinical use. We demonstrate that the mean FAZ area, vessel density, and flow measurements are reliable with higher repeatability by OCTA. We believe that repeatability results of different age groups are needed to determine strict standard values of OCTA measurements for diagnosis, follow-up, and treatment.

FAZ represents a non-capillary region in the center of the fovea and may vary significantly in dimension even among healthy individuals. The vascular layers in the retina end as they approach the center of the fovea. The FAZ area could be a useful parameter in the detection and follow-up of retinal vascular pathologies. FAZ area quantification has a significant positive correlation with the severity of capillary non-perfusion in various retinovascular diseases (14). Many devices used in clinical practice for ophthalmic evaluation have been discontinued because of low reliability. Studies that used FA to analyze the FAZ area showed that the FAZ area in healthy subjects varied greatly, ranging from 0.205 to 0.405 mm^2 (15-17). The high variability of FA measurements casts doubt on the reliability of this invasive technique used to measure the FAZ area (18). This method is also limited by overlaps in the capillary plexus and leaks during FA imaging. OCTA is non-invasive, can simultaneously image the retinal vasculature and microanatomy, and provides better FAZ measurement than FA imaging (19). This study investigates the repeatability of OCTA for measuring the FAZ area. Using automatic software, we found that the FAZ area had excellent reliability for ICC values for different age groups. For CV values, the FAZ area can be measured at a younger age with higher repeatability. OCTA repeatability studies have shown the greatest success for the FAZ area (7-9). FAZ is useful for detecting and monitoring the progression of retinovascular diseases, making OCTA useful for follow-ups of these diseases. The mean FAZ area was 0.28±0.10 mm^2; this result will guide us in determining the mean FAZ area of healthy individuals in the Turkish population. The FAZ area in the 30-39-year age group was significantly higher than in the other groups (p<0.001).

OCTA enables the observation of SCP and DCP of the retina with high resolution and three-dimensional

Table 3. ICC values of the OCTA parameters for different age groups

		20-29 years			30-39 years			40-49 years			50-59 years			≥60 years		
		ICC	95% CI		ICC	95% CI		ICC	95% CI		ICC	95% CI		ICC	95% CI	
			Min	Max		Min	Max		Min	Max		Min	Max		Min	Max
FAZ		**0.994**	0.988	0.998	**0.998**	0.997	0.999	**0.987**	0.973	0.994	**0.981**	0.959	0.993	**0.975**	0.949	0.989
VD of the SCP	Whole image	**0.789**	0.615	0.902	**0.809**	0.658	0.908	**0.600**	0.358	0.794	**0.867**	0.731	0.945	**0.859**	0.733	0.936
	Superior hemi	**0.782**	0.604	0.899	**0.795**	0.638	0.901	**0.591**	0.346	0.788	**0.881**	0.758	0.951	**0.885**	0.778	0.948
	Inferior hemi	**0.795**	0.624	0.905	**0.820**	0.675	0.913	**0.580**	0.335	0.781	**0.814**	0.639	0.921	**0.768**	0.586	0.891
	Fovea	**0.968**	0.935	0.986	**0.966**	0.931	0.985	**0.942**	0.885	0.974	**0.943**	0.878	0.977	**0.917**	0.836	0.964
	Parafovea	**0.775**	0.592	0.895	**0.843**	0.713	0.925	**0.584**	0.340	0.784	**0.892**	0.778	0.955	**0.893**	0.792	0.952
VD of the DCP	Whole image	**0.871**	0.761	0.945	**0.864**	0.749	0.936	**0.920**	0.843	0.964	**0.772**	0.572	0.901	**0.645**	0.412	0.824
	Superior hemi	**0.921**	0.843	0.965	**0.852**	0.729	0.930	**0.851**	0.689	0.941	**0.758**	0.552	0.894	**0.703**	0.491	0.856
	Inferior hemi	**0.876**	0.762	0.944	**0.858**	0.738	0.933	**0.881**	0.772	0.946	**0.818**	0.648	0.957	**0.560**	0.305	0.773
	Fovea	**0.961**	0.921	0.983	**0.989**	0.977	0.995	**0.979**	0.956	0.991	**0.946**	0.885	0.978	**0.939**	0.877	0.973
	Parafovea	**0.933**	0.866	0.971	**0.874**	0.781	0.957	**0.903**	0.812	0.956	**0.817**	0.643	0.923	**0.815**	0.612	0.917
VD of the optic disk	Whole image	**0.521**	0.262	0.747	**0.788**	0.625	0.897	**0.855**	0.729	0.933	**0.741**	0.519	0.887	**0.703**	0.491	0.856
	Inside disc	**0.548**	0.283	0.768	**0.768**	0.596	0.886	**0.850**	0.722	0.930	**0.693**	0.449	0.863	**0.786**	0.611	0.901
	Peripapillary	**0.535**	0.275	0.757	**0.730**	0.536	0.866	**0.846**	0.713	0.929	**0.747**	0.528	0.890	**0.810**	0.652	0.912
Flow area	Choriocapillaries	**0.864**	0.741	0.939	**0.856**	0.732	0.932	**0.857**	0.734	0.927	**0.860**	0.718	0.942	**0.840**	0.699	0.927

ICC: Intraclass correlation coefficient, VD: Vascular density, FAZ: Foveal avascular zone, SCP: Superficial capillary plexus, DCP: Deep capillary plexus, CI: Confidence interval, min: Minimum, max: Maximum
Interpretation for ICC agreement measures: 0.50, poor; between 0.50 and 0.75, moderate; between 0.75 and 0.9, good; greater than 0.9, excellent reliability

microcirculation imaging of the retina (20). Two-dimensional FA imaging methods can be used to explore the retina in just one plane. In contrast, OCTA provides a non-invasive approach for evaluating three-dimensional retinal microcirculation imaging (20,21). OCTA can also obtain individual images of the two vascular networks of the retina and indicate their morphology and characteristics, which is not possible using FA (22). The repeatability of VD measurements has been previously reported for the same and other OCTA devices. For the RS-3000 Advance OCTA device (Nidek) and its included measurement software, the CV and ICC for the SCP were 5.2% and 0.90, respectively (6). In a study of healthy participants using the RTVue device and an automated measurement tool, the agreement was reported as 0.78-0.99 (23). In this study, VD of the SCP and DCP as obtained using the AngioVue Imaging System for image analysis showed good repeatability. However, the inferior hemi and parafoveal VD of the DCP showed moderate reliability with ICC values of 0.510 and 0.562, respectively. This study showed a higher vessel density in the DCP than in the SCP. These findings are consistent with those of previous studies using other techniques (24). The different age groups did not show any differences in repeatability. Owing to the interest in using OCTA-derived quantitative measurements in clinical trials and practice, it is important to determine its repeatability limits. The repeatability of SCP and DCP did not differ statistically for different age groups.

The VD of the optic disk was divided into different sectors; several studies have evaluated the repeatability of OCTA measurements of the peripapillary region (25-27). Wang et al. (27) evaluated the repeatability of the peripapillary vessel density of 15 healthy individuals and found that CV was 1.21%. Liu et al. (25) evaluated the repeatability of the mean peripapillary vessel density in two groups-12 normal subjects and 12 glaucoma patients- and found that CV was 1.9% and 4%, respectively. This study showed that VD measurements using OCTA are reliable and have higher repeatability. Peripapillary VD showed moderate reliability with CV <2.32% and ICC of 0.661-0.808. CV values of the peripapillary region for different age groups showed no differences in terms of repeatability.

Perfusion of retinal vessels within natural limits is vital for retinal function to be within normal limits (28). OCTA, first described in 2012, enables examination and measurement of macular. Studies are increasingly investigating new techniques for studying changes in macular perfusion. In the study with healthy participants, the evaluation of the choriocapillaris flow area with the RTVue XR device showed high repeatability (20). In this study, the choriocapillaris flow area in the different age groups showed good repeatability with no statistically significant difference. Yu et al. (29) reported that the RTVue XR device had a mean ICC of 0.925 for the macular perfusion flow index in healthy participants. Wei et al. (30) reported that OCTA could be used with high repeatability to measure macular perfusion in healthy individuals. Al-Sheikh et al. (31) reported that the flow index of the choriocapillaris had medium to high repeatability in the subfoveal region.

Study Limitations

One limitation of this study is that the population included only healthy subjects. Therefore, it may not be possible to obtain these reliability rates in patients with poor fixation owing to maculopathy. In algorithms using automatic segmentation, problems may occur in the detection of FAZ and vascular structures secondary to pathologies such as macular edema that cause changes in retinal thickness. Furthermore, the sample size was relatively small. To test the repeatability of OCTA measurements, a rigorously designed prospective study representing subjects of all ages is required.

Conclusion

Preliminary results suggest that RTVue XR with OCTA may be appropriate and reliable for evaluating changes in the follow-up and treatment of ocular diseases. It can be used as a non-invasive, reliable method to quantitatively determine changes in FAZ shape and size. Therefore, the measurement of retinal VD by OCTA is reliable; the flow area could be a valuable way to detect and follow up choroidal disease in the future.

Ethics

Ethics Committee Approval: This retrospective interventional comparative study was approved by the Local Ethics Committee (2023.06.236) and conducted in accordance with the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all participants before registration.

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Authorship Contributions

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