



Comparison of Microvascular and Electrophysiological Findings of Normal-Tension Glaucoma and Chronic Non-Arteritic Ischemic Optic Neuropathies

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Abstract

Objectives: To compare microvascular alterations in the optic nerve head between non-arteritic anterior ischemic optic neuropathy (NAION) and normal-tension glaucoma (NTG) and assess the correlation between vascular density (VD) and pattern electroretinography (PERG) changes.

Materials and Methods: Patients with NTG and NAION underwent comprehensive ophthalmologic examinations, including optic coherence tomography angiography and PERG imaging. Demographic and clinical data were collected, and groups were matched for age, intraocular pressure, mean deviation, and global retinal nerve fiber layer thickness.

Results: The study included 25 eyes from the NAION group, 24 eyes from the NTG group, and 30 eyes from the control group. VD was significantly lower in the peripapillary, inferior hemi, inferior temporal, and temporal inferior regions in NAION patients compared to NTG patients ($p=0.004$, $p=0.003$, $p=0.002$, $p=0.006$, respectively). Analysis of PERG parameters revealed that the P50 amplitudes in both NAION and NTG patients were lower than those in the control group ($p=0.001$,

$p=0.012$, respectively). A statistically significant difference between the NAION and NTG groups was observed only in N95 amplitude ($p=0.035$). N95 amplitude emerged as the most sensitive discriminator, while inferior temporal VD was the most specific discriminator. VD correlated with P50 latency, P50 amplitude, and N95 amplitude ($p<0.050$ for all).

Conclusion: Our findings highlight the diagnostic value of peripapillary vessel density and PERG parameters in distinguishing NAION from NTG. The observed correlations between PERG and VD suggest a complementary role for these measures in evaluating retinal ganglion cell function and microvascular alterations.

Keywords: Non-arteritic ischemic optic neuropathy, normal-tension glaucoma, optic coherence tomography angiography, pattern electroretinogram

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Introduction

Glaucoma and non-arteritic anterior ischemic optic neuropathy (NAION) are the two most common causes of irreversible optic nerve damage. NAION is characterized by sudden vision loss and optic disc edema during the acute phase, which progresses to a chronic phase marked by optic disc pallor and thinning of the retinal nerve fiber layer (RNFL).¹ NAION has been associated with decreased blood flow in the short posterior ciliary artery, the primary vessel supplying the optic nerve head (ONH).² Normal-tension glaucoma (NTG) differs from other types of glaucoma in both its pathophysiological and clinical characteristics. Although the cause remains a subject of debate, it is suggested that chronic retinal ganglion cell loss in NTG occurs in conjunction with reduced perfusion of the ONH.^{3,4} Although the mechanisms of retinal nerve fiber damage in NAION and NTG differ, detecting the underlying cause of damage during the chronic period can be difficult. Both optic disc changes and visual field alterations may mimic each other.

Recently, optical coherence tomography angiography (OCTA) has found increasing utility as a non-invasive method enabling observation of capillary circulation in the peripapillary

region. Several studies using OCTA have demonstrated a decrease in vascular density (VD) in patients with NAION and open-angle glaucoma when compared to normal subjects.^{5,6,7,8} Pattern electroretinography (PERG) is a sensitive method for evaluating retinal ganglion cell function. It has been observed that PERG amplitudes are reduced in NAION and NTG compared to normal subjects, indicating retinal ganglion cell damage in both NAION and NTG.^{9,10}

The study aims to identify and compare the microvascular changes occurring in the ONH in NAION and NTG, as well as to determine the correlation between VD and PERG changes.

Materials and Methods

Patients diagnosed with NTG and NAION at a tertiary eye care center between May and October 2022 were included in this prospective cross-sectional study. The study was conducted in accordance with the Helsinki Declaration after obtaining approval from the Balikesir University Faculty of Medicine Ethics Committee (decision number: 2022/101, date: 28.09.2022). Written informed consent was obtained from all patients.

All patients underwent a comprehensive ophthalmologic examination, including assessment of best corrected visual acuity (BCVA) using a Snellen chart, measurement of intraocular pressure with Goldman applanation tonometry, slit-lamp biomicroscopic examination, measurement of central corneal thickness, and dilated fundus examination. Additionally, all patients underwent standard automated perimetry (Humphrey visual field analyzer, Carl Zeiss Meditec, Dublin, CA, 24-2 Swedish Interactive Threshold Algorithm Standard Strategy), RNFL thickness measurement by optical OCT (AngioVue, Optovue Inc., Fremont, CA), OCTA (AngioVue, Optovue Inc., Fremont, CA) for peripapillary angiography, and PERG measurements (Monpack One, Metrovision, France).

Inclusion criteria for the study were as follows: spherical equivalent between -6.0 diopters (D) and +3 D, cylindrical equivalent <2 D, axial length <25 mm, presence of open angles on gonioscopy, and at least two reliable visual field tests.

Exclusion criteria included systemic medication use, history of antiglaucoma medication use prior to NTG diagnosis, presence of pathologies causing media opacity (e.g., cataract, vitreous hemorrhage), and history of intraocular surgery.

A diagnosis of NTG was established in patients with compatible glaucomatous defects in the optic disc, visual field, and open anterior chamber angles, with intraocular pressure measurements on different days <18 mmHg. NAION diagnosis was defined by sudden painless vision loss, optic disc edema, presence of compatible visual field defects, and exclusion of arteritic causes. Patients with chronic phase NAION, defined as occurring at least 6 months after resolution of optic disc edema, were included in the study.

The healthy control group consisted of volunteers who did not have any ocular symptoms, no history of intraocular surgery, intraocular pressure <18 mmHg, and no visual field defects. The NTG, NAION, and control groups were matched based on age, intraocular pressure, mean deviation (MD), and global RNFL thickness. Matching was performed within ± 5 years for age, ± 1 mmHg for intraocular pressure, ± 1 dB for MD, and $\pm 15 \mu\text{m}$ for global RNFL thickness.

All participants underwent OCTA imaging of the peripapillary region using a $4.5 \times 4.5 \text{ mm}^2$ scan pattern (Figure 1). The operational mechanism of the device has been explained elsewhere.¹¹ VD was calculated as the ratio of the area covered by small vessels extending from the optic disc boundary within a 1000 μm -wide elliptical area to the total area. Segmentation of the device was based on the area between the inner limiting membrane and the posterior boundary of the RNFL. VD was measured inside the disc as a whole and in the peripapillary region, the superior and inferior hemifields, and the nasal superior, nasal inferior, inferior nasal, inferior temporal, temporal inferior, temporal superior, superior nasal, and superior temporal sectors. Peripapillary RNFL thickness was also measured using the same device. We selected only images with a scanning quality of more than 7 and no motion-related artifacts.

PERG recordings were obtained in a room isolated from magnetic fields and sound. Briefly, electrical responses were

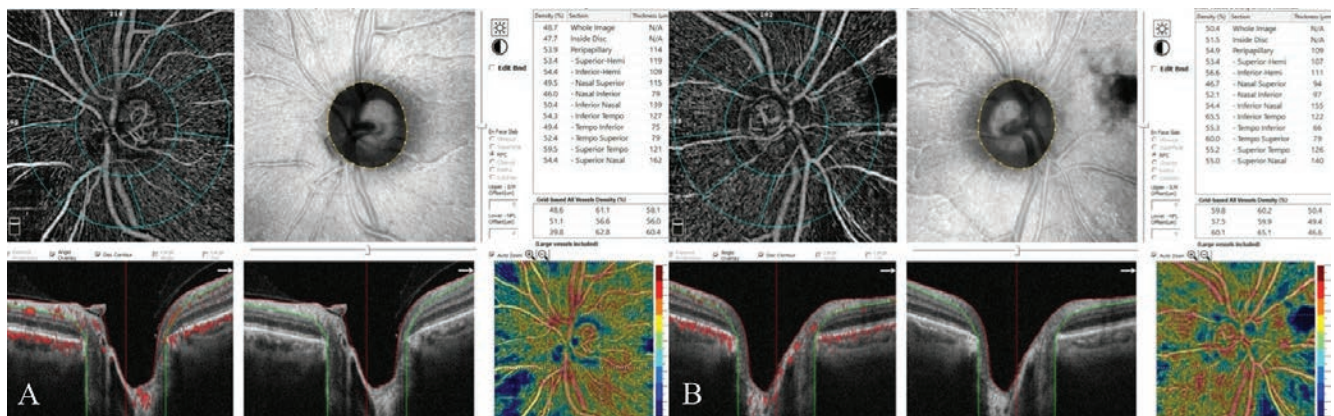


Figure 1. Representative optical coherence tomography angiography and optic nerve head (ONH) analysis outputs from (A) a patient with non-arteritic anterior ischemic optic neuropathy and (B) a patient with normal-tension glaucoma. Peripapillary vessel density and peripapillary retinal nerve fiber layer thickness were evaluated in the peripapillary annulus and inside-disc region, across 8 sectors, and in the superior/inferior hemifields using the ONH analysis software (Angio DiscVue)

recorded using five Dawson-Trick-Litzkow electrodes: one electrode for each eye was attached to the lower eyelid to face the cornea, and two reference electrodes were attached to the outer canthi of both eyes. A grounding electrode was placed on the forehead. We conducted the test under scotopic conditions at a distance of 30 cm, following 2 minutes of light adaptation under binocular vision with near-distance correction. Participants were instructed to focus on a red spot located at the center of a chessboard pattern displayed on the screen, and four PERG recordings were obtained for each eye and averaged. We expressed the results as a graph containing recorded electrical responses and analytical data about selected peak amplitudes. Amplitudes and latencies were recorded for the N35, P50, and N95 waves (peaks). Further details were provided in a separate study.¹²

Statistical Analysis

Normal distribution of the data was assessed using the Shapiro-Wilk test. Descriptive statistics were presented as mean \pm standard deviation for normally distributed variables and as number and percentage for categorical variables. Categorical data were analyzed using the chi-square test, while continuous variables were evaluated using one-way analysis of variance (ANOVA). Group differences were further assessed using Tukey's post-hoc multiple comparison test. Correlations between parameters were analyzed using Pearson's correlation coefficient. The discriminative ability of various parameters for group differentiation was evaluated using receiver operating characteristic (ROC) analysis. All statistical analyses were

performed using SPSS 25.0 software, with a p value of <0.05 considered statistically significant.

Results

The study included 30 eyes each from the NAION, NTG, and control groups. Five patients from the NAION group and six patients from the NTG group were excluded from the study due to inability to match RNFL thicknesses and visual field MD values. [Table 1](#) presents the demographic and clinical characteristics of the participants. There were no significant differences between the groups in terms of age, gender, intraocular pressure, and MD ($p>0.05$).

The optic disc parameters of OCTA are shown in [Table 2](#). VD was lower in NAION patients compared to the healthy control group in all regions except the inside disc ($p<0.05$). In NTG patients, VD was lower than in controls in all regions except for inside disc, inferior temporal, and temporal inferior values ($p<0.05$). Peripapillary, inferior hemifield, inferior temporal, and temporal inferior VD was lower in NAION patients than in NTG patients ($p=0.004$, $p=0.003$, $p=0.002$, $p=0.006$, respectively).

Upon examination of the PERG characteristics across the groups, the P50 amplitudes in NAION and NTG patients were significantly lower than those in the control group ($p=0.001$, $p=0.012$, respectively). There was a statistically significant difference between the NAION and NTG groups only in N95 amplitude ($p=0.035$). [Table 3](#) presents the PERG results between groups.

Table 1. Demographic and clinic features of patients with NAION, NTG, and healthy eyes

	NAION group (n=25)	NTG group (n=24)	Healthy group (n=30)	p value
Age (years)	59.68 \pm 11.49	60.33 \pm 8.75	59.93 \pm 8.10	0.971
Gender, female, n (%)	15 (60)	16 (66.7)	17 (56.7)	0.753
BCVA (logMAR)	0.54 \pm 0.34	0.35 \pm 0.41	0.03 \pm 0.05	$p^a=0.079$ $p^b<0.001$ $p^c<0.001$
CCT (μ m)	534.44 \pm 31.96	495.08 \pm 25.46	550.27 \pm 22.47	$p^a<0.001$ $p^b=0.079$ $p^c<0.001$
VF MD (dB)	-5.70 \pm 1.97	-5.20 \pm 2.77	-0.87 \pm 2.93	$p^a=0.639$ $p^b<0.001$ $p^c<0.001$
IOP (mmHg)	14.88 \pm 2.18	14.58 \pm 2.02	15.50 \pm 1.77	$p^a=0.861$ $p^b=0.485$ $p^c=0.218$
Cup to disc ratio	0.38 \pm 0.08	0.39 \pm 0.07	0.34 \pm 0.09	$p^a=0.885$ $p^b=0.269$ $p^c=0.111$
RNFL thickness, average (μ m)	83.84 \pm 4.78	82.41 \pm 7.66	100.03 \pm 3.99	$p^a=0.646$ $p^b<0.001$ $p^c<0.001$

p^a : NAION vs. NTG, p^b : NAION vs. healthy group, p^c : NTG vs. healthy group

NAION: Non-arteritic ischemic optic neuropathy, NTG: Normal tension glaucoma, BCVA: Best corrected visual acuity, logMAR: Logarithm of the minimum angle of resolution, CCT: Central corneal thickness, VF: Visual field, MD: Mean deviation, IOP: Intraocular pressure, RNFL: Retinal nerve fiber layer

Table 4 shows the significant discriminators of NAION and NTG according to ROC analysis. The most sensitive discriminator was N95 amplitude, and the most specific discriminator was inferior temporal VD.

The correlation analysis between N35, P50, and N95 parameters (latency and amplitude) and VD is shown in Table 5. The results include the whole image, inside disc, peripapillary, upper and lower hemifields, and different nasal and temporal regions. We did not obtain any statistically significant results for N35 latency, amplitude, or N95 latency across any regions. P50 latency had significant negative correlations with multiple

regions. In the superior nasal, inferior nasal, inferior temporal, inferior temporal, and superior nasal regions, P50 latency showed a statistically significant negative correlation ($p < 0.050$ for all). These results indicate that increasing latency is associated with reduced values in these regions. Various regions showed a positive correlation with the P50 amplitude. The highest positive correlation was in the superior hemifield ($r = 0.249$, $p = 0.027$). N95 amplitude showed significant negative correlations in several regions. The peripapillary region exhibited the strongest significant negative correlation ($r = -0.309$, $p = 0.006$).

Table 2. Comparison of peripapillary vascular density between groups

	NAION group (n=25)	NTG group (n=24)	Healthy group (n=30)	p value
Whole image (%)	41.90±6.51	44.80±6.16	50.97±2.39	$p^a = 0.132$ $p^b < 0.001$ $p^c < 0.001$
Inside disc (%)	49.84±5.26	51.77±4.36	52.23±3.84	$p^a = 0.295$ $p^b = 0.129$ $p^c = 0.927$
Peripapillary (%)	40.21±7.36	46.15±7.98	52.92±2.52	$p^a = 0.004$ $p^b < 0.001$ $p^c < 0.001$
-Superior hemi (%)	41.70±9.04	44.95±8.72	53.06±2.87	$p^a = 0.262$ $p^b < 0.001$ $p^c < 0.001$
-Inferior hemi (%)	42.56±9.01	47.58±7.93	52.62±2.42	$p^a = 0.003$ $p^b < 0.001$ $p^c = 0.024$
Nasal superior (%)	40.26±11.99	44.10±7.94	50.88±3.38	$p^a = 0.244$ $p^b < 0.001$ $p^c = 0.011$
Nasal inferior (%)	40.44±12.46	42.55±8.21	49.13±3.74	$p^a = 0.671$ $p^b = 0.001$ $p^c = 0.019$
Inferior nasal (%)	44.76±10.08	46.73±9.76	52.53±4.58	$p^a = 0.684$ $p^b = 0.003$ $p^c = 0.034$
Inferior temporal (%)	44.24±11.68	53.60±10.49	57.82±4.12	$p^a = 0.002$ $p^b < 0.001$ $p^c = 0.216$
Temporal inferior (%)	43.17±10.15	49.38±5.53	53.63±3.87	$p^a = 0.006$ $p^b < 0.001$ $p^c = 0.069$
Temporal superior (%)	43.79±8.08	47.74±11.81	56.15±3.43	$p^a = 0.218$ $p^b < 0.001$ $p^c = 0.001$
Superior temporal (%)	41.87±9.83	45.69±12.01	55.07±3.94	$p^a = 0.301$ $p^b < 0.001$ $p^c = 0.001$
Superior nasal (%)	39.76±13.31	41.13±10.22	51.60±5.51	$p^a = 0.880$ $p^b < 0.001$ $p^c = 0.001$

p^a : NAION vs. NTG, p^b : NAION vs. healthy group, p^c : NTG vs. healthy group
NAION: Non-arteritic ischemic optic neuropathy, NTG: Normal tension glaucoma

	NAION group (n=25)	NTG group (n=24)	Healthy group (n=30)	p value
N35 latency (ms)	29.12±10.38	28.34±14.42	25.07±5.31	p ^a =0.962 p ^b =0.325 p ^c =0.488
N35 amplitude (mV)	-0.13±1.11	-0.31±0.71	-0.39±0.96	p ^a =0.791 p ^b =0.101 p ^c =0.360
P50 latency (ms)	56.84±10.83	55.65±10.03	53.99±6.34	p ^a =0.892 p ^b =0.483 p ^c =0.783
P50 amplitude (mV)	3.56±1.22	4.05±1.39	5.63±2.67	p ^a =0.652 p^b=0.001 p^c=0.012
N95 latency (ms)	100.72±10.66	94.89±16.58	94.95±15.37	p ^a =0.341 p ^b =0.310 p ^c =0.998
N95 amplitude (mV)	-2.05±3.06	-4.12±1.60	-5.19±3.41	p^a=0.035 p^b<0.001 p ^c =0.368

p^a: NAION vs. NTG, p^b: NAION vs. healthy group, p^c: NTG vs. healthy group
NAION: Non-arteritic ischemic optic neuropathy, NTG: Normal tension glaucoma

	Cut-off point	AUC	95% CI	Sensitivity (%)	Specificity (%)	p value
Peripapillary VD (%)	44.2	0.724	0.578-0.871	70.8	72	0.007
Inferior hemi VD (%)	43.6	0.668	0.516-0.819	75	56	0.044
Inferior temporal VD (%)	54.75	0.731	0.587-0.874	66	80	0.006
Temporal inferior VD (%)	47.80	0.715	0.570-0.860	66	68	0.010
N95 amplitude (mV)	-5.25	0.272	0.127-0.417	75	12	0.006

NAION: Non-arteritic ischemic optic neuropathy, NTG: Normal tension glaucoma, ROC: Receiver operating characteristic, VD: Vascular density, CI: Confidence interval

	N35 latency (ms)		N35 amplitude (mV)		P50 latency (ms)		P50 amplitude (mV)		N95 latency (ms)		N95 amplitude (mV)	
	r	p	r	p	r	p	r	p	r	p	r	p
Whole image (%)	-0.175	0.123	-0.189	0.094	-0.231	0.040	0.226	0.045	-0.070	0.542	-0.259	0.021
Inside disc (%)	0.037	0.745	-0.114	0.319	0.138	0.237	0.175	0.124	0.074	0.517	-0.001	0.923
Peripapillary (%)	-0.089	0.436	-0.204	0.071	-0.216	0.055	0.221	0.050	-0.116	0.308	-0.309	0.006
-Superior hemi (%)	-0.164	0.148	-0.176	0.120	-0.232	0.040	0.249	0.027	-0.110	0.335	-0.212	0.061
-Inferior hemi (%)	-0.098	0.363	-0.190	0.093	-0.271	0.016	0.183	0.106	-0.071	0.533	-0.228	0.043
Nasal superior (%)	-0.141	0.214	-0.178	0.216	-0.278	0.013	0.223	0.049	-0.048	0.672	-0.293	0.009
Nasal inferior (%)	-0.118	0.299	-0.150	0.187	-0.255	0.023	0.124	0.278	-0.063	0.580	-0.229	0.042
Inferior nasal (%)	-0.054	0.635	-0.175	0.122	-0.186	0.101	0.092	0.420	-0.011	0.922	-0.166	0.143
Inferior temporal (%)	-0.094	0.409	-0.161	0.157	-0.263	0.019	0.239	0.034	-0.083	0.468	-0.225	0.047
Temporal inferior (%)	-0.119	0.298	-0.183	0.107	-0.250	0.026	0.190	0.094	-0.20	0.863	-0.210	0.063
Temporal superior (%)	-0.175	0.122	-0.096	0.399	-0.215	0.057	0.236	0.037	-0.135	0.237	-0.106	0.354
Superior temporal (%)	-0.158	0.164	-0.088	0.441	-0.129	0.259	0.235	0.037	-0.076	0.507	-0.151	0.185
Superior nasal (%)	-0.148	0.194	-0.199	0.079	-0.225	0.046	0.190	0.094	-0.068	0.552	-0.133	0.241

Discussion

This study revealed that N95 amplitude decreased more significantly in the NAION group than in the NTG group. A significant reduction in VD was seen, particularly in the inferior region of the peripapillary area. Furthermore, we identified a statistically significant association among N95 amplitude, P50 amplitude, P50 latency, and VD. Our findings indicate that NAION and NTG can be differentiated by a decrease in VD.

Decreased perfusion is a well-established factor in the pathophysiology of both NTG and NAION, with several studies showing reduced perfusion in both conditions.^{13,14} In line with previous research, our study confirmed a decrease in VD in both NAION and NTG when compared to the control group. In NAION, the short posterior ciliary artery, which supplies the ONH, is the primary artery affected during the acute phase. In NTG, one of the strongest hypotheses suggests that retinal ganglion cell damage occurs due to chronically decreased blood flow in the ONH. OCTA studies that compared NAION and NTG found that NAION had a bigger decrease in peripapillary VD.^{5,6} Our results support this observation. A few studies, however, have claimed that the reduction in peripapillary VD is greater in NTG and primary open-angle glaucoma.^{7,8} This disparity may be due to differences in RNFL thickness between groups in one study and the fact that the primary open-angle glaucoma group in the other study was receiving antiglaucoma treatment. Patients receiving beta-blocker therapy may also experience significant changes in VD and blood flow due to RNFL thickness. Kim et al.⁶ compared VD in NTG and NAION and found that in NTG, VD was lower at the prelaminar tissue and lamina cribrosa levels, while in NAION, it was lower at the peripapillary level, but this difference was not statistically significant. The evidence suggests that peripapillary region involvement increases as NAION progresses into the chronic phase. The short posterior ciliary artery supplies both the prelaminar ONH and parapapillary choroid. Ischemic damage to these areas stops the transport of axons and causes retinal ganglion cell damage that worsens over time. As ischemic damage persists, the reduction in VD may spread across all quadrants. Shin et al.⁵ found a significant reduction in VD in NAION patients compared to NTG patients, particularly in the temporal region. They also saw that the choroidal microvascular dropout area was bigger in NAION patients, with more involvement in the temporal area in NAION patients and less involvement in NTG patients.⁵ Similarly, Liu et al.¹⁵ reported lower peripapillary VD in NAION compared to POAG across all quadrants except the inferior quadrant. In our study, VD in the temporal quadrant was significantly lower in NAION compared to NTG.

Electrophysiological tests provide important insights into diseases affecting the optic nerve and retinal ganglion cells. Both glaucoma and NAION patients have reported decreases in PERG parameters, particularly P50 and N95 amplitudes.^{9,10} Research indicates that PERG signals represent nerve injury and become compromised when macular ganglion cells are injured.^{16,17,18} Several studies have examined the relationship between PERG

and OCTA, with correlations identified between PERG and VD in the macular and peripapillary regions.^{18,19,20} In our study, we identified correlations between N95 amplitude, P50 amplitude, P50 latency, and VD. The correlations between P50 and N95 amplitudes and macular VD in the normal and early NTG groups suggests that these metrics may serve as indicators of retinal ganglion cell functionality prior to significant impairment.¹⁸ A similar interpretation may be applicable to NAION patients. Our findings suggest that differences in N95 amplitudes may aid in differentiating NAION from NTG patients. Although we matched the groups for age, IOP, global MD, and peripapillary RNFL thickness and restricted analyses to untreated NTG and chronic-phase NAION (≥ 6 months post-edema resolution) to minimize acute-phase effects, disease stage may still influence OCTA-derived peripapillary VD and PERG measurements. More advanced structural and functional loss, such as lower BCVA in NAION, is likely correlated with increased capillary loss and diminished PERG amplitudes. In contrast, earlier NTG may exhibit more subtle changes, potentially obscuring or amplifying intergroup differences despite matched MD.

Study Limitations

The most significant limitation of our study is the small sample size, which was required due to the need to match RNFL thickness and visual field parameters among groups. This standardization allowed for a more accurate comparison of VD changes. Another limitation was the inclusion of only patients with sufficient visual acuity to ensure optimal image quality in the imaging tests. Additionally, we were unable to include patients with advanced retinal nerve fiber damage, as we focused on newly diagnosed NTG patients before the initiation of antiglaucoma treatment. Consistent with the study's hypothesis, the matching of NAION and NTG patients based on MD, as well as the generally mixed visual field patterns of the current NAION patients, precluded an investigation into the relationship between peripapillary VD and visual field pattern. This relationship warrants investigation in a pre-specified, adequately powered study focused solely on NAION, incorporating a larger sample size and including all NAION patients. Nevertheless, the limited sample size in our matched design, certain variables (especially worse BCVA in NAION) may affect both OCTA-VD and PERG due to the basic nature of the diseases.

Conclusion

Peripapillary VD, particularly in the temporal region, provides valuable information for differentiating NAION from NTG. Changes in PERG parameters support this differentiation. There is also a significant correlation between peripapillary region VD and PERG parameters.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Helsinki Declaration after obtaining approval from the Balikesir University Faculty of Medicine Ethics Committee (decision number: 2022/101, date: 28.09.2022).

Informed Consent: Written informed consent was obtained from all patients.

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Declarations

Authorship Contributions

Surgical and Medical Practices: M.M.U., H.Y., Concept: M.M.U., Design: M.M.U., Data Collection or Processing: M.K.T., H.Y., Analysis or Interpretation: M.K.T., M.M.U., Literature Search: H.Y., Writing: M.K.T., H.Y.

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