

Longitudinal Optical Coherence Tomography Angiography Assessment of Retinal Microvascular Changes in Patients Receiving Oral Isotretinoin

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ABSTRACT

Objective: To determine whether oral isotretinoin therapy is associated with longitudinal changes in retinal structure and microvasculature measured by optical coherence tomography angiography (OCTA).

Methods: This prospective, longitudinal, bilateral-eye study was conducted at an anonymized Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital between 01 June 2021 and 01 June 2022. Fifty-four patients receiving oral isotretinoin for acne were enrolled; of these, 34 patients (68 eyes) completed baseline, month 1, month 3, and month 6 visits, forming the complete-case sample. All quantitative optical coherence tomography (OCT)/OCTA examinations were performed on the Nidek retinopathy study (RS)-3000 advance platform after topical tropicamide-induced dilation. Acquisitions were repeated until the image-quality index was at least 0.70; scans that did not reach this threshold or had clinically relevant artifacts were excluded. Longitudinal change was analyzed with patient-clustered Gaussian generalized estimating equations, adjusted for eye laterality, age, and sex. Time was modeled categorically. Endpoint-level multiplicity was controlled using the Benjamini–Hochberg false-discovery rate (FDR) procedure, and pairwise contrasts were assessed using the Holm procedure and a global FDR correction.

Results: The analytic dataset included 34 patients, 68 eyes, and 272 eye-time observations. Mean age was 20.71 ± 3.33 years; 22 patients (64.7%) were women and 12 (35.3%) were men. No OCT or OCTA endpoint showed an FDR-confirmed time effect; no significant change was detected in any parameter. The lowest omnibus time-effect P value was observed for inner-nasal Early Treatment Diabetic Retinopathy Study 9 (ETDRS9) perfusion (Wald $\chi^2 = 9.786$, $df = 3$, $p = 0.0205$), but this finding did not survive FDR adjustment ($q = 0.5284$). The smallest pairwise contrast was the inner-temporal ETDRS9 perfusion between months 6 and 1 (difference = 1.338, SE = 0.532, $p = 0.0119$), which was not significant after the within-endpoint Holm correction ($p = 0.0715$) or the global FDR correction ($q = 0.5934$). Patient-averaged models, non-parametric repeated-measures tests, and time-by-eye interaction models were consistent with the primary analysis.

Conclusion: Over 6 months of oral isotretinoin therapy, no retinal structural or OCTA microvascular parameters exhibited changes that remained significant after correction for multiple testing. Small nominal fluctuations should be interpreted as exploratory signals rather than confirmatory evidence of retinal microvascular toxicity.

Keywords: Acne vulgaris, isotretinoin, optical coherence tomography angiography, OCTA, retinal microvasculature, foveal avascular zone, generalized estimating equations



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INTRODUCTION

Oral isotretinoin remains a mainstay of systemic treatment for severe, scarring, or treatment-resistant acne vulgaris.¹ Its mucocutaneous and anterior-segment ocular adverse effects are well recognized, but retinal and microvascular effects are less clearly defined.² Quantitative optical coherence tomography (OCT) and OCT angiography (OCTA) allow non-invasive assessment of retinal structure and perfusion that may not be evident on routine clinical examination.^{3,4}

OCTA can quantify the retinal and choroidal microvasculature without dye injection. However, OCTA metrics are sensitive to acquisition quality, eye movement, segmentation errors, projection artifacts, and device-specific processing algorithms.^{5,6} For longitudinal studies of systemic medications, strict image-quality control and statistical modeling of repeated bilateral-eye data are essential.

Published OCT studies of isotretinoin have been inconsistent. Several studies reported no meaningful short-term change in central macular thickness, choroidal thickness, or retinal nerve fiber layer measurements,^{7–9} whereas other work described choroidal vascular changes in acne patients under isotretinoin therapy.¹⁰ Dedicated longitudinal OCTA data during treatment remain limited.¹¹ We therefore evaluated retinal structural outcomes and OCTA perfusion outcomes over 6 months of oral isotretinoin therapy using patient-clustered longitudinal models and multiplicity-adjusted interpretation.

MATERIALS AND METHODS

Study Design and Setting

This prospective longitudinal bilateral-eye study was conducted at Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital between 01 June 2021 and 01 June 2022. Patients receiving oral isotretinoin for acne vulgaris underwent bilateral OCT/OCTA at baseline and at months 1, 3, and 6 of treatment. Visits were scheduled relative to the first isotretinoin dose and accepted within a window of ± 7 days for

month 1 and ± 14 days for months 3 and 6. Of the 54 enrolled patients, 20 did not complete all scheduled visits and were excluded from the complete-case analysis. The final analytic dataset therefore comprised 34 patients, 68 eyes, and 272 eye-time observations. Reporting followed STROBE principles where applicable.¹²

Eligibility Criteria

Eligible patients were aged 16 years or older, had acne vulgaris for which oral isotretinoin had been prescribed by the dermatology department as part of routine care, and were able to undergo repeated dilated imaging. Patients were excluded if they had any prior ocular surgery or laser treatment, glaucoma or ocular hypertension, uveitis or other intraocular inflammation, retinal or optic nerve disease, corneal or lens opacity sufficient to degrade image quality, diabetes mellitus, hypertension or any other systemic disease known to affect retinal microvasculature, or use of systemic vasoactive or antiangiogenic medications. Patients who had previously received isotretinoin and those from whom imaging of adequate quality could not be obtained at baseline were also excluded. Enrollment was consecutive among patients meeting these criteria during the study period.

Isotretinoin Exposure

The dermatology department prescribed and monitored isotretinoin according to the standard institutional regimen; dosing was not modified for this study. Treatment began at 0.5 mg/kg/day and was raised, after several weeks and according to tolerance, to between 0.5 and 1.0 mg/kg/day, with a target cumulative dose of 120 to 150 mg/kg and a usual course length of 16 to 24 weeks. Individual daily and cumulative doses were not recorded in the analytic dataset. Exposure is therefore described at the level of the prescribing regimen rather than as a measured per-patient variable; consequently, no dose-response analysis was possible, which is acknowledged as a limitation. Because the usual course is shorter than the observation period, some patients are likely to have completed or to be completing treatment by the month-6 visit, so that the visit reflects the end of the treatment course or shortly afterwards rather than mid-treatment.

Ethical Approval

The study was approved by the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (meeting date: 26.04.2021, meeting number: 06, decision number: 06/05, official document number: E-21142744-804.99-77544, 04.05.2021). The approved protocol title concerned retinal microvascular changes in patients receiving oral isotretinoin. The study was planned prospectively and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. All authors worked at this university's hospital during the study.

Ophthalmic Imaging Protocol

All imaging was performed by a single experienced operator on the Nidek RS-3000 Advance platform (Nidek Co., Ltd., Gamagori, Japan) using NAVIS-EX software version 1.4.0.1. Pupils were dilated with topical tropicamide 1% before each

MAIN POINTS

- Whether oral isotretinoin affects the retina and its microvasculature is still unclear, and longitudinal optical coherence tomography angiography (OCTA) data collected during treatment remain scarce.
- Over 6 months of treatment in 34 patients (68 eyes), no structural or microvascular endpoint on optical coherence tomography or OCTA showed a significant change after correction for multiple testing.
- Patient-clustered generalized estimating equations that account for both eyes and repeated visits, combined with false discovery rate and Holm correction, guard against inflated significance that can arise when many correlated imaging metrics are tested simultaneously.
- The few nominal fluctuations observed are best interpreted as exploratory signals rather than evidence of isotretinoin-related retinal microvascular toxicity.

session, and scanning began after adequate mydriasis. To limit diurnal variation, each patient was imaged at approximately the same time of day at every visit. Structural OCT used the device's macular map protocol centred on the fovea, from which Early Treatment Diabetic Retinopathy Study (ETDRS) sectoral retinal thickness, central macular thickness, and ganglion cell measurements were exported. OCTA was performed using a 6×6 mm macular scan centred on the fovea. Perfusion and foveal avascular zone (FAZ) metrics were derived from the superficial retinal capillary plexus, which was segmented by the device as the layer between the internal limiting membrane and the posterior boundary of the inner plexiform layer; the manufacturer's default projection-artifact removal was applied. Automatic segmentation was reviewed for every scan, and scans with segmentation errors were excluded rather than being manually corrected. All values used in this report are device-derived outputs; no third-party or custom software was used for quantification.

Image-Quality Control

Each acquisition was reviewed for signal quality, motion artifacts, segmentation reliability, and other clinically relevant artifacts. Scans were repeated until the image-quality index was at least 0.70. Images with a quality index below 0.70, those that could not reach this threshold despite repeated acquisition, or those with clinically relevant artifacts or unreliable segmentation were excluded.

Outcomes

The outcomes were the device-derived OCT and OCTA parameters, defined as follows: Superior and inferior ganglion cell measurements are the mean ganglion cell complex thicknesses in the corresponding hemifields, expressed in micrometers. FAZ area (mm^2) and FAZ perimeter (mm) denote the area and boundary length of the device-delineated avascular zone in the superficial capillary plexus. The FAZ circularity index is the dimensionless ratio $4\pi A/P^2$, which equals 1 for a perfect circle and decreases as the boundary becomes more irregular. Central macular thickness and the ETDRS sectoral retinal thickness values are full-thickness retinal measurements within the standard ETDRS subfields, expressed in micrometers. ETDRS9 sectoral perfusion is the device-reported vessel area density within each ETDRS subfield of the superficial capillary plexus, expressed as the percentage of the subfield area occupied by the flow signal. Twenty-seven such endpoints were analysed. Because no single primary OCTA endpoint was prespecified before analysis, the OCT/OCTA variables were treated as an exploratory outcome family and were interpreted primarily using multiplicity-adjusted results.

Statistical Analysis

Continuous variables are reported as mean $\pm$ standard deviation with median and interquartile range (IQR), and categorical variables as counts and percentages. The main longitudinal analysis used Gaussian generalized estimating equation (GEE) with patient-level clustering to account for both eyes of each patient and repeated visits.¹³ An exchangeable working correlation structure was specified, and robust sandwich

standard errors were used. Time was entered as a categorical factor with baseline as the reference, and models were adjusted for eye laterality, age, and sex. These three covariates were prespecified on structural rather than empirical grounds: laterality, because the unit of analysis is the eye and both eyes of each patient contribute, so a systematic right-left offset would otherwise enter the residual variance; and age and sex, because both are established determinants of macular thickness, ganglion cell thickness, and OCTA perfusion density in healthy eyes, and adjusting for them reduces residual variance and thereby sharpens the time contrasts. None of the three is on the causal path between isotretinoin exposure and retinal outcome; therefore, their inclusion cannot bias the time effect. The systemic disease variable was not included because it was constant across the analytic cohort. Overall time effects were tested using Wald tests with 3 degrees of freedom. Endpoint-level multiplicity across the 27 endpoints was controlled with the Benjamini–Hochberg FDR.¹⁴ Pairwise time contrasts, 6 per endpoint and 162 in total, were corrected within each endpoint with the Holm¹⁵ method and across all endpoint-contrast combinations with a global FDR. Sensitivity analyses included patient-averaged GEE models, patient-averaged Friedman tests, and GEE models with time-by-eye interaction terms. Analyses were performed in Python using the statsmodels and SciPy libraries; an equivalent IBM SPSS Statistics GENLIN/GEE syntax was prepared for local verification of the reported models.

RESULTS

Study Population and Data Completeness

Of the 54 patients initially enrolled and assessed at baseline, 20 did not complete all four scheduled visits and were excluded from the complete-case analysis (Figure 1). All 20 were lost to follow-up because they did not attend one or more

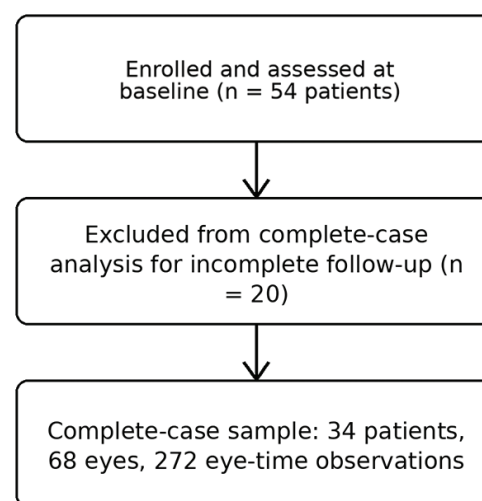


Figure 1. Flow of participants through the study. Of 54 patients enrolled and assessed at baseline, 20 were excluded from the complete-case analysis because they did not attend all four scheduled visits. The final analytic sample included 34 patients, 68 eyes, and 272 eye-time observations.

scheduled visits; no further reason was documented. None were withdrawn by the investigators, and none discontinued because of an ocular adverse event or any other ocular finding. The final dataset included 34 patients who completed the baseline, month 1, month 3, and month 6 visits, corresponding to 68 eyes and 272 eye-time observations, with no missing visits. Mean age was 20.71 ± 3.33 years, with a median of 20.5 years (IQR, 18.0–23.8). Of the patients, 22 (64.7%) were female and 12 (35.3%) were male. No additional systemic disease was recorded in the analytic dataset, and baseline laterality was balanced, with 34 right and 34 left eyes included (Table 1).

Descriptive OCT/OCTA Trends

Descriptive values changed little across the four visits. Selected structural, FAZ, and perfusion parameters are summarized in Table 2 and should be interpreted in conjunction with the clustered longitudinal models.

Primary Longitudinal GEE Analysis

In the primary patient-clustered GEE analysis, no endpoint remained significant after FDR correction; i.e., no significant change over time was detected for any endpoint. The smallest omnibus time-effect P value was observed for inner-nasal ETDRS9 perfusion (Wald $\chi^2 = 9.786$, $df = 3$, $P = 0.0205$), which did not survive FDR adjustment ($q = 0.5284$) (Table 3).

Exploratory Pairwise Time Contrasts

A small number of pairwise comparisons crossed the nominal 0.05 threshold, but none met confirmatory criteria after Holm correction within the endpoint or after global FDR correction across endpoints. The strongest nominal contrast was inner-temporal ETDRS9 perfusion between months 1 and 6 (difference = 1.338, $SE = 0.532$, $P = 0.0119$; Holm $P = 0.0715$; global FDR $q = 0.5934$) (Table 4).

Table 1. Study Characteristics and Data Structure

Characteristic	Value
Study design	Prospective longitudinal bilateral-eye study
Study site	Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital
Study period	01 June 2021 to 01 June 2022
Patients enrolled, n	54
Patients analyzed (completed all visits), n	34
Eyes, n	68
Eye-time observations, n	272
Scheduled visits	Baseline, month 1, month 3, month 6
Age, mean $\pm$ SD	20.71 ± 3.33 years
Age, median (IQR)	20.5 (18.0–23.8) years
Female/male, n (%)	22 (64.7%) / 12 (35.3%)
Additional systemic disease	None recorded in the analytic dataset
Baseline laterality	34 OD and 34 OS eyes
Image quality rule	OCT/OCTA images with quality index <0.70 were excluded

SD, standard deviation; IQR, interquartile range; OD, oculus dexter; OS, oculus sinister; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography.

Table 2. Selected OCT/OCTA Parameters Across Follow-up (Eye-Level Mean $\pm$ SD)

Endpoint	Baseline	Month 1	Month 3	Month 6
Superior ganglion cell thickness (μm)	109.16 ± 5.63	109.50 ± 5.40	109.01 ± 6.24	109.10 ± 6.32
Inferior ganglion cell thickness (μm)	110.24 ± 5.79	108.96 ± 5.40	110.01 ± 5.67	110.07 ± 6.35
FAZ area (mm^2)	0.30 ± 0.05	0.29 ± 0.05	0.30 ± 0.05	0.29 ± 0.05
FAZ perimeter (mm)	2.97 ± 0.36	2.95 ± 0.32	3.03 ± 0.30	2.98 ± 0.33
FAZ circularity index (dimensionless, $4\pi A/P^2$)	0.47 ± 0.05	0.47 ± 0.06	0.47 ± 0.06	0.45 ± 0.05
Central macular thickness, CMT (μm)	260.26 ± 10.68	258.19 ± 13.25	258.43 ± 13.74	259.62 ± 12.29
Outer ETDRS mean perfusion (% vessel area density)	53.43 ± 2.05	53.27 ± 1.61	53.06 ± 1.67	53.24 ± 1.87
Inner ETDRS mean perfusion (% vessel area density)	40.18 ± 2.22	40.28 ± 2.04	40.24 ± 2.36	40.22 ± 2.34
Outer ETDRS mean retinal thickness (μm)	332.53 ± 6.57	333.09 ± 7.43	333.05 ± 7.03	333.11 ± 6.03
Inner ETDRS mean retinal thickness (μm)	342.08 ± 6.83	342.57 ± 6.71	342.20 ± 6.24	342.87 ± 6.03

Perfusion values are vessel area density in the superficial capillary plexus, expressed as the percentage of subfield area occupied by flow signal; thickness values are in micrometers; FAZ area is in mm^2 and FAZ perimeter in mm; the FAZ circularity index is dimensionless. FAZ, foveal avascular zone; ETDRS, Early Treatment Diabetic Retinopathy Study; OCTA, optical coherence tomography angiography.

Sensitivity Analyses

Sensitivity analyses were concordant with the primary analysis. Neither patient-averaged GEE models nor patient-averaged Friedman tests detected an FDR-confirmed time effect at the endpoint level; time-by-eye interaction models did not suggest a laterality-specific pattern (Table 5).

Effect Estimates and Confidence Intervals

To complement hypothesis testing, the ten largest nominal pairwise contrasts were expressed as model-based differences with Wald 95% confidence intervals. Differences were small

in native units: approximately 1 to 3 percentage points for sectoral perfusion, a few micrometers for retinal thickness, and approximately 0.02 for the dimensionless FAZ circularity index. Contrasts whose confidence intervals excluded zero were nominally significant, but none remained significant after the Holm or global FDR correction. When interpreted as estimates rather than tests, the intervals place an upper bound on what may have been missed: across all endpoints and contrasts, the data exclude sectoral perfusion changes larger than about 2.9 percentage points, retinal thickness changes larger than about 5.8 μm , and FAZ circularity changes larger than about 0.04 (Table 6).

Table 3. Primary GEE Overall Time-Effect Results with the Smallest Nominal *P* values

Endpoint	Wald χ^2	df	Raw <i>P</i>	FDR <i>q</i>	FDR-significant
ETDRS9 perfusion, inner nasal	9.786	3	0.0205	0.5284	No
ETDRS9 perfusion, outer nasal	7.655	3	0.0537	0.5284	No
ETDRS9 perfusion, inner temporal	7.455	3	0.0587	0.5284	No
ETDRS retinal thickness, outer inferior	6.701	3	0.0821	0.5540	No
FAZ circularity index	5.834	3	0.120	0.6028	No
ETDRS9 perfusion, inner superior	5.580	3	0.134	0.6028	No
ETDRS9 perfusion, outer superior	4.967	3	0.174	0.6721	No
ETDRS retinal thickness, inner superior	3.055	3	0.383	0.9913	No

Models used Gaussian GEE with patient-level clustering, exchangeable working correlation, robust sandwich standard errors, and adjustment for eye laterality, age, and sex. FDR significance was defined as $q < 0.05$.

ETDRS, Early Treatment Diabetic Retinopathy Study; FAZ, foveal avascular zone; GEE, generalized estimating equation; FDR, false-discovery rate.

Table 4. Exploratory Pairwise Contrasts With the Smallest Nominal *P* values

Endpoint	Contrast	Estimate	SE	Raw <i>P</i>	Holm <i>P</i>	Global FDR <i>q</i>
ETDRS9 perfusion, inner temporal	Month 6–Month 1	1.338	0.532	0.0119	0.0715	0.5934
ETDRS retinal thickness, outer inferior	Month 6–Baseline	3.221	1.305	0.0136	0.0817	0.5934
ETDRS9 perfusion, inner nasal	Month 1–Baseline	1.603	0.657	0.0146	0.0878	0.5934
ETDRS9 perfusion, outer nasal	Month 3–Baseline	-1.412	0.584	0.0157	0.0942	0.5934
FAZ circularity index	Month 6–Month 1	-0.020	0.009	0.0290	0.1738	0.5934
ETDRS9 perfusion, inner superior	Month 3–Month 1	-1.279	0.588	0.0294	0.1767	0.5934
ETDRS9 perfusion, outer superior	Month 3–Month 1	0.956	0.439	0.0296	0.1776	0.5934
ETDRS9 perfusion, inner temporal	Month 1–Baseline	-1.221	0.565	0.0309	0.1544	0.5934
ETDRS9 perfusion, outer nasal	Month 6–Month 3	1.279	0.600	0.0330	0.1648	0.5934
ETDRS9 perfusion, inner superior	Month 3–Baseline	-1.324	0.648	0.0411	0.2056	0.6660

Pairwise estimates are model-based differences between the two visits shown. Holm *P* values are within-endpoint adjusted; global FDR *q* values are calculated across all endpoint-contrast combinations.

ETDRS9, Early Treatment Diabetic Retinopathy Study 9; FAZ, foveal avascular zone; FDR, false-discovery rate.

Table 5. Summary of Sensitivity Analyses

Analysis family	Endpoint with smallest raw <i>P</i>	Smallest raw <i>P</i>	Smallest FDR <i>q</i>	No. $q < 0.05$
Patient-averaged GEE overall time	ETDRS9 perfusion, inner nasal	0.0205	0.5284	0
Patient-averaged Friedman test	ETDRS9 perfusion, inner superior	0.118	0.9920	0
Time $\times$ eye interaction GEE	Superior ganglion cell thickness	0.1457	0.9118	0

ETDRS9, Early Treatment Diabetic Retinopathy Study 9; GEE, generalized estimating equation; FDR, false-discovery rate.

Table 6. Effect Estimates and 95% Confidence Intervals for the Largest Nominal Pairwise Contrasts

Endpoint	Contrast	Estimated difference	95% CI
ETDRS9 perfusion, inner temporal	Month 6-Month 1	1.338	0.30 to 2.38
ETDRS retinal thickness, outer inferior	Month 6-Baseline	3.221	0.66 to 5.78
ETDRS9 perfusion, inner nasal	Month 1-Baseline	1.603	0.32 to 2.89
ETDRS9 perfusion, outer nasal	Month 3-Baseline	-1.412	-2.56 to -0.27
FAZ circularity index	Month 6-Month 1	-0.020	-0.038 to -0.002
ETDRS9 perfusion, inner superior	Month 3-Month 1	-1.279	-2.43 to -0.13
ETDRS9 perfusion, outer superior	Month 3-Month 1	0.956	0.10 to 1.82
ETDRS9 perfusion, inner temporal	Month 1-Baseline	-1.221	-2.33 to -0.11
ETDRS9 perfusion, outer nasal	Month 6-Month 3	1.279	0.10 to 2.46
ETDRS9 perfusion, inner superior	Month 3-Baseline	-1.324	-2.59 to -0.05

Differences are model-based GEE contrasts expressed in native units: percentage points for ETDRS9 perfusion, micrometers for ETDRS retinal thickness, and dimensionless units for FAZ circularity index. The 95% confidence interval is the Wald interval (estimate $\pm$ 1.96 $\times$ SE). None of these contrasts remained significant after within-endpoint Holm or global FDR correction.

ETDRS, Early Treatment Diabetic Retinopathy Study; GEE, generalized estimating equation; FDR, false-discovery rate; FAZ, foveal avascular zone; CI, confidence interval; SE, standard error.

DISCUSSION

In this prospective, longitudinal bilateral-eye study, no retinal structural or OCTA microvascular changes that survived correction for multiple testing were detected over 6 months of oral isotretinoin therapy. A few endpoint-level and pairwise comparisons were nominally significant, but these findings were not robust after FDR or Holm adjustments. The most cautious interpretation is that the observed sectoral changes represent small longitudinal fluctuations rather than reproducible evidence of treatment-related retinal microvascular toxicity. We emphasise that this is a statement about what was detected, not a demonstration that no effect exists; the distinction matters in a study of this size and is developed below.

These findings are broadly aligned with OCT studies that reported no clinically meaningful short-term change in central macular thickness, choroidal thickness, or retinal nerve fiber layer parameters during isotretinoin treatment.^{7,9} They do not exclude the possibility of subtle changes in selected patients or specific vascular compartments, because choroidal vascular shifts and OCTA-derived signals have been reported in other cohorts.^{10,11} Differences in imaging platform, segmentation algorithm, scan-quality threshold, cumulative dose, treatment duration, and patient selection may explain some of the heterogeneity across studies.

The patient-clustered GEE approach is important in this setting. Treating two eyes from the same patient as independent can underestimate standard errors and overstate statistical significance. The models used in this study account for bilateral-eye and repeated-visit correlations while adjusting for age, sex, and eye laterality. Multiplicity control is also central because OCT/OCTA devices generate multiple, correlated structural and perfusion endpoints; interpretation based solely on nominal *P* values would overemphasize isolated sectoral findings.

Statistical significance and clinical relevance are separate questions, and it is worth stating what the observed magnitudes would mean if they were real. The largest nominal

perfusion contrast was 1.34 percentage points in vessel area density, observed against a sectoral baseline of roughly 40 to 53 percentage points, which corresponds to a relative change of approximately 3%. The largest retinal thickness contrast was 3.2 μ m for a sectoral baseline above 330 μ m; this value is close to 1% and is below the test-retest repeatability generally reported for spectral-domain OCT thickness measurements. The FAZ circularity change of 0.02 falls within the visit-to-visit variability of that index. None of these approaches the magnitude at which retinal perfusion loss becomes clinically visible or alters management, and none was accompanied by a change in visual acuity or the structural parameters that would ordinarily accompany true ischemic remodelling. The upper confidence bounds reported above are the more informative quantities: they indicate that any change escaping detection in this cohort would itself have been small.

The image-quality rule is clinically relevant. Motion, segmentation error, projection artifact, and low signal can distort OCTA-derived parameters.^{5,6} Requiring an image-quality index of at least 0.70 reduces the likelihood that apparent temporal changes reflect acquisition noise. Nonetheless, device-derived perfusion measures should be interpreted in relation to the scan protocol, segmentation reliability, and the absence of an untreated control group.

Study Limitations

The study has several limitations; two merit particular consideration. First, there was no parallel control group of untreated acne patients. Every participant received isotretinoin; therefore, the design cannot separate a treatment effect from seasonal, diurnal, developmental, or device-related drift over the same 6 months. In a young cohort followed for a calendar year, such drift is non-negligible, and its absence from our estimates reflects an assumption rather than an observation. A concurrent untreated acne group measured on the same schedule would have made the comparison internal and would have been the single change most likely to strengthen a future study. Second, axial length and refractive error were

not recorded. OCTA measurements are scaled by ocular magnification: longer eyes yield systematically larger apparent FAZ dimensions and lower apparent vessel density unless a magnification correction is applied. Because our analysis is longitudinal and within-eye, magnification is constant within each patient and cannot generate a spurious time effect; therefore, the primary comparison is protected. What is lost is the ability to interpret absolute values, to compare them with cohorts of different refractive composition, or to test whether myopic eyes respond differently. Additionally, the sample was small and predominantly composed of young participants. No a priori power calculation was available; therefore, the absence of an FDR-confirmed change should be interpreted as indicating that no change was detected rather than as proof that none occurred. The complete-case design may introduce selection bias because 20 of the 54 enrolled patients did not complete all visits and therefore were excluded. Individual cumulative isotretinoin dose, acne severity, and diurnal variation in perfusion measurements were not available in the analytic dataset; therefore, a dose-response relationship could not be examined. Because no single primary OCTA endpoint was prespecified, the study should be considered exploratory.

CONCLUSION

Using a longitudinal, patient-clustered analytical framework, this prospective study detected no FDR-confirmed retinal structural or OCTA microvascular changes over 6 months of oral isotretinoin therapy. The sectoral fluctuations observed were small and did not withstand correction for multiple comparisons. Within the limits of sample size, follow-up, and imaging protocol, the study provides no reproducible OCT/OCTA evidence that oral isotretinoin alters retinal microvascular parameters during treatment, but remains unable to exclude effects smaller than the bounds reported above.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (meeting date: 26.04.2021, meeting number: 06, decision number: 06/05, official document number: E-21142744-804.99-77544, 04.05.2021).

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

Footnotes

Author Contributions:

Concept Design – A.M.S., E.İ.; Data Collection or Processing – A.M.S., M.G.K.; Analysis or Interpretation – E.İ., M.G.K.; Writing, Reviewing and Editing – A.M.S., E.İ., M.G.K.

Declaration of Interests: The authors declare no conflict of interest.

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