Analysis of the Identification Value and Quadrant Characteristics of Iris OCTA Blood Flow Quantification in Type 2 Diabetic Neovascular Glaucoma

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Abstract of Research Protocol

Project Title	Quantitative Analysis of Iris Blood Flow via Iris OCTA for Identifying Neovascular Glaucoma in Type 2 Diabetes Mellitus and Its Quadrant Characteristics
Research Objective	This study adopts Optical Coherence Tomography Angiography (OCTA) to quantitatively analyze the iris blood flow fraction at six quadrants of the pupillary margin among four groups: healthy subjects, patients with Type 2 Diabetes Mellitus (T2DM) without fundus lesions, T2DM patients complicated with Diabetic Retinopathy (DR), and T2DM patients suffering from both DR and Neovascular Glaucoma (NVG). It systematically explores the microcirculatory characteristics of the iris across progressive stages of ocular complications in T2DM, identifies gender heterogeneity and quadrant specificity of inter-group blood flow differences, and provides objective imaging evidence for clinical evaluation of diabetic ocular complications.
Research Hypothesis	Significant differences exist in pupillary margin iris microcirculation among T2DM patients at distinct stages of ocular complications, especially those with T2DM-related neovascular glaucoma. The iris blood flow fraction quantified by OCTA can reflect pathological changes associated with disease progression, and such differences present quadrant specificity or gender heterogeneity.
Study Design	Cross-sectional study
Study Participants	**Inclusion Criteria** - Aged ⩾18 years of any gender; with or without T2DM and its associated ocular complications; clear consciousness and ability to cooperate with iris OCTA examination (able to stabilize the head to acquire interpretable images). Voluntarily participate in this study and sign written

	informed consent. - No history of intraocular surgery on the examined eye (including cataract surgery, glaucoma filtering surgery, retinal surgery, etc.). - General physical conditions: no acute disease episodes within the past 3 months (e.g., acute myocardial infarction, stroke, diabetic ketoacidosis); no severe hepatic or renal dysfunction (ALT and AST ≤ 2 times the upper limit of normal; serum creatinine ≤ 133 μmol/L). **Exclusion Criteria** - The examined eye has other ocular disorders that may interfere with imaging results besides the diseases specified in Inclusion Criterion 1 (e.g., uveitis, retinal vein occlusion, high myopic fundus lesions); invasive ocular treatments (e.g., retinal laser photocoagulation, intravitreal injection) administered to the examined eye within the past 6 months. - Complicated with Type 1 diabetes, gestational diabetes or other specific types of diabetes; with autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus) or hematological diseases (e.g., leukemia, aplastic anemia); administered medications interfering with ocular microcirculation (e.g., alprostadil, nitrates) within the past 3 months and unable to discontinue such medications. - Severe head tremor and inability to maintain a stable position during examination, resulting in substandard OCTA image quality (interpretable area < 80%); presence of mental disorders or cognitive impairment that prevent understanding and cooperation with examination procedures.
Diagnostic Criteria	1.**Normal Control Group**: No history of diabetes mellitus (fasting blood glucose < 6.1 mmol/L, 2-hour postprandial blood glucose < 7.8 mmol/L, glycated hemoglobin < 5.7%);

	no ocular diseases that may affect examination results (e.g., glaucoma, retinal vein occlusion) confirmed by fundus examination (fundus photography + optical coherence tomography/OCT). 2. **T2DM Non-fundus Lesion Group**: Meeting the 1999 WHO diagnostic criteria for Type 2 Diabetes Mellitus (T2DM) (fasting blood glucose ≥ 7.0 mmol/L, or 2-hour postprandial blood glucose ≥ 11.1 mmol/L, or glycated hemoglobin ≥ 6.5%) with a T2DM disease duration of ≥ 1 year; no diabetic retinopathy (DR) (no apparent retinal lesions on DR staging) and no other ocular diseases interfering with examination results confirmed by fundus examination. 3. **T2DM Fundus Lesion Group**: Meeting the 1999 WHO diagnostic criteria for T2DM with a disease duration of ≥ 1 year; confirmed non-proliferative diabetic retinopathy (NPDR, mild to severe stage on DR staging) via fundus examination (fundus fluorescein angiography/FFA supplemented when necessary), with no other ocular diseases affecting examination results. 4.**T2DM Neovascular Glaucoma Group**: Meeting the 1999 WHO diagnostic criteria for T2DM with a disease duration of ≥ 1 year; confirmed proliferative diabetic retinopathy (PDR) and definitive diagnosis of T2DM-related neovascular glaucoma (NVG) via fundus examination, with no other ocular diseases interfering with examination results.
Sample Size and Calculation Basis	This is a cross-sectional study aiming to compare the differences in iris OCTA blood flow fraction among four groups (normal control group, T2DM non-fundus lesion group, T2DM fundus lesion group, and T2DM-related NVG group), and to clarify the correlation between different disease states and iris microcirculatory

	characteristics. Based on relevant published studies on iris OCTA and preliminary experimental data of this study, the mean iris OCTA blood flow fraction of the normal control group is approximately (50.3±8.27). The minimum clinically significant difference (δ) in blood flow fraction between the NVG group and the normal control group was set as 4.0 points, and the overall standard deviation (σ) of iris blood flow fraction across the four groups was uniformly set as 8.5 points (combined data from literature and preliminary experiments). The effect size *f* calculated by the one-way ANOVA formula was approximately 0.24. With a two-tailed α of 0.05 and a statistical power (1-β) of 0.8, PASS 2021 software calculation showed that the basic required sample size for each group was 18 cases. Considering the low clinical incidence of T2DM-related NVG and the difficulty of patient recruitment, an unequal proportional sample size design was adopted in this study: the minimum effective sample size ensuring the efficacy of inter-group comparison was set for the NVG group, while the sample sizes of the other three groups were appropriately increased to compensate for the overall test efficacy. A 15% dropout rate and unqualified image rate was preset. Finally, the planned enrollment size was 28 cases in the normal control group, 28 cases in the T2DM non-fundus lesion group, 28 cases in the T2DM fundus lesion group, and 12 cases in the T2DM-related NVG group, with a total of 96 cases. Verified by PASS 2021 software, this unequal sample size design achieved an overall statistical power of 81.2% for one-way ANOVA, which meets the preset statistical requirements.
Major Exposure Factors	N/A

Outcome Variables	OCTA-quantified blood flow fractions of the iris pupillary margin at six quadrants (superior, superotemporal, inferotemporal, inferior, inferonasal, superonasal).
Follow-up Plan	N/A
Sample Collection	No
Non-medical Examination Purposes	No
Statistical Methods	Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) software. Descriptive statistics were first conducted for iris blood flow fractions of each group, including mean value, standard deviation, extreme values, and missing value proportion, with complete case analysis adopted to handle missing data. Before inter-group comparison, the Shapiro-Wilk test was used for normality testing, and Levene's test for homogeneity of variance. Parallelism tests were performed to verify the interaction effects of covariates (age, gender) with grouping to confirm the applicability of analysis of covariance (ANCOVA). One-way analysis of variance (ANOVA) was applied to compare the differences in iris blood flow fractions among the four groups if all covariates passed the parallelism test. If the parallelism test failed, stratified analysis based on unqualified covariates combined with ANCOVA was performed. Welch's test or Brown-Forsythe test was adopted for correction when the homogeneity of variance assumption was violated. For pairwise multiple inter-group comparisons, the LSD test was used for homogeneous variance, and Tamhane's T2 test for heterogeneous variance. For repeated measurement data of six iris quadrants, Box's test for equality of covariance matrices and Mauchly's sphericity test were conducted to verify the preconditions for analysis. Two-way

	repeated-measures ANCOVA was performed to analyze the main effects and interaction effects of iris quadrants, followed by pairwise inter-group comparisons. A two-tailed P value < 0.05 was considered statistically significant in this study.
Self-evaluation of Research Risks	**Low-risk study:** This is a cross-sectional observational study. Only routine non-invasive, non-radiative ocular OCTA examination data and basic clinical information of subjects are collected for statistical analysis throughout the study. No experimental intervention is applied, and the routine clinical diagnosis and treatment of subjects will not be changed, resulting in no physical injury risk related to the study. All subject information is de-identified, desensitized and encrypted for storage throughout the research process, with fully controllable privacy risks. No fees are charged to subjects, and no additional medical burden or psychological distress is caused. The research results can provide objective references for the clinical evaluation of diabetic ocular complications, and the potential benefits far outweigh the negligible minimal risks.

1. Research Background

Type 2 diabetes mellitus (T2DM) is a major global chronic non-communicable disease. China has the world's largest number of T2DM patients, with the prevalence of diabetes among adults exceeding 11%. Diabetic ocular complications represent the leading cause of irreversible blindness in working-age populations. Among them, diabetic retinopathy (DR) and neovascular glaucoma (NVG) are the most severe ocular outcomes of T2DM. They not only severely impair visual function and reduce patients' quality of life but also impose a heavy medical burden on families and society.

Ocular microcirculation disturbance is the core pathological basis for the occurrence and progression of T2DM-related ocular complications. Iris microcirculation abnormalities and iris neovascularization are hallmark changes of advanced DR and the initiating factors of NVG pathogenesis. These microvascular alterations usually occur earlier than clinically visible neovascularization and elevated intraocular pressure, serving as critical targets for disease progression assessment and therapeutic efficacy monitoring.

Optical coherence tomography angiography (OCTA) is a rapidly developing non-invasive ocular microcirculation imaging technology in recent years. It features non-radiativity, non-mydriasis, high resolution, and quantitative detection capabilities, overcoming the limitations of traditional fundus fluorescein angiography which is invasive and incapable of precise quantitative evaluation of microperfusion. OCTA has been gradually applied to microcirculation assessment in ophthalmic diseases.

Current domestic and international OCTA studies on diabetic ocular microcirculation mainly focus on blood flow changes in the macular and peripapillary regions, while systematic research on iris microcirculation remains insufficient. First, most existing studies are cross-sectional observations of a single disease stage, lacking full-gradient comparison across healthy individuals, T2DM patients without fundus lesions, T2DM patients with DR, and T2DM patients with NVG. Therefore, the dynamic pattern of iris perfusion changes with the progression of diabetic ocular complications cannot be clarified. Second, studies on the spatial distribution characteristics of iris blood flow (quadrant-specific sensitivity) and population heterogeneity (e.g., gender modification effects on diabetic iris microcirculatory changes) are inadequate, making it difficult to screen specific target regions for clinical disease evaluation. Third, quantitative OCTA

analysis of iris blood flow changes before and after anti-vascular endothelial growth factor (anti-VEGF) treatment in NVG patients is scarce, failing to provide objective imaging indicators for therapeutic efficacy monitoring.

Accordingly, this study adopts a cross-sectional observational design and enrolls four groups of subjects with different disease statuses. OCTA is performed to quantitatively measure iris blood flow fraction at six pupillary margin quadrants. The study systematically analyzes iris microcirculatory characteristics at different progressive stages of T2DM and its ocular complications, clarifies the gender heterogeneity and quadrant specificity of inter-group differences, and explores iris blood flow alterations in NVG patients before and after anti-VEGF treatment. This study has solid theoretical and clinical significance. Theoretically, it supplements evidence on full-course iris microcirculatory changes in T2DM ocular complications and improves the understanding of the pathological mechanisms of diabetic ocular microvascular injury. Clinically, it provides non-invasive, quantifiable imaging biomarkers for disease stratification, progression risk early warning, and anti-VEGF efficacy monitoring in diabetic ocular complications, supporting early identification of high-risk patients and optimization of individualized diagnosis and treatment strategies.

2. Research Questions and Objectives

This study aims to adopt optical coherence tomography angiography (OCTA) to quantitatively analyze the pupillary margin iris blood flow fraction at six quadrants among healthy individuals, T2DM patients without fundus lesions, T2DM patients with diabetic retinopathy (DR), and T2DM patients complicated with DR and neovascular glaucoma (NVG). It systematically explores the iris microcirculatory characteristics across different stages of T2DM ocular complications, clarifies the gender heterogeneity and quadrant specificity of inter-group blood flow differences, and provides objective imaging evidence for the clinical evaluation of diabetic ocular complications.

3. Research Methods

3.1 Study Design

This is a cross-sectional study. Subjects with or without T2DM and its associated ocular complications were enrolled and divided into four groups according to disease status: normal control group, T2DM non-fundus lesion group, T2DM fundus lesion group, and

T2DM-related neovascular glaucoma group. OCTA was used to quantitatively analyze the iris blood flow fraction at six pupillary margin quadrants (superior, superotemporal, inferotemporal, inferior, inferonasal, and superonasal).

3.2 Research Setting and Participants

Inclusion Criteria

(1) Aged ≥ 18 years, regardless of gender; with or without T2DM and related ocular complications; clear consciousness and capable of cooperating with iris OCTA examination (stable head posture to ensure qualified and interpretable images);

(2) Voluntary participation with signed written informed consent;

(3) No history of intraocular surgery on the examined eye (e.g., cataract surgery, glaucoma filtering surgery, retinal surgery);

(4) Good general systemic condition: no acute disease episodes (e.g., acute myocardial infarction, stroke, diabetic ketoacidosis) within the past 3 months; no severe hepatic or renal dysfunction (ALT and AST ≤ 2 times the upper limit of normal; serum creatinine ≤ 133 μmol/L).

Exclusion Criteria

(1) The examined eye has other ocular diseases that may interfere with imaging results (e.g., uveitis, retinal vein occlusion, high myopic fundus lesions); received invasive ocular treatments (e.g., retinal laser photocoagulation, intravitreal injection) within the past 6 months;

(2) Complicated with type 1 diabetes, gestational diabetes, or other specific types of diabetes; with autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus) or hematological diseases (e.g., leukemia, aplastic anemia); received medications affecting ocular microcirculation (e.g., alprostadil, nitrates) within the past 3 months and unable to discontinue medication;

(3) Unstable head posture with obvious tremor during examination leading to substandard OCTA images (interpretable area < 80%); presence of mental disorders or cognitive dysfunction that affects examination cooperation.

Subject Information

(1) Baseline information: age, gender, examined eye, medical history of T2DM and related ocular lesions;

(2) Imaging examination: iris optical coherence tomography angiography (OCTA);

(3) Recruitment procedure: research responsibilities were clearly defined for team members. Recruitment was conducted through online media, outpatient and inpatient screening, hospital referral, patient database screening, and patient health education activities. The recruitment period was from March 12, 2026, to April 30, 2026.

Subject Termination and Withdrawal Criteria

(1) Poor examination compliance and failure to complete required examinations;

(2) Occurrence of adverse events that may endanger the subject’s health.

3.3 Research Variables and Measurements

OCTA-quantified iris blood flow fractions at six pupillary margin quadrants (superior,

superotemporal, inferotemporal, inferior, inferonasal, superonasal).

3.4 Research Outcomes

Significant differences in pupillary margin iris microcirculation exist among T2DM patients at different ocular complication stages, especially in patients with T2DM-related NVG. OCTA-quantified iris blood flow fraction can reflect pathological changes associated with disease progression, and such differences present quadrant specificity.

3.5 Sample Size Calculation

This cross-sectional study aimed to compare iris OCTA blood flow fractions among four groups and clarify the association between disease status and iris microcirculatory characteristics. Based on published OCTA studies and preliminary experimental data, the mean iris blood flow fraction of the normal control group was 50.3 ± 8.27. The minimum clinically significant difference (δ) between the NVG group and the normal group was set at 4.0, and the overall standard deviation (σ) was set at 8.5. The calculated effect size f was approximately 0.24. With a two-tailed α of 0.05 and a statistical power (1- β) of 0.8, PASS 2021 software indicated a basic sample size of 18 cases per group.

Considering the low clinical incidence of NVG and recruitment difficulties, an unequal sample size design was adopted. The NVG group retained the minimum effective sample size to ensure statistical efficacy, while the other three groups had enlarged sample sizes to compensate for overall test power. A 15% dropout and unqualified image rate was preset. Finally, 28 cases were enrolled in the normal control group, T2DM non-fundus lesion group, and T2DM fundus lesion group respectively, and 12 cases in the NVG group, with a total of 96 cases. PASS 2021 verification confirmed that the overall statistical power reached 81.2%, meeting the statistical requirements.

3.6 Data Collection and Management

OCTA examination was performed to acquire iris blood flow fractions at six pupillary quadrants. SPSS 20.0 software was used for statistical analysis.

3.7 Statistical Analysis Methods

SPSS software was used for statistical analysis. Descriptive statistics (mean, standard deviation, extreme values, and missing rate) were performed for all groups, and complete case analysis was used to handle missing data. Before inter-group comparison, the Shapiro-Wilk test was used for normality testing, Levene’s test for homogeneity of variance, and parallelism tests were conducted to verify the interaction between covariates (age, gender) and grouping to determine the feasibility of ANCOVA.

One-way ANOVA was applied for inter-group comparison if all covariates passed the parallelism test; otherwise, stratified ANCOVA was performed based on unqualified covariates. Welch’s test or Brown-Forsythe test was used for correction when variance heterogeneity existed. For pairwise comparisons, the LSD test was used for homogeneous variance, and Tamhane’s T2 test for heterogeneous variance.

For repeated measurement data of six iris quadrants, Box’s test for covariance matrix

equality and Mauchly’s sphericity test were performed prior to analysis. Two-way repeated-measures ANCOVA was adopted to analyze the main effects and interaction effects of iris quadrants and conduct pairwise inter-group comparisons. A P value < 0.05 was defined as statistically significant.

3.8 Quality Control

The study was strictly implemented in accordance with the research protocol. The study protocol, all revisions, and informed consent forms were approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) before study initiation.

All serious protocol violations that may significantly affect patient safety, physical and mental integrity, or the scientific validity of the study must be reported immediately to the research team.

All research personnel hold qualified professional credentials, adequate educational backgrounds, professional training, and practical experience. Researchers with disciplinary sanctions, scientific misconduct, or academic fraud (e.g., revoked medical licenses) were excluded from this study.

4. Feasibility Analysis

(1) OCTA is a mature, widely applied non-invasive imaging technique in ophthalmic clinical practice and research. The quantitative iris blood flow detection method is standardized and stable. No experimental intervention is applied, ensuring high safety and operability.

(2) The research team has rich experience in the diagnosis, treatment, and clinical research of diabetic ocular complications. Professional imaging technicians are proficient in standardized OCTA examination, and all required equipment is fully equipped.

(3) The Department of Ophthalmology, Qilu Hospital of Shandong University, is a National Key Clinical Specialty and Shandong Provincial Key Clinical Specialty. It has abundant target patient resources and smooth recruitment channels, ensuring smooth implementation of the project.

5. Research Schedule

May 2026 – July 2026: Project initiation, patient enrollment, and data collection

August 2026: Data sorting and statistical analysis

September 2026 – December 2026: In-depth data analysis, manuscript writing and

publication

6. References

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