Nicotinamide Riboside as a Neuroprotective Therapy for Glaucoma Study Protocol for a Randomized, Double-blind, Placebo-control Trial

Christopher Kai-shun Leung (✉ cksleung@gmail.com)

Chinese University of Hong Kong <https://orcid.org/0000-0003-4862-777X>

Seraph Tianmin Ren

The Chinese University of Hong Kong

Poemen Pui-man Chan

The Chinese University of Hong Kong

Kelvin H Wan

The Chinese University of Hong Kong

Aziz Kam

The Chinese University of Hong Kong

Gilda Lai

The Chinese University of Hong Kong

Vivian Chiu

The Chinese University of Hong Kong

Match Ko

University of Hong Kong

Cedric Yiu

HK PolyU: The Hong Kong Polytechnic University

Marco Yu

Singapore Eye Research Institute

Study protocol

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Abstract

Background Whereas lowering the intraocular pressure (IOP) can slow optic nerve degeneration in glaucoma, many patients with glaucoma continue to develop progressive loss in vision despite significant reduction in IOP. No treatment has been shown to be effective for neuroprotection in glaucoma. We set out to conduct a randomized controlled trial to investigate whether nicotinamide riboside (NR), a nicotinamide adenine dinucleotide precursor, is effective to slow optic nerve degeneration in patients with primary open-angle glaucoma (POAG). We hypothesize that patients treated with NR have a slower rate of progressive retinal nerve fiber layer (RNFL) thinning compared with those treated with placebo.

Methods This is a randomized, double blind, placebo controlled, parallel group, multi-center study including 125 patients with POAG. Patients will be randomized to receive 300mg NR or placebo for 24 months. Clinical examination, optical coherence tomography imaging of the RNFL, and visual field (VF) test will be performed at the baseline, 1 month, 4 months, and then at 2-month intervals until 24 months. The primary outcome measure is the rate of RNFL thinning measured over 24 months. The secondary outcome measures include (1) time to VF progression, (2) time to progressive RNFL/ganglion cell inner plexiform layer (GCIPL) thinning, and (3) the rate of change of VF sensitivity over 24 months (to investigate neuroprotection) and 1 month (to investigate neuroenhancement). The rates of RNFL thinning and VF sensitivity decline between treatment groups will be compared with linear mixed modeling. Survival analysis will be performed to compare the differences in time from baseline to VF progression and time from baseline to progressive RNFL/GCIPL thinning between treatment groups using Cox proportional hazards models.

Discussion Outcome measures in glaucoma neuroprotection trials have been predicated on detection of VF progression, which may take years to develop and confirm. In addition to addressing whether NR has a neuroprotective/neuroenhancement effect in glaucoma patients, this study will demonstrate the feasibility of studying neuroprotection in a relatively short trial period (24 months) by comparing the rates of progressive RNFL thinning, a more reproducible and objective outcome measure compared with VF endpoints, between treatment groups.

Introduction

Background and rationale

Glaucoma, characterized by progressive degeneration of the optic nerve or the axons of retinal ganglion cells, is the leading cause of irreversible blindness worldwide with 76 million patients worldwide in 2020.^{1,2} Although lowering the intraocular pressure (IOP) can decrease the rate of visual field (VF) sensitivity worsening, many patients with glaucoma continue to show progressive loss in vision despite significant reduction in IOP.^{3,4} The need to investigate treatment to protect the optic nerve other than IOP reduction is pressing. At present, there is no neuroprotective therapy approved for treatment of glaucoma.

Nicotinamide riboside (NR) is a precursor of nicotinamide adenine dinucleotide (NAD), which is a key cofactor in redox metabolism including glycolysis, citric acid cycle, and oxidation phosphorylation as well as in non-redox cell signaling and gene regulation.⁵⁻⁷ Increasing evidence has shown that axon degeneration is associated with a decline in NAD and that supplementation of NAD or NAD precursors is effective to delay axon degeneration.⁷⁻¹⁰ In an experimental study, Williams and colleagues demonstrated oral administration of nicotinamide was able to increase the levels of NAD in the retina and protect retinal ganglion cells from degeneration in a mouse model of glaucoma.¹¹ Remarkably, 93% of eyes did not develop glaucoma despite elevated IOP in animals treated with a high dose of oral nicotinamide (2000mg/kg/day). However, high dose nicotinamide can induce hepatotoxicity and painful flushing.¹² Nicotinamide riboside, by contrast, is more orally bioavailable than nicotinamide to increase tissue levels of NAD.⁶ It is a well-tolerated NAD precursor with a high safety profile.^{13,14} Although oral administration of NAD precursors affords protection against glaucoma and neurodegenerative diseases in animal models, lost in translation to clinical practice is common.^{15,16} A randomized controlled trial is needed to inform whether NR can protect the integrity of optic nerve in patients with glaucoma.

Objectives

The study aims to investigate if oral supplementation of NR is effective to decrease the rate of progressive retinal nerve fiber layer (RNFL) thinning in glaucoma.

Trial design

This study is a double-blind, parallel group (1:1), randomized controlled trial. The trial will randomize 125 patients with primary open-angle glaucoma (POAG) with evidence of progressive RNFL thinning or ganglion cell inner plexiform layer (GCIPL) thinning without visual field (VF) progression in at least one eye to receive NR 300 mg/day or placebo. Patients will be followed up at 1 month, 4 months, and then every 2 months until 24 months for comparisons of the rate of RNFL thinning and the rate of change of VF sensitivity between treatment groups. The same IOP-lowering regimen as prescribed before study recruitment will be provided throughout the 24-month study period unless a safety end-point (described below) is reached. Change in VF sensitivity at 1 month will be compared between treatment groups to investigate neuroenhancement. We hypothesize that patients treated with NR have a slower rate of RNFL thinning and a slower rate of VF sensitivity decline compared with those treated with placebo over 24 months and that an improvement in VF sensitivity might be detected in the NR-treated group at 1 month. The study flow chart is shown in **Figure 1**.

Methods: Participants, Interventions And Outcomes

Study setting

Patients with primary open-angle glaucoma will be consecutively recruited from Chinese University of Hong Kong Eye Clinic, Hong Kong Eye Hospital, and Prince of Wales Hospital eye clinic.

Eligibility criteria

Inclusion criteria are as follows: age ≥ 18 years; best corrected VA $\geq 20/40$; IOP ≤ 28 mmHg; and evidence of progressive RNFL/GCIPL thinning detected by Guided Progression Analysis (GPA) or Trend-based Progression Analysis (TPA) in one or both eyes.¹⁷⁻²⁰ Exclusion criteria are as follows: pathological myopia; diseases that may cause visual field loss or optic disc abnormalities other than glaucoma; inability to perform reliable visual field; suboptimal quality of OCT images; diabetic retinopathy/maculopathy; and a history of abnormal liver function within 12 months.

Who will take informed consent?

The principal investigator will take informed consent after explaining the potential benefits and downsides of participation, the details of clinical investigations performed during the study follow-up, and the right to withdraw from the study. Patients will be given at least one week to consider the participation and raise any questions at their concern.

Additional consent provisions for collection and use of participant data and biological specimens

Not applicable

Interventions

Explanation for the choice of comparators

Patients randomized to the control arm will be provided a placebo containing corn starch. The placebo capsule has identical color, appearance, and packaging as the capsule containing NR.

Intervention description

During the 24-month study period, patients randomized to the NR treatment group will have oral administration of 3 capsules of NR every morning, each containing 100mg NR; patients randomized to the controlled group will have oral administration of 3 capsules of placebo every morning. Patients will be

followed up at 1 month, 4 months, and then every 2 months for clinical examination, VF testing, and OCT imaging of the RNFL.

Criteria for discontinuing or modifying allocated interventions

The safety endpoints are: (1) development of visual field progression (defined by the Early Manifest Glaucoma Trial criteria);²¹ (2) decrease in visual acuity ≥ 2 lines; and (3) IOP > 30 mmHg on 2 consecutive visits. Patients will exit the study and receive appropriate treatment if any of the safety end-points is reached. Patients are allowed to withdraw from the study for any reasons at any time.

Strategies to improve adherence to interventions

The capsules (NR or placebo) are packed in weekly packets (each packet contains 21 capsules – 3 capsules per day for 7 days) labelled with patient's name. Patients are requested to bring back the packets at each follow-up visit. Capsules left in the packets will be counted to check and remind patients' compliance to the treatment.

Relevant concomitant care permitted or prohibited during the trial

Patients will continue the existing IOP-lowering regimen as prescribed before study enrollment. No additional IOP-lowering intervention will be provided unless a safety end-point is reached (i.e. detection of VF progression; decrease in visual acuity ≥ 2 lines; or IOP > 30 mmHg on 2 consecutive visits). They are allowed to take other medications for medical conditions during the trial. Details of the medications used during the study period will be recorded and reported.

Provisions for post-trial care

The no-observed-adverse-effect-level of NR was 300 mg/kg/day.¹⁴ We do not expect to observe any adverse effect with an oral intake of NR at 300 mg/day.

Outcomes

The primary outcome measure is the rate of change of RNFL thickness measured by OCT over 24 months of study follow-up. Secondary outcome measures include (1) time to VF progression, and (2) time to progressive RNFL/GCIPL thinning, and (3) the rate of change of VF sensitivity over 24 months (to

investigate neuroprotection) and 1 month (to investigate neuroenhancement). Change in inner retinal density over the parapapillary region and the macula measured by OCT angiography over 24 months will be evaluated as an exploratory outcome measure.

Participant timeline

Study enrollment started on 9 March 2020 and the expected completion date of recruitment is 8 March 2021. Patients will be follow-up at 1 month, 4 months, and then every 2 months until 24 months for clinical examination, IOP measurement, OCT imaging of the RNFL, and VF testing (**Figure 2**).

Sample size

In our pilot study following 236 glaucoma patients every 4 months for ≥ 3 years, the mean rate of RNFL thinning was $1.2\mu\text{m}/\text{year}$ ($P < 0.001$) for eyes with evidence of progressive RNFL thinning detected by TPA; the slope variance was $1.39(\mu\text{m}/\text{year})^2$ and the residual variance was $3.83\mu\text{m}^2$.²⁰ We aim to detect a difference of at least $0.70\mu\text{m}/\text{year}$ in the rate of RNFL thinning between treatment groups, a rate slightly greater than the mean rate of age-related RNFL thinning, which has been reported to be approximately $0.52\mu\text{m}/\text{year}$.²² Taking reference from the method of sample size calculation for linear mixed modeling to compare the rates of change of a parameter of interest between two treatment groups described by Ard and Edland,²³ 53 patients per study arm are needed for serial RNFL thickness measurements at baseline, 1 month, 4 months, and then every 2 months for 24 months (3 separate measurements will be collected at the baseline, 1-month, and 24-month follow-up visits) with a power of 80% and an alpha of 5%. Assuming a default rate of 15% during study follow-up, a total of 125 patients will be recruited.

Recruitment

Eligibility for study inclusion will be reviewed by the attending ophthalmologists. Eligible patients will be referred to the investigators to cross check if the admittance criteria are met. The principal investigator will then explain the study procedures to the patients. Those who agree to participate in the study will come back on a scheduled visit for baseline investigation.

Assignment of interventions: allocation

Sequence generation

Patients were randomly allocated (1:1) at the baseline visit to receive NR or placebo within 1 month of enrollment. Randomization with minimization by age (< 60 years vs ≥ 60 years), gender (male vs female),

VF MD (<-6 dB vs ≥ -6 dB), spherical equivalent (<-6.0 D vs ≥ -6.0 D), mean IOP measurement over the past 3 years (≤ 21 mmHg vs >21 mmHg), and number of glaucoma medications (0,1,2,3, or ≥ 4) will be performed using an open source computer program MinimPy.²⁴ A random element was introduced in the minimization procedure (i.e. weighted randomization of 0.8) to make the randomization more unpredictable.²⁵

Concealment mechanism

Patients and investigators are unaware of treatment allocations and have no access to the randomization sequence and codes, which are stored in a password-protected file in a computer. The NR and placebo tablets are in identical appearance and uniformly packaged.

Implementation

The principal investigator will enroll participants. Two masked research technicians will generate the allocation sequence with randomization by minimization and assign participants to interventions.

Assignment of interventions: Blinding

Who will be blinded

The attending ophthalmologists, the participants, the outcome assessors, and data analysts will be blinded after assignment to intervention.

Procedure for unblinding if needed

The blinding will be maintained until serious adverse event occurs. The unblinded participant will exit the trial and the ocular and/or medical conditions will be managed accordingly. The management results will be recorded on the clinical report form and reported to the Kowloon Central / Kowloon East Cluster Research Ethics Committee.

Data collection and management

Plans for assessment and collection of outcomes

Patients will receive clinical examination, OCT imaging of the RNFL, and VF testing at baseline, 1 month, 4 months, and then every 2 months for 24 months. Three repeated OCT and VF measurements will be collected at the baseline, 1-month, and 24-month visits.

Clinical examination

Clinical examination includes slit-lamp biomicroscopy of the anterior and posterior segments, and Goldmann applanation tonometry. Two IOP readings will be obtained to calculate the mean. A third reading will be obtained if the difference between the first two is $>2\text{mmHg}$ and the median is recorded. Axial length will be measured with partial coherence laser interferometry (IOLMaster, Carl Zeiss Meditec). Subjective and objective refraction will be performed at the screening and the last follow-up visits. Central corneal thickness will be measured with ultrasound pachymetry. Dilated fundus examination for color optic disc stereophotography will be performed at the screening visit and then yearly.

Optical coherence tomography imaging

The RNFL will be imaged with the Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, USA) for comparison of the rates of RNFL thinning (primary outcome measure) between treatment groups. The RNFL/GCIPL will also be imaged by the Triton OCT (Topcon, Tokyo, Japan) to determine the time to progressive RNFL/GCIPL thinning detected by TPA for an individual eye (secondary outcome measure). RNFL-GCIPL thickness data of individual pixels in serial RNFL/GCIPL thickness maps collected over 24 months will be exported for TPA. The algorithm of TPA has been described.^{17,18,20} In brief, TPA performs pixel-by-pixel linear regression analysis between RNFL/GCIPL thickness and time for evaluation of progressive RNFL/GCIPL thinning after registering and aligning serial OCT scans in corresponding retinal locations of an eye. To minimize type I errors consequential to multiple testing in an eye, the RNFL-GCIPL thickness maps will be condensed from 512×256 pixels to 128×64 superpixels with a false discovery rate (FDR) controlled at 5%. Low test-retest variability of Fourier-domain OCT measurements has been shown.^{26,27} Images with a poor signal-to-noise ratio, motion artifact, poor centration, segmentation error or missing data (e.g. blinking) will be discarded; re-scanning will be performed in the same visit. Peripapillary and macular inner retinal vessel density will be measured with OCT angiography.

Visual field test

Visual field test will be performed with the Humphrey Field Analyzer II 24-2 SITA standard strategy (Carl Zeiss Meditec). A reliable VF test has fixation losses $<20\%$ and false positive rate $<15\%$. Unreliable tests will be repeated on the same day. Visual field progression is identified when three test locations show significant reduction in VF sensitivity greater than the test-retest variability observed on at least two

(possible progression) or three (likely progression) consecutive tests, according to the Early Manifest Glaucoma Trial (EMGT) criteria.²¹

Plans to promote participant retention and complete follow-up

Investigators will phone contact or send text message to remind the participants for follow-up before each study visit. Participants who default a scheduled appointment will be contacted by the investigators to re-arrange another appointment within 2 weeks.

Data management

Clinical data will be collected and recorded by two designated technicians. The data will be stored in password-protected computers. The study will be conducted in compliance with Good Clinical Practices to ensure the rights and well-being of the participants and that the data collected are complete and verifiable from source documents. Data validation is performed with cross-system consistency checks by another technician.

Confidentiality

No personal information will be recorded on the data sheets or electronic data files. A study code will be assigned to each participant. The document containing the information of the study code and the identity of the patient will be kept separate from the study data files and data sheets.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use

There are no plans for collection of biological specimens in this trial.

Statistical methods

Statistical methods for primary and secondary outcomes

(1) The rates of RNFL thinning (primary outcome measure) between treatment groups will be compared with linear mixed modeling after adjusting for covariates including age, axial length, glaucoma severity (baseline RNFL thickness), IOP levels during following-up, OCT signal strength, multiple testing, and

clustering between fellow-eyes. (2) Changes in VF sensitivity (measured in dB) at (A) one month and (B) 24 months between treatment groups will be compared with linear mixed models after adjusting for covariates. (3) Survival analysis will be performed to compare the differences in (A) time from baseline to VF progression (determined by EMGT criteria) and (B) time from baseline to progressive RNFL/GCIPL thinning (determined by TPA) between treatment groups using Cox proportional hazards models with shared-frailty to adjust for clustering between fellow eyes.

Interim analyses

Interim analyses will be performed by a statistician after data collection at (A) 4 months and (B) 12 months. The rates of change of VF sensitivity and RNFL thickness will be compared between treatment groups. The participants and investigators will remain masked to the study groups. The trial steering committee will stop the trial if the rate of VF sensitivity decline and the rate of RNFL thinning were significantly worse in the NR-treated group than the placebo-treated group.

Methods for additional analyses (e.g. subgroup analyses)

Detection of progressive RNFL thinning and VF sensitivity decline has been shown to be more difficult in moderate to advanced glaucoma compared with early glaucoma. Subgroup analysis will be performed to determine if the rates of change of RNFL thickness and VF sensitivity would be different between treatment groups in patients with early glaucoma versus those with moderate to advanced glaucoma. Subgroup analysis will also be performed to investigate if the responses of NR supplementation would be different between old and young patients.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data

Data will be analyzed on an intention-to-treat basis to include all patients who receive the randomized treatment and have at least two follow-up visits. Multiple imputation by chained equations (MICE) algorithm will be applied for estimation of missing data.²⁸

Plans to give access to the full protocol, participant level-data and statistical code

No later than 3 years after the completion of data collection, the protocol and deidentified dataset will be available from the principal investigator upon reasonable request.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee

The coordinating center is located at Hong Kong Eye Hospital. The trial steering committee is directed by the principal investigator and includes two independent researchers and one statistician, who are not part of the same institution as any of the members of the study team. The trial steering committee is responsible to ensure the protocol is implemented as planned, review study progress on a regular basis, and uphold good clinical practice at all times. The coinvestigators, research assistants and technicians of the trial will be responsible for all aspects of logistics and organization of the trial.

Composition of the data monitoring committee, its role and reporting structure

An independent data monitoring committee comprises two independent experts in statistics, who are not part of the same institution as any of the members of the study team. They will be responsible to inspect clinical data collected during the study period, review interim analysis, and report back to the investigators if any action is required.

Adverse event reporting and harms

Any adverse event will be recorded whether they are related to the study or not. A serious adverse event will be reported to the Kowloon Central / Kowloon East Cluster Research Ethics Committee within 24 hours of the event. The principal investigator will be responsible to follow the management of the serious adverse event until resolution or conclusion. The investigators and the trial steering committee will determine whether an adverse event or serious adverse event is related to the study drug.

Frequency and plans for auditing trial conduct

A team of clinical research coordinators independent of the investigators will monitor the data reported in the clinical research forms are complete and accurate, ensure all adverse events and serious adverse events are recorded and reported, and confirm study drugs to be stored and distributed according to the good clinical practice every 3 months.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees)

Any amendment to the protocol will be submitted by the principal investigator to be approved by the Kowloon Central / Kowloon East Cluster Research Ethics Committee and Hong Kong Food and Health Bureau Research Secretariat before implementation.

Dissemination plans

Trial results will be presented at international scientific conferences and published in peer-reviewed scientific journals.

Discussion

Glaucoma neuroprotection trials have been centered on event analysis of VF for evaluation of treatment outcome, which may take 4 to 5 years to complete.^{4,29} We engineered various study designs to enable the detection of a potential treatment effect in 2 years; these include the adoption of (A) a structural (i.e. progressive RNFL thinning) instead of a functional biomarker (e.g. VF progression) as the primary outcome measure, (B) a clustered paradigm to collect multiple measurements at the beginning and at the end of the trial,³⁰ (C) a frequent follow-up schedule at 2-month intervals, and (D) a trend analysis instead of an event analysis to compare the change in RNFL thickness between treatment groups,³¹ as well as (E) the enrollment of progressing (i.e. eyes with progressive RNFL/GCIPL thinning evident in GPA/TPA) instead of stable glaucoma patients.

Daily NR intake at 300mg/day is considered to be safe because 300mg/kg/day represents the no-observed-adverse-effect-level.¹⁴ Whereas many ongoing clinical trials investigate NR at a dosage of ≥ 1000 mg/day, we prescribe NR at 300mg/day because oral administration of NR at 300mg has been shown to attain similar levels of NAD elevation in the blood at 24 hours compared with oral administration of NR at 1000mg in healthy volunteers.⁶

Lowering the IOP is currently the only treatment option for glaucoma patients. Our clinical trial has the potential to demonstrate the feasibility of neuroprotection in glaucoma, which is pivotal to prevent or slow glaucoma blindness in patients with progressive loss in vision despite adequate control of IOP.

Trial status

Study recruitment started on 9 March 2020 and the recruitment completion date is 8 March 2021. Protocol Version 5 (8 Nov 2020).

Abbreviations

EMGT, early manifest glaucoma trial

FDR, false discovery rate

GCIPL, ganglion cell inner plexiform layer

GPA, guided progression analysis

IOP, intraocular pressure

NAD, nicotinamide adenine dinucleotide

NR, nicotinamide riboside

OCT, optical coherence tomography

POAG, primary open-angle glaucoma

RNFL, retinal nerve fiber layer

SITA, Swedish interactive thresholding algorithm

TPA, trend-based progression analysis

VF, visual field

Declarations

Acknowledgements

Not applicable

Authors' contributions

CL is the Principal Investigator; he conceived the study, led the proposal and protocol development. PC, KW, AK, and CL contributed to examination and recruitment of study participants. GL and VC contributed to data acquisition. CY, MY, and CL contributed to study design in statistics. All authors read and approved the final manuscript.

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Availability of data and materials

The Principal Investigator will have access to the final trial dataset

Ethics approval and consent to participate

Hong Kong Hospital Authority Kowloon Central / Kowloon East Cluster Research Ethics Committee approved the study on 16 January 2019 (reference number: KC/KE-18-0098/FR-1). Informed consent will be obtained from all study participants.

Consent for publication

Not applicable.

Competing interests

CL has a patent on Trend-based Progression Analysis (Detection of disease-related retinal nerve fiber layer thinning – U.S. Patent No. US9117121B2). He has received research support in the form of instruments from Carl Zeiss Meditec (Dublin, CA, USA).

Authors' information (optional)

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Figures

Figure 1

Flow chart of study design. POAG: primary open-angle glaucoma; RNFL: retinal nerve fiber layer; GCIPL: ganglion cell inner plexiform layer; GPA: Guided Progression Analysis; TPA: Trend-based Progression Analysis; IOP: intraocular pressure; VF: visual field; OCT: optical coherence tomography; NR: nicotinamide riboside

Study Phase	9-3-2020 to 8-3-2021	9-3-2021 to 8-3-2023	9-3-2023 to 9-9-2023
I. Recruitment			
II. Study Follow-up			
III. Data analysis and reporting			

	Clinical Examination	OCT RNFL-GCIPL Imaging	Perimetry	Remarks
Screening	✓	✓	✓	Identify patients with progressive RNFL-GCIPL thinning by TPA
Baseline	✓	✓	✓	Collect three sets of RNFL-GCIPL thickness and VF MD measurements
Month 1	✓	✓	✓	Collect three sets of RNFL-GCIPL thickness and VF MD measurements
Month 4	✓	✓	✓	
Month 6	✓	✓	✓	
Month 8	✓	✓	✓	
Month 10	✓	✓	✓	
Month 12	✓	✓	✓	
Month 14	✓	✓	✓	
Month 16	✓	✓	✓	
Month 18	✓	✓	✓	
Month 20	✓	✓	✓	
Month 22	✓	✓	✓	
Month 24	✓	✓	✓	Collect three sets of RNFL-GCIPL thickness and VF MD measurements

Figure 2

Study timeline. Clinical examination includes visual acuity measurement, Goldmann applanation tonometry (GAT), slitlamp biomicroscopy of the anterior & posterior segments. OCT: optical coherence tomography; RNFL: retinal nerve fiber layer; GCIPL: ganglion cell inner plexiform layer; TPA: Trend-based Progression Analysis; VF: visual field; MD: mean deviation