MEDICAL UNIVERSITY

"PROF. DR. PARASKEV STOYANOV" - VARNA

Faculty of Medicine

Department of "Eye Diseases and Vision Sciences"

Dr. Konstantina Grigorova Kancheva-Bandramalieva

Place and role of nutritional supplements in the ophthalmology practice

ABSTRACT

ON DISSERTATION PAPER

TO OBTAIN AN EDUCATIONAL AND SCIENTIFIC DEGREE

"DOCTOR"

SCIENTIFIC SPECIALTY "OPHTHALMOLOGY",

CODE 03.01.36

Scientific supervisor

Prof. Dr. Z. Zlatarova, MD, PhD

Official Reviewers:

Art.-cor. Prof. Dr. H. Grupcheva, MD FEBO, FICO (Hon), FBCLA

Varna, 2024

The dissertation contains 173 pages, including 10 tables and 28 figures. 465 literary sources are cited, of which 5 are in Cyrillic and 460 are in Latin. 22 more tables have been added to the annex. 7 chapters are presented, corresponding to the purpose and tasks and meeting the requirements for the layout of the dissertation work.

The dissertation work was discussed and proposed for defense to the departmental council of the Department of Eye Diseases and Visual Sciences at the MU "Prof. Dr. Paraskev Stoyanov" – Varna on 14.10.24.

Scientific council:

External members:

1. Assoc. Prof. Desislava Nikolaeva Koleva-Georgieva, MD, PhD , Medical University-Plovdiv
2. Assoc. Prof. Kalina Zlatkova Trifonova-Slaveykova, MD, PhD, Thracian University-Stara Zagora
3. Prof. Marieta Ivanova Konareva-Kostyaneva, MD, PhD, Medical University-Plovdiv

Internal members:

1. Prof. Christina Grupcheva, MD, PhD, DSc, FEBO, FICO(Hon), FBCLA, FIACLE, Department "Eye diseases and visual sciences" at Medical University Varna
2. Assoc. Prof. Binna Nikolaeva Nencheva, MD, PhD, Department " Optometry and occupational diseases at Medical University-Varna

The official defense of the dissertation work will take place 15.01.25 at an open meeting of the Scientific Jury at the Department of Eye Diseases and Visual Sciences of the University of Varna. The defense materials are available at the Scientific Department of the Medical University - Varna and are published on the website of the Medical University - Varna.

CONTENTS

Abbreviations used

POAG - Primary open-angle glaucoma

AMD - Age-related macular degeneration

AREDS - Age-related eye disease study

RGK - Retinal ganglion cells (Retinal ganglion cells)

IOP - Intraocular pressure

ONH - Optic nerve head

SAP - Standard Automated Perimetry

OCT - Optical Coherence Tomography

SD-OCT - Spectral-domain OCT

GCC - Ganglion cells complex

RNFL - Retinal nerve fiber layer

NFL - Neurofibrillary layer

FLV - focal loss volume

GLV - global loss volume

SITA - Swedish Interactive Threshold Algorithm

TD - Total deviation

PD - Pattern Deviation

GHT - Glaucoma Hemifield Test /Glaucoma Hemifield Test

MD - Mean deviation

PSD - Pattern Standard Deviation

VFI - Visual field index

GPA - Glaucoma Change Probability Maps

QoL - quality of life

VEP - visual evoked potentials

MPOD - Macular Pigment Optical Density

OAS - oral antioxidant supplements

BCACs - blackcurrant anthocyanins

PERG - Pattern electroretinography

mgCIPL - the inner plexiform layer of macular ganglion cells

DHA - docosahexaenoic acid

ERM - epiretinal membrane

Note: The numbering of figures and tables in the abstract corresponds to the numbering in the dissertation.

Note: The team and the principal investigator declare that they have no financial benefit or affiliation with any of the mentioned trademarks of the products used in the study, devices or sites cited.

Chapter I

Introduction

Blindness has been identified as the most severe disability. 80% of blindness is avoidable – 20% preventable and 60% treatable. According to the VISION 2020 global study, 1.1 billion people worldwide live with vision loss. Prognosis shows that vision loss will increase by 55%, or 600 million people, over the next 30 years, mainly due to two factors - an aging population and lifestyle changes. (1)

The global population is prognosed to grow by 25% by 2050, reaching 9.7 billion. By 2050, the number of people over 65 will almost double and those aged 80 or over will triple. This is concerning because the prevalence of vision loss increases with age due to the established association with increased prevalence of cataracts, age-related macular degeneration (AMD) and glaucoma. Not only population growth and aging, but also lifestyles directly affect the progression of vision loss. Increased urbanization and education, more sedentary indoor lifestyles, lower quality food sources and the resulting obesity have contributed to an increase in the prevalence of diabetes and myopia worldwide. (2) This means that the need for health services related to vision loss will increase in the coming years.

In the near future, it will be increasingly necessary to develop new therapeutic strategies to combat the leading causes of blindness, among which, according to VISION 2020, are cataract (15.2 million), followed by glaucoma (3.6 million), under corrected refractive anomaly (2.3 million), AMD (1.8 million) and diabetic retinopathy (0.86 million). (1,2) A possible and easily implemented concomitant therapeutic option would be the administration of dietary supplements with antioxidant and neuroprotective properties.

According to the US National Institutes of Health, nutritional supplements are already being used in ophthalmology practice. Findings from the Age-Related Eye Disease Studies (AREDS and AREDS2) suggest that supplementation with vitamins C and E, lutein and zeaxanthin, copper oxide, beta-carotene, and zinc may slow down the progression of AMD. There is also some evidence that for populations with more limited food resources, the carotenoids lutein/zeaxanthin may be associated with reduced cataract progression, but whether supplementation would be beneficial in such populations requires further study. Vitamin B12 supplements are not recommended for the treatment of cataracts, although there is some evidence that such supplements may slow down or prevent the development of the disease. There is some

limited evidence which suggest that omega-3 dietary supplements may play a role in the management of dry eye, but more research is needed. However, the current data are not so definitive when it comes to the use of dietary supplements in glaucoma. (3)

Almost 8 million people have vision loss due to glaucoma, of these: 3.6 million are blind and 4.2 million have moderate to severe visual impairment. Glaucoma is thus ranked as the second leading cause of blindness and the fourth leading cause of moderate and severe visual impairment, and therefore the most common cause of irreversible blindness and the second most common cause of irreversible moderate and severe visual impairment. (1,2)

Glaucoma as a term unites a group of diseases proceeding with degenerative optic neuropathy, which is characterized by typical structural pathological changes in the disc of the optic nerve and specific functional changes in the visual field. (4) According to the latest 5th edition of the European Glaucoma Society, the definition reads – Primary open-angle glaucoma is a chronic, progressive, irreversible eye disease that potentially leads to blindness, causing damage to the optic disc and neurofibrillary layer, with associated visual field defects. (5)

Nowadays, the fact that glaucoma is a disease with a multifactorial pathology, the consequence of which is apoptosis of the RGC, is obvious. This process occurs as a result of a number of pathogenetic mechanisms including not only increased IOP, but also a violation of autoregulation, deficiency of neurotrophic factors, ischemia, glutamate-induced toxicity, disorders of Ca2+ metabolism, oxidative stress, immunological disorders, etc. Elevated intraocular pressure (IOP) is the most observed risk factor and the only one that is currently treatable. IOP can be lowered by eye drops, laser treatment, and other forms of surgery, but this does not always prevent further degeneration, and visual field loss can progress even in individuals who have never had documented elevated IOP. For these reasons, there is a need to develop additional therapeutic strategies to delay and ideally prevent progressive damage to the RGC in glaucoma. The study of alternative methods of treatment and prevention of disease progression is of increasing interest among researchers. In this regard, neuroprotection appears to be a promising aspect in the fight against this insidious disease. Neuroprotection focuses on improving the survival and function of neurons. Furthermore, beyond neuroprotection, there is a need to develop approaches to aid in the restoration of vision in patients with glaucoma and other forms of optic nerve disease who have already lost vision due to RGC damage.

Loss of vision and changes in the visual field combined with the need for treatment and constant monitoring of disease progression correlate with low quality of life in glaucoma patients. The high frequency, the irreversibility of changes in visual functions and the associated high disability place glaucoma in the group of socially significant diseases.

It is important to develop new treatment strategies for glaucoma patients that stop or slow progression without impairing quality of life. Further clinical studies are needed to prove the place and role of neuroprotection in the management of glaucoma. If more clinical trials with neuroprotectants support the available laboratory evidence for the visual field preservation effect and prevention of glaucoma progression, the treatment paradigm will shift beyond IOP reduction and begin to focus on preserving patient's visual function, both through neuroprotection as well as through ocular hypotension.

Chapter II

Purpose and tasks

The purpose of the study is to investigate and document the use of the dietary supplements **Mielooptik** and **Citizin**, and to analyze the effect of their application as adjunctive therapy in patients with POAG.

To achieve this goal, the following tasks are set:

1. To track the functional and structural changes in the course of the POAG, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who also take the nutritional supplement Mielooptik according to a scheme for a period of 15 months.
2. To follow the functional and structural changes in the course of POAG, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who do not take a nutritional supplement for a period of 15 months.
3. To follow the functional and structural changes in the course of the POAG, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who also take the dietary supplement Citizin according to a scheme for a period of 15 months.

4. To compare the results reporting the progression of functional and structural changes in the three groups, using statistical methods, and to evaluate the effect of the use of nutritional supplements as adjunctive therapy in the treatment of POAG.

The present study received permission from the Research Ethics Committee of MU-Varna to conduct a scientific study on the topic "Influence of nutritional supplements on the progression of primary open-angle glaucoma" - Protocol/Decision No. 103/ 27.05.2021

Chapter III

Material and methods

3.1. Material

In the present paper, a clinical study was carried out. It included 180 eyes of 90 patients (45 women and 45 men) aged 50-75 years with POAG undergoing topical anti-glaucoma therapy. The patients were randomly divided into three groups of 30 people (15 men and 15 women) and followed for a period of 15 months. The IOP values of the respective patients were in the range of 14 - 21 mmHg.

The patients of the first group (GROUP A) took the combined dietary supplement Mielooptik with the following composition: Turmeric (100 mg), Uridine monophosphate (50 mg), Lutein (10 mg), Vitamin B3 (10 mg NE), Vitamin B6 (6 mg), Vitamin B1 (4 mg), Folic acid (400 μg), Vitamin B12 (10 μg).

The patients in the second group (GROUP B) did not take a nutritional supplement.

The patients of the third group (GROUP C) took 2 tablets of Citizin of 250 mg each, which represents the intake of Citicoline 500 mg/24h.

The nutritional supplements were applied in the form of a product approved for usage in Bulgaria:

Citizin (manufacturer - Laboratorios virens SL Spain, importer - Bioshield LTD Bulgaria)

Mielooptik (manufacturer - Plantapol Spain, importer - Naturpharma Bulgaria)

Applied in the standard dosage prescribed by the manufacturer:

2 x 1 tab./day Citizin 250 mg per os

1 ampoule/day Mielooptik per os

Study participants took the supplements for two 6-month periods, with a 3-month interval without taking them, and were followed for a total of 15 months.

Inclusion criteria:

- Primary open-angle glaucoma
- age 50-75 years
- administration of topical antiglaucoma therapy
- IOP values 14-21 mmHg
- signed informed consent to participate in the study

Exclusion criteria:

- best visual acuity with correction < 0.5
- reduced transparency (cataract, hemophthalmus)
- concomitant neurodegenerative diseases or diseases of the posterior eye segment that may affect the results of computer perimetry and optical coherence tomography
- poor quality of optical coherence tomography
- allergy or intolerance to the ingredients of the food supplements used
- refusal to participate in the study

3.2. Methods

Informed consent was obtained from all patients or their relatives after being given a detailed description of the aims of the study and the procedures to be performed.

Patients were matched for age and sex. The duration of the disease and the extent of glaucomatous damage varied between the groups, which the researchers believe gives a more representative sample of patients suffering from POAG.

Before starting the study, all patients underwent a complete ophthalmological examination, including:

- history of eye and general diseases
- visual acuity test with the best correction for each eye separately
- Goldman tonometry
- indirect ophthalmoscopy
- gonioscopy with Goldman lens
- pachymetry
- Standardized Computer Perimeter (SAP)
- Optical Coherence Tomography (OCT)

The same examinations were carried out on the 6th and 15th months from the beginning of the study.

The effect of the application of nutritional supplements was investigated by following the dynamics of the functional and structural changes occurring in the course of the POAG disease using standardized computerized perimetry (SAP) and optical coherence tomography (OCT) for a total period of 15 months, while maintaining IOP within constant limits.

3.2.1. In the present study, **Standardized Computer Perimetry (SAP)** was performed with a **Humphrey Field Analyzer 2 perimeter (Carl Zeiss Meditec).** (Figure 8.)

HFA 2 is the gold standard for the diagnosis and management of glaucoma. It includes various testing strategies including Full Threshold, FastPac, SITA Fast 24-2 and SITA Standard

24-2. SITA adapts to the patient's responses by making optimal use of the information contained in the patient's responses and continuously refines the measurements. This ensures an accurate and insightful analysis of the patient's visual field. HFA2 includes STATPAC-2 statistical software that compares test results to proprietary age norms and glaucoma databases. This allows expert analysis of changes in the patient's visual field over time. Its features include fixation control, Heijl-Krakau blind spot monitor, video eye monitor and gaze tracking. The software has the following features: Single Field Analysis (SFA), Glaucoma Hemifield Test (GHT), Visual Field Index (VFI), Progression Analysis (GPA).

Technical specifications

Maximum range (degrees)	89
Stimulus duration	200 ms
Visual field-testing distance	30 cm
Background lighting	31.5 ASB
Threshold Test Library	24-2, 30-2, 10-2, Macula, 60-4nasal step
Threshold test strategies	SITA Standard, SITA Fast, Full Threshold, FastPac
Stimulus	White on white
Stimulus dimensions	Goldman IV

Figure 8. Examination of the visual field with the Zeiss HFA 740i Visual Field Perimeter

In this case, the research for the study was conducted with stimulus size III, the program used was 30-2 and the Full Threshold strategy. Only reliable perimetric studies were selected for statistical evaluation in the study. Reliability was determined using standard criteria of fixation loss rate below 20%, false positive responses below 33%, and false negative responses below 33%. The mean visual field sensitivity was calculated by the machine software and presented as mean deviation (MD), pattern standard deviation (PSD), and VF index (VFI). In order to determine the effect of dietary supplements on the progression of POAG, in the present work, the global indices MD and PSD were analyzed. Only patients with already diagnosed and followed-up glaucoma were selected, and the procedure to perform SAP was not new to any of the patients.

Perimetric examinations were classified as glaucomatous according to the Hoddap-Parish-Anderson criteria:

- ✓ glaucoma hemifield test – outside the normal limits

- ✓ a combination of three or more non-endpoints in a location typical of a glaucomatous defect, all with decreased pattern deviation graph sensitivity p<5% and at least one with decreased pattern deviation graph sensitivity p<1%
- ✓ PSD of less than 5% in the healthy population

Defects must be confirmed by at least two visual field tests.

The study included patients in different stages of the disease, classified according to Hoddap's simplified classification (EGS 5), namely:

- ✓ early glaucomatous loss MD ≤ - 6 dB
- ✓ moderate glaucomatous loss - 6 dB > MD ≤ - 12 dB
- ✓ advanced glaucomatous loss MD > - 12 dB

3.2.2. **RTVue XR Model Avanti Scanner, Optovue, Version 2018.1.1.63** was used to determine structural changes in the present study with **Optical Coherence Tomography (OCT)**. (Figure 9) It is a latest generation spectral domain SD-OCT offering a full range of anterior and posterior eye segment examinations. It also features the AngioVue™ Imaging System, which allows simultaneous three-dimensional (3D) structural imaging of the retina and generation of en-face blood flow maps. **SharpVue technology** provides extremely detailed B-scans up to 12 mm using 70,000 scans per second, exclusive 2-phase noise reduction, real-time VTRAC tracking, 5 μm tissue resolution and DCI (Deep Choroidal Imaging).

Figure 9. Examination of morphological characteristics with the RTVue XR Model Avanti Scanner, Optovue

Technical Specifications Optovue RTVue XR OCT

OCT camera:	70,000 A-scans / second
Optical resolution: (in tissue) Axial resolution:	5 µm
Scan range:	Axial Image Depth: 2 to 3 mm (depending on the protocol)
Across:	2 mm to 12 mm
Image Size (Retina):	3x3mm, 6x6mm HD, 8x8mm

Image Size (Optical Disc)	4.5 × 4.5 mm HD, 6x6 mm HD
Field of view:	12x9 mm
Minimum pupil diameter	2.5mm
Wavelength of the scanning beam:	λ = 840 nm
Focus adjustment (diopters)	-15D to +20D

Research protocols depend on the eye pathology and the purpose of the research: AngioVue, Retina, Nerve Fiber, Cornea.

For diagnosis and follow-up of structural changes in glaucoma, the Nerve Fiber protocol is used, which covers 3D disc Analysis, ONH Analysis and GCC Analysis. ONH analysis includes measurements of RNFL (Average RNFL, Superior RNFL, Inferior RNFL) and measurements of ONH parameters (Cup/Disc Area Ratio, Cup/Disc V.Ratio, Cup/Disc H. Ratio, Rim Area, Disc Area, Cup Volume). Ganglion cell complex (GCC) analysis covers: Average GCC, Superior GCC, Inferior GCC, and focal volume loss (FLV%) and global volume loss (GLV%) parameters that increase the sensitivity and specificity of GCC analysis.

Optovue's patented algorithms enable accurate assessment of the optic nerve head and retinal nerve fibers. A comparison with a large normative database of ONH parameters is provided. A color-coded RNFL thickness map allows for a quick “first look”. In this thickness map, warm colors (red and orange) correspond to areas of thick RNFL, and cooler blues represent thinner areas.

The ONH profile curves provide a visual representation of the neuroretinal rim and RNFL thickness in a “TSNIT” model: starting with the temporal thickness, progressing to superior, nasal, inferior, and back to temporal for both rim and RNFL thickness. The TSNIT plot is generated by a circle with a diameter of 3.45 mm, which is centered on the ONH. Peripapillary RNFL thickness measurements are reported as 8 sector measurements. The neuroretinal edge curves and RNFL profile are overlaid on the color coding. Based on the reference database,

yellow pixels are considered "borderline" and mean that only 5% of norms fall within this range, and red pixels are considered "outlier" and represent less than 1% of the normative database. Figure 10. is a printout of the ONH analysis.

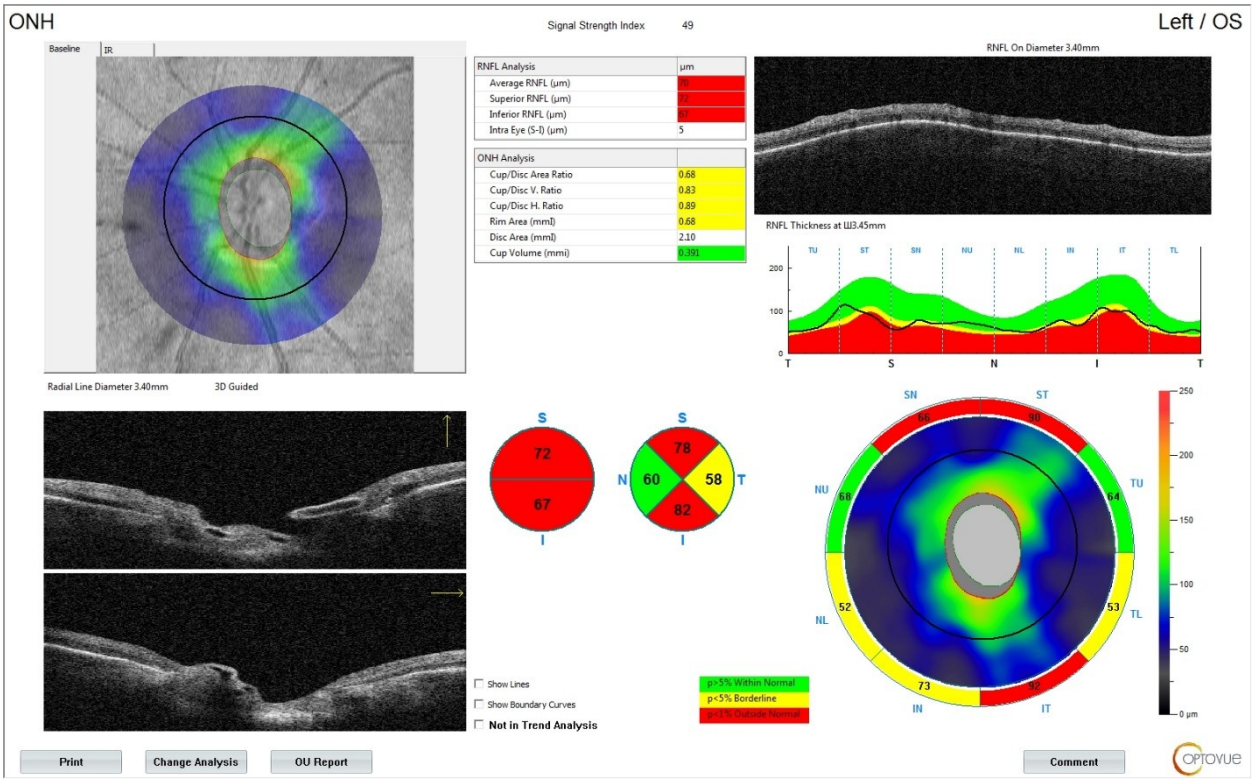

Figure 10. RTvue SD-OCT patient ONH analysis printout

The GCC topographic map was centered 0.75 mm temporal to the fovea, covering a 7 × 7 mm square grid on the macula and consisting of 15 B-scans spaced 0.5 mm apart. GCC thickness was calculated within a circular macular area 6 mm in diameter and encompassed the area from the internal limiting membrane to the outer border of the inner plexiform layer (ie, it measured the thickness of the inner 3 layers of the macula). Figure 11 shows a printout of the measured parameters from the GCC analysis.

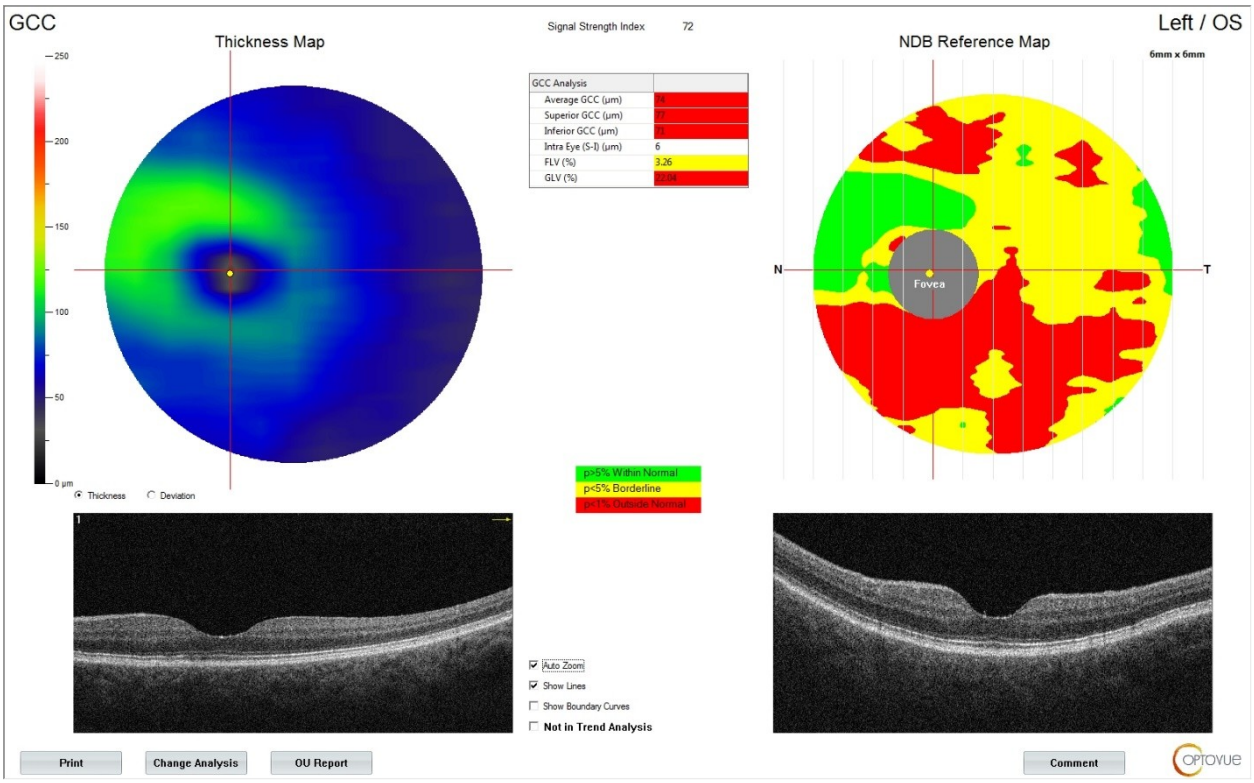

Figure 11. The RTvue SD-OCT patient GCC analysis printout

The summary report of nerve fiber changes with TREND analysis (Figure 12) provides a comprehensive data representation that facilitates disease assessment and progression. Proprietary algorithms define the optic disc boundary and vessel patterns to provide reliable assessment of RNFL, ONH, and GCC changes over time.

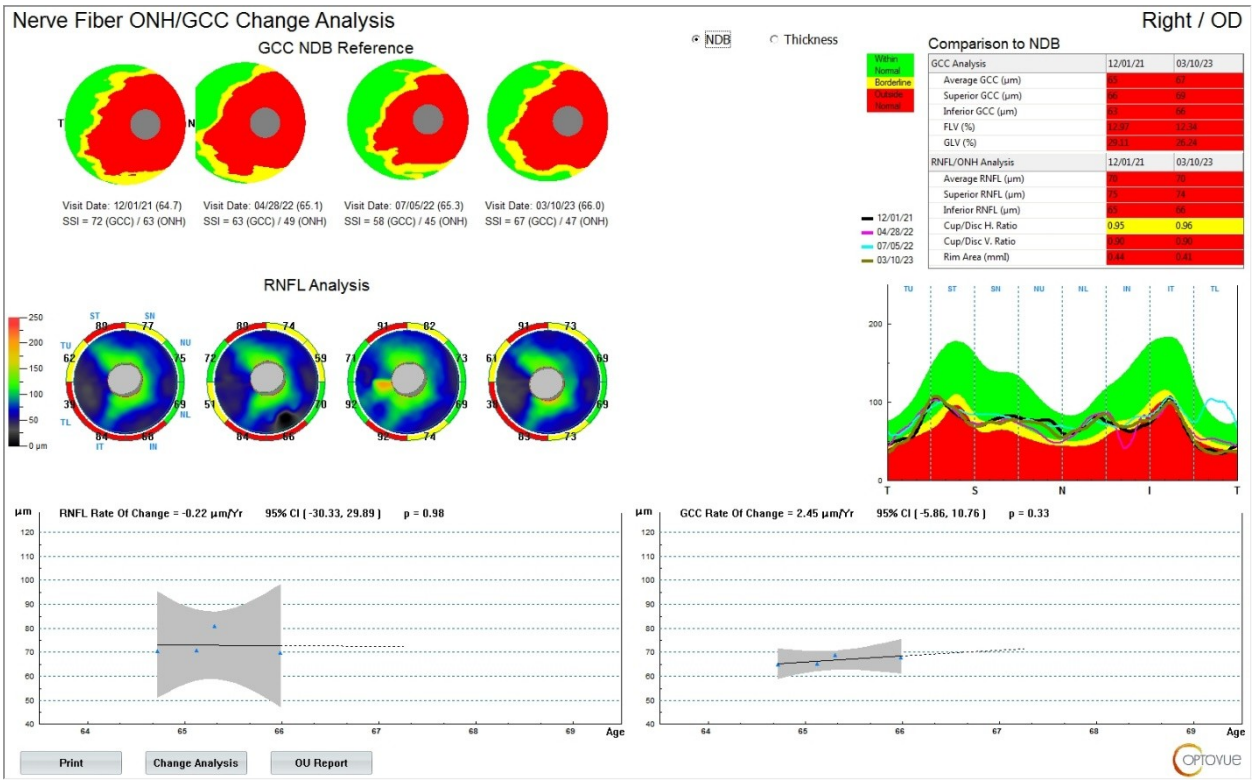

Figure 12. The ONH/GCC change report printout with RTvue SD-OCT

For the present study, from the summary analysis Summary ONH/GCC Report (Figure 13), the following parameters were taken into account: the average thickness of the peripapillary RNFL (RNFL Ave), the thickness of the RNFL in the upper half (RNFL Sup) and the thickness of the RNFL in the lower half (RNFL Inf). The parameters generated by the GCC module and analyzed by us in the present clinical study are the average thickness (GCC Ave), the thickness of the upper and lower halves of the GCC (GCC Sup and GCC Inf, respectively).

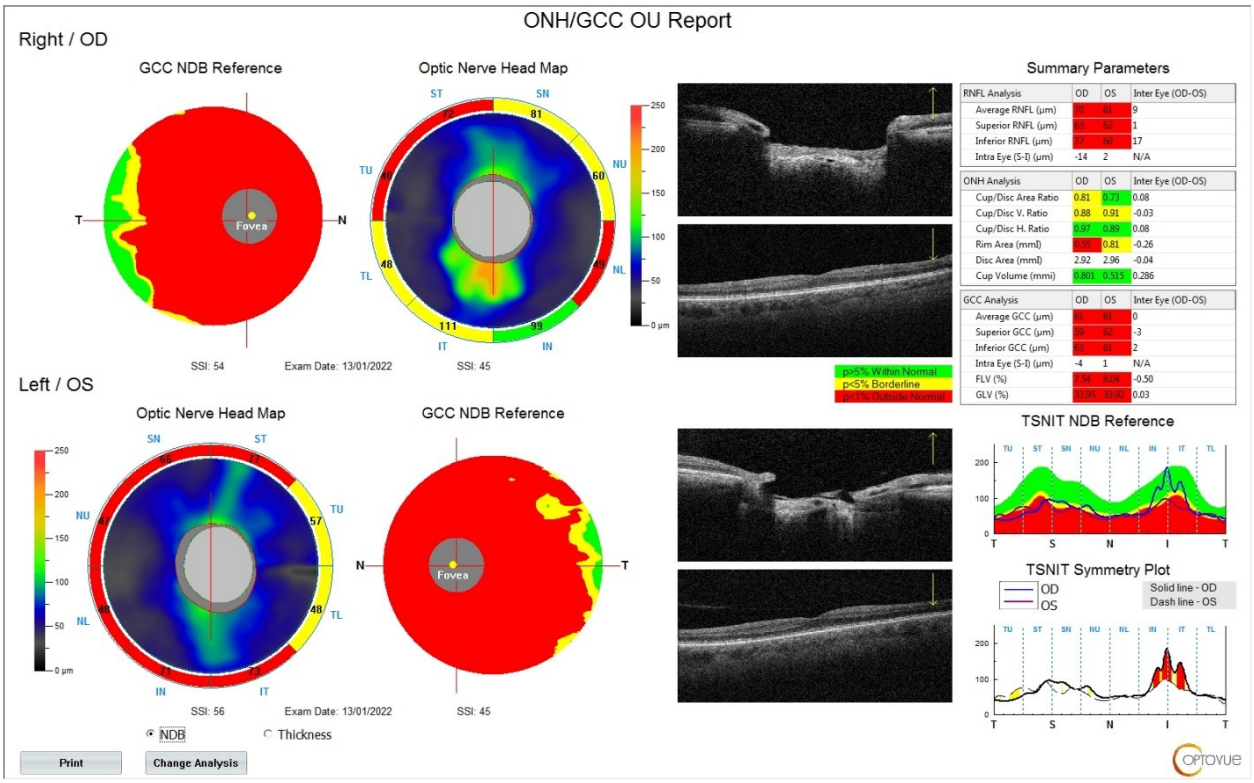

Figure 13. RTvue SD-OCT patient ONH/ GCC OU Summary Report printout

Immediately prior to OCT scans, artificial tears were applied as needed and patients were asked to blink repeatedly to improve the quality of the tear film and thus image quality.

Goldman tonometry was performed between 2 and 4 p.m., before the SAP and OCT examinations.

At months 6 and 15, all three groups underwent a comprehensive eye examination, including SAP and OCT. In addition, in groups A and C taking Mielooptik and Citizin supplements, possible side effects were assessed at each visit. Only the data of patients from the three groups who completed the 6-month and 15-month courses were compared and statistically analyzed.

The evaluated parameters are as follows: MD (dB) and PSD (dB) measured with SAP; average RNFL thickness (RNFL Ave); thickness in upper (RNFL Sup) and lower (RNFL Inf) halves; average thickness of GCC (GCC Ave), thickness in superior (GCC Sup) and inferior (GCC Inf) half measured by OCT.

Statistical evaluation

All analyzes were performed using SPSS software (IBM Corp. Armonk, NY) version 24.0. For all tests, the level of significance was set at $p < 0.05$.

The following methods were applied:

1. ***Descriptive analysis*** – the frequency distribution of the examined signs, broken down by research groups, is presented in tabular form.
2. ***Analysis of Variance*** – to assess the central tendency and scatter characteristics of the data.
3. ***Graphical analysis*** – for visualization of the obtained results.
4. ***Analysis of variance and*** t- test to compare differences between groups and within groups to test hypotheses for the presence of a relationship between the studied parameters.

The fulfillment of data requirements for parametric analysis (normality and homogeneity of variance) is assessed by specific tests (Kolmogorov-Smirnov and Levine). Groups A, B and C were compared by one-way ANOVA for each parameter. In addition, within-group temporal changes of parameters were assessed with the Student's t-test for repeated measures.

5. ***Pearson correlation analysis***

Correlations between baseline criteria and their variations after 6 and 15 months, respectively, were assessed using the parametric (Pearson) test.

Chapter IV

Results

Tables 1. and 2. present the demographic data and IOP values of the three groups of patients before the start of the study, at 6 and 15 months. All three groups had the same gender distribution, approximately the same age, and approximately the same IOP values, and no statistically significant changes were reported for the entire observation period. The average values of IOP for the entire 15-month period are Group A - an average of 14.7 mmHg, for Group B - an average of 14.1 mmHg and for Group C - an average of 14.4 mmHg. Table 3 presents the distribution of glaucoma stages in the three groups according to the simplified Hodapp classification (EGS 5). Table 4 presents the topical antiglaucoma therapy in the groups.

Table 1. Distribution of patients with POAG by age, gender for groups A, B and C before the start of the study, after 6 months and after 15 months

Age	in the beginning		after 6 months			after 15 months		
	Mean	SD	Mean	SD	p	Mean	SD	p
Group A	65,77	7,31	66,77	7,31	p > 0.05	67	7,31	p > 0.05
Group C	64,5	7,92	65.07	7,92	p > 0.05	65,57	7,92	p > 0.05
Group C	66,5	6,69	67	6,69	p > 0.05	67	6,69	p > 0.05
Gender (m/f)	15/15		15/15			15/15		

Table 2. Mean IOP values for groups A, B and C before the start of the study, after 6 months and after 15 months

IOP (mmHg)	- at the beginning		after 6 months			after 15 months		
	Mean	SD	Mean	SD	p	Mean	SD	p
Group A	14,2	1,14	14.9	1,67	p = 0.31	15	1,21	p = 0.21
Group C	13,9	1,31	14,4	1,55	p = 0.28	14,1	1,3	p = 0.38
Group C	14,5	1,2	15	1,05	p = 0.47	13,8	1,42	p = 0.61

Table 3. Distribution of the stages of patients with POAG in group A, B and C according to the simplified Hodapp classification

Stages	Number of eyes Group A	Number of eyes Group C	Number of eyes Group C
MD ≤ -6 dB	46	49	43
-6 < MD ≥ -12 dB	8	8	9
MD > -12 dB	6	3	8

Table 4. Topical therapy of patients with POAG in group A, B and C

Topical therapy	Number of patients Group A	Number of patients Group C	Number of patients Group C
Prostaglandin analog	11	12	14
Beta Blocker	2	3	0
Carbonic anhydrase inhibitor	0	1	1
Combination Carbonic anhydrase inhibitor + Beta Blocker	8	7	5
Combination Prostaglandin analogue + Beta Blocker	6	5	6
Combination Prostaglandin analogue + Beta Blocker + Carbonic Anhydrase Inhibitor	3	2	4

IV 1. Results for task 1.

To track the functional and structural changes in the course of the POAG disease, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who also take the nutritional supplement Mielooptik according to a scheme for a period of 15 months.

Table 5 shows the mean values of the MD and PSD parameters measured with SAP and the RNFL and GCC parameters measured with OCT for group A, before and after taking the Mielooptik food supplement.

Table 5. The observed parameters for group A for the follow-up period

Parameters	Group A at the start		Group A - after 6 months		Group A – after 15 months		
	Mean	SD	Mean	SD	Mean	SD	p
MD (dB)	- 5,35	4,83	- 4,76	4,47	-4.61	4,41	p<0.05
PSD (dB)	4,02	2,84	3,32	2,39	3,11	2,36	p<0.05
RNFL Ave (µm)	81,87	15,08	84,41	16,98	85,3	16,83	p<0.05
RNFL inf (µm)	80,93	14,89	83,53	16,52	85	16,46	p<0.05
RNFL sup (µm)	82,68	16,45	85	17,53	85,58	17,73	p<0.05
GCC Ave (µm)	84,76	12,97	87,15	16,56	88,38	16,76	p<0.05
GCC inf (µm)	84,43	13,89	87,38	17,11	88,47	17,32	p<0.05
GCC sup (µm)	84,58	13,9	86,87	16,86	88,3	16,85	p<0.05

The first 6-month follow-up period of **group A** patients showed that they had an effective improvement in the observed parameters, as the mean MD decreased by 11%, the mean PSD decreased by 17%, and the mean values of RNFL and GCC were statistically higher than the initial average by 2%-3% for 6 months. In the subsequent 6-month period (after a 3-month pause on Mielooptik) in group A, it was found that there was a statistically significant improvement in all investigated parameters, as the average MD decreased by 4%, the average PSD decreased by another 7%, a RNFL and GCC mean values were statistically higher than their 6-month values by an average of 1%-2% over 9 months.

From table 5. follows that after 15 months the mean MD values of group A decreased by 0.74 dB ($p<0.05$), and the average PSD values of group A decreased by 0.91 dB ($p<0.05$) compared to those at baseline. After 15 months, mean RNFL Ave values of group A increased by

3.43(μm) ($p<0.05$), and mean GCC Ave values increased by 3.62(μm) ($p<0.05$) compared to baseline.

IV 2. Task 2 results.

To follow the functional and structural changes in the course of POAG, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who do not take a nutritional supplement for a period of 15 months.

Table 6 shows the mean values of the MD and PSD parameters measured with SAP and the RNFL and GCC parameters measured with OCT of the patients in group B who did not take nutritional supplements during the 15-month follow-up.

Table 6. Observed parameters for group B for the follow-up period

Parameters	Group B - at the beginning		Group B - after 6 months		Group B – after 15 months		
	Mean	SD	Mean	SD	Mean	SD	p
MD (dB)	-3,78	3,29	-3,93	3,36	-3,99	3,34	p<0.05
PSD (dB)	3,07	2,08	3,3	2,19	3,41	2,29	p<0.05
RNFL Ave (μm)	88,56	13,25	87,5	13,31	85,2	13,38	p<0.05
RNFL inf (μm)	87,57	12,44	86,73	12,95	85,18	13,18	p<0.05
RNFL sup (μm)	90,92	12,57	89,25	12,76	86,78	13,19	p<0.05
GCC Ave (μm)	86,81	11,27	85,53	11,21	84,06	11,21	p<0.05
GCC inf (μm)	86,85	11,88	85,53	11,11	84,05	11,3	p<0.05
GCC sup (μm)	87,38	10,85	86,08	11,11	84,63	11,18	p<0.05

During the first 6-month follow-up period, patients in **group B** had a deterioration of the monitored parameters with a statistical significance of 95%, with mean MD worsening by 4%, mean PSD worsening by 7%, and all other parameters (RNFL Ave, RNFL inf, RNFL sup, GCC Ave, GCC inf, GCC sup) were statistically lower than baseline with RNFL Ave decreased by 1%, RNFL inf decreased by 1%, RNFL sup decreased by 1.84%, GCC Ave decreased by 1.45%, GCC inf – with 1.5% and GCC sup – with 1.5% for 6 months. In the subsequent 9-month period, patients in group B were found to have a statistically significant deterioration in all parameters

studied, with mean MD worsening by a further 1.7%, mean PSD worsening by a further 3%, and all others indicators (RNFL Ave, RNFL inf, RNFL sup, GCC Ave, GCC inf, GCC sup) were statistically lower than their 6-month values by an average of 1%-2% over 9 months.

From Table 6. follows that after 15 months the average MD values of group B deteriorated by 0.22dB ($p<0.05$) and the average PSD values deteriorated by 0.35dB ($p<0.05$) compared to the beginning. At the end of the follow-up period, the mean RNFL values of group B decreased by 3.37(μm) ($p<0.05$) and the mean GCC values of group B decreased by 2.75(μm) ($p<0.05$) compared to baseline.

IV 3. Results for task 3.

To follow the functional and structural changes in the course of the POAG, using computerized perimetry and optical coherence tomography, in patients with topical antiglaucoma therapy who also take the dietary supplement Citizin according to a scheme for a period of 15 months.

Table 7 shows the mean values of the MD and PSD parameters measured with SAP and the RNFL and GCC parameters measured with OCT for group C, before and after taking the food supplement Citizin.

Table 7. Observed parameters for group C for the follow-up period

Parameters	Group C - at the beginning		Group C - after 6 months		Group C – after 15 months		
	Mean	SD	Mean	SD	Mean	SD	p
MD (dB)	- 5,85	5,91	- 5,79	5,82	-5,53	5,6	p<0.05
PSD (dB)	4,06	2,53	4,05	2,56	3,66	2,35	p<0.05
RNFL Ave (μm)	80,98	14,99	80,91	15,13	82,26	14,75	p<0.05
RNFL inf (μm)	79	15	79,72	14,4	81,75	14,82	p<0.05
RNFL sup (μm)	83,38	15,87	83,17	15,58	83,53	15,44	p<0.05
GCC Ave (μm)	80,23	11,46	80,55	11,40	82,11	11,53	p<0.05
GCC inf (μm)	79,33	11	79,45	10,86	81,28	10,66	p<0.05
GCC sup (μm)	81,37	13	81,7	12,7	83,02	12,83	p<0.05

The first 6-month follow-up period for patients in **group C** showed that MD decreased by 0.9%, patients' mean PSD decreased by 0.3%, mean RNFL worsened by 0.1%, and mean GCC has improved by 0.4% in 6 months. In the follow-up 6-month Citizin intake (after a 3-month break) in group C patients, it was found that there was a statistically significant improvement in all observed parameters, with the mean MD decreased by 4.7%, the mean PSD decreased by another 10%, average RNFL improved 1.7% and average GCC improved 2%.

After 15 months, group C mean MD values improved by 0.31dB ($p<0.05$) and group C mean PSD values improved by 0.37dB ($p<0.05$) compared to baseline. At the end of the period, mean RNFL values of group C improved by 1.29(μm) ($p<0.05$) and mean GCC values of group C improved by 1.89(μm) ($p<0, 05$) compared to the beginning.

IV 4. Results for task 4.

To compare the results reporting the progression of functional and structural changes in the three groups, using statistical methods and to evaluate the effect of the use of nutritional supplements as an adjunctive therapy in the treatment of POAG.

- ## Descriptive analysis

A statistical comparison of demographic data and parameters monitored with SAP and OCT was performed for group A (glaucoma patients taking Mielooptik), group B (glaucoma patients not taking supplements) and group C (glaucoma patients taking Citizin) at the beginning of the study, after 6 and after 15 months. The three groups were comparable in terms of gender, age and measured IOP.

The following figure 14 presents a comparative analysis between the monitored parameters at baseline, after 6 and after 15 months.

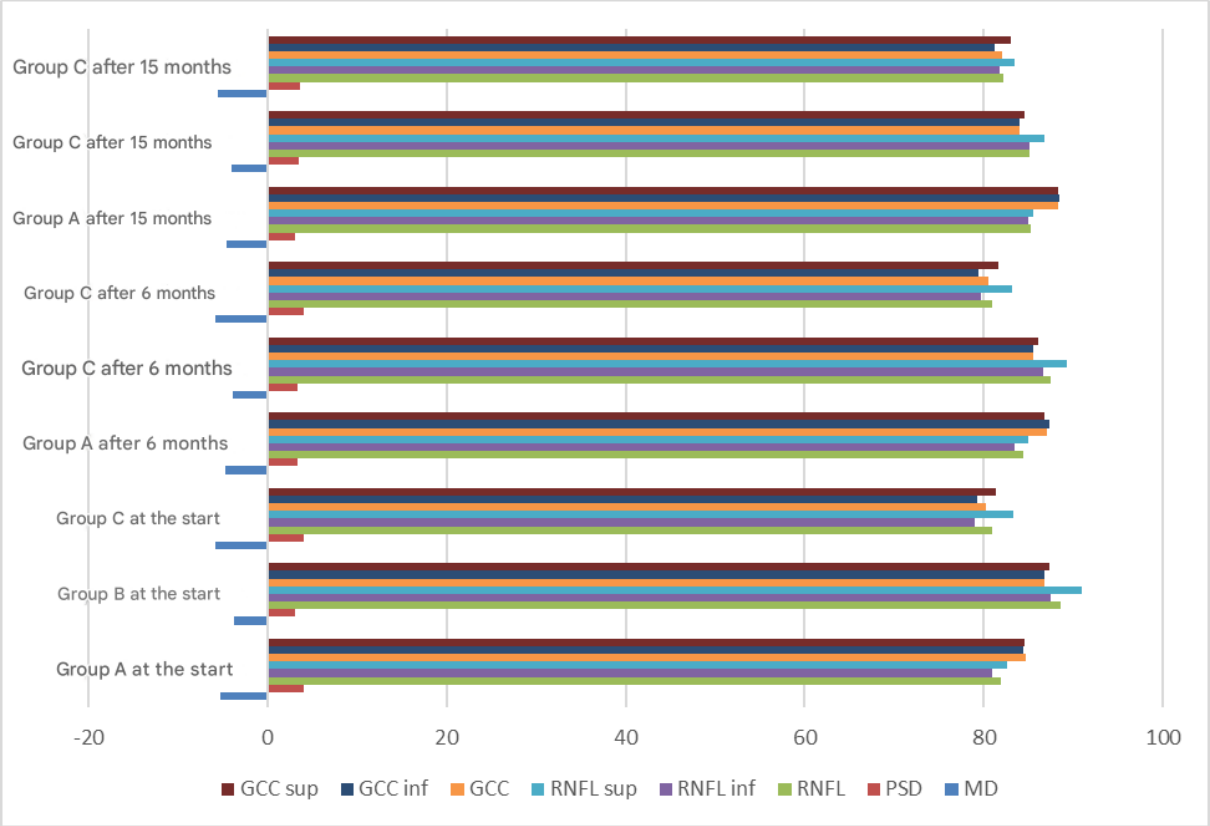

Figure 14: Comparative analysis between the parameters at the beginning, after 6 and after 15 months for the three studied groups

The next fig. 15 shows the mean values of MD (dB) measured by standard automated perimetry (SAP) on the vertical axis of patients taking Mielooptik (group A), of patients not taking any supplement (group B) and of patients taking Citizin (group C) before initiation of therapy, after 6-month and 15-month follow-up.

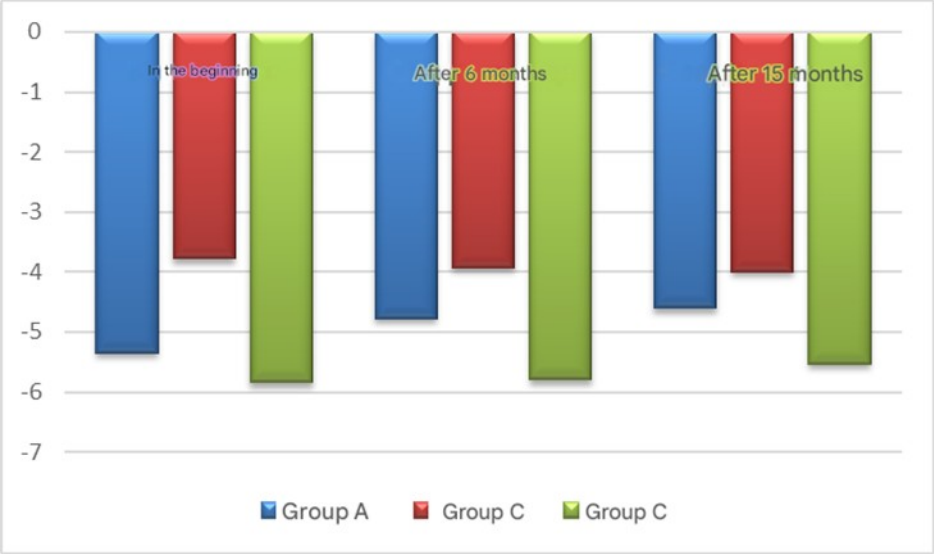

Figure 15. Comparison of mean MD values (dB) at baseline, after 6 months and 15 months of therapy for groups A, B and C

Fig. 15 shows a 15% improvement in mean MD after 15 months of Mielooptik supplementation, a 5.6% improvement in mean MD after 15 months of Citizin supplementation, and a 5.7% deterioration in group B patients after 15 months.

The following figure 16 presents a comparison of mean PSD values, on the vertical axis, of patients taking Mielooptik (group A), of patients not taking any supplement (group B) and of patients taking Citizin (group C) before starting therapy, after 6-month and 15-month follow-up.

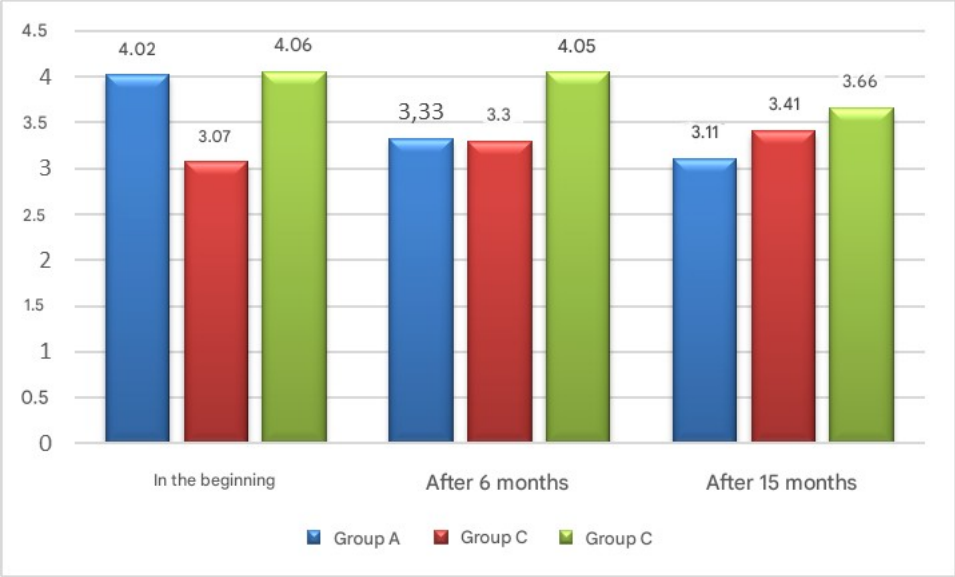

Figure 16. Comparison of mean PSD values (dB) at baseline, after 6 months and 15 months of therapy for groups A, B and C

Fig. 16 shows a 23% improvement in mean PSD after 15 months of Mielooptik supplementation, a 10.3% improvement in mean PSD after 15 months of Citizin supplementation, and a 10% deterioration in group B patients over the 15 months.

The following Figure 17 presents a comparison of the mean RNFL values, on the vertical axis, of patients taking Mielooptik (group A), of patients not taking the supplement (group B) and of patients taking Citizin (group C) before starting therapy, after 6-month and 15-month follow-up.

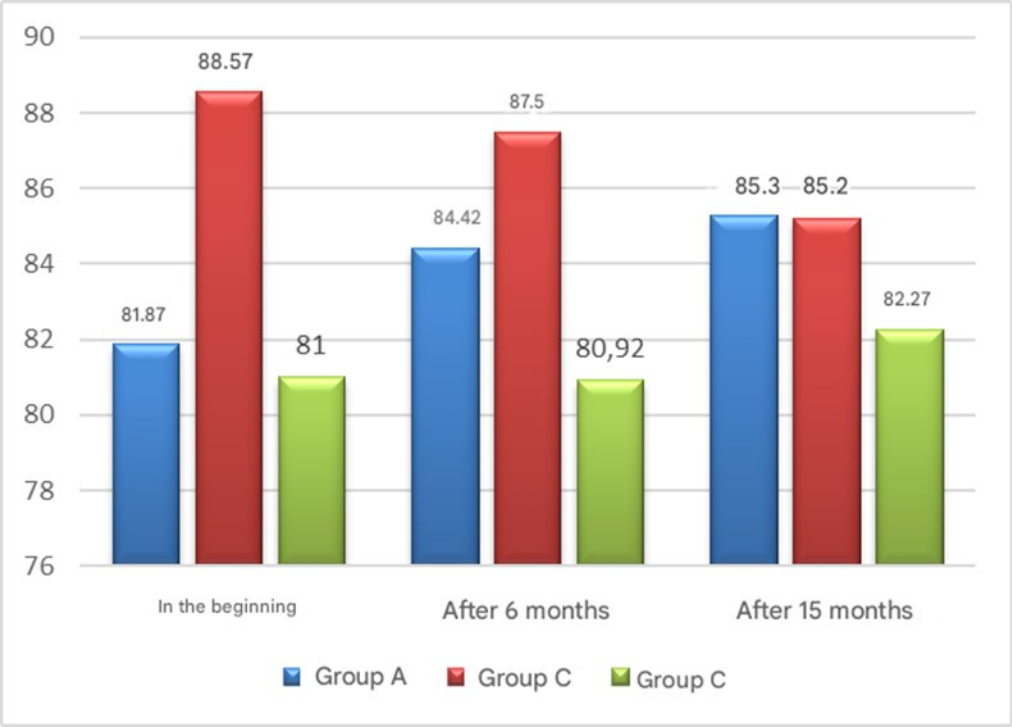

Figure 17. Comparison of mean RNFL values(μm) at baseline, after 6 months and 15 months of therapy for groups A, B and C

Fig. 17 shows a 4% improvement in mean RNFL after 15 months of Mielooptik supplementation, a 1.6% improvement in mean RNFL after 15 months of Citizin supplementation, and a 3.8% deterioration in group B patients over 15 months.

The following figure 18 presents a comparison of the mean values of GCC, on the vertical axis, of patients taking Mielooptik (group A), of patients not taking the supplement (group B) and of patients taking Citizin (group C) before starting therapy, after 6-month and 15-month follow-up.

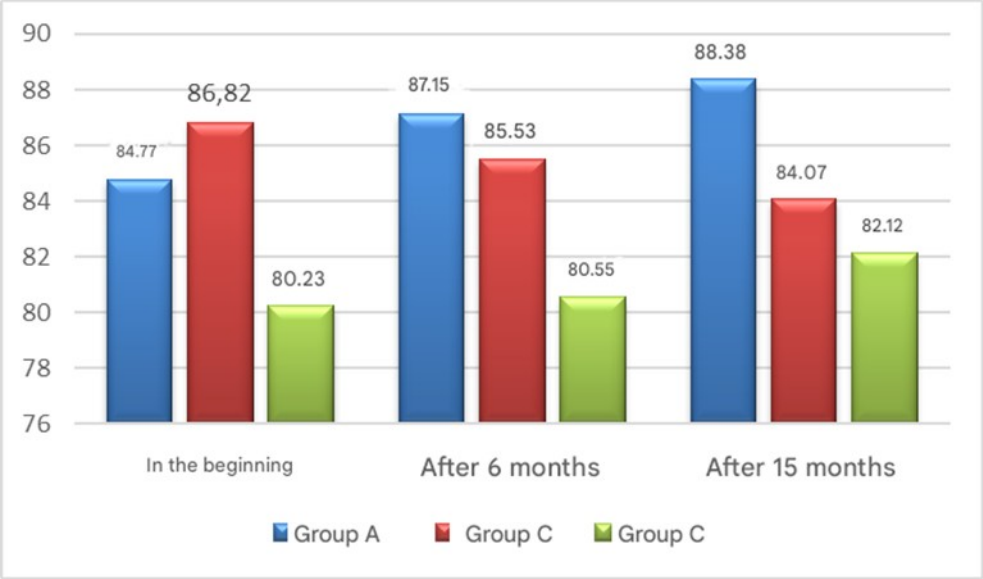

Figure 18. Comparison of mean GCC values(μm) at baseline, after 6 months and 15 months of therapy for groups A, B and C

Fig. 18 shows a 4% improvement in mean GCC after 15 months of Mielooptik supplementation, a 1.6% improvement in mean GCC after 15 months of Citizin supplementation, and a 3% deterioration in group B patients over 15 months.

- **Correlation analysis**

The analysis of the correlation between the values of the studied parameters at the beginning and the variations of the same parameters during the follow-up after 6 and after 15 months allows to assess whether the baseline values influence the detected variations.

MDs are in very high statistically significant correlation with 95% probability with their respective values after 6 and 15 months for all three groups – they are equal to 0.98 and 0.98 for group A, respectively; 0.99 and 0.99 for group B; 0.99 and 0.99 for group C.

The PSDs are in very high statistically significant correlation with 95% probability with their respective values after 6 and 15 months for all three groups – they are equal to 0.93 and 0.94 for group A, respectively; 0.99 and 0.99 for group B; 0.99 and 0.99 for group C.

RNFL Ave are in very high statistically significant correlation at 95% probability with their respective values after 6 and 15 months for all three groups – equal to 0.94 and 0.94 for group A, respectively; 0.99 and 0.98 for group B; 0.99 and 0.99 for group C.

GCC Ave are in very high statistically significant correlation with 95% probability with their respective values after 6 and 15 months for all three groups – they are equal to 0.89 and 0.88 for group A, respectively; 0.99 and 0.98 for group B; 0.99 and 0.99 for group C.

Cronbach's alpha coefficient was interpreted to calculate the reliability of the measurement scale. The coefficient has values above 0.9 on all criteria for all three groups, indicating that the scale is reliable.

Therefore, the positive change occurred for all patients of group A and group C due to the intake of Mielooptik and Citizin, respectively, and the deterioration of the monitored parameters occurred for all patients of group B within the observed period of 6 and 15 months. The parameters of the patients of group A improved to a higher extent than those of group C. A statistically significant strong correlation relationship was observed between the criteria pairs MD-PSD and RNFL Ave-GCC Ave for all three studied groups. Age, gender and IOP were non-deterministic for all three groups during the study.

- **Comparison of parameters between groups and within groups with Student's t-test, Wilcoxon and one-way analysis of variance**

From the statistical analysis of the differences between the evaluated parameters of each group with Student's t-test ($p < 0.05$), follows that for the patients of the three groups there is a statistically significant effect on all parameters after the 15-month observation period. After the first 6-month intake of Mielooptik for group A, we have a statistically significant effect on all parameters compared to the initial ones, and it is less pronounced on the parameters GCC Ave, GCC Inf and GCC Sup. A statistically significant effect in the direction of improvement for group C was observed after the second 6-month intake of Citizin. After the 15-month observation period, a statistically significant improvement of the monitored parameters was found in group A, which was stronger than the statistically significant improvement of the parameters found in group C. In group B, there was a clearly pronounced statistically significant effect in the direction of worsening of all parameters.

The results of the univariate analysis of variance showed that the three groups were not distinguishable in terms of the evaluated parameters, both at baseline and after 6 and 15 months. In the three groups, there is a change in the studied values, as in group A it is in the direction of moderate improvement, in group C - in the direction of slight improvement and in group B - in the direction of deterioration of all observed parameters.

The Friedman test is a nonparametric alternative to one-way analysis of variance for related samples. We apply this test to group A, B and C on all monitored parameters and it finds that there is a statistically significant difference between the observed parameters at baseline, after 6- and 15-month period for the three groups.

The Wilcoxon test compares pairwise follow-up parameters for a given group at 0 and 6 months, at 6 and 15 months, at 0 and 15 months and finds that there is a statistically significant difference between these pairs $(p \leq 0{,}05)$. There was a statistically significant improvement in all monitored parameters of group A, a statistically significant deterioration in all relevant parameters of group B. For group C, there was also a statistically significant improvement of the parameters according to the Wilcoxon test, but no statistically significant difference was found in the values of MD, PSD and RNFL Ave in the beginning and those after the first 6 months.

- **Statistical comparison of the rates of progression of parameters in group A (glaucoma patients taking Mielooptik), in group B (glaucoma patients not taking supplements) and in group C (glaucoma patients taking Citizin)**

Under the same conditions for conducting the research, the same groups by gender (15 men and 15 women for each group), approximately the same age (65.76 for group A; 64.5 years for group B; 65.7 for group C) and approximately the same IOP (Group A - average14.7 mmHg, for Group B - average 14.1mmHg and for Group C - average14.4 mmHg) for the entire 15-month period, the following rates of progression of the observed parameters by periods:

Table 8 shows the rate of progression of MD, PSD, and mean RNFL and GCC values for the first 6-month period for the three study groups. Figures 19-22 illustrate the relevant changes.

Table 8. Growth rate of observed parameters for group A, B and C after the first 6-month period

Growth rate at the end of a 6-month period	Group A				Group B				Group C			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
MD (dB)	-0,86	3,22	0,593	0,937	-0,88	0,05	-0,156	0,239	-0,35	0,58	0,05	0,19
PSD (dB)	-3,45	0,43	-0,688	1,091	0,00	0,88	0,229	0,275	-0,2	0,23	-0,01	0,09
RNFL (µm)	-4	29	2,55	5,97	-7	0,5	-1,07	1,65	-3	1,5	-0,07	0,94
GCC (µm)	-7,5	41	2,38	7,79	-5,5	0	-1,28	1,36	-0,5	1,5	0,32	0,59

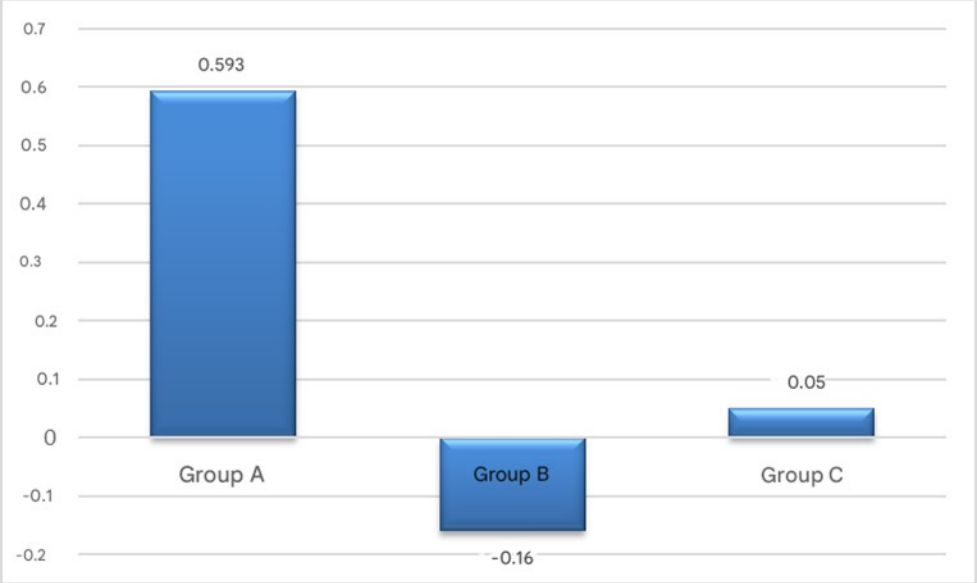

Figure 19. Average increase rate of in MD (dB) by group over 6 months

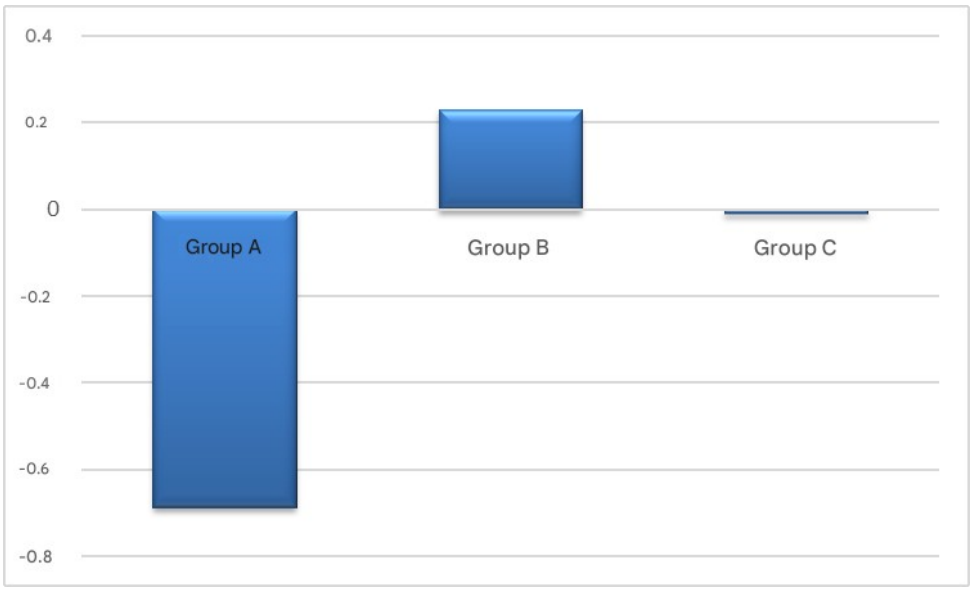

Figure 20. Mean increase rate of PSD (dB) by group over 6 months

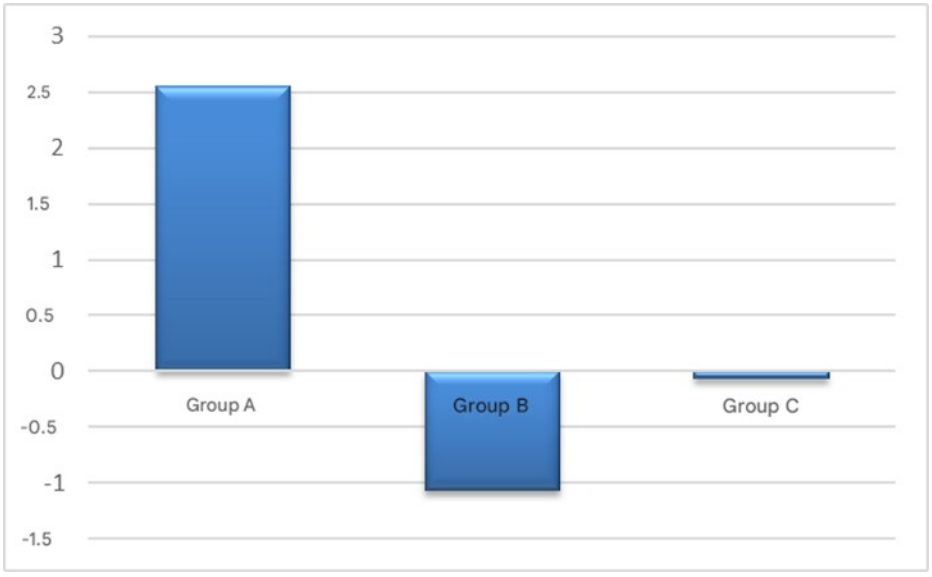

Figure 21. Mean increase rate of RNFL (μm) by group at 6 months

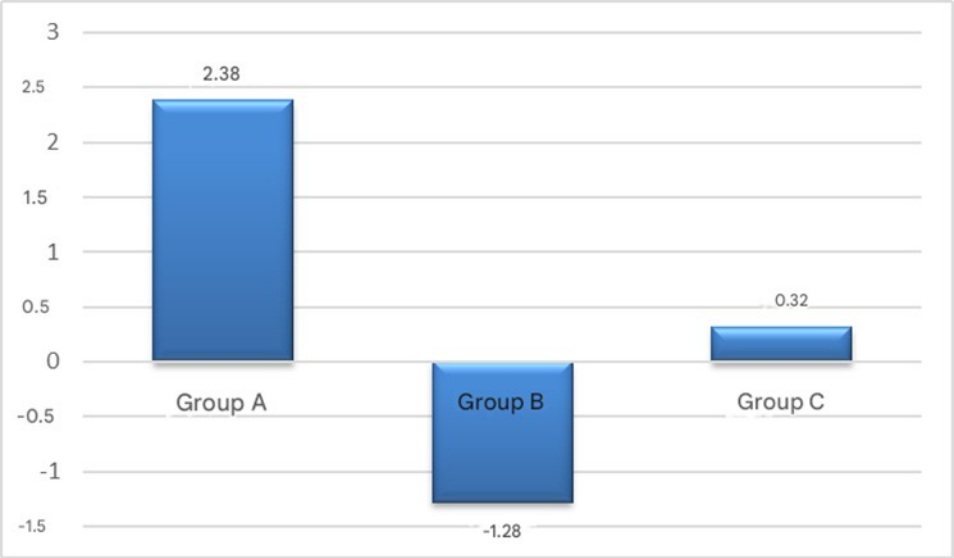

Figure 22. Mean increase rate of GCC(μm) by group at 6 months

For the first 6-month follow-up period, a moderate 0.59 dB improvement in MD was reported for group A. MD for group B deteriorated by 0.15 dB. The MD value for group C has improved by 0.05 dB.

The PSD for group A improved moderately by 0.69 dB in the first 6 months. The PSD for group B has deteriorated by 0.23 dB. The PSD for group C has improved by 0.01 dB.

The mean RNFL value for group A improved by 2.55(μm) /6 months, and RNFL Ave for group B deteriorated by 1.07(μm) for the first 6 months. RNFL Ave for group C worsened by 0.07(μm) /6 months.

The mean GCC value for group A improved by 2.38 (μm) for the first 6 months. GCC Ave for Group B has deteriorated by 1.28(μm) /6 months. GCC Ave for Group C improved by 0.32 (μm) / 6 months.

Table 9 shows the rate of progression of MD, PSD, and mean values of RNFL and GCC between the 6- and 15-month period for the three studied groups. Figures 23-26 illustrate the relevant changes.

Table 9. Rate of progression of MD, PSD, RNFL Ave and GCC Ave. for the three groups between the 6- and 15-month period

Growth rate between 6 and 15 months. period	Group A				Group B				Group C			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
MD (dB)	-0,3	0,73	0,16	0,25	-0,35	0,18	-0,06	0,11	-0,46	1,48	0,26	0,41
PSD (dB)	-1,05	0,25	-0,22	0,29	-0,38	0,95	0,12	0,22	-1,22	0,16	-0,39	0,4
RNFL (µm)	-1,5	3,5	0,88	1,2	-6,5	1	-2,3	1,88	-2	8	1,35	1,82
GCC (µm)	-1	4	1,23	1,28	-5	3	-1,47	1,54	-4	4,5	1,57	1,87

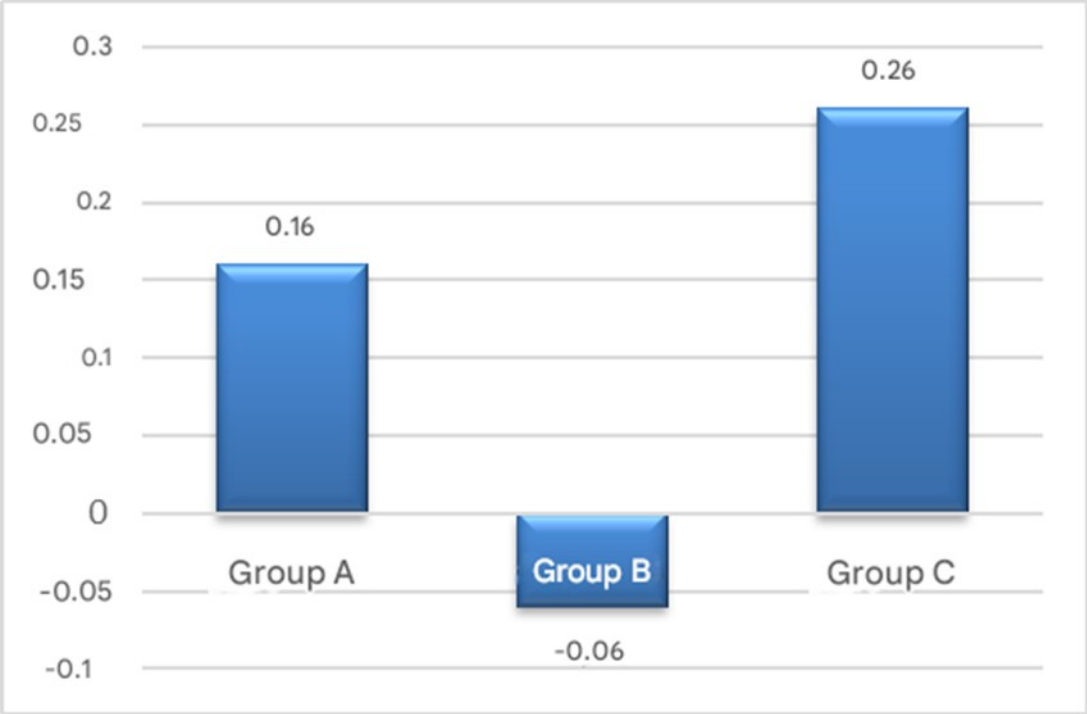

Figure 23. Mean increase rate of MD (dB) by group between the 6- and 15-month period

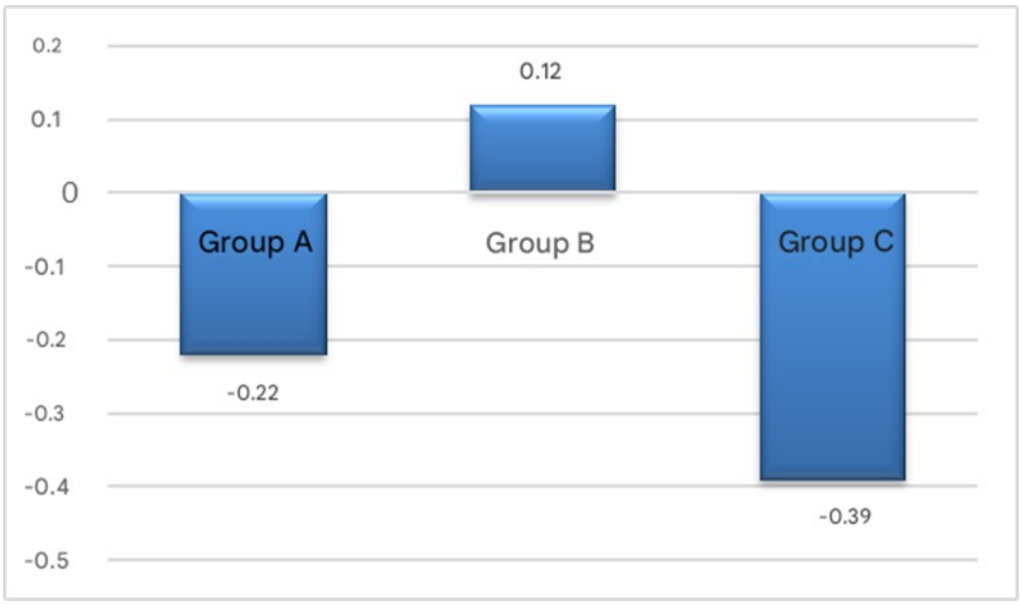

Figure 24. Mean increase rate of PSD (dB) by group between the 6- and 15-month period

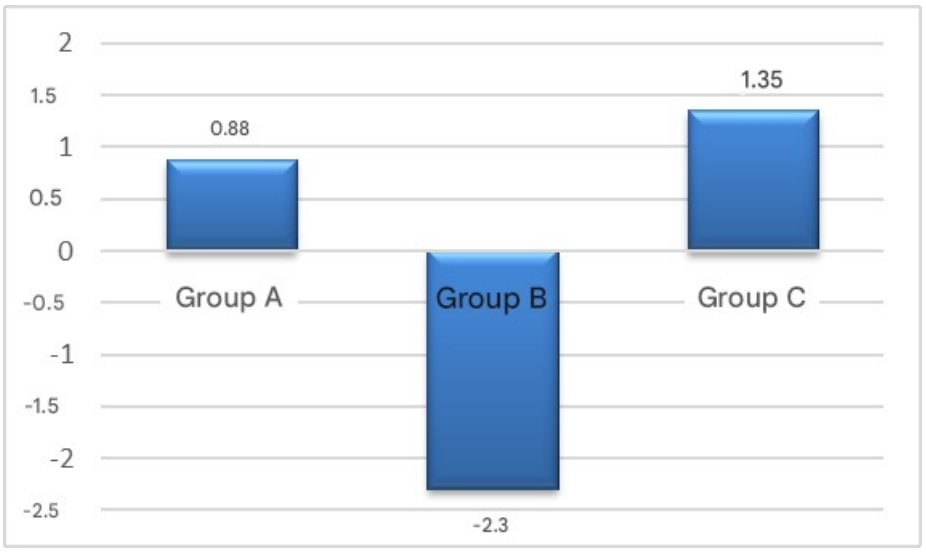

Figure 25. Mean RNFL Ave (μm) increase rate by group between the 6- and 15-month period

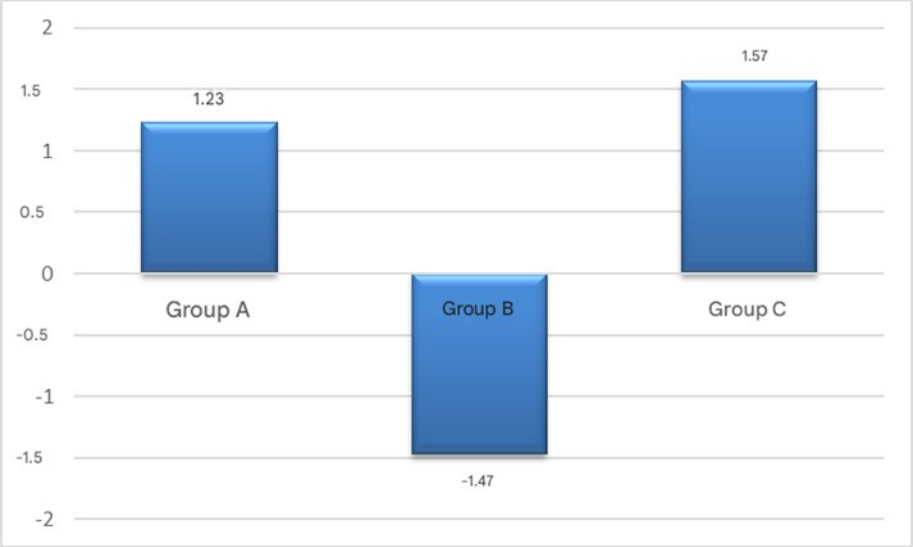

Figure 26. Average increase rate of GCC Ave (μm) by groups between the 6- and 15-month period

Over the follow-up period between 6 and 15 months, the MD for group A improved slightly by 0.16 dB/9 months, and the MD for group B worsened by 0.06 dB/9 months. MD for group C improved by 0.26 dB/9 months.

The PSD for group A has improved by 0.22 dB for the period between 6 and 15 months. PSD for group B deteriorated by 0.12 dB/9 months. PSD for group C improved by 0.39 dB/9 months.

The mean RNFL value for group A improved by 0.88(μm) for the period between 6 and 15 months, and the RNFL Ave for group B deteriorated by 2.3 (μm) /9 months. RNFL Ave for group C improved by 1.35 (μm) /9 months.

The mean GCC value for group A improved by 1.23 (μm) for the period between 6 and 15 months. GCC Ave for Group B has deteriorated by 1.47 /(μm) 9 months. GCC Ave for Group C has improved by 1.57(μm) /9 months.

The following table 10 and figures 27 and 28 show the average rates of progression of the monitored parameters for the 0-6-, 6-15- and 0–15-month periods.

Table 10. Average rates of progression of the parameters for the 0-6- and 6-15-month periods

		MD (dB)	PSD (dB)	RNFL Ave (µm)	GCC Ave (µm)	p
Group A	0-6 months	0,59	-0,69	2,55	2,38	p<0.05
	6-15 months	0,16	-0,22	0,88	1,23	p<0.05
	0-15 months	0,74	-0,91	3,43	3,62	p<0.05
Group C	0-6 months	-0,16	0,23	-1,07	-1,28	p<0.05
	6-15 months	-0,06	0,12	-2,3	-1,47	p<0.05
	0-15 months	-0,22	0,37	-3,37	-2,75	p<0.05
Group C	0-6 months	0,05	-0,01	-0,07	0,32	p<0.05
	6-15 months	0,26	-0,39	1,35	1,57	p<0.05
	0-15 months	0,31	-0,4	1,28	1,9	p<0.05

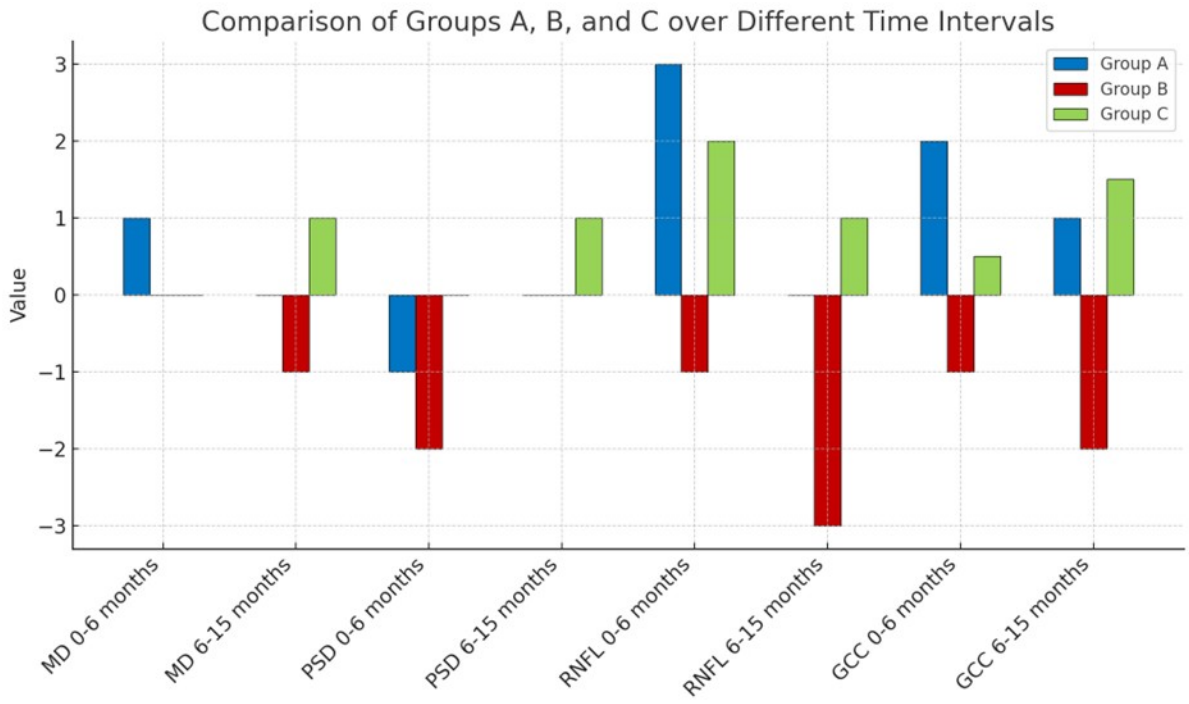

Figure 27. Average rates of progression of the parameters for the 0-6- and 6-15-month periods

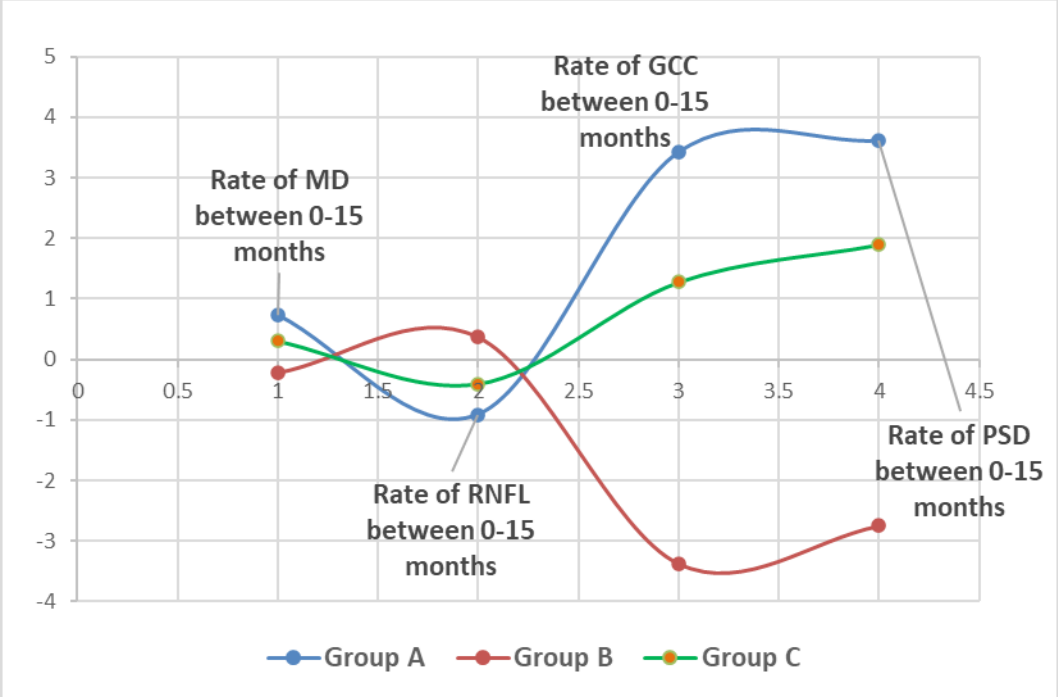

Figure 28. Rates of progression of the observed parameters for the 0–15-month period

Figure 28 shows the rate of progression of the observed parameters for the entire observation period. The following conclusions can be drawn from it:

MD for group A improved moderately by 0.74 dB and MD for group B progressed by 0.22 dB over 15 months. MD for group C improved by 0.31 dB over the observed period.

The PSD for group A improved moderately by 0.91 dB/15 months. PSD for group B has deteriorated by 0.37 dB/15 months. The PSD for group C improved by 0.4 dB over 15 months.

The mean RNFL value for group A improved by 3.43(μm) for the 15-month period, and the RNFL for group B deteriorated by 3.37 (μm) /15 months. RNFL for group C improved by 1.28 (μm) for the relevant period.

The mean GCC value for group A improved by 3.62 (μm) over the 15-month period. GCC for group B deteriorated by 2.75(μm) /15 months. GCC for group C improved by 1.9 (μm) during the observed period.

According to the results of the application of ANOVA on the rates of progression of the parameters for the three studied groups for the 15-month period, it is statistically significant ($p<0.05$) whether the type of supplement is accepted or not. In group A there is a statistically significant ($p<0.05$) positive change in the parameters, in group C there is also a statistically significant ($p<0.05$) improvement, but to a lesser extent than in group A. In group B there is a statistically significant ($p<0.05$) deterioration of the parameters for the studied 15-month period.

For all three univariate analyzes of variance, there was a statistically significant difference between groups. From the results of the Post-hoc tests that show exactly where the differences between the groups are, it is clear that there is a statistically significant difference between the rates of progression of MD for groups A and B, while for A and C it is not statistically significant. There is a statistically significant difference between the rates of progression of PSD for pairs of groups A and B; A and C; B and C. There is a significant difference between the rates of progression of mean RNFL for groups A and B, while for A and C it is not statistically significant. There is a significant difference between the rates of progression of the mean GCC value for groups A and B, while for A and C it is not statistically significant.

Chapter V
Discussion

In the current clinical ophthalmology, measurements which determine the status of the RGC are usually limited to non-invasive studies. Structural measurements of the RNFL and ONH are performed by OCT, and functional measurements of the visual field are performed with SAP or electroretinogram. (368) These examinations are also used in our study to follow the morphological and functional effects on the RGC after the administration of nutritional supplements in patients with POAG.

No publications on studies of the nutritional supplement Mielooptik and its effect in patients with POAG were found in the global database, but the effect of the constituent elements of the combined nutritional supplement in relation to neurodegenerative diseases is well studied.

Curcumin, due to its anti-inflammatory, antioxidant, and antitumor properties, has been widely studied in vitro and in vivo in the context of many inflammatory, autoimmune, and degenerative diseases of both the anterior and posterior segments of the eye, and has been proposed as an adjunctive therapy. (397) Wang L. et al demonstrate that Curcumin inhibits neuronal and vascular degeneration in the retina after ischemia and reperfusion injury. (397) Curcumin has been shown to reduce oxidative damage to microglia and reduce retinal ganglion cell loss. (423) Matteucci, A and co-authors (398) demonstrate its positive effect on mitochondrial dysfunction and its neuroprotective properties. Curcumin has been reported to prevent changes in the apoptotic cascade, preserving RGC survival and retinal thickness in mice. (424) But no parallel to our study with curcumin, which examines its effect on neurofibrillary layer thickness or its impact on the visual field in patients with POAG, was found. The therapeutic potential of curcumin in clinical ophthalmology is limited by several adverse factors, including extremely poor bioavailability and water solubility. (425) The active fraction of curcumin found in the blood is often not optimal, so increased doses are required to achieve the desired therapeutic effect. (426) To overcome these limitations, several approaches have been

reported and extensively reviewed in various studies such as: the use of enhancers or analogs and nanocarriers to provide a hydrophobic environment for poorly water-soluble curcumin. (427,428,429) These studies highlight the potential of curcumin to provide neuroprotective therapy in glaucoma. It should be noted that the use of nanocarriers is one of the most promising approaches to improve curcumin delivery. (430) In our case, in the composition of Mielooptik, it is not mentioned in what form the curcumin is used, it is only known that it is 100 mg, which, based on other studies that investigated the bioavailability and used curcumin 375 mg 3 times a day or 600 mg 2 times per day, is relatively small. (396)

Studies have also investigated the relationship between blood levels of vitamin B-complex and glaucoma, but again, there are no findings analogous to our study examining the effect of B vitamins on the visual field or thickness of the retinal layers in patients with POAG. Glaucoma patients have been found to be deficient in thiamine (vitamin B1). (408) Vitamin B1 has a protective effect in open-angle glaucoma. This statement is supported by the fact that in vitamin B1 deficiency ganglion cell degeneration and reduced GCC thickness (in rats) are found. (407,409,410) According to other studies, people with high intakes of vitamin B1 have about twice the risk of primary open-angle glaucoma compared to those with low intakes. (410) Studies on dietary B-complex vitamin intake and glaucoma have been inconclusive. For vitamin B1, the Rotterdam study reported a protective effect on POAG, although the Osteoporotic fracture study found no association between vitamin B1 and POAG. (394, 410, 422) Some studies have found no relationship between dietary intake of vitamin B3, B6, B9, or B12 and POAG. (408)

Nicotinamide B3 treatment exerts a neuroprotective effect on ganglion cells. (410, 431) According to a crossover, randomized clinical trial involving 57 glaucoma patients, oral vitamin B3 supplementation for 6 weeks at 1.5 g/day, then for 6 weeks at 3.0 g/day, improved RGC function but without affecting IOP and RNFL thickness. (432)

According to a study involving 594 participants with glaucomatous lesions from the National Health and Nutrition Examination Survey between 2005 and 2008, taking high doses of vitamin B12 may even promote the development of glaucoma. (433) Tűrkyilmaz and colleagues (2013) showed that patients with vitamin B12 deficiency had a thinner retinal nerve fiber layer compared to controls. (414)

Other findings show a strong inverse correlation between folate B9 blood levels and glaucoma. (434) Other authors reported no significant differences in plasma levels of vitamin B6 or serum levels of B9 or B12 in POAG patients compared to controls. (403,416,417,418,419)

We found 1 monocentric non-randomized experimental clinical study by Rolle T. and co-authors, which, similarly to ours, investigated the effect of taking a combined nutritional supplement, which, however, included homotaurine, carnosine, forskolin, vitamins B1, B2 and B6, folic acid and magnesium. The degree of progression of visual field loss in 31 patients with an average age of 70 years with progressive glaucoma was monitored. Patients with MD ranging from -2 dB to -15 dB and IOP values ≤18 mmHg were included. All patients underwent additional therapy for a period of 6 months. The first 2 months they took 2 tablets a day, and the next 4 months 1 tablet a day. Patients were evaluated before the start of treatment, after 2 and after 6 months. At each examination, patients underwent a complete eye examination including perimetry, RNFL and GCC using OCT, PERG, contrast sensitivity, and QoL assessment using the Glaucoma Symptom Scale Questionnaire and the National Eye Institute Visual Function Questionnaire. MD values measured by perimeter were found to have decreased compared to baseline. Patients also demonstrated a significant reduction in IOP, statistically significantly improved light and contrast sensitivity, and a better quality of life. However, no significant variations were reported with respect to RNFL and GCC measured by OCT. The conclusion of the study is similar to ours, namely that the combined nutritional supplement can slow the rate of progression of functional impairment and improve visual function after 2 and 6 months of daily intake. (435)

Lutein has been studied as a component of a combined dietary supplement in relation to glaucoma. Macular pigment optical density (MPOD) measured by autofluorescence using a Heidelberg Spectralis scanning laser ophthalmoscope was evaluated. The study included 62 (38 men, 24 women) with a diagnosis of POAG. Forty-two received a combined supplement containing the carotenoids Lutein, Zeaxanthin, and Meso-zeaxanthin, and 20 participants received a placebo. A statistically significant increase in MPOD volume and a statistically significant increase in mesopic contrast sensitivity were found, compared to no effect in the control group. The conclusion of the study is that the macular pigment can be increased in glaucomatous eyes by adding formula containing the carotenoids Lutein, Zeaxanthin and Meso-zeaxanthin. (436)

In other clinical trials, a protective trend was observed with increased dietary carotenoid consumption and glaucoma risk. Data suggests that carotenoid vitamin therapy has synergistic neuroprotective benefits and has the capacity to serve as an adjunctive therapy in the treatment of glaucoma. (406)

In another randomized controlled trial, Garcia-Medina JJ et al evaluated the effect of oral antioxidant supplements (OAS) on POAG over a 2-year follow-up period, also following MD, PSD and GCC and RNFL parameters like us. They followed 117 eyes of 117 patients with mild or moderate POAG and controlled IOP with topical antiglaucoma drugs, who were randomly divided into three groups according to the supplements: (1) 26 patients received OAS with (ICAPS R(®) - Alcon Laboratories); (2) 28 patients received OAS without ω-3 fatty acids (OFTAN MACULA(®) - Laboratories Esteve); and (3) a control group of 63 patients without OAS. All of them underwent visual field tests with Humphrey 24-2 and OCT (RTVue-100) at the beginning of the study and 2 years later. Mean deviation (MD), pattern standard deviation (PSD), peripapillary retinal nerve fiber layer (RNFL) and macular ganglion cell complex (GCC) parameters were considered for the analysis. Patients were also classified according to worsening of MD. As a result, they found that global visual field indices, peripapillary RNFL thickness, and macular GCC thickness showed insignificant differences between the groups at the beginning and end of follow-up, and their conclusion was that oral antioxidant supplementation with or without ω-3 fatty acids appear to have no benefit as adjunctive treatment of mild/moderate POAG in the short term. (388)

Ohguro H et al also followed the effect of a dietary supplement on visual field in glaucoma in a two-year randomized, placebo-controlled trial, but used blackcurrant anthocyanins (BCACs). They followed 38 patients with POAG treated with antiglaucoma drops, with 19 patients taking oral BCAC (50 mg/day) and 19 patients taking placebo once daily over a period of 24 months. Systemic blood pressure, pulse rate, intraocular pressure, ocular blood flow, and mean visual field deviation (MD) with Humphrey perimeter were measured over the 24-month period. They observed a statistically significant difference between groups in the mean change from baseline in MD after 24 months of therapy in favor of the dietary supplement group. Furthermore, BCAC administration showed an increase in ocular blood flow during the 24-month observation period, which did not lead to significant changes in systemic and ocular conditions, including IOP, during the 24-month period. It is concluded that oral administration of

BCACs may be a safe and promising adjunct for patients with POAG in addition to antiglaucoma medication. (437)

The non-reproductive potential of Citicoline in relation to POAG is better studied and studies similar to ours that track the effect of its intake are being found. A number of in vitro and in vivo studies have demonstrated the neuroprotective role of Citicoline through increased retinal dopamine levels, enhanced anti-apoptotic effect, limited retinal nerve fiber layer (RNFL) thinning, neurite regeneration, protection against glutamate excitotoxicity, minimization of RGC damage and corresponding visual field improvement. (368)

Already in 1989 J Pecori Giraldi et al evaluated the therapeutic value of citicoline administration by automated computerized perimetry. Beneficial effects on the visual field of patients suffering from open-angle glaucoma following intramuscular administration of Citicoline are indicated. The drug was administered in a dose of 1 g for ten consecutive days. The visual field was examined using central computerized perimetry and automated perimetry. All patients had well-controlled intraocular pressure with beta-blockers but had characteristic glaucomatous perimetry defects. Even then, scientists suggested that Citicoline could be used as a useful adjunct to conventional hypotensive therapy, as it has a positive effect on glaucomatous damage to the optic nerve. (357)

In1999, there was a publication about the use of Citicoline in POAG. (358) In this randomized clinical trial, 40 glaucomatous patients with a mean deviation (MD) between −2 and −6 dB (Humphrey 24-2 perimetry) were evaluated. Patients were randomly divided into two groups with similar characteristics - a Citicoline group (25 eyes) and a placebo group (15 eyes). Throughout the follow-up period, IOP was below 18 mmHg. As the patients took Citicoline, in a dose of 1000 mg/day intramuscularly/ or placebo according to a scheme with periods of intake and pause. In all patients, RGC function was assessed by recording PERG (Pattern Electroretinogram) responses. In patients after treatment with Citicoline, a significant improvement ($p<0.01$) was found in the values of PERG indicators compared to those of placebo patients, which means that, according to the authors, the application of Citicoline induces an increase in the function of the RGC with a subsequent enhancement of the neural conduction along the visual roads.

Of these 40 patients included in the previous study, 24 glaucoma patients were included in a second study in which the follow-up period was extended to 8 years. (360) Twelve patients

were treated, in addition to ocular hypotensive therapy, with Citicoline, at a dose of 1000 mg/day intramuscularly (Neuroton, Nuovo Consorzio Sanitario, Rome, Italy) every 2 months, followed by a 4-month break (16 cycles in total); and exclusively ocular hypotensive therapy was performed in 12 patients. The result was a greater improvement in PERG values compared to baseline and patients not taking Citicoline. Enhancement of PERG induced enhancement of VEP responses, suggesting that increases in RGC function result in enhancement of neural conduction throughout the visual pathways. The reduction in dysfunction of the glaucomatous visual pathways due to Citicoline treatment also causes a reduction in visual field defects. Like us, they found that at the end of follow-up (in this case a total of 96 months), HFA 24–2 perimetry MD values were reduced relative to those observed at baseline. Changes in MD were statistically significantly associated (Pearson's test) with improvements in PERG.

Virno et al (366) observed during a 10-year follow-up period that glaucomatous patients treated with citicoline showed a stable improvement in the "unperceived zone" on perimetric examination.

A comparison of oral and intramuscular Citicoline treatment in 60 glaucoma patients (361) with moderate visual field defects and IOP less than 18 mmHg found a significant improvement in RGC function, and no significant differences were found between groups in PERG values.

Administration of Citicoline in an oral solution with a bioavailability of about 98% (438) in patients with POAG who had a documented history of disease progression despite controlled IOP was observed to significantly reduce the mean rate of MD progression to -0. 15 (±0.3) dB/year after 2 years of treatment with Citicoline (4 cycles of 4 months each followed by a 2-month break). (363)

When administered as eye drops, Citicoline has been shown to reach the vitreous, and this gives reason to believe that this molecule may have an effect on glaucomatous damaged ganglion cells. (364) A study evaluating the effects of citicoline eye drop treatment on RGC function and optic nerve conduction (365) with 47 POAG patients with moderate visual field defects and IOP below 18 mmHg confirmed all previously seen with intramuscular or oral Citicoline treatment. It was also found that after treatment with Citicoline eye drops, an improvement in the visual field (increase in MD of more than 1 dB) was observed in 71% of glaucomatous eyes.

Chițu I and co-authors (439) observed the development of various parameters in glaucoma patients treated with Citicoline. 22 patients were included in the study and received oral citicoline in addition to ocular hypotensive therapy. Examinations were performed at the beginning of the study, then at 3 months and 6 months and included, in addition to a complete ophthalmological examination and determination of IOP, OCT and VEP. They reported that RNFL thickness showed a positive correlation with the amplitude of P100 and P2 waves. Like us, this study reported that RNFL thickness had statistically significantly higher values at the 6-month visit and found a slight increase in GCC thickness between consecutive visits. However, the researchers attributed this to an artifact of the examination because they believe they are not clinically possible. Like us, they report that Citicoline has a positive effect in glaucoma in certain aspects.

Analogous to our study, Lanza et al. (440) demonstrated a neuroprotective effect of oral citicoline that delayed the progression of POAG. 60 patients were randomly divided into two groups of 30 participants each. Age, sex, and disease duration were matched between groups. Despite stable IOP, slow disease progression was observed in all patients. All patients underwent a complete eye examination, including measurements of IOP, SAP, retinal nerve fiber layer (RNFL) thickness, and ganglion cell complex (GCC) thickness measurements with optical coherence tomography (OCT) before initiation of Citicoline treatment, at 6-, 12-, 18- and 24-months follow-up. The results of this study largely overlapped with ours, namely: after 18 months, there was a significant increase in the mean values, mean deviation (MD) of the Citicoline group, and these values were stable at subsequent visits. In the antihypertensive-only group, mean MD values continued to decrease significantly over time. Mean RNFL and GCC thicknesses in the citicoline group were significantly higher at 12 months and appeared stable at later visits, while the latter group's values decreased significantly over time. The conclusion of the study is that Citicoline appears to be effective in slowing the progression of POAG.

Rossetti L et al (441) also conducted a randomized, double-masked, placebo-controlled, multicenter, 3-year trial to demonstrate whether adjunctive therapy with citicoline eye drops could slow glaucoma progression in patients with progression and an IOP below 18 mmHg. Patients were randomized to receive Citicoline eye drops or placebo 3 times daily for 3 years and were followed up every 3 months. Visual field with 24-2 and 10-2 strategies and RNFL assessment with OCT were investigated. Progressive worsening of MD and RNFL thinning was

significantly less in the Citicoline group compared to the placebo group. It follows that additional treatment with Citicoline eye drops to IOP-lowering treatment may reduce disease progression in patients with progressive glaucoma despite IOP ≤18 mmHg.

Sahin AK et al (442) also evaluated the short-term effects of oral citicoline therapy on the retinal nerve fiber layer (RNFL) and macular ganglion cell inner plexiform layer (mgCIPL) in patients with primary open-angle glaucoma. Fifty-four eyes of 54 glaucoma patients were included in the study. In addition to topical hypotensive, 250 mg of oral citicoline was administered to 27 patients, while 27 patients were designated as the control group. RNFL and mgCIPL values were measured using optical coherence tomography (OCT) 1 day before treatment and 3 months after the start of treatment. At the third month visit, Citicoline treatment was discontinued, and drug-free control measurements were obtained at the fourth month in the Citicoline group. They, like us, reported significantly higher mean RNFL thickness values at month 3 from baseline in the Citicoline group. According to them, this improvement partially regressed after a 1-month break period. No statistically significant changes in RNFL were observed in the superior, nasal, temporal, and inferior quadrants at months 3 and 4. The change in mean thickness and lower quadrant RNFL thickness in the Citicoline group at 3 months was significantly greater than the control group. There were no significant differences between groups according to change in mgCIPL thickness and superior, nasal, and temporal quadrant RNFL thickness. These results indicate, according to the authors, that with oral treatment with Citicoline, loss of the average RNFL is prevented in patients with POAG in the short term. Data from the study suggests that Citicoline may have a significant impact on slowing the progression of glaucoma, which may have a potential neuroprotective effect.

Anton A et al (443) investigated the role of dietary supplements in the treatment of glaucoma by evaluating the effect of citicoline, vitamin C, and docosahexaenoic acid (DHA) administration in glaucoma patients in a prospective, randomized trial. Glaucoma patients were randomized into one of four groups and treated for 3 months with vitamin C, DHA, Citicoline, or a combination of DHA and Citicoline. A complete ophthalmological examination was performed, and visual fields were examined monthly. Changes in visual field indices (VFI) were assessed and compared in each group. Seventy-three people were included in the study. As a result, the mean defect (MD) was reported to have significantly improved (p= 0.001) from -9.52 ± 4.36 to -7.85 ± 4.36 dB during the study period in subjects taking DHA + Citicoline. Similarly,

mean VFI significantly improved (p= 0.001) in the respective group. The only treatment group that showed a statistically significant improvement (p =0.006) in MD (from -0.1041 ± 0.2471 to 0.1383 ± 0.2544 dB/month) and VFI was the DHA-treated group + Citicoline. Therefore, according to the authors, the combination of oral treatment with DHA + Citicoline significantly improved visual field parameters and in glaucoma patients after 3 months of treatment.

On the other hand, Prinz J and co-authors (444) published in September 2023 a systematic review that contradicts the conclusions of previous studies. After identifying 10 clinical trials investigating the efficacy of Citicoline on IOP, mean deviation of the 24-2 visual field test (MD 24-2), retinal nerve fiber layer (RNFL) and electroretinogram (PERG) pattern P50-N95 amplitude in glaucoma patients in PubMed, Web of Science, Google Scholar and Embase, they reported no significant differences in IOP amplitude, MD 24-2, RNFL or PERG P50-N95 between patients receiving Citicoline and the control group and lack sufficient evidence to support that citicoline slows the progression of glaucoma.

From everything exposed abovemis clear that in the world literature there are many studies in which improvements in the parameters measured by computer perimetry (358,360,365,435,437,438,440,443) after taking dietary supplements by glaucoma patients are noted, but only a few studies report improvements in the parameters measured by OCT. (439,440,441,442) On the one hand, it is suggested that no increase in the thicknesses of OCT parameters should be clinically possible (439), but on the other hand, there are reported factors that could affect the measurements. (445,446,447)

Research shows that artefacts that affect the quality and subsequently the utility of OCT images are common in clinical practice. The results of a 2016 review and analysis of this topic indicate that more than 25% of patients are expected to have SD-OCT artifacts in glaucoma testing that could lead to false-positive or false-negative interpretation. (446) Another study by Asrani et al (448) found that there were artifacts associated with SD-OCT images in 15.2%–36.1% of patients evaluated for glaucoma. Macula segmentation and GCC complex measurements are prone to error and misinterpretation, especially in eyes with pathology (e.g., ARMD, retinal edema). Therefore, nearly a quarter of patients have artifacts from the RNFL and/or GCL analysis with SD-OCT. It is important to distinguish artifacts from genuine changes, especially when interpreting POAG disease progression. (445)

Several independent studies have shown that scans with greater signal strength are associated with higher RNFL thickness measurements, and that decreased signal strength is associated with reduced RNFL thickness, which may be misinterpreted as the presence of glaucomatous damage or progression when comparing multiple OCT scans over time. (449-453) Therefore, signal strength values should always be considered when evaluating RNFL thickness measures. (446)

Any obstruction in the path of the light beam can reduce the signal-to-noise ratio and interfere with the ability to identify the borders of the RNFL or key features of the optic nerve head, such as the optic disc and cup rims, resulting in unreliable measures. (446) The most common causes of ocular media opacities that reduce signal strength and compromise retinal layer segmentation are dry eye, corneal opacities, cataracts, and impaired vitreous structure. (446) The small pupil could also potentially reduce signal quantity and quality. (447) In our study, we did not account for lens capsule density, nor did we account for the presence of cataract or intraocular lens as study confounders, due to the relatively short follow-up period and the relatively small group of patients followed.

Conditions such as glaucoma, dry eye, and cataract often coexist due to their prevalence in the aging population. (454) In addition, ocular surface disease and dry eye syndrome are common in patients using topical hypotensive medications. (455) Studies have shown that dry eye and/or cataract decrease the scan quality index and decrease RNFL thickness measures. (456-459) This effect should always be considered during RNFL assessment, given the potential risk of false positives in the detection of glaucoma or disease progression. (447) In our study, artificial tears were placed on patients and patients were encouraged to blink several times immediately before scanning to ensure even distribution of the tear film and to maintain adequate scan quality. (447)

Marked opacity at the vitreoretinal interface could also cause errors in segmentation of RNFL thickness and could be misidentified as the internal limiting membrane, leading to artificial thickening of the RNFL. (446)

The effect of large refractive errors and the associated long or short axial length of the eye should be taken into account because it may go unrecognized in patients who have undergone refractive surgery or cataract extraction with intraocular lens implantation. (446) In the medical history, which we performed at the beginning of the study, the presence of operative

interventions was documented, but the axial length was not taken into account when performing the OCT examination.

Studies have shown increased RNFL thickness in eyes with epiretinal membrane (ERM). (460,461) RNFL segmentation errors in SD-OCT measurement occurred significantly more frequently (35.8%) in the presence of ERM compared to eyes without any retinal pathologies. (462) Due to the fact that ERMs are included in the segmented RNFL, there is artificial increase in RNFL thickness. (448) Therefore, caution is warranted when interpreting RNFL thickness measurements in eyes with ERM. In our study, the presence of ERM was not documented during the observation period.

Gliosis of the optic nerve and retinal axons after optic nerve injury can lead to an increase in RNFL thickness. The presence of a layer of myelinated nerve fibers can also cause an increase in RNFL thickness, which can lead to an overestimation of the number of axons in the corresponding location. Acute edema of the inner retina due to arterial occlusions can cause an increase in RNFL thickness associated with hypodensity in the outer retina due to attenuation of the signal in this layer. (447) Bayraktar S et al investigated the effect of peripapillary retinoschisis on thickness measurements of RNFL by using SD-OCT in glaucomatous eyes. They observed an increase in RNFL thickness in the presence of retinoschisis. It is important to examine circumpapillary RNFL images of glaucoma patients so that RNFL thickness is not overestimated. (463) In our study, none of the above reasons for increased RNFL and GCC thickness were documented.

Kim S et al investigated the impact of uncomplicated cataract surgery on SD-OCT RNFL measurement in patients with POAG and reported a significant increase in RNFL and IOP reduction after phacoemulsification. RNFL thickness was significantly higher in eyes with POAG compared to normal eyes and was associated with postoperative IOP reduction and preoperative mean visual field deviation. (464) Scientists also documented the change in RNFL and ONH thickness using Stratus OCT after trabeculectomy in adult glaucoma patients and reported a temporary increase in RNFL thickness and a decrease in cup area postoperatively. Values returned to normal within 3 months. (465) In our study, no phacoemulsification or trabeculectomy was documented in the patients during the observation period.

OCT lens opacities, such as those from fingerprints or accidental contact with the patient's periocular region or face (e.g. nose), can reduce image quality and directly affect RNFL thickness measurements. (446)

On the one hand, the RTVue OCT (Optovue Inc., Fremont, CA) has built-in eye tracking systems, making it less affected by blink or movement artifacts. New SD-OCTs map each subsequent OCT image to a reference baseline scan using landmarks such as retinal vessel position and optic disc location, which reduces variability between subsequent scans. (445) On the other hand, dry eye, cataracts, floating opacities, changes in scan quality index, OCT lens opacities, or axial misalignment are possible to affect parameter measurements with different SD-OCT devices. (447)

Chapter VI

Summary

In the current dissertation, the effect of the application of nutritional supplements was studied by following the dynamics in the functional and structural changes occurring in the course of the disease POAG. In the study, 90 patients with bilateral POAG were included and randomly divided into three groups of 30 people each. Group A was taking the dietary supplement Mielooptik, Group B was not taking nutritional supplements, Group C was taking the dietary supplement Citizin. Patients were evenly distributed by gender (15 women and 15 men in each group) and similar age (50-75 years). Disease duration and degree of glaucomatous damage varied between groups. The observation of the patients lasted 15 months, and the nutritional supplements were taken according to a scheme for two periods of 6 months with a 3-month break between them. Patients received topical antiglaucoma therapy and IOP values were kept stable with good compliance (14 - 21mmHg). At the beginning of the study, at the 6th and at the 15th month, the patients underwent a complete ophthalmological examination. Goldman tonometry was performed between 2 and 4 p.m. Functional changes were examined with Standardized Computer Perimetry (SAP) performed with a Humphrey Field Analyzer perimeter.

To determine structural changes, Optical Coherence Tomography (OCT) was used, performed with RTVue Avanti, Optovue. The evaluated parameters were as follows: MD (dB) and PSD (dB) measured with SAP; average RNFL thickness (RNFL Ave); thickness in upper (RNFL Sup) and lower (RNFL Inf) halves; mean GCC thickness (GCC Ave), thickness in superior (GCC Sup) and inferior (GCC Inf) half measured by OCT. No side effects or adverse reactions were reported as a result of taking the nutritional supplements.

The results of the specific study were observed under the same conditions for conducting the research, the same groups according to gender (15 men and 15 women for each group), approximately the same age (65.76 for group A; 64.5 years for group B; 65 .7 for group C) and approximately the same IOP (Group A - average 14.7 mmHg, for Group B - average 14.1 mmHg and for Group C - average 14.4 mmHg) for the entire 15-month period.

After the first 6-month intake of Mielooptik for group A, we had a statistically significant effect on all parameters compared to baseline, and it was less pronounced on the parameters GCC Ave, GCC Inf and GCC Sup. For group C, there was also a statistically significant improvement in parameters, but there was no statistically significant difference in MD, PSD and RNFL Ave values after the first 6 months admission compared to baseline. In group B, in the absence of dietary supplements, there was a clearly pronounced statistically significant effect in the direction of deterioration of all parameters.

For the entire observation period, an improvement in the parameters studied with SAP (MD and PSD) was reported in the group receiving Mielooptik and in the group receiving Citizin. In the group that had not taken nutritional supplements, a deterioration of the relevant indicators was reported. The parameters measured by OCT (RNFL and GCC) also improved at the end of the observation period in the patients taking dietary supplements Mielooptik and Citizin, and in group B a worsening of the corresponding parameters was reported again.

It is interesting to note that the effect of taking Mielooptik was already noticed after the first 6-month period, while taking Citizin it was not so obvious in the first 6 months. In the next follow-up period, a more tangible effect of taking Citizin was also reported, suggesting that this type of treatment needs time to show relevant clinical results.

There is no way to establish the long-term effect of the application of nutritional supplements with neuroprotective properties on functional and morphological characteristics in

POAG, but in any case, it would not harm to add them to anti-glaucoma therapy. The placebo effect should not be overlooked for patients suffering from this chronic progressive disease.

The results observed in our study could be due to the fact that the patients strictly adhered to the antiglaucoma and concomitant therapy. Patients participating in the study were instructed at each visit on the importance of strict adherence to therapy. One of the limitations of this study is the heterogeneity of glaucoma stage in the included patients, as the study was not limited to a specific glaucoma stage. On the other hand, this feature provides a more realistic representation of the patient population. Another limitation of the study is the small sample analyzed.

In conclusion, although these results need to be confirmed by further studies with a longer follow-up period and performed on a larger population, and it would be useful to add other studies on optic nerve function and visual pathways, the data from our study show that the dietary supplements Mielooptik and Citizin have a positive effect on the progression of POAG at different stages of the disease.

Chapter VII
Conclusions

1. The intake of the dietary supplement Mielooptik by patients with POAG showed a statistically significant improvement in all the parameters monitored by the study. The data observed in this study showed that there was a modest improvement in MD and PSD values measured by SAP and a modest increase in RNFL and GCC thickness measured by OCT at 6 and at 15 months. The study showed that the supplement Mielooptik has a positive effect on the progressive damage of the optic nerve in patients with glaucoma.

2. In the absence of nutritional supplementation during the observed period, a statistically significant worsening of the parameters measured with SAP and OCT was reported, despite the compensation of IOP with topical hypotensive treatment, which testifies to the progressive damage of the optic nerve in patients with POAG.

3. Taking the food supplement Citizin leads to a minimal but statistically significant improvement in the parameters MD and PSD, measured by SAP and a slight increase in RNFL and GCC thickness measured by OCT over the observation period. During the first 6-month admission period, no statistically significant effect was reported for MD, PSD and RNFL Ave parameters. The change in the observed parameters is more noticeable after the second 6-month intake of Citizin, which probably means that a longer period is needed to improve the condition and, respectively, slow down the progression of POAG.

4. Correlation analysis proved the statistically significant positive change for all patients of group A and group C due to the intake of Mielooptik and Citizin, respectively, and the worsening of indicators occurred in all patients for group B within the observed 6- and 15-month period. The parameters of group A patients improved to a higher extent than those of group C. A statistically significant strong correlation was observed between the pairs of criteria MD-PSD and the mean values of RNFL-GCC for all three studied groups.

5. The results of the analysis of variance showed that the three groups were not distinguishable in terms of the evaluated parameters, both at baseline and after 6 and 15 months. Age, gender and IOP were not significant for the three groups during the study period. In the three groups, there was a change in the studied parameters, with group A in the direction of moderate improvement, in group C - in the direction of slight improvement, and in group B - in the direction of deterioration of the indicators.

Chapter VIII
Contributions

Contributions of a cognitive nature:

1. An in-depth and analytical literature review of the available literature devoted to the pathogenetic factors, functional and structural changes occurring in the course of POAG was made.

2. A detailed literature review of imaging and functional studies, as well as available and potential medication options for POAG, was made.

Contributions of a scientific and applied nature:

1. For the first time in Bulgaria, a prospective, long-term study was conducted with the follow-up of functional and structural changes in patients with POAG who take the nutritional supplements Mielooptik and Citizin.

2. A comparative analysis was made of the rates of progression of POAG in patients with and without dietary supplements.

3. The correlations of the observed parameters during the course of the study were investigated.

Contributions of a confirmatory nature:

1. The benefit of using nutritional supplements with neuroprotective and antioxidant properties as an additional option to delay progression in patients with POAG is proven.

2. Disease progression has been confirmed to occur, even with well-controlled IOP.

3. The diagnostic capabilities of OCT and SAP in the diagnosis and follow-up of POAG have been confirmed.

Chapter IX.
Publications and participation in scientific forums

Publications related to the dissertation work

1. Kancheva K., Zlatarova Z., Role of neuroprotection in complex antiglaucoma therapy Glaucoma ISSN 1314-7692 Volume X, Issue 2, 2021
2. Kancheva K., Zlatarova Z., Morphological and functional benefits of using nutritional supplements in the treatment of primary open-angle glaucoma - first results, "Bulgarian Ophthalmological Review" 2023;67(1)
3. Kancheva K., Zlatarova Z., Modern understanding of the pathogenesis of primary open-angle glaucoma, Glaucoma ISSN 1314-7692 Volume XII, issue 1 / 2023
4. Kancheva K., Zlatarova Z., Morphological and functional benefits of using citicoline in the treatment of primary open-angle glaucoma, Bulgarian Ophthalmological Review 2024;68(2):11-20

Participation in scientific forums on the subject

1. XXI Symposium of the Bulgarian Glaucoma Society 17-18.03.2023, electronic pOCTer on the topic "Functional results of the action of neuroprotectors"
2. XXII Symposium of the Bulgarian Glaucoma Society March 22-24, 2024, electronic pOCTer on the topic "MicrOCTructural changes in the retina in patients with primary open-angle glaucoma after taking nutritional supplements"
3. Annual conference of the Bulgarian Society of Ophthalmology "Ophthalmology and interdisciplinary solutions" 03-06.10.2024, report on the topic "Morphological and functional benefits of using citicoline in the treatment of primary open-angle glaucoma"

Chapter X

Summary

Purpose**:** The purpose of the study is to investigate and document the use of the nutritional supplements Mielooptik and Citizin, and to analyze the effect of their application as adjunctive therapy in patients with POAG.

Materials and methods: This study includes the results from 180 eyes of 90 patients (45 women and 45 men) aged 50-75 years with POAG, undergoing topical anti-glaucoma therapy. Patients were randomly divided into three groups of 30 (15 women and 15 men in each group) and followed for a period of 15 months. The IOP values of the patients were kept stable with good compliance in the range of 14 - 21 mmHg. The patients of group A took the dietary supplement Mielooptik according to a scheme. Those in group B did not take any nutritional supplements that could have affected the results of the study during the observation period. Group C patients took the dietary supplement Citizin according to a scheme. The nutritional supplements were taken in two periods of 6 months with a 3-month break between them.

At the beginning of the study, at the 6th and at the 15th month, all patients underwent a complete ophthalmological examination: examination of visual acuity with the best correction for each eye separately, Goldmann tonometry, indirect ophthalmoscopy, gonioscopy, pachymetry, Standardized Computer Perimetry (SAP) and Optical Coherence Tomography (OCT). The evaluated parameters are as follows: MD (dB) and PSD (dB) measured with SAP; average RNFL thickness (RNFL Ave); thickness in superior (RNFL Sup) and inferior (RNFL Inf) half; mean GCC thickness (GCC Ave), thickness in superior (GCC Sup) and inferior (GCC Inf) half measured by OCT.

Results**:** The results of the study were observed under the same conditions for conducting the research, the same groups according to gender (15 men and 15 women for each group), approximately the same age (65.76 for group A; 64.5 years for group B; 65 .7 for group C) and approximately the same IOP (Group A - average 14.7 mmHg, for Group B - average 14.1 mmHg and for Group C - average 14.4 mmHg) for the entire 15-month period.

After the first 6-month intake of Mielooptik for group A, there was a statistically significant effect on all parameters compared to baseline, and it was less pronounced on the parameters GCC Ave, GCC Inf and GCC Sup. For group C, there was also a statistically significant improvement in parameters, but there was no statistically significant difference in MD, PSD and RNFL Ave values after the first 6-month admission compared to baseline. In group B, in the absence of dietary supplements, there was a clearly pronounced statistically significant effect in the direction of deterioration of all parameters.

Over the entire observation period, the mean MD values of group A decreased by 0.74 dB ($p<0.05$) and the mean PSD values decreased by 0.91 dB ($p<0.05$) compared to those at the beginning. After 15 months, mean RNFL Ave values of group A increased by 3.43(μm) ($p<0.05$) and mean GCC Ave values increased by 3.62(μm) ($p<0.05$) compared to the initial ones.

At 15 months, MD values of group B worsened by 0.22dB ($p<0.05$) and mean PSD values worsened by 0.35dB ($p<0.05$) compared to baseline. At the end of the follow-up period, the mean RNFL values of group B decreased by 3.37(μm) ($p<0.05$) and the mean GCC values of group B decreased by 2.75(μm) ($p<0.05$) compared to baseline.

After 15 months, group C mean MD values improved by 0.31dB ($p<0.05$) and mean PSD values improved by 0.37dB ($p<0.05$) compared to baseline. At the end of the period, mean RNFL

values of group C improved by 1.29(μm) ($p<0.05$) and mean GCC values of group C improved by 1.89(μm) ($p<0, 05$) compared to the beginning.

An interesting observation was that the effect of taking Mielooptik was reported already after the first 6-month period, while when taking Citizin the impact was not so obvious in the first 6 months. In the next follow-up period, a more tangible effect of taking Citizine was reported, suggesting that this type of treatment needs time to show relevant clinical results.

According to the results of the application of ANOVA on the rate of progression of the parameters for the three studied groups for the 15-month period, it is statistically significant ($p<0.05$) whether the type of supplement is accepted or not. In group A there is a statistically significant ($p<0.05$) positive change in the parameters, in group C there is also a statistically significant ($p<0.05$) improvement, but to a lesser extent than in group A. In group B there is a statistically significant ($p<0.05$) deterioration of the parameters for the studied 15-month period.

Conclusion: These results suggest that the intake of the nutritional supplements Mielooptik and Citizin (Citicoline), as a concomitant antiglaucoma therapy, has a positive effect on the progression of POAG at different stages of the disease. There is no way to establish the long-term effect of the application of nutritional supplements with neuroprotective properties on functional and morphological characteristics in POAG, but in any case it would not harm to add them to the anti-glaucoma therapy. In the absence of intake of nutritional supplements within the observed period, a statistically significant deterioration of the parameters measured with SAP and OCT is reported, which testifies to the progressive damage of the optic nerve in patients with POAG. Further studies with a longer follow-up period and performed on a larger population are needed to confirm the results of this pilot study.

Keywords: glaucoma, Mielooptik, citicoline, neuroprotection, SAP, OCT

ACKNOWLEDGMENTS

This dissertation work is dedicated to Doc. Dr. Konstantin Kanchev, my grandfather, who, I believe, would be proud of me today.

I would like to express huge gratitude to my family for their endless love, support and faith in me!

Heartfelt thanks for the dedication of my supervisor, Prof. Dr. Zornitsa Zlatarova, MD, PhD, who was always by my side! Thanks to her help and support, valuable experience and advice, this dissertation became a reality.

And last but not least, I sincerely thank an incredible person, mentor and role model,

Art.-cor. Prof. Dr. Hristina Grupcheva, MD, FEBO, FICO(Hon), FBCLA, FIACLE,

with whose support my whole professional growth became possible.