ORIGINAL RESEARCH

Oral citicoline: Influence of long-term therapy on perimetric glaucoma defects

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Abstract

INTRODUCTION: Glaucoma is a chronic-degenerative optical neuropathy, characterized by gradual loss of ganglion cells and thinning of the retinal nerve fiber layer. The increase of intraocular pressure is the main risk factor, but the damage can also progress with other mechanisms. Currently the research is aimed at the discovery of drugs able to inhibit the mechanism that leads to the apoptosis of the ganglion retinal cells. Among these, Citicoline is the one with the most important scientific evidence.

PURPOSE: This study evaluated the effects of long-term therapy of Citicoline in oral solution, on perimetric defects in patients with glaucoma in good tonometric compensation (IOP ≤ 18mmHg). The study lasted 3 years.

MATERIALS AND METHODS: 60 patients (120 eyes) affected by chronic glaucoma, with perimetric alterations classified at 4th stage with mixed localization defects (according to GGS2 classification) were selected. Patients were randomly divided into two groups composed by 30

patients (60 eyes). One group (TG) was treated with Citicoline in oral solution at a dosage of 500 mg/day for 60 days, followed by 30 days of suspension; the other group, without Citicoline therapy, constituted the control group (CG). Both groups underwent perimetry at T0, T12 months, T24 months and T36 months. The variations of the MD and PSD indices and of the staging of the perimetric defects were analyzed. Visus, tone and refraction variations were observed. The results obtained were related to a 3rd group of 30 healthy patients (60 eyes). This study was approved by the Sapienza-University of Rome, Ethics Board (Pro-tocol No. 1076/14). This study will be performed in accordance with the Declaration of Helsinki. Dissemination plans include presentations at scientific conferences and publication in scientific journals. Trial registration: ClinicalTrials.gov, identifier: NCT02257333 on October 6, 2014.

RESULTS: After 12 months, a statistically significant improvement in MD was observed in the GT (Δ = 21%), while PSD seemed steady (Δ = <1%). At T24 the MD improvement was confirmed linear (Δ = 35%) while the PSD value started to decrease (Δ = -7%). At T36 MD stabilized at Δ = 35%, while PSD maintained its decreasing trend (Δ = -16%), with values confirming the statistical significance. In the GC MD was progressively decreasing and PSD was slowly increasing. The stage, initially common to the two groups, underwent a gradual improvement in GT, up the 3rd stage with defects located at the T36, compared to a slight deterioration in GC that at the T36 confirmed itself at the 4th stage with mixed defects.

CONCLUSION: Long-term therapy with Citicoline determined an improvement in perimetric indices in GT: this would indicate a neuroprotective effect in chronic glaucoma, even in long-term treatments.

Abbreviations in this article:

RGCs: Retinal Ganglion Cells

IOP: IntraOcular Pressure

MD: Mean Deviation

PSD: Pattern Standard Deviation

GSS2: Glaucoma Staging System 2

TG: Therapy Group

CG: Control Group

HG: Healthy Group

Introduction

Glaucoma is a chronic-degenerative optic neuropathy, characterized by a gradual loss of RGCs and a thinning of the retinal nerve fiber layer, responsible for the progressive reduction of the visual field to blindness[1,2,3,4,5,6]. Many literature studies[7,8,9,10,11] and the clinical experience agree that the treatment of this pathology can't be usually limited to the traditional, but always fundamental, hypotoning treatment, which purpose is to affect the main risk factor, the IOP: this concept has developed from the evidence that, despite good tonometric compensation, a percentage of patients have a visual damage progression. This highlighted the role of other risk factors, especially those related to neurodegeneration. In fact, it is assumed that due to a trigger event, such as a raise of the ocular tone, a neuronal damage occurs (caused to vascular or mechanical insults) which can subsequently progress by secondary degeneration, even when the hypertone, is removed[7].

Therefore, the research of molecules and neuroprotective drugs, capable of inhibiting the mechanism that brings all the RGCs to apoptosis, is increasing. This therapy should always be associated with the traditional hypotonic one. Among these molecules with hypothesized neuroprotective activity, Citicoline is the one studied for at least 20 years, which has collected a series of interesting clinical evidences[7,12,13,14].

Citicoline, or CDP-choline, is an organic molecule belonging to the nucleotide group, produced endogenously but which needs a dietary supplement[13,15]. Its neuroprotective action seems to unfold through different modalities: it's an intermediary in the synthesis of phosphatidylcholine, an important phospholipid particularly present in neuronal cells[13,15,16,17]. It seems to have a trophic effect on cellular membranes, increasing the metabolism of brain structures and inhibiting phospholipid degradation[15,16,17,18]. Moreover, it performs a functional activity related to the bioavailability of neurotransmitters and neuromodulators, among which acetylcholine and dopamine[18,19, 20, 21], present in the retinal, post-retinal and visual cortex, stand out.

The purpose of this study is the evaluation of a long-term treatment (3 years) with Citicoline in oral solution at the dosage of 500 mg/day, taken through 2-month consecutive cycles with 1-month suspensions on perimetric defects, variable psycho-physical indicator of glaucomatous damage. All these patients are affected with chronic glaucoma in good tonometric compensation (IOP ≤ 18 mmHg), obtained with hypotonizing drugs. The role and efficacy of

drugs with neuroprotection function in the treatment of glaucomatous patients have been for a long-time subject of debate in the scientific community. Among all these molecules, Citicoline stands out for its importance, with numerous evidences of efficacy. The present study is part of this kind of scientific analysis, but it adds the aspect of long-term follow-up, up to 3 years, an element that makes it peculiar compared to many studies conducted in shorter time intervals. The evaluation was performed using Humphrey 30-2 Perimeter Tests, analyzing MD (and PSD parameters and subsequently staging all the patients using the Glaucoma Staging System 2 (GSS2).

Materials and methods

In this study, 60 patients were selected (66 men, 54 women), for a total of 120 eyes (average age = 69.2 years, minimum age = 44 years, maximum age = 86 years).

A post hoc sample size calculation (statistical software SPSS, version 22.0 for windows) indicate that 30 patients per treatment arm would have been sufficient to demonstrate a neuroprotective effect in patients, assuming a power of 80% and an alpha of 0.05.

The inclusion criteria were: presence of chronic open-angle glaucoma (4^{th} grade angle according to Schaffer classification); good tonometric compensation (IOP <18 mmHg) with hypotonizing drugs (to exclude that the progression of the damage was due to changes in endocular pressure); perimetric alterations caused by glaucoma; corneal pachymetry in the normal range (> 520µm and <550µm).

The exclusion criteria were: positivity for concomitant ocular pathologies and opacity of the dioptric means.

All patients underwent: anamnestic evaluation, complete ophthalmological examination, slit-lamp biomicroscopic examination, Goldmann tonometry, gonioscopy with Goldmann mirror lens, corneal pachymetry (with ACCUPACH V ultrasound pachymeter), examination of the fundus with Schepens lens, computerized perimetry (Humphrey program 30-2 HFA II and SITA Standard threshold strategy) and classification of perimetric alterations through Glaucoma Staging System 2.

The study was conducted through a retrospective analysis (case-control study) and was approved by the Sapienza-University of Rome, Ethics Board (Pro-tocol No. 1076/14). This study will be performed in accordance with the Declaration of Helsinki. Dissemination plans include

presentations at scientific conferences and publication in scientific journals. Trial registration: ClinicalTrials.gov, identifier: NCT02257333 on October 6, 2014. Written informed consents were obtained.

The overall population was randomly divided into two groups: the first, called Therapy Group (TG), composed of 30 elements treated with oral Citicoline 500mg/day in consecutive 2-month cycles followed by a month of suspension and hypotonic therapy; the other 30 elements, constituting the Control Group (GC), exclusively in hypotonic therapy. The two groups comply with the criteria listed above and are homogeneous for age, sex, tone, pachymetry and perimetric defects according to GGS2: all patients present a stage with mixed localization defect and both TG and CG have an average stadium at time T0 of the 4^{th} grade. Both groups were subjected to perimetric examination with perimeter Humphrey 30-2 at time T12 months, T24 months and T36 months, thus carrying out a long-term monitoring.

In addition there is a third group, called Health Group (HG), consisting of 30 patients not affected by chronic glaucoma, hypertension, diabetes mellitus or other major diseases worthy of note, with standard visual fields.

The analysis took into consideration MD and PSD parameters of each patient, obtaining from these the corresponding stage according to the GSS2 classification during the whole follow-up, comparing the TG values with those of the CG to evaluate the therapeutic efficacy of the drug.

A statistical analysis was conducted on the two groups of glaucomatous patients (therapy and control) and on the healthy sample, through the calculation of the mean (± SD), median (IQR, max and min) and the comparison between the values of the parameters of study (linear correlations). For the comparison between the three independent groups medians, a paired statistical test t of Student or Wilcoxon-Mann-Whitney Test was used, in case of non-parametric distribution.

If the test shows a non-normal distribution and/or an inhomogeneous variance, the comparison is performed on the medians, using the Kruskal-Wallis test.

Differences between group with $P<0,05$ were considered significant. The values of the P were expressed in two queues.

Results

TG and CG are similar and homogeneous; therefore, they can be compared through statistical analysis.

In the TG there was a statistically significant improvement of MD at T12 and even more at T24 (increasing linear trend); MD, which at T0 had a value of -13dB, at T12 went to -10 dB, until -8dB at T24. After T24 there was a stabilization of MD, not dued to drug saturation, but to minimal inter-patient variation (figure A).

In GC the progression of MD during follow-up was decreasing (MD with a T0 value of -13dB and T36 of over -14 dB) and outlined a statistically significant deterioration compared with the TG (p-value <0.05) (figure A).

In comparison with the HG, these characteristics were even more significant as a general trend over time (table A, table B).

In TG there was a gradual but statistically significant decrease (p-value <0.05) of PSD from T12 over the entire follow-up trend (decreasing linear trend). The PSD has shown a rise in the reduction of the value, above all starting from T12, going from 12 dB up to less than 11 dB at T36 (figure B).

In the CG the trend of the PSD was slowly increasing, and it resulted statistically significant compared with the group in therapy only after T36 (p-value <0.05) (figure B).

In the comparison with the HG these characteristics were more significant as a general trend over time (table C, table D).

Through the acquisition of the MD and PSD values, it was possible to visualize the progress of the patients' stage according to the GSS2. This analysis (figure C) showed that at T0 the TG was located in the 4th mixed stage. At T12 it improved, going on to 3rd stage with mixed defects; at T24 it decreas slightly below 3rd stage, always with mixed defects. Finally, at T36 has reached the 3rd stage with localized defects, thus improving the visual field. Through the improvement of MD and the reduction of PSD, we therefore see a statistically significant improvement (p-value <0.05) of the patient's retinal sensitivity during follow-up (figure D).

In the CG the stage during the follow-up was increasing with worsening at the T24, as if there was a decrease in RGCs which would reduce the patients' retinal sensitivity despite the good tonometric compensation (figure E).

The comparison with the HG further highlights these differences (table E, table F).

During this study, other parameters closely related to the perimetric defects of the patient, such as visus and refraction were evaluated. The average of the ocular tone values was also investigated, to exclude the possible bias of the worsening of the visual field due not to the chronic course of the illness, even if in good tonometric compensation, but to abrupt increases of the tone that can cause a sudden deterioration of the visual field.

During the follow-up, in the TG an increase of the visus was found, more marked between T12 and T24, passing from an initial value of 9/10 to T0, up to a value exceeding 10/10 of average at the end of the observation (figure D).

Conversely, in CG patients visus tended to progressively decrease during observation, while in HG it remained stable over time (figure D).

Considering the results previously exposed concerning the improvement of the visual field, it's reasonable to suppose that in the TG this increase in visual acuity was recorded thanks to the neuroprotective effect of the Citicoline on RGCs.

Analyzing refraction trend in TG with Citicoline, a linear increase in the value in analysis was found, which exceeded -2 to T24, while initially, at T0, was just under -1.5. Subsequently, the refraction presented a considerable decrease which led it to values lower than the starting ones (figure E).

In the CG the refraction remained stable at the approximate value of -0.5, then declined from T24 to T36 up to the value of -1.5 (figure E).

Considering what previously reported about visus, also refraction could have benefited from the neuroprotective influence of the Citicoline.

Regarding the analysis of tone values, these were found constantly below the value of 18 mmHg in the three groups, indicating a good tonometric compensation, achieved through antiglaucomatous therapy. (figure F). The minimum fluctuation of values wasn't statistically significant.

Discussion

Focusing on the results obtained from the data in our possession, Citicoline seems to have determined in patients in therapy an improvement, statistically significant, of the perimetric indexes. MD has improved in TG, going from an initial value at T0 of -14 dB to a value of -8.8 dB at T36 (35% variation compared to T0); instead, PSD recorded a 16% decrease compared to T0, from an initial value of 13 dB at T0 and a final value of 11 dB at T36; all this, in the face of a worsening of the two variables in CG patients.

There is no doubt that the normalization of IOP (main glaucomatous risk factor) must continue to be the essential basis of glaucoma therapy. However, it has been observed in clinical practice that, despite a significant reduction in intraocular pressure, glaucomatous damage continues, even in the absence of conditions that may have caused it. To curb this harmful progression, therefore, a neuroprotective therapeutic approach using Citicoline has begun to be used, in addition to the hypotonic therapy. Considering what has been previously illustrated about the actions carried out by the Citicoline, the improvement of retinal sensitivity[9], the reduction of the scomatose areas[22], the arrest of the progression of the campimetric damage[9,22,23,24], the increase of electrophysiological parameters of PERG and PEV [11,12] observed in a high percentage of treated glaucomatous patients, can be explained.

There are numerous experimental studies on the possibility of protecting RGCs from degeneration by administering Citicoline and they have also interesting results, therefore they suggest the validity of the use of Citicoline in addition to the traditional glaucoma therapy.

Conclusions

Analyzing the results obtained in our study, Citicoline in oral solution at a dose of 500mg/day, seems to play a neuroprotective effect in chronic glaucoma.

The efficacy of this drug can be seen already after a year and it maintains its trend of improvement of campimetric defects up to 3 years, affecting MD, PSD and staging according to the Glaucoma Staging System 2. The only parameter that appears to have a sort of stabilization after 3 years is the MD, but, having found some inter-patient variability, it would be appropriate to continue the observation over time to verify the possibility of maintaining the trend of the improving of the clinical picture.

In addition, the absence of side effects during treatment with Citicoline guarantees an excellent

risk/benefit ratio.

Our results also seem to exclude that the improvement of perimetric defects would be linked to the functional activity of the Citicoline on the increase in the bioavailability of neurotransmitters and neuromodulators (among which dopamine stands out, widespread in the retinal, post-retinal and visual cortex levels), that would act on the psychophysical variables of the visual field, since in the monthly suspension periods there should have been a regression of the improvement of MD and PSD and their stabilization at the starting values.

Finally, this study focuses on the conception of glaucoma not only as a chronic course, but also as a degenerative disease of the visual structures: so, the reduction of IOP, the main therapeutic moment, must be accompanied by a neuroprotective treatment, with the awareness that the increase in intraocular pressure is just one of several factors that contribute to the damage of RGCs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Data availability

All data used to support the findings of this study are included within the article.

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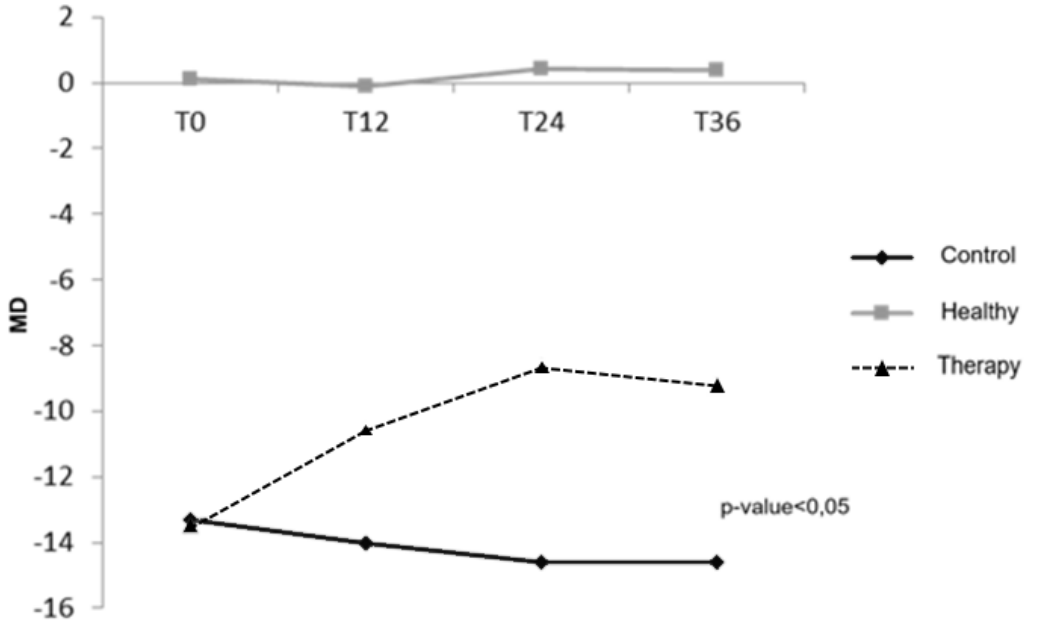


Figure A. MD trend in function of time and therapy.

Abbreviation: MD, mean deviation.

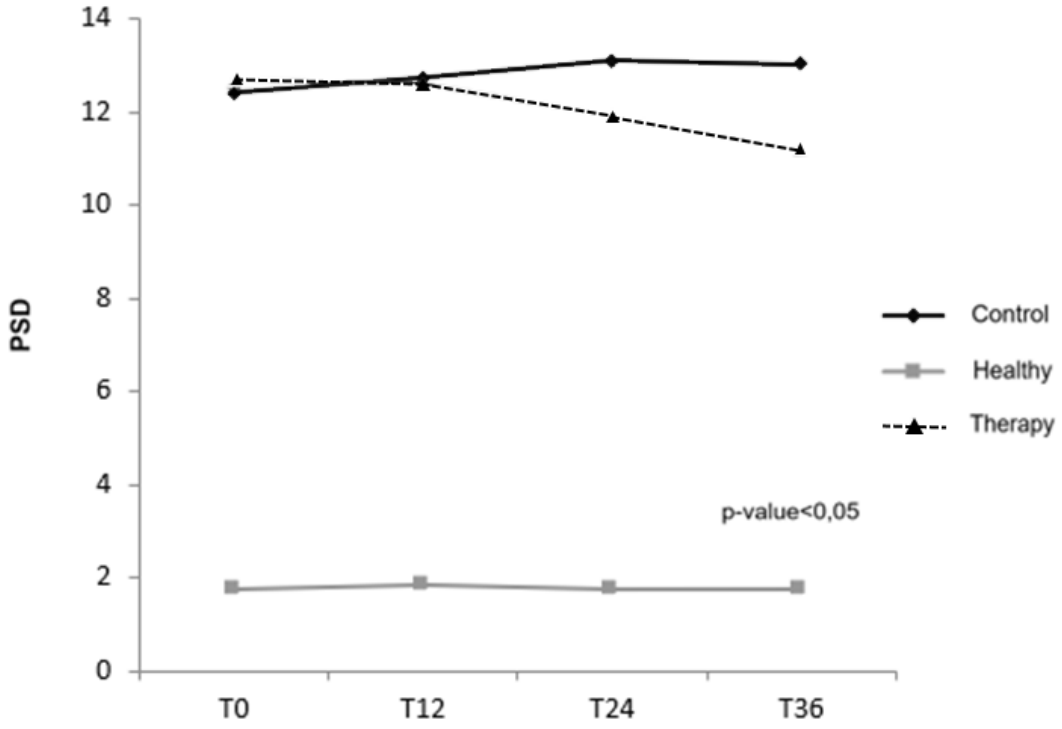


Figure B. PSD trend in function of time and therapy.

Abbreviation: PSD, pattern standard deviation.

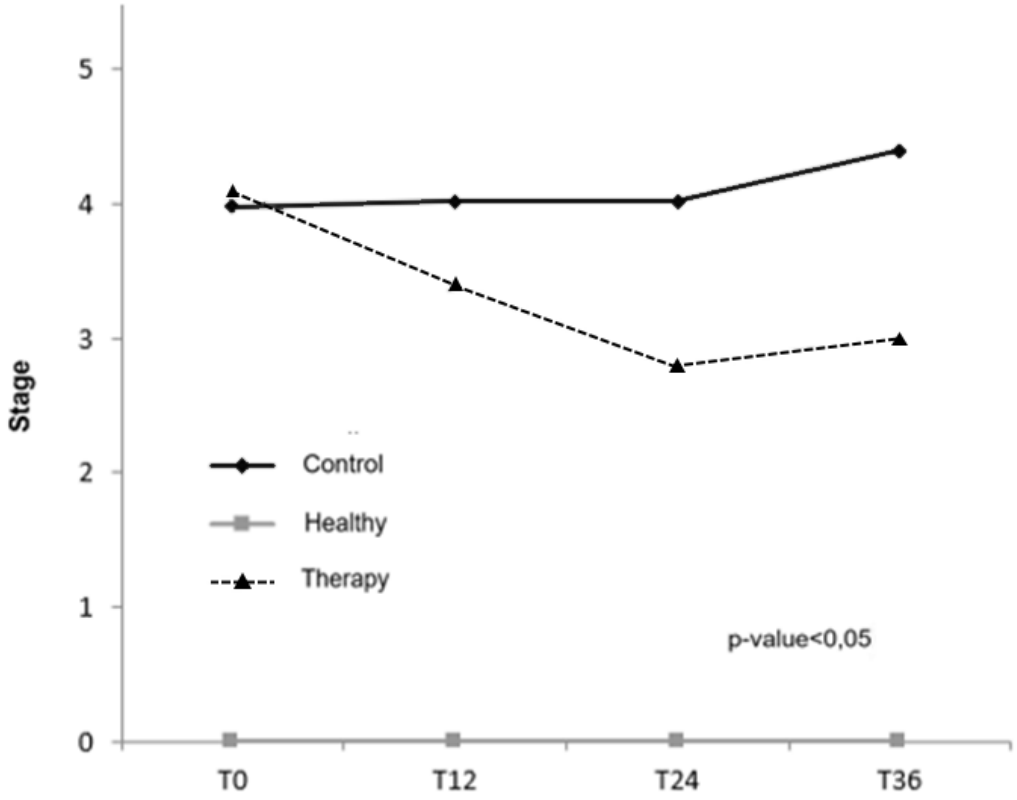


Figure C. Stage trend in function of time and therapy.

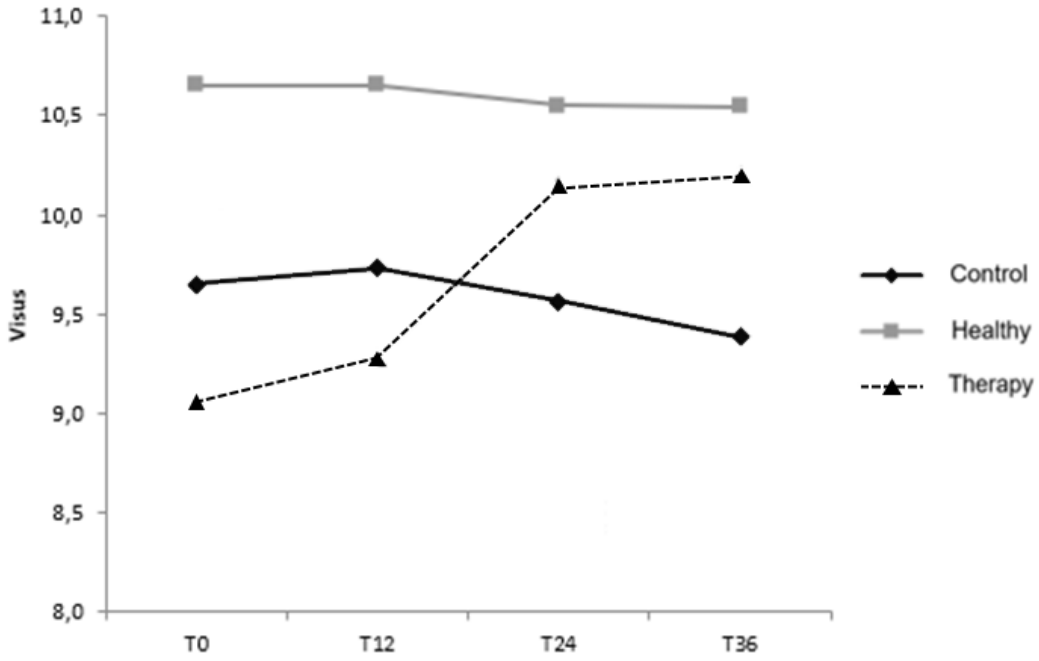
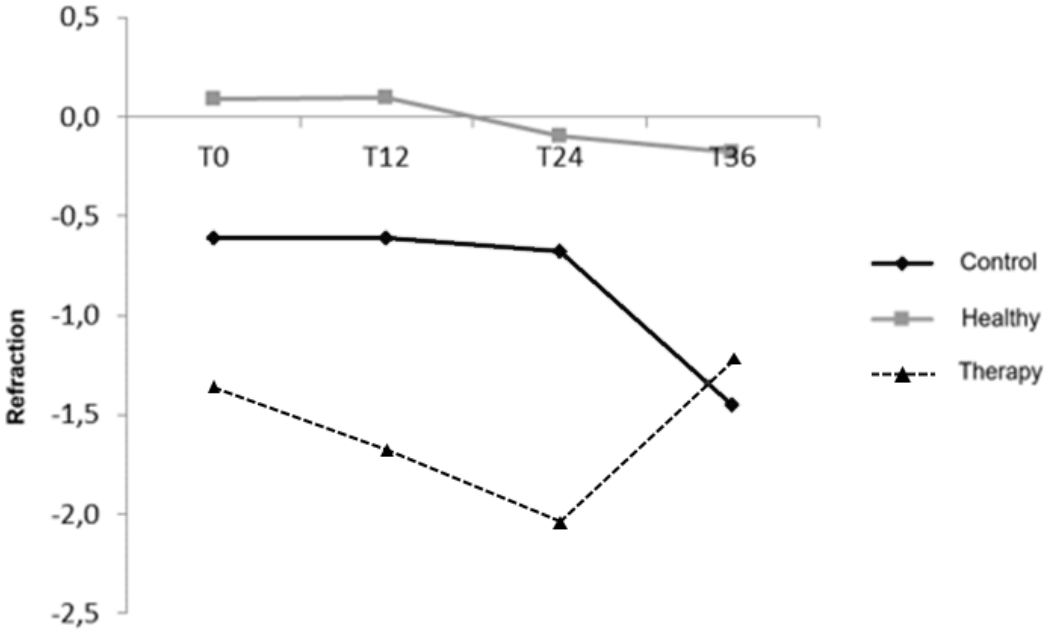


Figure D. Visus trend in function of time and therapy.



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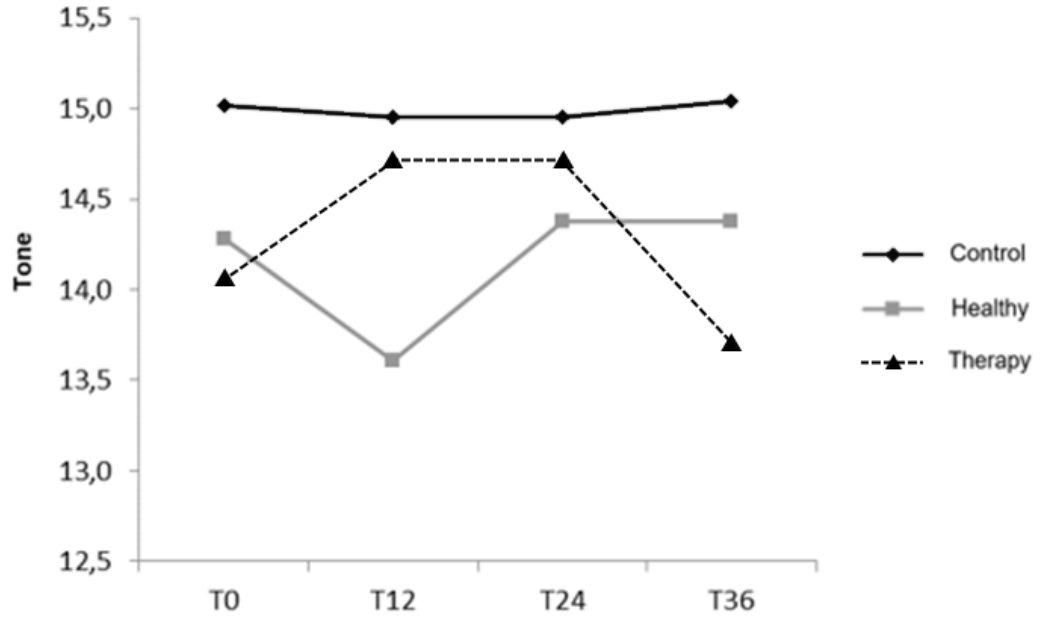
320 **Figure E.** Refraction trend in function of time and therapy.



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322 **Figure F.** Tone trend in function of time and therapy.

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Parameter MD	T0 (mean ± SD)	T12 (mean ± SD)	T24 (mean ± SD)	T36 (mean ± SD)
Control	-14±6.1	-14±3.9	-15±4	-15±4
Healthy	0.1±1	0.2±1.1	0.2±0.7	0.1±0.6
Therapy	-14±3.2	-11±2.4	-9±4	-8.8±3.8

Table A. Comparison of MD parameter between the groups: Control, Health and Therapy. The table shows the mean ± standard deviation (SD).

Abbreviations: MD, mean deviation; SD, standard deviation.

Parameter MD	T0-T12 (Δ)	p-value	T0-T24 (Δ)	p-value	T0-T36 (Δ)	p-value
Control	-7%	0.151	-6%	0.211	-7%	0.151
Healthy	<1%	0.333	<1%	0.353	<1%	0.233
Therapy	21%	**0.001(*)**	35%	**0.001(*)**	35%	**0.001(*)**

Table B. Relative percentage differences (Δ) between the times divided by groups. Student's t test application for paired samples. (*). A p value>0.05 is statistically significant.

Abbreviation: MD, mean deviation.

Parameter PSD	T0 (mean ± SD)	T12 (mean ± SD)	T24 (mean ± SD)	T36 (mean ± SD)
Control	13±3.9	13±4	13±2.8	13±3
Healthy	2±0.6	2±0.1	2±0.7	2±0.3
Therapy	13±3.2	13±2.6	12±4.2	11±3

Table C. Comparison of PSD parameter between the groups: Control, Health and Therapy. The table shows the mean ± standard deviation (SD).

Abbreviations: PSD, pattern standard deviation; SD: standard deviation.

Parameter PSD	T0- T12 (Δ)	p-value	T0- T24 (Δ)	p-value	T0- T36 (Δ)	p-value
Control	<1%	0.654	<1%	0.222	<1%	0.151
Healthy	<1%	0.432	<1%	0.272	<1%	0.295
Therapy	<1%	0.234	-7%	0.752	-16%	**0.001(*)**

Table D. Relative percentage differences (Δ) between the times divided by groups. Student's t test application for paired samples. (*). A p value>0.05 is statistically significant.

Abbreviation: PSD, pattern standard deviation.

Parameter Stage	T0 (mean ± SD)	T12 (mean ± SD)	T24 (mean ± SD)	T36 (mean ± SD)
Control	4±1.5	4±1.6	4±1.1	4.2±1.6
Healthy	0	0	0	0
Therapy	4.1±1.4	3.4±1.3	2.7±1.2	3±1.3

Table E. Comparison of the stage parameter between the groups: Control, Health and Therapy. The table shows the mean ± standard deviation (SD).

Abbreviations: SD, standard deviation.

Parameter Stage	T0- T12 (Δ)	p-value	T0- T24 (Δ)	p-value	T0- T36 (Δ)	p-value
Control	<1%	0.255	<1%	0.512	+2%	0.413
Healthy	<1%	0.125	<1%	0.212	<1%	0.313
Therapy	-12.8%	0.068	-30%	**0.001(*)**	-35%	**0.001(*)**

Table F. Relative percentage differences (Δ) between the times divided by groups. Student's t test application for paired samples. (*). A p value>0.05 is statistically significant.