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Article

Evaluation of retinal ganglion cell layer in patients with macular edema treated with intravitreal anti-VEFG and corticosteroid

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Abstract. *Purpose***:** The aim of the study is to detect the effect of the different intravitreal therapies (anti-VEFG drugs and corticosteroids) on the single layer of retinal ganglion cells (GCL) in patients affected by macular edema.
Methods: Forty (17 males/23 females) Caucasian patients (40 eyes) affected by macular edema treated with intravitreal injections (anti-VEGF and steroids) are included. Spectralis HRA-OCT device with a specific protocol for the acquisition and evaluation of the GCL layer has been used. Macular GCL thickness is assessed with automated retinal segmentation based on ETDRS grid rings. Retinal layer automated segmentation is carefully assessed and manually corrected for any misalignment. GCL measurements are correlated with best-corrected visual acuity (BCVA) measured with Early Treatment Diabetic Retinopathy Study (ETDRS), central macular thickness (CMT) and intraocular pressure (IOP). All patients have been evaluated at baseline and at the subsequent follow-up: 4 weeks, 8 weeks and 12 weeks.

Results: Neither anti-VEFG drugs (Bevacizumab, Ranibizumab and Aflibercept) nor corticosteroids (Dexametasone) has a significant effect on GCL layers during three months of follow up compared to long-term treatments. A significant correlation between GCL and ETDRS has found (p=0.0453, R2=0.023).
Conclusions: This study shows that anti-VEFG drugs and corticosteroids do not significantly affect GCL layer over three months of treatment. This study also demonstrates that the ganglion cell layer is correlated to visual acuity, an important factor for future investigations on the effects of new treatments.

Keywords: retinal ganglion cells; macular edema; intravitreal injections; anti-VEGF; corticosteroids; spectral domain optical coherence tomography

Introduction

Macular edema (ME) is caused from an exceptional fluid accumulation in the central retina. It represents a common event to several retinal and ocular disorders [1-5]. The development of macular edema affects visual function, causes a persistent visual loss and impairs the quality of life in working age patients even if it results very difficult to correlate the occurrence and extent of macular edema with altered visual function.
Significant progress has been made in the last few years in the treatment of macular disorders by introducing intravitreal injections of anti-VEGF and corticosteroids [6-8], trying to actually deal with the symptoms. However, only few studies aimed to analyze the potential toxicity of these intravitreal therapies on retinal cells, and the possibility of creating alternative rehabilitation therapies [9].

The measurement of the thickness of the layer of the retinal ganglion cells is gaining great interest as a sensitive index of early retinal damage. Quigley and colleagues have shown that large ganglion cells predominantly localized in the macular region and their large axons are more susceptible to the adverse effects of glaucoma than smaller ganglionic cells and thinner nerve fibers [10]. In addition, death of ganglionic cells causes alterations in the retinal nerve fiber layer (RNFL), constituted by the axons of the ganglion cells [11,12].
In humans, the reduction in the thickness of the RFNL was evaluated by optical coherence tomography after a series of intravitreal injections of ranibizumab [13]. However, at the end of the treatment, no alteration of the function of the retinal ganglion cells at the macular and peripheral levels, and no electroretinography alterations were observed [14,15].

Based on these data, we analyzed the thickness variations of the single layer of retinal ganglion cells (GCL) in the macular region in patients undergoing intravitreal therapy with different drug molecules (Bevacizumab, Ranibizumab, Aflibercept and Dexamethasone). The aim was to detect the effect of the different intravitreal therapies on the single layer of GCL, since a deterioration of retinal GCL would result in an alteration of the spatial frequency contrast discrimination. We used the Spectralis HRA-OCT device of Heidelberg Engineering (version 6.1) with a specific protocol for the acquisition and evaluation of the GCL layer. In addition, we correlated the GCL measurements with

best-corrected visual acuity (BCVA) measured with Early Treatment Diabetic Retinopathy Study (ETDRS), central macular thickness (CMT) and intraocular pressure (IOP) values in order to establish the presence of positive or negative correlation among these parameters.

Methods

Patients

The present study retrospectively included 40 (17 males/23 females) Caucasian patients (40 eyes) with mean age 63 years (SD: 17), affected by macular edema and treated with intravitreal injections (anti-VEGF and steroids), examined at the Department of Ophthalmology of the University Sapienza in Rome from April 2009 to September 2015 (**Table 1**). The study was performed in accordance with the tenets of the Declaration of Helsinki for research in human subjects and informed consent was obtained for all patients.

Table 1. Demographic characteristics and treatments.

Demographic characteristics	**Treatment**			
	Bevacizumab (Bevacizumab)	*Ranibizumab (Ranibizumab)*	*Aflibercet (Aflibercept)*	*Dexamethasone (Dexametasone)*
Number	10	10	10	10
Age (years)	73±11.35	78.4±5.42	73.4±8.69	66.3±16.35
Sex	3F/7M	8F/2M	7F/3M	5F/5M

Data are the mean ±standard deviation. F=female; M=male

Inclusion criteria

Criteria for inclusion were the following: (i) subjects older than 18 years affected by macular edema; (ii) CMT ⊚ 285 ⊚m measured by optical coherence tomography (OCT); (iii) BCVA between 5 and 40 letters to the ETDRS at T0; (iv) Naïve patients, who had not undergone any previous nor intravitreal or laser treatment.

Exclusion criteria

The exclusion criteria were as follows: intravitreal injections with anti-VEGF and steroids before macular edema, bilateral exudative AMD, or atrophic AMD in the treated or similar eye; ocular hypertension; corneal abnormalities such as corneal leucomas, Fuchs corneal endothelial dystrophy; glaucoma; severe cataract with lens opacity in agreement with Lens Opacity Classification System (LOCS) > 2; retinal photocoagulation laser; any intraocular surgery within 6 months. , or any systemic disease, eye disease or drug therapy which could affect thickness values and spherical equivalent over 3 diopters.

Group of treatment

Each group consisted of 10 eyes and was homogeneous for age, sex, and pathology (Table 1). In particular, patients treated with bevacizumab 1.25 mg, ranibizumab 0.5 mg and aflibercept 2 mg were affected by AMD, with ongoing anti-VEGF therapy administered with a pro-rata regimen in one eye only. Patients treated with dexamethasone 0.7 mg, had macular edema following retinal vein occlusion (RVO).

Evaluations

All patients at the time of enrollment (T0) and subsequent follow-up T1 (4 weeks), T2 (8 weeks) and T3 (12 weeks) were subjected to the following examinations: Evaluation of BCVA through ETDRS tables placed at a distance of 4 m, by slit lamp biomicroscopy; ocular tonometry (using a Goldman applanation tonometer); fundus biomicroscopy; optical coherence tomography (OCT) for measurement of macular thickness and Ganglionar Cells Layer (GCL) layer thickness, using a Spectralis HRA-OCT produced by Heidelberg Eye Explorer V1.9.10.0.

SD-OCT (Spectralis OCT Family Acquisition Module, V 6.0.11.0 Heidelberg Engineering, Germany) records included near infrared images and macular scans with the raster 20°x 20°, 25-line scan protocol with an interval between scans of 250 μmand 100 frames averaged for each scan. The automated segmentation protocol of the Spectralis OCTwas used to measure GCL thickness [16], from the area delineated between the outer border of the macular retinal nerve fibre layer and the inner border of the inner plexiform layer (**Figure 1**).

Two experienced investigators evaluated the automated segmentation and manually corrected for any misalignment. The average of measurements was recorded with the ETDRS macular grid at 1, 3, and 6 mm, and color fundus photography.

Figure 1.

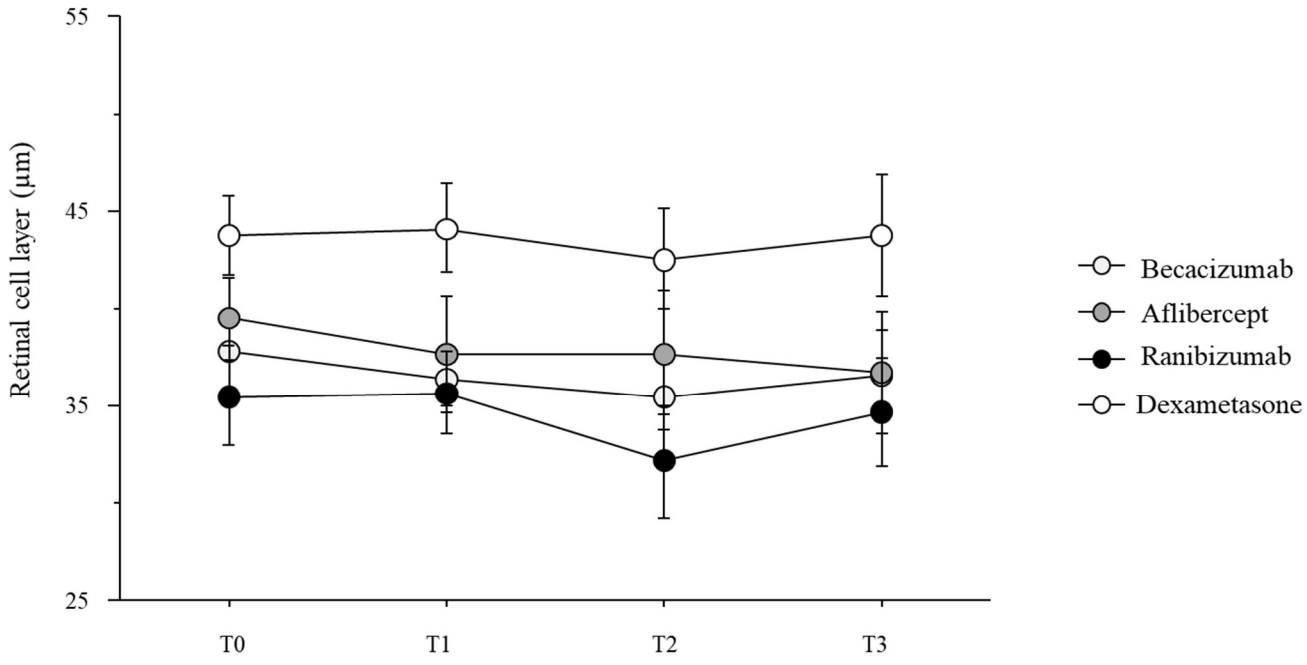

Figure 1. Retinal ganglion cell layer (GCL) in patients affected by macular edema and treated with anti-VEFG drugs (Bevacizumab, Ranibizumab and Aflibercept) or corticosteroids (Dexametasone) over three months. Data are the mean± SEM. Values are expressed in mm.

Intravitreal Injections

The same operator performed intravitreal injections in the operating room, with the patient in the supine position. Following the placement of a sterile drape and sterile blepharostat to spread the eyelids, the periocular and conjunctival skin were disinfected with topical solution of 5% iodopovidone solution and topical anesthesia with ossibuprocain ocular 0.4 %.

Intravitreal injections were performed via pars-plana in the inferior-temporal area, 4 mm apart from the limbus being all phakic patients, leading to the center of the eye in the vitreous chamber. No complications such as retinal tears, detachment or endophthalmitis occurred during the course of anti-VEGF and corticosteroid treatment.

Each patient was subjected from the day before the injection and for the following seven days to the following topical therapy according to the guidelines: antibiotic eye drops netilmicin 0.3% in the eye affected.

Statistical analysis

Data were analyzed by ANOVA with repeated measures. Post-hoc comparisons were performed with Fisher's Protected Least Significant Difference (PLSD) post-hoc test. Correlation analysis between the measurement of retinal ganglion cell layers and the other visual parameters (EDTRS, IOP, and CMT) was performed with simple regression.

A p-value <0.05 was considered statistically significant. Statistical analysis was performed using the Statview software from SAS Institute.

Results

Retinal cell layer measurement in patients treated with anti-VEFG and dexamethasone drugs

Retinal cell layer measurements in patients treated with anti-VEFG and dexamethasone drugs are shown in Fig. 1. ANOVA for repeated measures showed that Dexametasone (p=0.812), Bevacizumab (p=0.531), Ranibizumab (p=0.219) and Aflibercept (p=0.354) treatments had no significant effect over time on retinal cells.

Early Treatment Diabetic Retinopathy Scale (ETDRS) in patients treated with anti-VEFG and dexamethasone drugs

ETDRS in patients treated with anti-VEFG and dexamethasone drugs are shown in **Fig. 2**. ANOVA for repeated measures showed a significant effect of Dexametasone (p=0.0064), Bevacizumab (p=0.0067), Ranibizumab (p=0.010) and Aflibercept (p=0.0002) over time on ETDRS values.

Post-hoc comparison showed that Dexametasone increased ETDRS at T1 (p=0.034), T2 (p=0.006), and T3 (p=0.031) as compared to baseline (T0). Similarly, Ranibizumab also induced an increase in ETDRS at T1 (p=0.021), T2 (p=0.013), and T3 (p=0.0015) as compared to T0.

Bevacizumab increased ETDRS at T3 as compared to T0 (p=0.0063) and T1 (p=0.0012). Aflibercept induced an increase in ETDRS at T2 (p=0.0008) and T3 (p<0.0001) as compared to T0.

Figure 2.

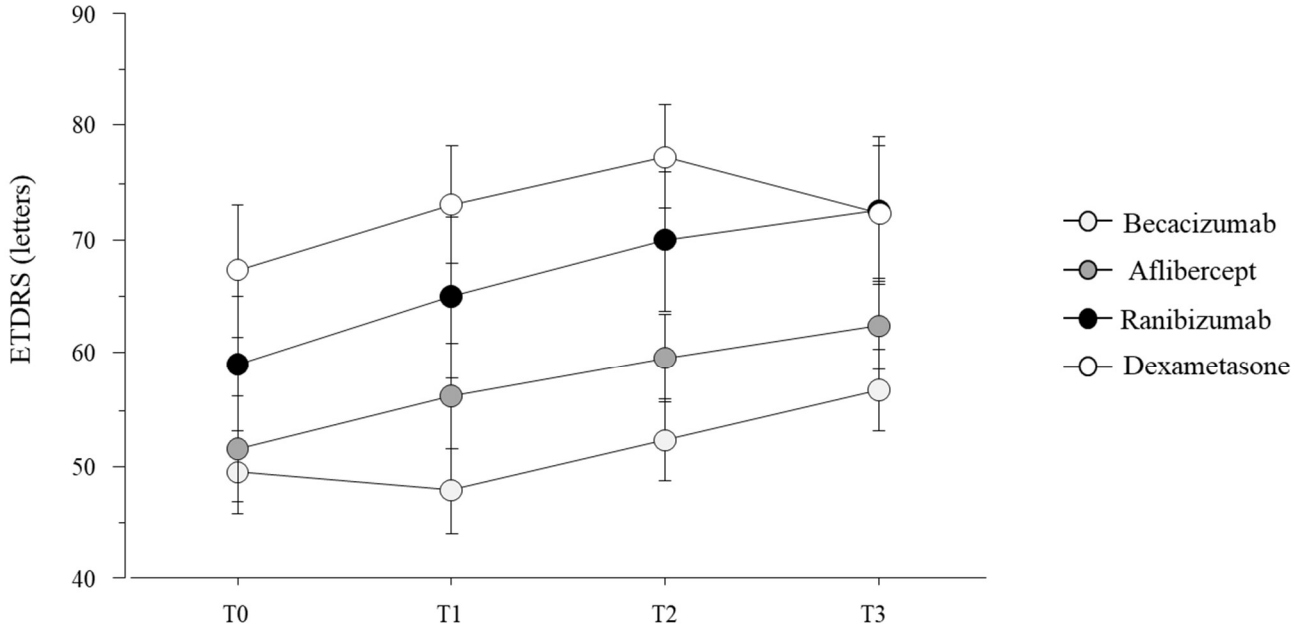

Figure 2. Early Treatment Diabetic Retinopathy Study (EDTRS) in patients affected by macular edema and treated with anti-VEFG drugs (Bevacizumab, Ranibizumab and Aflibercept) or corticosteroids (Dexametasone) over three months. Data are the mean± SEM. Values are expressed in letters.

Central macular thickness (CMT) in patients treated with anti-VEFG and dexamethasone drugs

CMT in patients treated with anti-VEFG and dexamethasone drugs are shown in **Fig.3**. ANOVA for repeated measures showed a significant effect of Ranibizumab (p=0.0444) and Aflibercept (p=0.0001) over time on CMT values. Post-hoc comparison showed that Ranibizumab reduced CMT at T2 (p=0.0098) and T (p=0.0211) as compared to T0. Aflibercept reduced CMT at T1 (p=0.0048), T2 (p=0.0001), and T3 (p=0.0001) as compared to T0. No significant effects of Dexametasone (p=0.095) and Bevacizumab (p=0.308) on CMT over time were observed.

Figure 3.

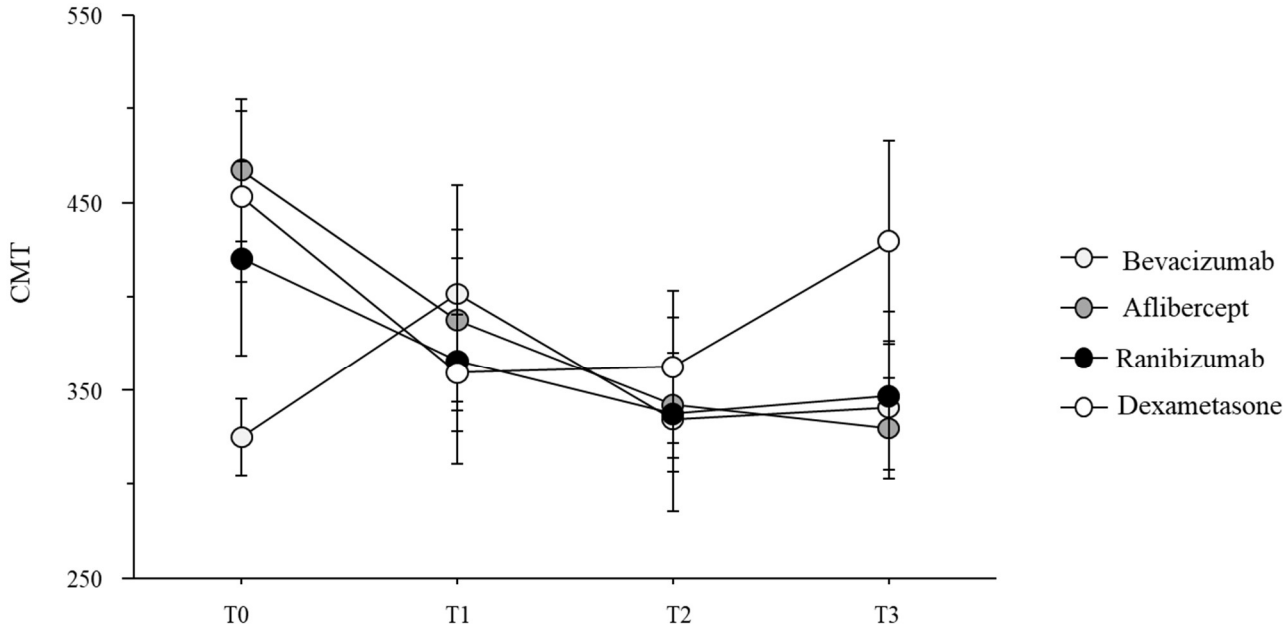

Figure 3. Central macular thickness (CMT) in patients affected by macular edema and treated with anti-VEFG drugs (Bevacizumab, Ranibizumab , and Aflibercept) or corticosteroids (Dexametasone) over three months. Data are the mean± SEM. Values are expressed in μm.

Intraocular pressure (IOP) in patients treated with anti-VEFG and dexamethasone drugs

IOP in patients treated with anti-VEFG and dexamethasone drugs are shown in **Fig.4**. IOP was not significantly altered over time by Aflibercept (p=0.941), Bevacizumab (p=0.383), Ranibizumab (p=0.885) and Dexametasone (p=0.142).

Figure 4.

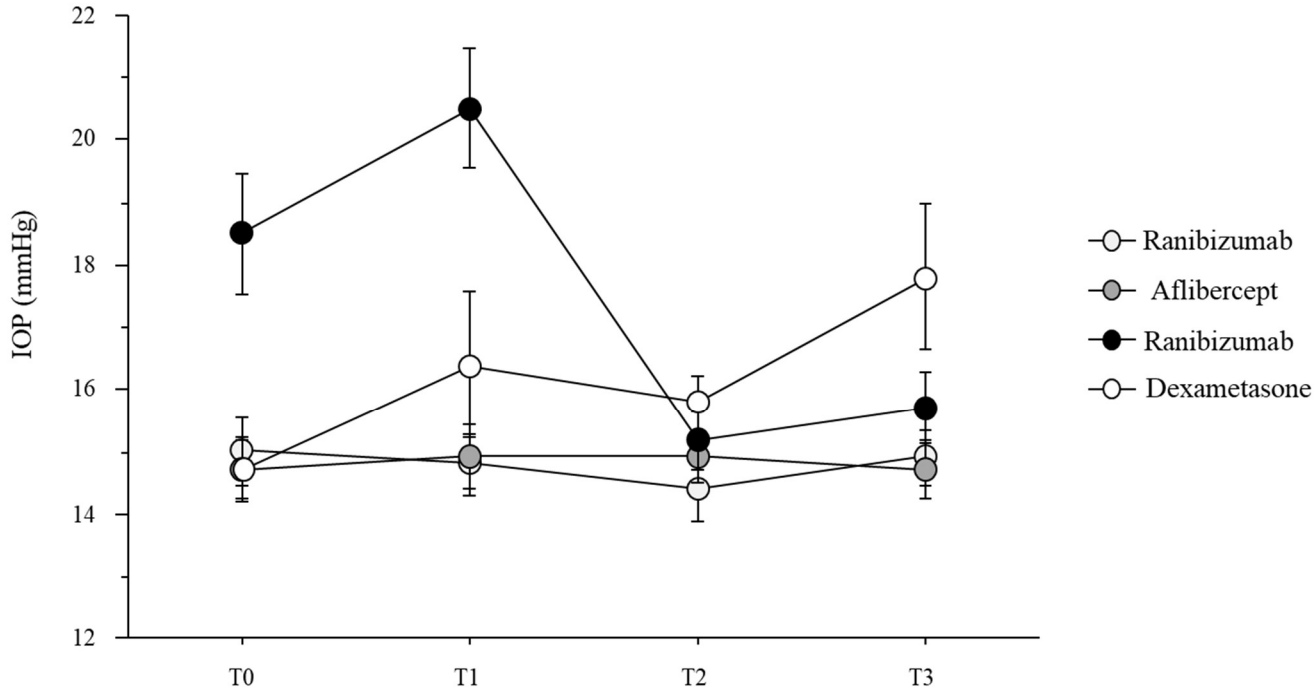

Figure 4. Intraocular pressure (IOP) in patients affected by macular edema and treated with anti-VEFG drugs (Bevacizumab, Ranibizumab , and Aflibercept) or corticosteroids (Dexametasone) over three months. Data are the mean± SEM. Values are expressed in mmHg. Asterisk (*) indicates significant difference between the groups. **p<0.01; ***p<0.001.

Correlation analysis between Retinal cell layer measurement and the other visual parameters (ETDRS, IOP, and CMT)

Correlation plots are shown in **Fig. 5**. Simple regression analysis showed a significant correlation between Retinal cell layer and ETDRS (p=0.0453, R2=0.023). No correlation of retinal cell layer and IOP (p=0.813, R=0.00029). or CMT (p=0.09, R2=0.014). was found.

Figure 5.

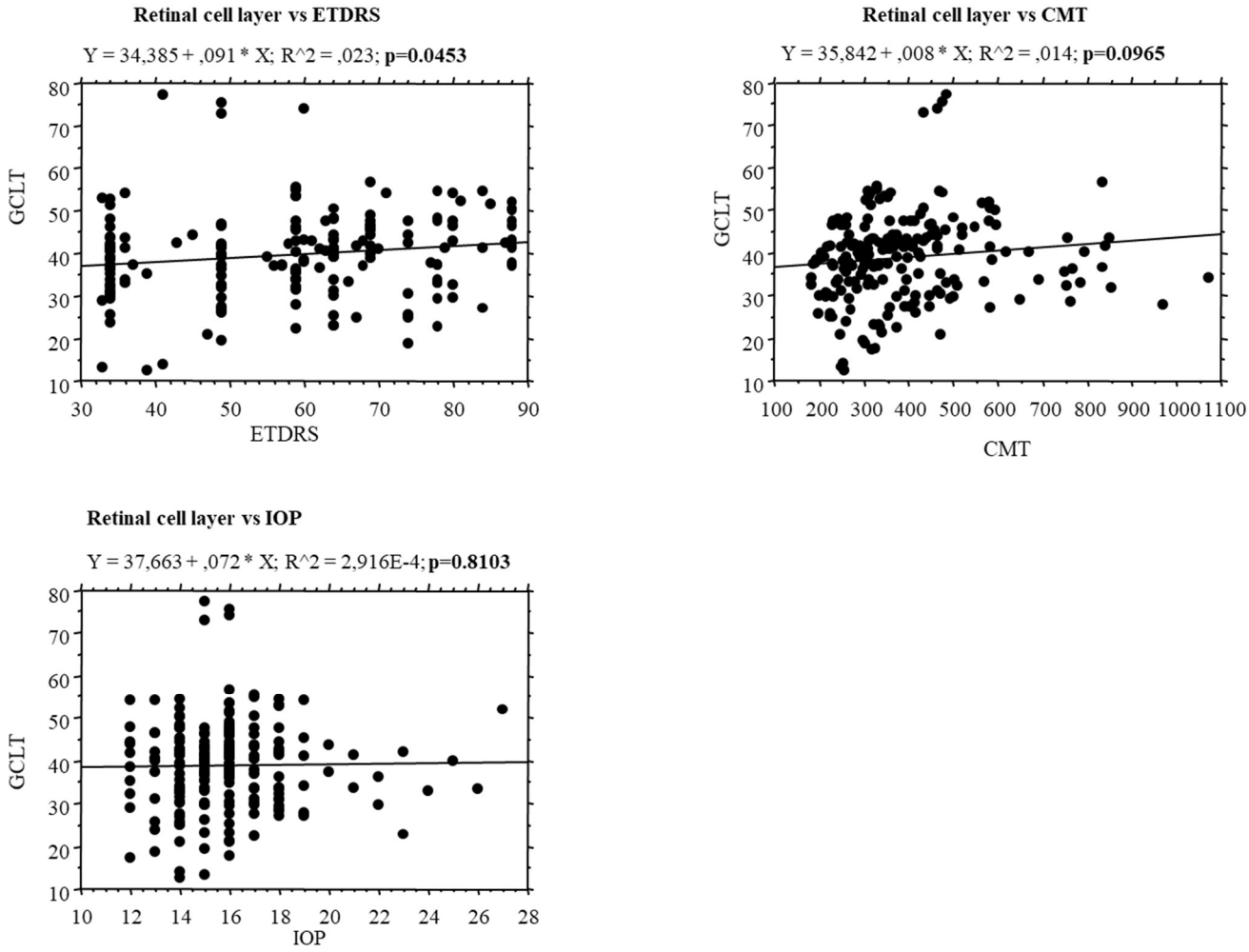

Figure 5. Correlation plots of retinal ganglion cell layer (GCL) with Early Treatment Diabetic Retinopathy Study (EDTRS), central macular thickness (CMT), and intraocular pressure (IOP). R2=coefficient of correlation.

Discussion

This study was performed to determine whether different intravitreal pharmacological agents (Anti-VEGF and Corticosteroids) for the treatment of macular edema might affect the layer of retinal ganglion cells (GCL) over time. The measurement of GCL layer in the macular area has become an important index of toxicity during pharmacological treatment and of early damage in retinal pathologies. To achieve this goal, we used a device of Optical Coherence Tomography, the Spectralis

HRA-OCT device of Heidelberg Engineering (version 6.1) with a specific protocol that makes possible a precise and automatic segmentation for the acquisition of retinal layers.

Our results showed that neither anti-VEFG drugs (Bevacizumab, Ranibizumab , and Aflibercept) nor corticosteroids (Dexametasone) had a significant effect on GCL layers during three months of follow up. Our data are in line with those of other studies that demonstrated both the efficacy and safety of anti-VEGF and corticosteroid drugs used for the treatment of macular disorders.

Although in some experimental studies, a decrease in the thickness of ganglion cells has been observed after intravitreal injections of certain drugs such as Ranibizumab (Bevacizumab) [15], in our study and others these effects were not found, with intravitreal therapies for short periods.

This difference between human and animal experimentation has not yet been clarified. In any case, Quigley studies have clearly shown that ganglionic retinal cells are susceptible to glaucoma damage and that their disappearance is reflected on the thickness of the layer of these cells [10].

Our data on GCL layer are important if referred to the other evaluation parameters examined. In fact, in absence of effects on ganglion cells, we observed an improvement in visual acuity measured with ETDRS in patients treated with these drugs. In addition, a reduction in CMT was observed and, therefore, a reduction in macular edema while intraocular pressure remained constant. These data do not significantly add much to the literature on the efficacy of these drugs, but it is important to note that the positive effects on macular disorders are associated with maintenance of the retinal ganglion layer [17].

In particular, GCL modifications may be closely dependent on the toxic action of anti-VEGF drugs due to the inhibition of the neuroprotective function of VEGF-A, or to temporarily increase intraocular pressure (IOP) caused by intravitreal injection. In fact, it is known that the increase in IOP is due to the increase in the ocular volume, and ischemic events occurring in ganglionic cells are complications of intravitreal injections [18-20]. Nonetheless, it has been observed that usually, after an intravitreal injection, only few minutes (around 30 min) are needed to gain a normalization of IOP without the need for therapy [21].

In our study, IOP values were stable from 24 hours after injection and the successive follow-up (T1, T2 and T3). In addition, the OCT examination showed that the thickness of the retinal ganglion layer did not change significantly at the end of follow-up, confirming the safety of these drugs.

Another important factor is the positive correlation between visual acuity measured by ETDRS and the ganglion cell layer. We observed that increasing the ETDRS value corresponds to an increase in the thickness of the retinal ganglion cells. This confirms the importance of maintaining a correct thickness of these cells for better visual acuity.

Conclusions

In conclusion, this study shows that anti-VEFG drugs and corticosteroids do not significantly affect GCL layer over three months of treatment. This study also demonstrates that the GCL measurement is correlated to visual acuity. Thus, our data suggest that the measurement of the retinal ganglion cell layer through OCT represents a valid method of evaluation to determine the efficacy and safety of present and future drugs for macular pathologies.

Acknowledgements

None

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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