UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Logo d'une autre institution éventuelle
(CHUV, UNIGE, HES-SO, etc.)

Mémoire de Maîtrise en médecine No

Evaluation of inner retinal function assessed with a novel handheld instrument (RETeval) using flicker electroretinography after anti-VEGF treatment in patients with diabetic macular oedema

Etudiant
Abdulghafor Mouna

Tuteur
Wolfensberger Thomas Jona
Hôpital Ophtalmique Jules-Gonin

Co-tuteur
Konstantinidis Lazaros
Hôpital Ophtalmique Jules-Gonin

Expert
Borruat François-Xavier
Hôpital Ophtalmique Jules-Gonin

Lausanne, 18.02.2019

Evaluation of inner retinal function assessed with a novel handheld instrument (RETeval) using flicker electroretinography after anti-VEGF treatment in patients with diabetic macular oedema

Mouna Abdulghafor

Aki Kawasaki

Mohamed Wardani

Theodor Stappler

Thomas J. Wolfensberger

Lazaros Konstantinidis

Jules Gonin Eye Hospital
Department of Ophthalmology
University of Lausanne
Fondation Asile des Aveugles
Switzerland

Correspondence:

Dr. Lazaros Konstantinidis
Jules Gonin Eye Hospital
University of Lausanne
Fondation Asile des Aveugles
Ave de France 15
CH-1000 Lausanne 7
Switzerland
Tel: +41-21-626 8099
Fax: +41-21-626 8144
e-mail: lazaros.konstantinidis@fa2.ch

Abstract

Background

Electroretinographic studies have suggested that anti-VEGF treatment apart from its positive impact on the diabetic macular oedema may have a more generalised impact on the entire retinal function.

A portable, handheld, flash and flicker electro retinography (ERG) recording system has recently been developed (RETeval™ LKC Technologies, Gaithersburg, MD) that makes it possible to record ERGs more rapidly and less invasively than the conventional ERG systems.

We evaluate ERG measurements by the RETeval device before and after treatment with intravitreal anti-VEGF injections in eyes with diabetic macular oedema associated with mild nonproliferative diabetic retinopathy.

Methods

Prospective pilot study of 7 eyes of 5 patients with type II diabetes treated for macular edema by intravitreal ranibizumab injections in Jules Gonin University Eye Hospital in Lausanne, Switzerland. ERGs were recorded with the RETeval system before treatment and 1 month after the 3rd injection. Measurements included responses to photoptic flicker and ERG recording according to various protocols including photopic negative response (PhNR), ERGs recorded by sinusoidal light stimulus and the RETeval specific diabetic retinopathy Assessment Protocol. Additionally, fluorescein angiography was performed on each patient before and after treatment.

Results

All patients demonstrated significant improvement of visual acuity and resolution of macular oedema after treatment. We found no significant differences of the amplitudes and implicit times of ERGs after intravitreal injection of anti-VEGF with most of the protocols applied bar ERGs recorded by sinusoidal light stimulus. The mean implicit time of the flicker ERGs recorded by sinusoidal light stimulus was shortened significantly from 30.2 msec (SD: 0.6) at baseline to a mean of 29 msec (SD:0.76,) after the last intravitreal injection ($p< 0.05$; paired t-test).

Additionally, the RETeval specific diabetic retinopathy Assessment Protocol designed to aid in the detection of vision threatening diabetic retinopathy including clinically significant macular oedema, could detect improvement in 71% of our cases.

Conclusion

In the present study we found no significant difference of the amplitudes and implicit times by most protocols applied bar shortening of the implicit times after ranibizumab treatment using a sinusoidal light stimulus. It is believed that sinusoidal stimulus is sensitive to inner-retinal function though its significance is not yet clearly elucidated. Additionally, the RETeval specific DR Assessment Protocol could detect improvement in the majority of our cases. The RETeval device offers a novel, quick and easily accessible method of objective electroretinographic measurements though the significance of its clinical role warrants further studies.

Keywords: Diabetic retinopathy, Diabetic macular oedema, ranibizumab, Flicker electroretinogram, RETeval

Introduction

Diabetic retinopathy (DR) is the leading cause of blindness in adults of working age and diabetic macular oedema (DMO) is the most common vision-threatening manifestation of diabetic retinopathy (1-3).

The complex pathophysiology of DMO has been under investigation in recent years. In individuals with diabetic retinopathy, fluid can accumulate within the central retina as a result of a breakdown in the blood–retinal barrier. Hyperglycaemia is associated with detrimental effects on the retinal vasculature (4) causing capillaries occlusion and ischemia with subsequent local hypoxia (5). Vascular endothelial growth factor (VEGF) plays a pivotal role in the retinal microvascular complications of diabetes (6-7).

In parallel, several studies showed that neurodegeneration is also part of the physiopathology of RD and includes among others neural apoptosis, retinal ganglion cell loss, reactive changes in macroglia and thinning of the inner retina (8-10).

Interestingly several studies have advocated that neurodegeneration appears in its early stages and it has even been suggested that inner retina alterations happen in type 2 diabetes, even before visible vascular signs of DR (11).

It has been advocated that VEGF, apart to its role in the retinal microvascular complications of diabetes, can also display neurodegenerative and neuroprotective characteristics (12-13).

Currently, therapeutic proteins designed to inhibit and/or neutralize VEGF have become the leading treatment modality for diabetic macular oedema (14-16).

Interestingly, it has been suggested by some ERG studies that anti-VEGF treatment may have - apart from the anatomic and functional improvement of the macula in the cases of diabetic macular oedema - an impact on the entire retinal function (17-19).

A portable, handheld, flash and flicker ERG recording system has recently been developed (RETeval™ LKC Technologies, Gaithersburg, MD) that use adhesive skin electrodes as the active electrodes. This system makes it possible to record ERGs more rapidly and less invasively than the conventional ERG systems with contact lens electrodes. The RETeval device combines flicker electroretinogram (ERG) and pupillary responses (20).

Patients with diabetes may have an altered response to flicker stimulation through several mechanisms (21) such as the dysfunction of neurons and photoreceptors

and the reduced vasodilatator capacity of the retinal vessels in response to flicker stimulation (22).

Thus, the aim of this pilot study was to compare ERG measurements recorded by the RETeval device before and after treatment with intravitreal anti-VEGF injections in eyes with diabetic macular oedema associated with mild nonproliferative diabetic retinopathy. We also evaluated angiographic findings and as well functional and anatomic changes assessed with ocular coherence tomography during follow-up. We also aimed to assess the specific diabetic Assessment Protocol.

Methods

Subjects

Seven eyes of 5 patients with type II diabetes, who attended the diabetic retinopathy clinic of the Jules-Gonin University Eye Hospital in Lausanne, Switzerland were enrolled in this pilot monocentric prospective nonrandomized study. Inclusion criteria comprised macular oedema in the context of DR who were eligible for intravitreal ranibizumab injections and who were receiving this treatment for the first time.

The exclusion criteria were the following: 1. Other co-existing or previous ocular disease that, in the opinion of the recruiting ophthalmologist, could affect the electrophysiologic test (such as optic neuropathy and vascular retinopathies), 2. known allergy to anticholinergic mydriatic drugs, 3. current or previous use of systemic drug with potential retinal toxicity, 4. previous retinal laser treatment, 5. history of photosensitive epilepsy , 6. inability to follow procedures, for example dementia.

A written study information leaflet was provided to all participants while all patients gave an informed written consent regarding their participation in the study and their treatment.

Our study respects the tenets of Declaration of Helsinki while approval for the study was obtained from the local ethic committee.

Procedures

At the time of the first examination (baseline), a short entry questionnaire permitted to record the duration of diabetes, type of diabetes, type of diabetes treatment and levels of glycosylated haemoglobin. Subsequently, the patients underwent a clinical examination, which included visual acuity testing, intraocular pressure measurements, slit lamp and fundus examination. Auxiliary

tests included OCT, wide field fundus colour photography and fluorescein angiography. Each study participant had a complete ocular examination and repeated all auxiliary tests four weeks after the last injection.

Intravitreal injections of 0.5 mg ranibizumab were given three times, every fourth week, starting within 1 week of the baseline examination. All patients underwent the same procedure: 0.5 mg ranibizumab was administered in a standardized way.

Electrophysiologic testing

The electrophysiologic testing of the retina was performed using a commercially available and CE-approved device: RETeval (LKC Technologies, Inc., UK). The device was used following the manufacturer's recommendations. It consists of a hand-held, battery powered stimulator and recorder. The instrument uses disposable sensor strips placed 2 mm away from the lateral half of the lower eyelid margin. The lead is connected to the sensor strip to measure the eye's electrical response.

The RETeval device tested each eye with 16, and 32 Td·s flicker stimuli (28.3 Hz) that each lasted between 5 and 15s, depending on the standard error of mean for the implicit time measurement. Skin electrodes were used to pick up the ERGs that were elicited by white light delivered at the frequency of 28.3 Hz.

The RETeval device enables the changing of the stimulus conditions supporting different protocols. Additional protocols applied included photopic negative response (PhNR), ERGs recorded by sinusoidal light stimulus and the RETeval specific diabetic retinopathy assessment protocol.

For the PhNR test the stimulus consisted of brief (<4 ms), red-flashes (1.6 cd.s/m2) on a steady blue background (photopic 10 cd/m2).

Concerning the sinusoidal test sinusoidal stimuli can be generated with LKC's patented Troland-based technology for providing consistent retinal illumination independent of pupil size.

The amplitudes and implicit times were automatically extracted and displayed by the RETeval system using an embedded algorithm.

The DR Assessment Protocol is designed to aid in the detection of vision threatening diabetic retinopathy (DR), which is defined as severe non-proliferative DR, proliferative DR or clinically significant macular edema. The DR Assessment Protocol uses sets of flickering white stimuli (28.3 Hz) with no background light. The number of sets is determined by the device's internal precision metrics. The RETeval-DR device measures the pupil size in real time and continuously adjusts the flash luminance to deliver the desired amount of light into the eye regardless of the size of the pupil.

The score produced by the DR Assessment Protocol correlates with the presence and severity of diabetic retinopathy and clinically significant macular edema (20).

The significance of the differences in the amplitudes and implicit times between before and after IVR was determined by paired t-tests. A commercially available software (SPSS v.25 for mac; SPSS Inc., Chicago, IL) was used for all statistical analyses. A $p < 0.05$ was considered significant.

Results

The pilot study included 7 eyes of 5 male patients with a mean age of 69.5 years (range, 56 to 82 years). All patients, had type-2 diabetes on insulin treatment. All eyes presented mild non-proliferative diabetic retinopathy. Duration of diabetes was years 17 years (SD: 9 years).

Fasting blood glucose and blood pressure levels did not change significantly between the examinations.

At baseline, the mean visual acuity was 0.27 logMAR units (0.54 decimal equivalent) with a range of 0.4 to 0.2 logMAR units. Mean, baseline foveal thickness evaluated by OCT was 440 μm with a range of 308 to 731 μm. On the last follow-up examination one month after the last intravitreal injection, the mean visual acuity improved significantly to 0.029 logMAR units (0.93 decimal equivalent) with a range of 0.1 to 0 logMAR units. The mean foveal thickness determined by OCT was reduced to 319 μm with a range of 229 to 452μm.

We found no significant difference of implicit times or the amplitudes of the flicker ERG before and after the IVR with neither of the different flicker stimuli used (16, and 32 Td·s).

Regarding the stimulus flash retinal illuminance of 16 photopic Td-s, the mean implicit time was 32.6 msec, SD: 0.69) at baseline versus 32.8 msec, SD: 0.75 after treatment p: 0.7, paired t-test)

For the PhNR test the mean implicit time for the a-wave was 14.1ms SD:05, at baseline versus 14ms SD:0.9 after treatment. (p:0.663 paired t-test) The mean a-wave amplitude was 4.2uV, SD:3.1 at baseline versus 4.3uV, SD:2.9 (p:0.844 paired t-test).

The mean b-wave, mean implicit time was 33.8ms at baseline SD:1.4 ms versus 33.5ms SD:1.4ms after treatment. (p:0.681 paired t-test). The mean b-wave amplitude was 21uV, SD:0.7 at baseline versus 20uV, SD:0.8 (p:0.576 paired t-test).

The mean implicit time of the flicker ERGs recorded using the sinusoidal test
at baseline by the RETeval system was 30.2 msec (SD: 0.6, msec, MSE: 0.2).
On the last follow-up examination one month after the last intravitreal injection, the implicit times of the flicker ERGs on the treated eye recorded with the RETevalTM system were significantly shortened to a mean 29 msec (SD:0.76, SEM:0.28 msec) ($p< 0.05$; paired t-test). (Figure 1, Figure 2) The difference of the amplitudes of the flicker ERG before and after the IVR was not statistically significant 9.6 (SD:5.2) versus 9.1 (SD:4.5); paired t-test, $p = 0.67$

The score produced by the DR Assessment Protocol was significantly reduced in 5 of the 7 eyes. (Figure 3).

Discussion

Numerous studies have shown improvement in visual acuity and decrease in central retinal thickness (CRT) after intravitreal injections of ranibizumab in diabetic patients presenting macular oedema (23).

Emerging data suggest that ranibizumab may have, apart from its beneficial effect on CRT reduction, a favourable effect in DR severity indicating a beneficial effect on the entire retina (24-25).

Full-field electroretinography is an objective method for registration of the overall retinal function. Nevertheless, very few ERG studies after ranibizumab treatment exist in the medical literature. The flicker ERG is a response from the cone system, and is therefore representative of the whole retina. While cone density in the fovea show a 40-fold increase, the area of the fovea is quite small (3.6 mm2 out of 1000 mm2) and the rest of the retina is relatively uniform in cone density. Changes in these ERG parameters have been correlated with DR severity. (20-26) More precisely, DR progression has been associated with a prolongation of the implicit time and a decrease of the amplitude (20).

In 2015, Holm K. et al. found a significant decrease in cone implicit time with 30-Hz flicker 1 month after the third injection of ranibizumab in patients with diabetic macular oedema using a visual evoked response imaging system according to the standardized protocol for clinical electroretinography by the International Society for Clinical Electrophysiology of Vision (ISCEV) (17).

On the other hand, in 2014 Comyn O. et al in a randomized trial to assess functional and structural effects of ranibizumab versus laser in diabetic macular edema demonstrated that global retinal function, measured by full-field ERG, showed no significant change after treatment (27).

Changes in 30-Hz flicker have also been described in other retinal diseases.

In central vein occlusion, a correlation between retinal ischemia and delayed 30-Hz flicker implicit time has been shown (28-29). It has been hypothesized that these studies may indicate that an increased 30-Hz flicker implicit time indicates damaged cone function of the entire retina in the form of ischemia (17).

Pedersen et al. examined the effect on the electroretinogram after injections of bevacizumab in patients with age-related macular degeneration and they found significant improvement in the implicit time of the cone flicker response at 6-month follow-up (30).

In another study Neubauer et al. (31) reported a significant decrease of peripheral ischaemia 1 month after intravitreal anti-VEGF (bevacizumab) injection in eyes with diabetic retinopathy. It has been suggested that improvement of the peripheral retinal ischaemia after anti-VEGF therapy may be accountable for the shortening of the implicit times of the flicker ERGs recorded by RETeval devise after anti-VEGF treatment in eyes presenting central retinal vein occlusion (32).

In the present study we have used a novel flicker ERG recording system allowing to record ERGs more rapidly and less invasively in diabetic retinopathy than the conventional ERG systems which are cumbersome and which require a contact lens on the cornea after local anaesthesia. Previous studies concluded that the novel RETeval device is a reliable tool with reasonable accuracy in comparison with the conventional ERG (33).

In the present study, we did not find a significant decrease in 28-Hz of the cone flicker response implicit time 1 month after the third injection of ranibizumab in diabetics treated for macular oedema.

Maybe this result reflects the fact that our study included only patients presenting mild non-proliferative diabetic retinopathy with no signs of ischemia as demonstrated by the Optos wide field angiography. Consequently, no improvement of ischemic retinal areas could take place and therefore no shortening of the cone implicit time could be detected. Furthermore, in a recent study McAnny et al. suggested that flicker ERG

of approximately 30 Hz, the frequency recommended by international

standards may not be able to detect ERG changes in mild nonproliferative DR and that high-frequency flicker ERG may be more appropriate (34).

Two of the protocols applied in this pilot study are supposedly related to inner retinal function; the PhNR test and the sinusoidal test.

Several studies have suggested that neurodegenerative alterations of the inner retina happen in type 2 diabetes, even before visible vascular signs of DR (11). Animal studies also suggested that neurodegeneration leading to inner retinal thinning is not limited to cell death and tissue loss but also includes changes in neuronal morphology, reduced synaptic protein expression and alterations in neurotransmission, including changes in expression of neurotransmitter receptors as well as neurotransmitter release, reuptake and metabolism (35).

Bressel et al. showed that individuals receiving ranibizumab therapy for diabetic macular edema may have favourable changes in DR severity concomitant with sequential reduction in anti-vascular endothelial growth factor therapy (25).

Joachim, S. C. et al. demonstrated that photoreceptors and retinal ganglion cell number are protected by ranibizumab treatment (12).

The photopic negative response (PhNR) of the full-field electroretinogram (ERG) is a slow negative potential following the a- and b-waves that has been reported to originate primarily from the retinal ganglion cells (RGCs) and their axons (36).

In the present study we did not find any difference in photopic negative response before and after treatment in our patients. It has been recently suggested that some corrections should be incorporated into signal processing protocols to improve PhNR recording and enhance its clinical

use (37). Therefore, it could be possible that our PhNR results were not optimally recorded and their validity might have been compromised.

Kim et al. demonstrated that PhNR amplitude was reduced and the implicit time was prolonged in DR patients compared to those of normal subjects (38).

Similarly, Chen et al found significant reduction of PhNR amplitude in diabetic compared to the control group. However, they found that this decrease in PhNR amplitude was not significantly different among groups with different stages of diabetic retinopathy (39). However, Kizawa et al found that PhNR progressively decrease with the progression of diabetic retinopathy (40).

Further studies may be warranted to determine optimal signal processing protocols of PhNR recordings and its clinical significance in diabetic retinopathy.

Interestingly though, the implicit times of the flicker ERGs recorded using the sinusoidal test of the RETeval system were significantly shortened after treatment to all patients.

The harmonics of flicker ERG test results elicited from a sinusoidal light stimulus is believed to originate in the inner retina (41), and therefore this finding may indicate an improvement of the inner retinal function. Though, the role of flicker ERG test results elicited from a sinusoidal light stimulus has not been extensively studied and consequently its significance is not yet clearly elucidated.

We furthermore evaluated ERG recordings applying the RETeval specific diabetic retinopathy assessment protocol that was developed using measurements of 467 people with diabetes aged 23 – 88 (20). It has been advocated that the score produced by the DR Assessment Protocol correlates with the presence and severity of diabetic retinopathy and clinically significant macular edema. In

our cases the DR Assessment Protocol of the RETeval devise could detect improvement in about 71% of our cases although it failed to detect the improvement in 2 of the 7 cases.

One limitation is the limited number of patients included in this pilot study. Although previous studies concluded that the novel RETeval device is a reliable tool with reasonable accuracy in comparison with the conventional ERG, further studies are warranted in order to establish its accuracy in a wider context and to evaluate the significance of its various protocols in clinical practice.

In conclusion, in the present study we found no difference of the amplitudes and implicit times of flicker ERGs with any of the protocols applied bar shortening of the implicit times after ranibizumab treatment applying a sinusoidal light stimulus. These findings could theoretically indicate changes to inner-retinal function after intravitreal injection of ranibizumad in our diabetic patients, though the significance of the sinusoidal test is not yet clearly elucidated and these results should be interpreted with caution.

The DR Assessment Protocol of the RETeval devise could detect improvement in about 71% of our cases.

The RETeval devise offers a novel, quick and easily accessible method of objective electroretinographic measurements though its clinical significance warrants further studies.

Bibliography

1. Klein R. Retinopathy in a population-based study. Transactions of the American Ophthalmological Society. 1992;90:561-94.

2. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. Ophthalmology. 1984;91(12):1464-74.

3. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XV. The long-term incidence of macular edema. Ophthalmology. 1995;102(1):7-16.

4. Khurana RN, Do DV, Nguyen QD. Anti-VEGF therapeutic approaches for diabetic macular edema. International ophthalmology clinics. 2009;49(2):109-19.

5. Nguyen QD, Shah SM, Van Anden E, Sung JU, Vitale S, Campochiaro PA. Supplemental oxygen improves diabetic macular edema: a pilot study. Investigative ophthalmology & visual science. 2004;45(2):617-24.

6. Do DV, Nguyen QD, Boyer D, Schmidt-Erfurth U, Brown DM, Vitti R, et al. One-year outcomes of the da Vinci Study of VEGF Trap-Eye in eyes with diabetic macular edema. Ophthalmology. 2012;119(8):1658-65.

7. Caldwell RB, Bartoli M, Behzadian MA, El-Remessy AE, Al-Shabrawey M, Platt DH, et al. Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. Diabetes/metabolism research and reviews. 2003;19(6):442-55.

8. Lieth E, Gardner TW, Barber AJ, Antonetti DA. Retinal neurodegeneration: early pathology in diabetes. Clinical & experimental ophthalmology. 2000;28(1):3-8.

9. Kern TS, Barber AJ. Retinal ganglion cells in diabetes. The Journal of physiology. 2008;586(18):4401-8.

10. Lorenzi M, Gerhardinger C. Early cellular and molecular changes induced by diabetes in the retina. Diabetologia. 2001;44(7):791-804.

11. Chhablani J, Sharma A, Goud A, Peguda HK, Rao HL, Begum VU, et al. Neurodegeneration in Type 2 Diabetes: Evidence From Spectral-Domain Optical Coherence Tomography. Investigative ophthalmology & visual science. 2015;56(11):6333-8.

12. Joachim SC, Renner M, Reinhard J, Theiss C, May C, Lohmann S, et al. Protective effects on the retina after ranibizumab treatment in an ischemia model. PloS one. 2017;12(8):e0182407.

13. Foxton RH, Finkelstein A, Vijay S, Dahlmann-Noor A, Khaw PT, Morgan JE, et al. VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma. The American journal of pathology. 2013;182(4):1379-90.

14. Martinez-Zapata MJ, Marti-Carvajal AJ, Sola I, Pijoan JI, Buil-Calvo JA, Cordero JA, et al. Anti-vascular endothelial growth factor for proliferative diabetic retinopathy. Cochrane Database Syst Rev. 2014;11:CD008721.

15. Virgili G, Parravano M, Menchini F, Evans JR. Anti-vascular endothelial growth factor for diabetic macular oedema. Cochrane Database Syst Rev. 2014;10:Cd007419.

16. Virgili G, Parravano M, Menchini F, Brunetti M. Antiangiogenic therapy with anti-vascular endothelial growth factor modalities for diabetic macular oedema. Cochrane Database Syst Rev. 2012;12:Cd007419.

17. Holm K, Schroeder M, Lovestam Adrian M. Peripheral retinal function assessed with 30-Hz flicker seems to improve after treatment with Lucentis in patients with diabetic macular oedema. Documenta ophthalmologica Advances in ophthalmology. 2015;131(1):43-51.

18. Karacorlu M, Ozdemir H, Senturk F, Arf Karacorlu S, Uysal O. Macular function by multifocal electroretinogram in diabetic macular edema after intravitreal triamcinolone acetonide injection. European journal of ophthalmology. 2008;18(4):601-8.

19. Leozappa M, Micelli Ferrari T, Grossi T, Pace V, Rinaldi ML, Battista D, et al. Prognostic prediction ability of postoperative multifocal ERG after vitrectomy for diabetic macular edema. European journal of ophthalmology. 2008;18(4):609-13.

20. Maa AY, Feuer WJ, Davis CQ, Pillow EK, Brown TD, Caywood RM, et al. A novel device for accurate and efficient testing for vision-threatening diabetic retinopathy. Journal of diabetes and its complications. 2016;30(3):524-32.

21. Lecleire-Collet A, Audo I, Aout M, Girmens JF, Sofroni R, Erginay A, et al. Evaluation of retinal function and flicker light-induced retinal vascular response in normotensive patients with diabetes without retinopathy. Investigative ophthalmology & visual science. 2011;52(6):2861-7.

22. Garhofer G, Zawinka C, Resch H, Kothy P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. The British journal of ophthalmology. 2004;88(7):887-91.

23. Zucchiatti I, Bandello F. Intravitreal Ranibizumab in Diabetic Macular Edema: Long-Term Outcomes. Developments in ophthalmology. 2017;60:63-70.

24. Stewart MW. A Review of Ranibizumab for the Treatment of Diabetic Retinopathy. Ophthalmology and therapy. 2017;6(1):33-47.

25. Bressler SB, Odia I, Glassman AR, Danis RP, Grover S, Hampton GR, et al. CHANGES IN DIABETIC RETINOPATHY SEVERITY WHEN TREATING DIABETIC MACULAR EDEMA WITH RANIBIZUMAB: DRCR.net Protocol I 5-Year Report. Retina (Philadelphia, Pa). 2018;38(10):1896-904.

26. Degirmenci MFK, Demirel S, Batioglu F, Ozmert E. Role of a mydriasis-free, full-field flicker ERG device in the detection of diabetic retinopathy. Documenta ophthalmologica Advances in ophthalmology. 2018;137(3):131-41.

27. Comyn O, Sivaprasad S, Peto T, Neveu MM, Holder GE, Xing W, et al. A randomized trial to assess functional and structural effects of ranibizumab versus laser in diabetic macular edema (the LUCIDATE study). Am J Ophthalmol. 2014;157(5):960-70.

28. Kjeka O, Bredrup C, Krohn J. Photopic 30 Hz flicker electroretinography predicts ocular neovascularization in central retinal vein occlusion. Acta ophthalmologica Scandinavica. 2007;85(6):640-3.

29. Larsson J, Bauer B, Andreasson S. The 30-Hz flicker cone ERG for monitoring the early course of central retinal vein occlusion. Acta ophthalmologica Scandinavica. 2000;78(2):187-90.

30. Pedersen KB, Moller F, Sjolie AK, Andreasson S. Electrophysiological assessment of retinal function during 6 months of bevacizumab treatment in neovascular age-related macular degeneration. Retina (Philadelphia, Pa). 2010;30(7):1025-33.

31. Neubauer AS, Kook D, Haritoglou C, Priglinger SG, Kampik A, Ulbig MW, et al. Bevacizumab and retinal ischemia. Ophthalmology. 2007;114(11):2096.

32. Yasuda S, Kachi S, Ueno S, Piao CH, Terasaki H. Flicker electroretinograms before and after intravitreal ranibizumab injection in eyes with central retinal vein occlusion. Acta ophthalmologica. 2015;93(6):e465-8.

33. Liu H, Ji X, Dhaliwal S, Rahman SN, McFarlane M, Tumber A, et al. Evaluation of light- and dark-adapted ERGs using a mydriasis-free, portable system: clinical classifications and normative data. Documenta ophthalmologica Advances in ophthalmology. 2018;137(3):169-81.

34. McAnany JJ, Park JC. Temporal Frequency Abnormalities in Early-Stage Diabetic Retinopathy Assessed by Electroretinography. Investigative ophthalmology & visual science. 2018;59(12):4871-9.

35. Barber AJ, Baccouche B. Neurodegeneration in diabetic retinopathy: Potential for novel therapies. Vision research. 2017;139:82-92.

36. Wu Z, Hadoux X, Hui F, Sarossy MG, Crowston JG. Photopic Negative Response Obtained Using a Handheld Electroretinogram Device: Determining the Optimal Measure and Repeatability. Translational vision science & technology. 2016;5(4):8.

37. Tang J, Hui F, Coote M, Crowston JG, Hadoux X. Baseline Detrending for the Photopic Negative Response. Translational vision science & technology. 2018;7(5):9.

38. Kim HD, Park JY, Ohn YH. Clinical applications of photopic negative response (PhNR) for the treatment of glaucoma and diabetic retinopathy. Korean journal of ophthalmology : KJO. 2010;24(2):89-95.

39. Chen H, Zhang M, Huang S, Wu D. The photopic negative response of flash ERG in nonproliferative diabetic retinopathy. Documenta ophthalmologica Advances in ophthalmology. 2008;117(2):129-35.

40. Kizawa J, Machida S, Kobayashi T, Gotoh Y, Kurosaka D. Changes of oscillatory potentials and photopic negative response in patients with early diabetic retinopathy. Japanese journal of ophthalmology. 2006;50(4):367-73.

41. Viswanathan S, Frishman LJ, Robson JG. Inner-retinal contributions to the photopic sinusoidal flicker electroretinogram of macaques. Macaque photopic sinusoidal flicker ERG. Documenta ophthalmologica Advances in ophthalmology. 2002;105(2):223-42.

Annex

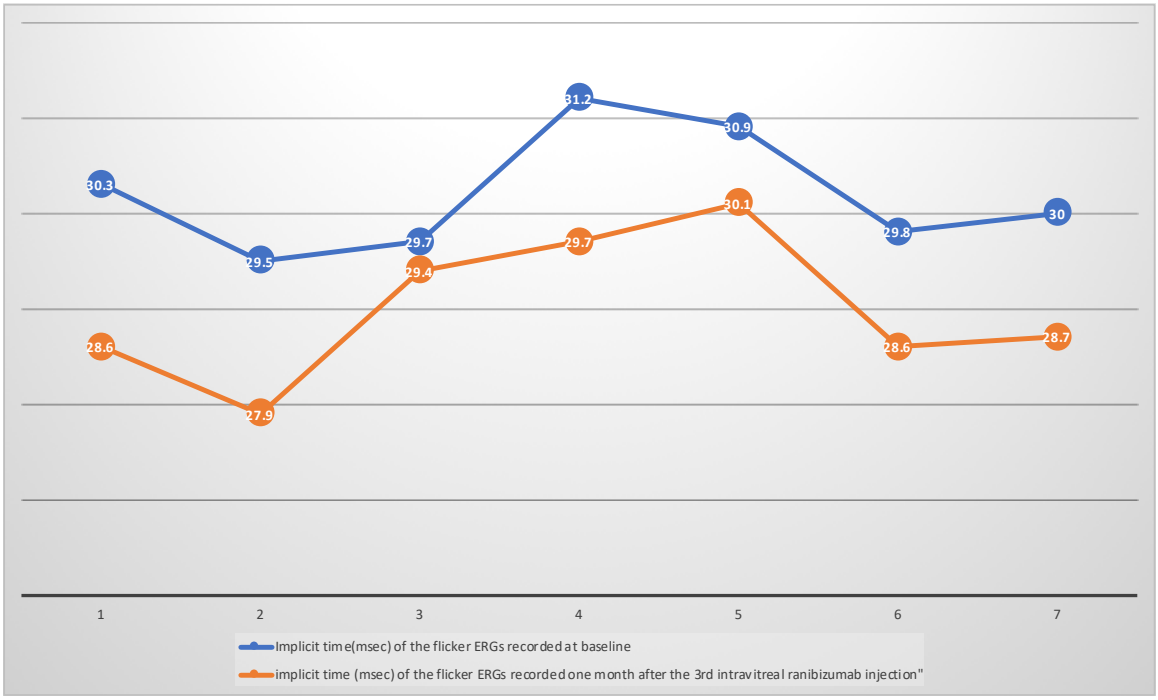

Figure 1. Implicit times of the flicker ERGs before and after IVR recorded with the sinusoidal test of RETevalTM system. One month after the 3rd IVR, the implicit times of the flicker ERGs recorded by the RETevalTM system were shortened for all 7 patients.

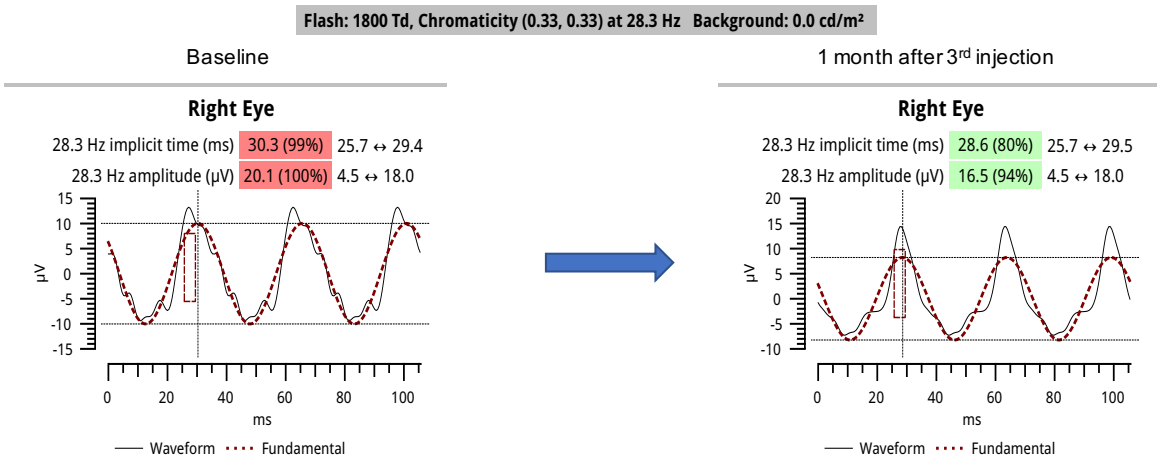

Figure 2. ERG of patient 1 recorded with the sinusoidal test the RETevalTM system at baseline and one month after the 3rd intravitreal injection of ranibizumab. Implicit time reduced from 30.3

ms to 28.6 ms. The black curve represents the electrical response of the eye to the flickering light (Waveform). The red dashed curve represents the fundamental of the electrical response. The rectangular box represents reference intervals that enclose 95% of the data in the visually-normal test population. Measurements of times and amplitudes are evaluated from the best-fitting sine wave to the waveform at the stimulus frequency (the fundamental of the response).

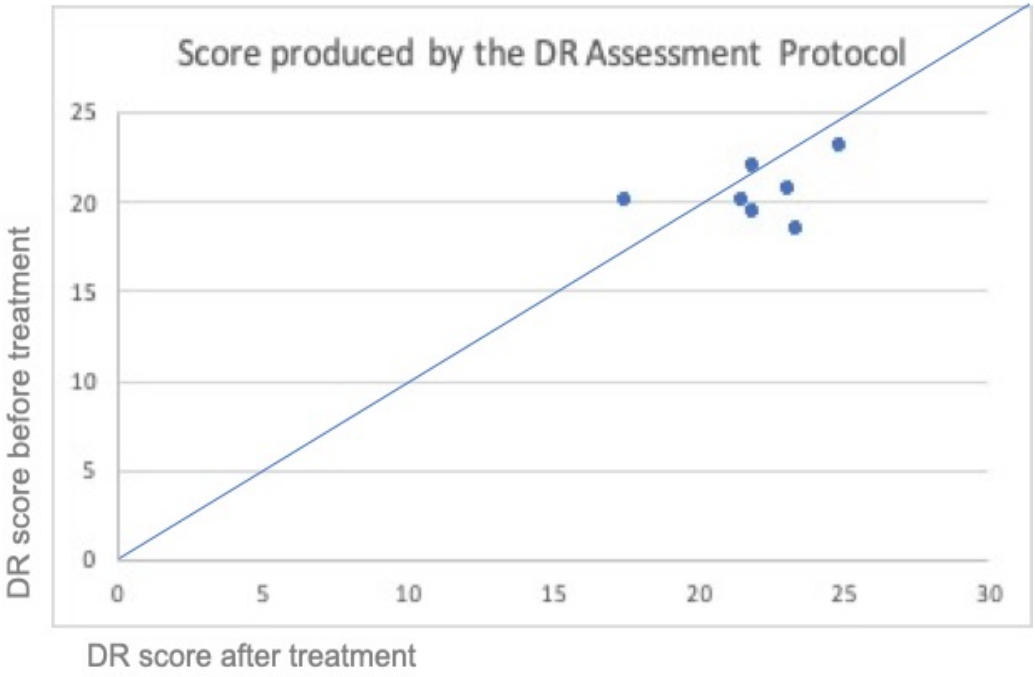

Figure 3. The score produced by the DR Assessment Protocol was significantly reduced to 5 of the 7 eyes.