Current Eye Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/icey20

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To cite this article: Zeki Baysal & Hamidu Hamisi Gobeka (2023) Electrophysiological Evaluation of Macular Photoreceptor Functions in Patients with Choroidal Neovascular Membranes, Current Eye Research, 48:4, 425-431, DOI: 10.1080/02713683.2022.2159982

To link to this article: https://doi.org/10.1080/02713683.2022.2159982

Published online: 21 Dec 2022.

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CURRENT EYE RESEARCH
2023, VOL. 48, NO. 4, 425–431
https://doi.org/10.1080/02713683.2022.2159982

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Electrophysiological Evaluation of Macular Photoreceptor Functions in Patients with Choroidal Neovascular Membranes

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ABSTRACT

Purpose: To evaluate changes in cone functions using light-adapted (LA) 30 Hz flicker and LA 3.0 electroretinography (ERG) in intravitreal ranibizumab (IVR)-treated naïve neovascular age-related macular degeneration (nAMD) patients.

Materials and methods: This retrospective interventional study reviewed the medical records of 32 nAMD patients (32 eyes) who received monthly IVR between January 2019 and January 2021. A comprehensive ophthalmic examination, including best-corrected visual acuity (BCVA) testing and slit-lamp biomicroscopy, was performed as part of their clinical care, followed by LA 30 Hz flicker and LA 3.0 ERGs, optical coherence tomography, and fundus fluorescein angiography. All measurements were taken before IVR (baseline), as well as at months 6 and 12 later. Treatment was resumed for up to 12 months if recurrence occurred.

Results: Compared to baseline, visual acuity improved significantly at months 6 and 12, respectively, coinciding with a significant decrease in central macular thickness ($p < 0.05$ for all). LA 30 Hz flicker ERG b-wave amplitude decreased significantly between baseline and months 6 and 12, respectively ($p < 0.05$ for both). There were no significant changes in LA 3.0 ERG a- and b-wave amplitudes between baseline and month 6 ($p > 0.05$ for both), but a significant decrease existed between baseline and month 12 ($p < 0.05$ for both). While LA 3.0 ERG a-wave implicit time increased significantly ($p < 0.05$ for both) between baseline and months 6 and 12, respectively, b-wave implicit time did not ($p > 0.05$ for both). Also, LA 30 Hz flicker ERG b-wave implicit times did not differ significantly between baseline and months 6 and 12, respectively ($p > 0.05$, for both).

Conclusions: IVR was associated with long-term electrophysiological changes in cone functions, as measured by LA 30 Hz flicker and LA 3.0 ERGs.

ARTICLE HISTORY
Received 13 March 2022
Accepted 13 December 2022

KEYWORDS
Electroretinography; photoreceptor; age-related macular degeneration; choroidal neovascular membranes; intravitreal ranibizumab

Introduction

Choroidal neovascular membranes (CNVM) are new, damaging blood vessels that grow and breach the retinochoroidal barrier, causing painless vision loss and blank spots in central vision once they leak. Vision may also be distorted, with lines appearing bent, crooked, or irregular. Choroidal neovascular membranes are most common in people over 50, with the risk increasing with age, and they are frequently associated with a variety of serious ocular diseases, most notably neovascular age-related macular degeneration (nAMD), one of the leading causes of severe, irreversible visual impairment in developed countries.[1–3] Depending on the underlying disease, anti-VEGF drugs, thermal laser therapy, or photodynamic therapy may be used to treat CNVM.

Vascular endothelial growth factor-A (VEGF-A) is a primary mitogenic factor implicated in pathological neovascular ocular disorders such as AMD due to its involvement in retinal physiology and retinal vascular disorders.[4] All biologically active VEGF-A isoforms are inhibited by anti-VEGF-A monoclonal antibodies. This may explain why anti-VEGF therapy has proven to be so effective in the treatment of nAMD-related CNVM.[5] Intravitreal therapies, which involve delivering medications directly to the retina and choroid, have been used to treat a wide range of ocular diseases. Since the FDA approved pegaptanib, an anti-VEGF aptamer, in 2004, the clinical use of intravitreal therapies has grown. Several anti-VEGF agents, in particular, have been used in the treatment of nAMD.[6] Ranibizumab, a recombinant, humanized monoclonal anti-VEGF-A antibody with antigen-binding fragment (Fab), has been shown to significantly improve central vision in AMD patients.[7,8] Further, evidences from clinical trials indicate that multiple intravitreal ranibizumab (IVR) injections are required to maintain visual improvement.[9]

Full-field electroretinography (ERG) is a technique that objectively reflects a generalized cone system function from the whole retina under standard conditions with varying flash light intensities produced by a Ganzfeld ("full field") stimulator.[10] Around 6 million cone cells are found in the human retina. High spatial acuity is achieved due to the

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high density of cones (with low convergence) in the fovea. Furthermore, the presence of three cone types in the human retina that have different peak spectral sensitivities allows for a diverse range of color perceptions.[11] The primary goal of intravitreal anti-VEGFs in nAMD is to treat CNVM in the macula. These agents may, however, have an impact on the function of macular cone cells. While animal and clinical studies have shown that IVR has no effect on retinal function as measured by ERGs,[12,13] other studies have shown that repeated intravitreal anti-VEGFs may be toxic to the retina.[5,7–9] In addition to systemic complications,[14] intravitreal therapy has also been linked to severe ocular adverse effects.[15] All these findings point to anti-VEGFs having both local and systemic toxicity, which warrants further investigation. However, the long-term effects of anti-VEGFs, particularly on retinal electrophysiological functions in nAMD, have received little attention.

Thus, the current study, which we believe is the first of its kind, aimed to evaluate long-term electrophysiological changes in cone functions as measured by light-adapted (LA) 30 Hz flicker and LA 3.0 ERGs in treatment-naïve patients with nAMD-related CNVM who received IVR therapy.

Materials and methods

Study design and patient selection

This single-centered retrospective interventional study abided by the ethical standards of the Helsinki Declaration and obtained full approval from the Institutional Review Boards of the Batman Training and Research Hospital Ethics Committee (Approval ID: 2021-284). This included a review of patient medical records from the hospital database between January 2019 and January 2021. Initially, the medical records of 40 treatment-naive nAMD patients were retrieved and reviewed retrospectively; however, only the records of 32 patients who met the inclusion criteria for the study were deemed eligible for analysis. Patients who had previously received other intravitreal anti-VEGFs, thermal laser therapy, and/or photodynamic therapy were not studied.

Ophthalmic examination

All patients underwent a comprehensive ophthalmic examination, which included best-corrected visual acuity (BCVA) testing in logarithm of the minimum angle of resolution (logMAR), as well as anterior and posterior slit-lamp biomicroscopy before and after pupil dilation with tropicamide 1% and phenylephrine 10%. LA 30 Hz flicker and LA 3.0 ERGs (Metrovision Ophthalmic Device, France) were then performed with a dilated pupil, followed by optical coherence tomography (OCT, Heidelberg Engineering Inc, Heidelberg, Germany), and fundus fluorescein angiography (FFA) with confocal scanning laser ophthalmoscopy (HRA-2, Heidelberg Engineering Inc, Heidelberg, Germany). Measurements were taken before IVR therapy (baseline) and at monthly follow-up visits; however, only data obtained from medical records at baseline, as well as months 6 and 12, were analyzed.

Anti-VEGF therapy and follow-up regimen

The "loading phase" involved IVR therapy to all patients on a monthly basis for three months. The same procedure was followed for all patients under topical anesthesia with 4% lidocaine. An experienced ophthalmologist injected ranibizumab at a dose of 0.5 mg/0.05 ml intravitreally 3.5 mm posterior to the superior temporal limbus using a 30-gauge needle in a standardized manner at the operating room. Before inserting a sterile lid speculum, the conjunctival sac was cleaned with 10% povidone-iodine and 0.05% chlorhexidine gluconate. This was followed by topical antibiotic drops (levofloxacin, 5 mg/ml) and, finally, intravitreal injection *via* the pars plana. The eye was patched with a sterile eyepatch after antibiotic eye drops (5 mg/ml levofloxacin) were applied.

Monthly intravitreal injections were administered until visual stability (a one Snellen line improvement in BCVA compared to baseline) and anatomical stability (as determined by OCT for CMT and intra-or sub-retinal fluids, and FFA for any CNVM-related leakage) were achieved, at which point treatment was discontinued based on a careful collective decision of the study authors. If there was a recurrence, which was indicated by a loss of more than one Snellen line in the BCVA test, as well as recurring or persistent intra- or sub-retinal fluid involving fovea on OCT and CNVM-related new retinal hemorrhages and leakage on FFA, treatment was resumed and continued for up to 12 months.

Full-Field electroretinography

All ERG shots were taken by the same technician using the same device. The recordings were performed in accordance with International Society for Clinical Electrophysiology of Vision standards [ISCEV-(2022 update)][16] using the Ganzfeld ("full field") stimulator (Visual-Evoked Response Imaging System). Responses were obtained with a 10 K external gain and a bandwidth at 1 and 1000 Hz. Isolated cone responses were recorded after 10 min of light adaptation in the Ganzfeld with a standard light-adaption background of 30 candelas per meter squared ($cd.m^{-2}$). The LA 30 Hz flicker ERG was then acquired at a stimulus strength of 2.51 candela-second per meter squared ($cd.s.m^{-2}$) and a single LA 3.0 ERG at a stimulus strength of 2.58 $cd.s.m^{-2}$. The LA 3.0 ERG a- and b-wave amplitudes, as well as implicit times calculated by averaging five 10-second interval flashes, were recorded for analysis, as were the LA 30 Hz flicker ERG b-wave amplitude and implicit time.

Data analysis

Statistical analysis was carried out using the IBM SPSS Statistics software for Windows, version 18 (SPSS, Inc, Chicago, IL, USA). The data was analyzed using frequency and descriptive statistical tests. The statistical validity was confirmed using Mauchly's sphericity test and Kolmogorov-Smirnov analyses. The repeated measures were compared using a general linear model with Bonferroni correction. Statistical significance was defined as a p value less than 0.05.

Results

The current study evaluated data of the remaining 32 eyes of 32 treatment-naïve nAMD patients at baseline, as well as at months 6 and 12 after IVR therapy. No patients had IVR in both eyes during the same study duration. There were 19 males and 13 females among the patients, with an average age of 73.6 ± 8.05 years (range: 52–85). Occult, predominantly classic, and minimally classic CNVMs were found in 59.3%, 21.8%, and 18.7% of the patients, respectively. The majority of the accompanying drusen types were soft drusen (46.9%). Mean number of injections was 8.3 ± 1.75. Table 1 summarizes the demographics and ocular characteristics of the patients.

Table 1. Demographics and ocular characteristics of treatment-naïve patients with nAMD-related CNVM who received IVR ($n = 32$).

Parameters		Number of patients (eyes)
Laterality	Right eye	19
	Left eye	13
Lens status	Phakic	21
	Pseudophakic	11
Type of nAMD	Occult CNVM	19
	Predominantly classic CNVM	7
	Minimal classic CNVM	6
Drusen types (majority)	Reticular	7
	Soft	15
	Hard	10

nAMD: Neovascular age-related macular degeneration; IVR: Intravitreal ranibizumab; CNVM: Choroidal neovascular membrane; *n*: Number of patients.

Visual and anatomical outcomes

Compared to baseline, logMAR BCVAs improved significantly at months 6 and 12, respectively ($p < 0.001$, for both), which coincided with a significant decrease in central macular thickness (CMT) ($p = 0.004$ and <0.001, respectively) (Table 2).

Electroretinography findings

Compared to baseline, there were significant decreases in LA 30 Hz flicker ERG b-wave amplitude at months 6 and 12 after IVR, respectively ($p < 0.05$ for both). LA 3.0 ERG a- and b-wave amplitudes increased non-significantly at month 6 compared to baseline ($p = 0.956$ and 0.156, respectively). However, there were significant decreases in both a- and b-wave amplitudes at month 12 compared to baseline ($p = 0.002$ and 0.036, respectively) (Figure 1).

There were no significant changes in LA 30 Hz flicker ERG b-wave implicit times between baseline and months 6 and 12, respectively ($p > 0.05$, for both). LA 3.0 ERG a-wave implicit time increased significantly between baseline and months 6 ($p < 0.001$) and 12 ($p = 0.002$), respectively. However,

Table 2. Visual and anatomical outcomes of treatment-naïve patients with nAMD-related CNVM who received IVR ($n = 32$).

	Duration (mean ± SD)			*p* Value	
Parameters	Baseline	Month 6	Month 12	Baseline-Month 6	Baseline-Month 12
logMAR BCVA	0.63 ± 0.28	0.38 ± 0.33	0.39 ± 0.20	<0.001	<0.001
CMT (μm)	402 ± 101	330 ± 80	325 ± 85	0.004	<0.001

IVR: Intravitreal ranibizumab; nAMD: Neovascular age-related macular degeneration; CNVM: Choroidal neovascular membrane; BCVA: Best-corrected visual acuity; logMAR: Logarithm of the Minimum Angle of Resolution; CMT: Central macular thickness; SD: Standard deviation; μm: Micrometer; *n*: Number of patients.

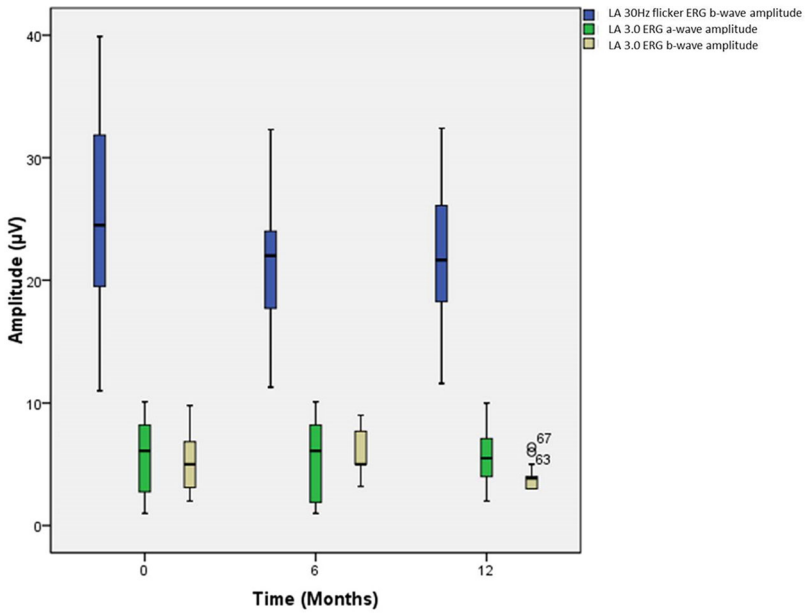

Figure 1. Amplitude variations (in microvolts) in light-adapted 30 Hz flicker electroretinography b-wave (*blue boxes*), as well as light-adapted 3.0 electroretinography a- and b-waves (*green and yellow boxes, respectively*) of treatment-naïve patients with nAMD-related CNVM who received IVR during the study.

Table 3. Changes in LA 30 Hz flicker and LA 3.0 ERGs of treatment-naïve patients with nAMD-related CNVM who received IVR ($n = 32$).

Amplitude (μV) (mean ± SD)				*p* Value	
	Baseline	Month 6	Month 12	Baseline-Month 6	Baseline-Month 12
LA 30 Hz flicker ERG					
b-wave	25.67 ± 7.58	21.70 ± 6.07	21.56 ± 5.51	0.031	0.015
LA 3.0 ERG					
a-wave	5.71 ± 3.07	6.05 ± 2.05	4.08 ± 1.86	0.956	0.002
b-wave	5.02 ± 2.53	6.02 ± 1.77	3.98 ± 1.00	0.156	0.036
Implicit time (ms) (mean ± SD)				*p* value	
	Baseline	Month 6	Month 12	Baseline-Month 6	Baseline-Month 12
LA 30 Hz flicker ERG					
b-wave	40.97 ± 2.95	41.75 ± 3.12	41.30 ± 2.69	0.565	0.580
LA 3.0 ERG					
a-wave	20.22 ± 4.11	24.22 ± 2.75	23.44 ± 3.30	<0.001	0.002
b-wave	38.83 ± 4.21	39.50 ± 2.97	39.82 ± 3.50	0.571	0.451

nAMD: Neovascular age-related macular degeneration; CNVM: Choroidal neovascular membrane; IVR: Intravitreal ranibizumab; LA: Light-adapted; ERG: Electroretinography; μV: Microvolt; ms: Millisecond; Hz: Hertz; *n*: Number of patients; SD: Standard deviation.

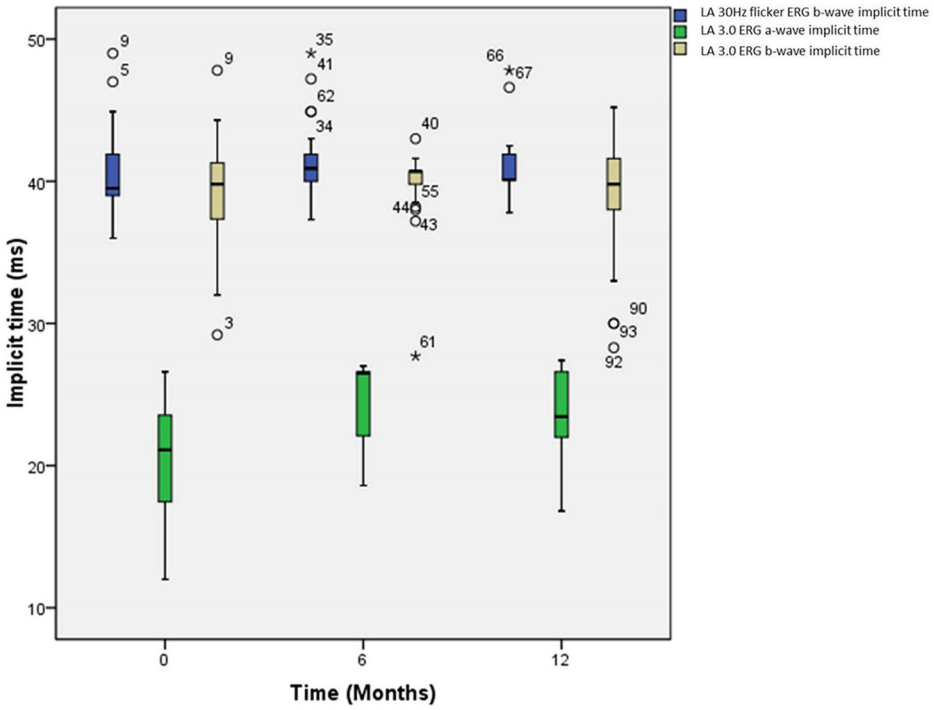

Figure 2. Implicit time variations (in milliseconds) in light-adapted 30 Hz flicker electroretinography b-wave (*blue boxes*), as well as the light-adapted 3.0 electroretinography a- and b-waves (*green and yellow boxes, respectively*) of treatment-naïve patients with nAMD-related CNVM who received IVR during the study.

compared to baseline, there were no significant changes in LA 3.0 ERG b-wave implicit time at months 6 ($p = 0.571$) and 12 ($p = 0.451$), respectively (Table 3, Figure 2).

Discussion

ERG is a valuable tool for neuro-retinal integrity and function assessment. In the current study, changes in cone electrophysiological functions in LA 30 Hz flicker and LA 3.0 ERGs were retrospectively evaluated in treatment-naïve nAMD patients receiving an average of 8.3, 0.50 mg IVR injections over a 12-month period. IVR therapy resulted in a significantly improved logMAR BCVA, which corresponded to a significant decrease in CMT. This could be due, in part, to the fact that the current study was conducted on patients with naïve nAMD who had never received laser therapy, and thus had no prior retinal ultrastructural damage.

Meanwhile, varying results of ERG amplitude and implicit time were revealed during the study. Compared to baseline, LA 30 Hz flicker ERG b-wave amplitudes decreased significantly at months 6 and 12, respectively. LA 3.0 ERG a- and b-wave amplitudes increased non-significantly at month 6 when compared to baseline. These amplitudes, however, were significantly lower at month 12 when compared to baseline. Moreover, only changes in LA 3.0 ERG a-wave implicit time reached statistical significance at months 6 and 12, respectively, when compared to baseline. Nonetheless, there was a general trend for implicit times in both ERGs to increase, albeit non-significantly, between baseline and

month 6, only to decrease non-significantly between baseline and month 12. These results could reflect a varying degree of IVR suppressive effect on cone electrophysiological functions, particularly over time.

Ultimately, even though CMT decreased during the course of the study, both ERGs showed a trend with varying changes in amplitude and implicit times. Of note, CMT was only studied quantitatively in the current study, not morphologically. It decreased most significantly during the first 6 months, with only minor reductions observed at month 12. Photoreceptor degeneration can be confused with an abnormally thicker central macula.[17] Consequently, the subsequent IVR therapy could have treated dry AMD rather than nAMD. This could explain the reduced amplitude and varying implicit time elongation. It is important to remember that, while logMAR BCVA increased dramatically at month 6, this increase was only marginally visible at month 12, indicating that ERG changes occurred during the course of the study. This appears to be noteworthy in light of the ever-increasing medical use of anti-VEGFs in patients with CNVM-related diseases.

Based on the literature, IVR is associated with improvements in BCVA and decreases in CMT, which is consistent with the current study. Despite this, there is no clear relationship between BCVA or CMT and ERG results.[12] The variable ERG responses in the study could be explained by the possibility of posterior pole geographic atrophy, which has been identified as a negative effect of anti-VEGF treatment for AMD.[18] As a result, learning more about nAMD is critical in order to make more informed decisions about when to initiate and discontinue treatment. In this situation, ERG may be a rational and reasonable way to proceed with this task.

In one randomized study of 48 rabbits, ERG evaluation of retinal function 8–9 weeks after intravitreal injection of bevacizumab [1.25 mg (0.05 ml, 25 mg/ml)], ranibizumab [0.5 mg (0.05 ml,10 mg/ml)], pegaptanib [0.3 mg (0.09 ml)], or a balanced saline solution (BSS, 0.05 ml) revealed that anti-VEGFs were associated with lower amplitudes of the dark adapted b-wave rod-mediated response to dim light compared to BSS. Aside from an unchanged a-wave amplitude in a single LA 3.0 ERG, no significant changes in combined responses, a-b wave amplitudes, or LA 30 Hz flicker b-wave amplitudes were observed.[19] An *ex vivo* model in which 0.2 mg/ml ranibizumab was administered to isolated bovine retinas revealed non-significant a- and b-wave amplitude reductions of 4% and 22.32%, respectively, in dark adapted rod responses. Unlike the above study, this model demonstrated the efficacy of the administered ranibizumab concentration by simply demonstrating stability of the ERG-amplitudes, excluding substantial neuro-retinal dysfunction after up to 1 mg IVR.[13] High-dose intravitreal anti-VEGFs are thought to affect photoreceptor-mediated retinal function as early as two months after administration, highlighting the importance of comprehending drug action and planning in new areas such as retinopathy of prematurity, where vitreous volume is markedly smaller than in the adult eye.[19]

Despite the lack of rod response assessment in the current study, which included more than half of the patients who were phakic with soft drusen predominance (46.9%), LA 30 Hz flicker ERG b-wave amplitudes were significantly lower at months 6 and 12 after 0.5 mg/0.05 ml IVR, findings that differed from prior short-term animal studies[13,19] with LA 3.0 cone ERGs. The ERG changes directly and exclusively demonstrated long-term IVR toxic effects on electrophysiological functions, with a reduced b-wave denoting definite neuronal dysfunction of the retina.

Changes in LA 30 Hz flicker ERG have been reported in other retinal diseases. There has been evidence of a link between retinal ischemia and LA 30 Hz flicker implicit time increment in central retinal vein occlusion,[20] as well as a propensity for shorter LA 30 Hz flicker implicit time after retinal detachment surgery.[21] These findings may imply that LA 30 Hz flicker implicit time increment signifies impaired cone function of the entire retina, possibly due to ischemia or a detached retina.

Other relatively short-term contradictory reports of significant reductions in implicit times in LA 30 Hz flicker ERG amplitudes in central retinal vein occlusion[22] and diabetic macular edema[23] one and two months, respectively, after IVR have been published. Further, despite a significantly improved cone flicker ERG implicit time at month 6 in one study of intravitreal bevacizumab-treated AMD patients, others reported no indications of changes in cone functions with IVR in diabetic patients after about a year. Furthermore, the retinal function evaluation before and immediately after IVR or intravitreal aflibercept in 79 patients with various diseases, including AMD ($n = 37$), diabetic macular edema ($n = 24$), and retinal vein occlusion-related macular edema ($n = 18$), revealed no significant change in LA 30 Hz flicker ERG amplitude in nAMD patients 24 h after IVR, while implicit time was significantly increased.[15] This study is similar to the current study in terms of IVR application in nAMD; however, it differs in terms of short-term duration and the absence of LA 3.0 ERG.

In another photopic negative response ERG study that examined the IVR safety on retinal ganglion cell function in 32 eyes of 32 AMD patients, no significant changes in the amplitude and implicit time of the red full field cone ERG responses were observed one year after IVR.[4] The current study procedure and design were comparable to those described above. Nonetheless, the current study evaluated cone electrophysiological functions in LA 30 Hz flicker and LA 3.0 ERGs, revealed significantly reduced cone functions after IVR over a 12-month period. The previous explanation for the suppression of cone electrophysiological functions in IVR-treated naïve nAMD patients, combined with increased implicit time, could possibly indicate impaired cone function of the entire retina as a result of potential ranibizumab toxicity. Aside from a potential ultrastructural toxic effect of ranibizumab *via* apoptosis in the retinal photoreceptors, there could also be temporary choriocapillaris damage[12] as a result of RPE-secreted VEGF blockade,[24,25] which is required for choriocapillaris development and maintenance.

Additionally, the current study findings could also be explained by the macular ultrastructural anatomy, which has an increased amount of cones in the foveal centralis, and the fact that the macula only accounts for about 10% of the global retinal cone photoreceptors. In this context, when evaluating retinal electrophysiological function, the pathogenesis of retinal diseases, i.e. vascular versus degenerative, and the disease's natural course, characterized by progressive loss of these retinal photoreceptors, must also be taken into account, not to mention the lack of a control group, as we are unaware of natural electrophysiological changes.

Furthermore, long-term changes in cone electrophysiological functions were evaluated in the current study, which included the majority of patients with occult CNVM, to determine the efficacy of IVR on treatment-naïve nAMD patients aged 73.6 ± 8.05 years on average. When compared to baseline, LA 30 Hz flicker ERG b-wave implicit time increased slightly at month 6, but then decreased with no significant differences at month 12. While LA 3.0 ERG a-wave implicit time increased significantly after IVR, there was no significant change in b-wave implicit time. Variations in implicit times over the course of the study could be attributed to reversible ultrastructural changes in choriocapillaris after therapy.[26]

Long-term use of anti-VEGFs, as in the current study, may however result in permanent choriocapillaris damage, contrary to past studies.[4,12] The retinal pigment epithelium (RPE) atrophy in CNVM-related retinal diseases is preceded by choriocapillaris damage.[26–28] The current study findings support prior reports that choriocapillaris regeneration is reduced when anti-VEGFs block RPE-secreted VEGFs.[27,29] The RPE functions deteriorate with decreasing regeneration as long as choriocapillaris damage persists. This impairs photoreceptor viability and the removal of cellular debris.[30] The loss of these photoreceptor functions is reflected in full-field cone ERG waves.

Inhibiting VEGFs alone can halt CNVM progression, resulting in anatomical improvement.[6] But the RPE secretes a slew of other growth factors in addition to VEGFs.[30] Therefore, inhibiting VEGFs alone, without affecting other growth factors, prolongs the inflammatory process, albeit slowly, decreasing RPE functions and, as a result, photoreceptor functions. Since anti-VEGFs do not inhibit the effects of other pro-inflammatory mediators, the RPE may be compromised, resulting in reduced photoreceptor functions, as observed in the current study. Long-acting drugs targeting multiple growth factors may reduce the need for frequent injections while also protecting choriocapillaris, RPE, and photoreceptor functions. Furthermore, using ERG to monitor nAMD patients receiving anti-VEGFs may provide clinical data about photoreceptor functions, and treatment modality may be modified if deterioration occurs.

The current study has limitations, including a relatively small number of patients and a lack of monthly ERG follow-up records. Long-term large-scale clinical studies on neuro-retinal functions after anti-VEGF therapies may yield more clinically relevant findings on this topic.

Conclusions

The current study revealed long-term significant ERG changes in cone functions in both LA 30 Hz flicker and LA 3.0 ERGs, indicating that IVR could affect retinal photoreceptor electrophysiology. The findings in both ERG forms could have brought about no conclusive evidence of long-term IVR toxicity. However, as the long-term effects of treating patients multiple times are unknown, supplemental monitoring of both central and full neuro-retinal electrophysiology is required. In this regard, ERG may be a useful and practical tool, especially in patients with nAMD who are receiving anti-VEGFs like ranibizumab. Nonetheless, additional long-term clinical studies are required to determine the effects of specific anti-VEGFs on neuro-retinal electrophysiological functions in this patient population.

Ethical approval

This single-centered retrospective interventional study abided by the ethical standards of the Helsinki Declaration and obtained full approval from the Institutional Review Boards of the Batman Training and Research Hospital Ethics Committee (Approval ID: 2021-284), which included a review of patient medical records from the hospital database between January 2019 and January 2021.

Author contributions

Both ZB and HHG contributed significantly to the conception and design of the current study, acquisition, analysis and interpretation of the data, the drafting and essential revision of the manuscript for relevant intellectual content and the endorsement of the final version of the manuscript. ZB = Zeki Baysal; and HHG = Hamidu Hamisi Gobeka.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data are available from the corresponding author on reasonable request.

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