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RESEARCH ARTICLE

Multifocal electroretinogram analysis of the effectiveness of anti-VEGH treatment for clinically significant macular edema treatment

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Abstract

This study aimed to compare multifocal Electroretinogram (mfERG) between before and after the treatment of clinically significant macular edema (CSME). Cross-sectional comparative study was performed on the mfERG measurements of four patients (54 ± 11 years old) diagnosed with CSME by an ophthalmologist (retina subspecialty). The subjects were examined within 1 to 2 months before and after the anti-vascular endothelial growth factor (anti-VEGF) CSME treatment. The procedure of mfERG adhered to the standard recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV). Parameters included in this investigation were N1 amplitude (μV), N1 implicit time (ms), P1 amplitude (μV), and P1 implicit time (ms). Further analysis was performed by dividing the retina area into five rings zone (1st Ring, 2nd Ring, 3rd Ring, 4th Ring, and 5th Ring with a subtended surface area of 0–2°, 2–5°, 5–10°, 10–15°, and >15°, respectively). The paired sample T-test was used to compare the mfERG between before and after Anti-VEGF intervention. Overall, mean differences were observed before and after the CSME treatment with the anti-VEGF, but not statistically different for the N1 amplitude (μV), N1 implicit time (ms), P1 amplitude (μV), and P1 implicit time (ms). Further analysis results based on rings was revealed to be not statistically different, except N1 amplitude (μV) at 5 to 10° surface area (3rd Ring), which the N1 amplitude became less negative after the treatment, -24.95 μV, (95% CI, -37.44 to -12.46), t (3) = -6.36, $p < 0.05$]. There was no statistically significant difference in mfERG before and after between treatment of CSME except for N1 amplitude (μV) for 3rd Ring after 1 to 2 months follow up. In the future, it is recommended that similar investigation should be conducted by involving more patients and performing a series of follow up.

Keywords: MfERG, CSME, rings, anti-VEGF, retina

INTRODUCTION

According to Ali (1997), almost 10 % of patients with diabetes developed diabetic macular edema (DME) during their lifetime. Clinically significant macular edema (CSME) is a form of DME as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS) (Musat *et al.,* 2015). ETDRS has established a method for classifying and diagnosing DME and determining when treatment was required (Kertes and Johnson, 2007). In order to diagnose CSME, one of the following characteristics must be presented on clinical examination: retinal thickening within 500 microns of the center of the macula, hard exudates within 500 microns of the center of the macula with adjacent retinal thickening, and retinal thickening at least 1-disc area in size, any part of which is within 1-disc diameter from the center of the macula. The determination of the presence of CSME is a clinical diagnosis based on a retinal biomicroscopic examination of the patient, and not based on fluorescein angiography (Saxena *et al.*, 2000).

The traditional treatment of DME took two approaches. Prevention of DME was ideal, and through stringent metabolic control of blood glucose levels, the onset of diabetic retinopathy was indeed delayed, and progression was slowed (American Diabetes Association, 2011). Unfortunately, prevention does not always work. Diabetic retinopathy and DME were often the initial presenting signs of diabetes (Park and Roh, 2015). Once CSME happened, treatment was recommended. The EDTRS study demonstrated that timely treatment with pan-retinal photocoagulation (PRP) significantly reduced vision loss associated with diabetic retinopathy. Laser photocoagulation was considered the standard of care in the treatment of DME primarily because of the findings of the EDTRS. It is a significant risk for those patients with lesions close to the fovea as macular photocoagulation might induce paracentral scotomas, accidental foveal photocoagulation, subfoveal fibrosis and choroidal neovascularization at sites of laser scars (Luttrull & Dorin, 2012). More recently, treatment strategies focused on inhibition of vascular endothelial growth factor (VEGF) and numerous therapies became a topic of interest in age-related macular degeneration.

In mfERG, the first-order kernel was represented by N1 and P1 components, which the most extensive response and most commonly used in the clinical setting for diagnosis. N1 amplitude was measured from the baseline to the N1 trough, and the P1 amplitude was measured from the N1 through to the P1 peak. Implicit times were measured from the onset of the stimulus to the peak of the waveform (Lai *et al.,* 2007). The response dominated by cells of the outer retina, such as the photoreceptors and the on and off-bipolar cells (Hood *et al.,* 2002). Compared the first-order kernel was the second-order kernel, which smaller in the waveform, and challenging to measure because of the poor signal-to-noise ratio. This signal is referred to as

inner retinal activity from the retinal ganglion cells (Lai *et al.,* 2007). The anti-VEGF is used to stop a protein called vascular endothelial growth factor (VEGF) produced by cells in the retina from working. The new blood vessel growth is a significant problem and this revolutionized treatment is beneficial for exudative age-related macular degeneration (AMD) and holds great promise for diabetic macular edema, branch and central retinal vein occlusions, and retinopathy of prematurity (Stewart, 2012). Using of intravitreal pegaptanib and intravitreal ranibizumab have shown short-term benefit in visual acuity (Nicholson and Schachat, 2010). Various study have showed that effectiveness of treatment averaged 5.7 ± 2.8 months (Nicholson and Schachat, 2010).

The new multifocal Electroretinogram (mfERG) paradigm is a fast and sensitive test for the detection of early functional changes in the diabetic retina (Chan and Siu, 2003). It has been reported that there were significant mfERG implicit time differences between controls and patients with diabetes, controls and diabetics without retinopathy, and between controls and diabetics with retinopathy (Tyrberg, 2010). Although no improvement in visual function was reported, improvements in retinal structures was found using mfERG after treatment on diabetic macular edema (Greenstein *et al.,* 2000). Therefore, this study focused on the effectiveness of the treatment of clinically significant macular edema and explored the level of mfERG abnormality before and after the treatment of Clinically Significant Macular Edema.

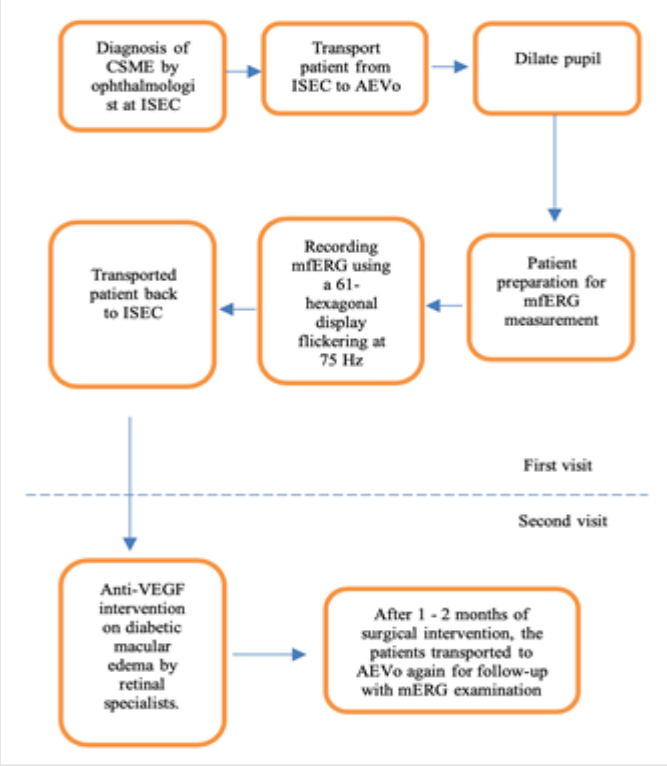

Fig. 1 Summary of the research flow. The pre- and post-mfERG measurements were done during the first visit and second visit, respectively.

EXPERIMENTAL

Study design

A cross-sectional comparative study was chosen as the investigation study design. The study was located at the Advanced Electrophysiology for Vision Laboratory (AEVo Lab), Centre for Research in Optometry & Visual Science (iROViS), Universiti Teknologi MARA (UiTM), Malaysia by following the standard methodology recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV). Four patients consisted of two males and two females with diabetic macular edema were recruited, screened, and treated at the International Specialist Eye Centre (ISEC), MidValley, Malaysia. Multifocal Electroretinogram before and after the treatment of CSME was measured and compared based on the implicit time (ms) and amplitude (μV) of mfERG in patients. Fig. 1 summarized the flow chart of the study. This study adhered to the Declaration of Helsinki and was approved by the institutional ethics review board. Informed consent was obtained from all subjects before participating in the present study.

Sample size calculation

A study on the prevalence and risk factors for diabetic retinopathy showed that that 26.7 % of 217 Malaysian diabetic patients had evidence of maculopathy (Tajunisah *et al.,* 2006). Laser photocoagulation and surgical intervention were the mainstays of diabetic retinopathy treatment. Moderate visual loss due to DME might be reduced by approximately 60 % after a single session of grid or focal laser treatment. Four patients with diabetic macular edema were recruited based on diagnosis by the retina specialist of International Specialist Eye Centre (ISEC). The following calculation was based on the estimation of 60 % improvement shown by photocoagulation studies. The confidence level was 95 %, and the confidence interval was 10 %. Determination of sample size was done using the formula:

$$\text{Sample size, } n = \frac{z^2 * (p) * (1-p)}{c^2} \qquad (1)$$

n = 4 samples.

Patients selection criteria

Diabetic patients with cystoid macular edema or clinically significant macular edema as defined by the EDTRS were recruited. Patients were required to undergo full eye examination, which included history taking, entrance tests, colour vision test, refraction, slit lamp examination, and funduscopy. The refractive errors for the patients ranged between +6.00D and -6.00D. Patients with dilated pupil size less than 5 mm were excluded. Patients with a history of colour vision defect, strabismus, glaucoma, previous intraocular surgery, and the systemic disease that known to affect the retina and the presence, or a history of retinal detachment were also excluded. The procedure of anti-VEGH commenced by the ISEC retinal specialist which involved injection for centre subfield. All patients have not received similar treatment before.

The procedure of mfERG assessment

The mfERG was recorded using a 61-hexagonal display flickering at 75 Hz with subtending 45° in the posterior pole centred on the fovea using the Espion (Diagnosys LLC) multifocal ERG system. Binocular mfERG was recorded with dilated pupils using a DTL-Plus™ micro-conductive fibre electrode positioned on the limbal margin and held in place with adhesive pads placed near the nasal and temporal canthi. The recording time of four minutes was divided into 30 seconds recording segments (using m=14 sequence) and blink artefact was automatically rejected. The response amplitude of the mfERGs was determined and compared to an age-match normative database. The mfERG statistical deviation z-score was derived to express the degree of normality/abnormality for mfERGs throughout the 45-degree field. The mfERG was used to provide an objective functional evaluation of CSME rather than patient's functional evaluation such as Visual Acuity (VA) or Visual Field (VF). Our study used the values of mfERG to investigate the changes in the retinal pattern before any detectable clinical changes in CSME before and after treatment for early detection of Diabetic Macular Edema. Parameters of investigation included N1 implicit time (ms), N1 amplitude (μV), P1 implicit time (ms), and P1 amplitude (μV) (Fig. 2). Further analysis was performed by dividing the retina area into five rings zone [1st Ring, 2nd Ring, 3rd Ring, 4th Ring and 5th Ring subtended the surface area of 0–2°, 2–5°, 5–10°, 10–15°, and >15°, respectively] (Fig. 3).

Statistical analysis

The paired sample T-test was used to compare the parameters before and after the Anti-VEGF intervention. The average values of

N1 and P1, in amplitude and implicit time in 1st to 5th rings, were averaged and analysed. Then, further descriptive and statistical analysis for every ring was compared before and after the intervention.

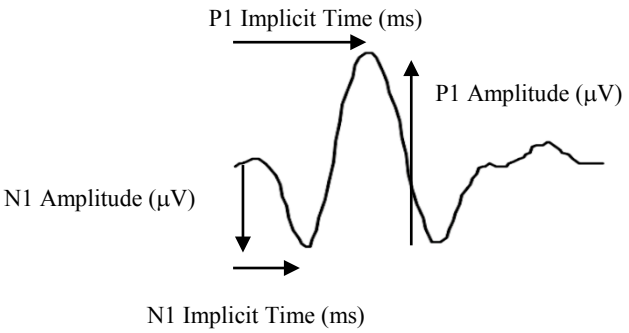

Fig. 2 Illustration of four parameters in our mfERG investigation: N1 amplitude (μV), N1 implicit time (ms), P1 amplitude (μV), P1 implicit time (ms), and N2 amplitude (μV).

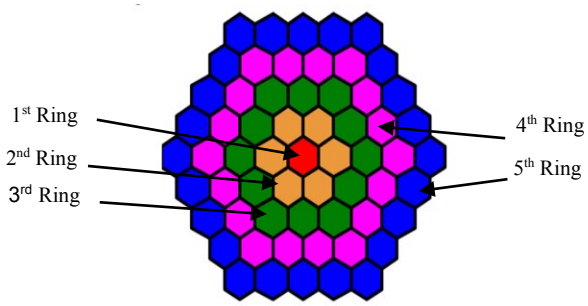

Fig. 3 The locations of five rings zones in our mfERG measurements: 1st Ring (innermost ring), 2nd Ring, 3rd Ring, 4th Ring and 5th Ring (outer most ring).

RESULTS AND DISCUSSION

As the average ring from 1st to 5th ring, N1 amplitude showed more minus reading before (mean = -70.05 ± 18.49 μV) than after (mean = -41.70 μV ± 49.59) CSME treatment, but not statistically significant (t-test = -0.409, $p > 0.05$). N1 implicit time did not display any significant difference before (mean = 17.22 ± 1.31 ms) and after (mean = 16.46 ± 1.44) the CSME treatment (t-test = 3.03, $p > 0.05$). Meanwhile, P1 amplitude showed higher reading before (mean = 214.05 ± 51.39 μV) than after (mean = 195.28 ± 67.19 μV) CSME treatment, but not statistically difference (t-test = 0.660, $p > 0.05$). P1 implicit time revealed no significant difference before (mean = 34.93 ± 3.78 ms) and after (mean = 35.55 ± 4.16 ms) the CSME treatment (t-test = 0.932, $p > 0.05$).

The changes varied according to the locations of the ring showed in Table 1. To achieve further on certainty of the efficacy of CSME treatment, macular edema should show significant differences in amplitude and implicit time in N1 and P1 for the entire five ring zones before and after study. However, there was no statistically significant difference of the mfERG measurements before and after treatment of CSME except for N1 amplitude for the 3rd ring [N1 amplitude less negative -24.95 μV, (95 % CI, -37.44 to -12.46), $t(3) = -6.36$, $p < 0.05$].

As the average for all the rings, there were no statistically significant changes in amplitude of P1 and N1 and implicit time of P1 and N1 before and after 1 to 2 months of the Anti-VEGF treatment. This support the previous finding that no significant variation in pattern electroretinogram (PERG) and mfERG after 3 and 6 months of treatment (Nowacka *et al.*, 2016). Analysis of P1 amplitude and implicit time also showed no significant after the third injection and first level (Holm *et al.,* 2015). However, the significant variation reported for visual acuity and macular thickness in both studies reveals the effectiveness of the treatment for the CSME. In this study, further investigation was extended to N1 amplitude and implicit time, and no changes for N1, also in P1. The structural and resolution function changes might give an earlier treatment effect rather than an electrophysiology effect when dealing with Anti-VEGF, which contradict to Greenstein study (Greenstein *et al.,* 2000).

Table 1 The mean and standard deviation (SD) of 4 parameters of mfERG measurements.

	N1 amplitude (μV) (mean ± SD)		N1 implicit time (ms) (mean ± SD)		P1 amplitude (μV) (mean ± SD)		P1 implicit time (ms) (mean ± SD)	
	Before	After	Before	After	Before	After	Before	After
1st Ring	-84.70 ± 50.12	-143.23 ± 126.70	16.43 ± 1.94	16.00 ± 2.08	271.05 ± 57.27	266.70 ± 51.24	103.33 ± 141.80	43.08 ± 18.51
2nd Ring	-65.53 ± 30.39	-70.85 ± 14.77	16.00 ± 1.85	15.38 ± 0.85	238.35 ± 36.73	221.33 ± 45.48	33.08 ± 3.62	32.85 ± 4.81
3rd Ring	-74.60 ± 24.89	-49.65 ± 27.27	17.45 ± 2.26	16.00 ± 2.18	230.90 ± 39.29	197.40 ± 39.81	33.28 ± 2.94	34.13 ± 4.57
4th Ring	-68.15 ± 17.89	-36.45 ± 57.15	18.30 ± 2.77	16.23 ± 4.81	213.85 ± 42.53	183.28 ± 47.86	34.73 ± 3.44	35.35 ± 3.97
5th Ring	-70.05 ± 18.49	-41.70 ± 49.59	17.90 ± 1.75	18.70 ± 4.11	214.05 ± 51.39	195.28 ± 67.19	34.93 ± 3.78	35.55 ± 4.16

Grey highlight Indicated the significant difference between before and after CSME treatment with Anti-VEGF, with $p < 0.05$.

Within the 1 to 2 months interval, the changes of mfERG were only found in 5° to 10° from the macula, which might indicate the deterioration of the parafovea area. Information in the parafovea can affect the processing of a scene, which was the guide for image resolution, such as in visual acuity (Thibaut *et al.,* 2014). It was correlated with morphological changes revealed by OCT and with multifocal ERGs (Yamamoto *et al.,* 2001). A previous study demonstrated that retinal function was significantly worse in diabetic eyes with CSME than healthy eyes (Tehrani *et al.,* 2015). The electroretinographic findings showed abnormalities in foveal cone responses in the eyes with non-proliferative diabetic retinopathy (NPDR), particularly in the presence of CSME (Weiner *et al.,* 1997). CSME was a clinical diagnosis defined as retinal thickening within 500 μm of the macular centre and hard exudates within 500 μm of the macular centre with an adjacent retinal thickening. Histopathological observations show retinal swelling initiates intracytoplasmic swelling of Müller cells, and that the outer plexiform layer or Henle's fibrer layer was markedly swollen in diabetic eyes. This condition might

result in higher standard deviation on mfERG results. The duration of edema may significantly influence both anatomical and functional results (Yamamoto *et al.,* 2001).

CONCLUSION

We focus on the objective assessment before and after the CSME treatment, which the objective evaluation on average and independent rings was establish. Electrophysiology changes were found in terms of N1 amplitude (μV) for third ring, while other parameters and ring did not show any significant difference within 1 to 2 months before and after treatment of anti-VEGF for Clinically Significant Macular Edema (CSME) treatment. Also, the effectiveness of anti-VEGF treatment for clinically significant macular edema was not observed from the mfERG analysis due to the short interval of the current study. As for the recommendation the future investigation may want to increase the duration of observation and take into account the changes of acuity to see which changes have early effect after the treatment.

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