Anatomical and Functional Evaluation of Laser Photocoagulation and Intravitreal Bevacizumab in Diabetic Macular Edema

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ABSTRACT

 Purpose: to evaluate the effect of combined laser photocoagulation and intravitreal Bevacizumab (IVB) on diabetic macular edema (DME) and assess the function of the retina by multifocal electroretinogram.

Study design: prospective, interventional case series study.

Methods: 30 eyes of 30 patients with clinical significant macular edema (CSME) were examined and focal or grid macular laser photocoagulation was done as indicated by fluorescein angiography. One week later, Bevacizumab (1.25 mg/0.1 ml) was injected intravitreally. The main outcome measures included best-corrected visual acuity, fundus fluorescein angiography, macular edema map values of optical coherent tomography (OCT) and multifocal electroretinogram (mf-ERG) before and one month after treatment (laser and intravitreal injection).

Results: The visual acuity increased significantly from 0.49±0.33 to 0.25±0.23 (logMAR) after one month treatment (P<0.001). The central macular thickness values significantly decreased from 446.46±73.42 to 256.73±46.53 µm, (P<0.001). Multifocal-ERG showed significant reduction of latency with increase in amplitude of P1 wave as compared to pretreatment values, (P<0.001).

Conclusion: Laser photocoagulation and early intravitreal Bevacizumab injection provided significant improvement in visual acuity of diabetic patients and clinical course of macular edema, and may therefore be a promising approach in the treatment of diabetic macular edema.

Key words: Diabetic edema, Bevacizumab, Laser photocoagulation

INTRODUCTION

Diabetic Macular edema (DME) is a common cause of visual loss in diabetic patients with subsequent legal blindness. The pathogenesis of diabetic macular edema was mainly attributed to breakdown of the inner blood-retinal barrier due to loss of retinal capillary pericytes with increased vascular permeability, which may occur at any stage of non-proliferative or proliferative diabetic retinopathy (PDR).[1] Macular edema is divided into focal, diffuse and cystoid edema. Focal macular edema is caused by focal leakage from microaneurysms and dilated retinal capillaries with abnormal permeability. In diffuse macular edema, generalized leakage from dilated capillaries is observed throughout the posterior pole.[2]

Early Treatment Diabetic Retinopathy Study Research Group (ETDRS) demonstrated that laser treatment could prevent moderate visual loss (loss of 15 letters or three lines on the standard ETDRS visual acuity chart) in 24% eyes, compared to 12% in untreated controls, after 3 years.[3] After macular laser treatment, the results of the psychophysical tests suggested improvement in local ERG (electroretinogram) responses with increase in implicit time and decrease in amplitude.[4] Focal laser was suggested to reduce hypoxic areas and directly occlude leaking microaneurysms. Grid laser could exert its effects by thinning the retina, bringing retinal vessels closer to choroidal vessels and permitting the retinal vessels to close by auto regulation. However, some eyes may not respond to laser photocoagulation due to diffuse or cystoid macular oedema. Therefore, other treatment modalities, such as intravitreal triamcinolone acetonide (IVTA) injection,[5] dexamethasone intravitreal implant (Ozurdex)[6], pars plana vitrectomy or treatment with anti-VEGF (Anti Vascular endothelial growth factor) therapies may be required.[2]

Vascular endothelial growth factor (VEGF) is an important mediator of neovascularization and vascular hyperpermeability that is increased in eyes with DME.[7] Anti-VEGF mediators such as Bevacizumab and ranibizumab which inhibits all isoforms of VEGF-A has been used for treatment of diabetic macular edema with improved visual outcomes compared to laser photocoagulation alone.[7&8] Bevacizumab is a full length,
recombinant, humanized monoclonal antibody against VEGF. Its intravitreal injection in patients with choroidal neovascularization, iris neovascularization, and macular oedema showed beneficial effects.[9]

Various combination treatments were tried in cases of DME but with limited success[10&11]. Lee et al. reported unfavourable results of intravitreal Bevacizumab combined with grid laser[11]. To our knowledge, a little work was done to evaluate sequential combination treatment in a series of patients with diabetic macular edema. Therefore, this study investigated the efficacy of combined laser photocoagulation and intravitreal Bevacizumab for treatment of DME using the OCT (optical coherence tomography) and multifocal ERG (mfERG) as a measure to structural and functional success.

PATIENTS & METHODS

This study was carried out in Memorial Institute of Ophthalmic Research during 2013, approved by the Institutional Ethics Committee and a written informed consent was obtained from each patient. For this interventional, prospective, case series study, we examined 30 patients (30 eyes) with diabetic macular edema (DME). The patients were Type 2 diabetes mellitus and well controlled on oral hypoglycemic agents. Excluded were cases of DME with diffuse retinal thickening, hard exudates involving the center of the macula (clinically significant DME as defined by the ETDRS[3] on slit-lamp biomicroscopic examination with diffuse leakage on fluorescein angiography (FA). Also, cases with significant hyporeflective cystic spaces of the outer retinal layers and/or subretinal fluid collection as detected by optical coherence tomography (OCT) were included.

Exclusion criteria included eyes with macular ischemia, intraocular inflammation, uncontrolled intraocular pressure, cataract surgery or a prior history of vitreoretinal surgery, and the presence of epiretinal membrane or vitreomacular traction. Also patients with uncontrolled hypertension or thromboembolic history or myocardial infarction were excluded. Ethical aspects: The study followed ethical rules in collecting data, analysis and keeping honesty and informed consent was taken. All identifiable information about patients' health status, medical condition, diagnosis, prognosis and treatment and all other information of a personal kind, were kept confidential.

Ophthalmologic examination

Ophthalmologic clinical examination was performed immediately before laser photocoagulation and Bevacizumab injection (baseline) and one month post treatment. Each patient underwent complete ophtalmic examination including best corrected visual acuity (BCVA) measurement with Snellen charts (values were converted to a logarithm of the minimum angle of resolution (logMAR) scale for analysis) and mydriatic slit-lamp biomicroscopy. Baseline and one month post-treatment fluorescein angiography and central retinal morphology and thickness were analyzed by OCT (3D OCT2000 - FA plus, Version8.02, Topcon) using 3D macula scan through a dilated pupil.

Electrophysiological tests

Multifocal ERG was done before and one month post-treatment following the international standard clinical electrophysiology of vision (ISCEV) protocol using the Reti-com system (Roland-Consult) as previously described. [12] Pupils were fully dilated with tropicamide 1% eye drops (Alcon, Egypt) and phenylephrine 2.5% eye drops (Misr, Egypt), instilled twice over 15 minutes. Subjects were adapted in ordinary room light for 15 minutes before testing. Electrical responses were recorded with a corneal electrode and signals were amplified and filtered. The retina was stimulated with an array of 61 hexagonal elements. The filter setting band pass was 5 to 100 Hz and the duration of the data acquisition was 4 minutes divided into eight sessions of 30 seconds. The mfERG stimulus locations and anatomic areas corresponded roughly as follows: ring 1 to the fovea, ring 2 to the parafovea, and ring 3 to the perifovea. The recordings used the first-order kernel component of the mfERG, which is the largest mfERG response derived with a biphasic waveform characterized by an initial negative deflection (N1), followed by a positive peak (P1). This study included the summed amplitude and implicit time of P1 in rings 1 and 2. P1 Amplitudes (nV/deg²) were measured from the lowest point of the negative peak of a-wave to the positive peak of the b-wave (P1). P1
Latencies (ms) were measured from the beginning of stimulus to the following positive peak of the b-wave. Figure 1 showed a trace array and 3 D map of a normal mf ERG.

**Laser treatment**

Patients with focal, diffuse and cystoid edema were subjected to focal and/or laser therapy, as previously described. Briefly, Focal direct argon green laser (Zeiss argon laser, Germany) spots of 50 to 100 µm applied to leaking microaneurysms with whitening at the microaneurysm site. The focal-grid pattern treatment consisted of 100 to 200 light burns of 50 to 200 µm spot size lasting 0.1 seconds, spaced one burn width apart in a ‘C’ shaped zone between 500 and 3000 µ from the foveal center sparing the papilla-macular bundle.

After laser treatment, patients received topical steroid eye drops (Prednisolone eye drops, Predfort, Allergan, Egypt), tropicamide 1% eye drops (Mydriacyl, Alcon, Egypt), and B blocker eye drop (Timolol 0.5%, Eipico, Egypt) for 3-4 days.

**Intravitreal injection**

One week after laser treatment, intravitreal Bevacizumab was done in the operating theatre. After the eye had been prepared using 5% povidone-iodine (Pharaonia, Egypt), an eyelid speculum was used to stabilize the eyelids. Intravitreal injection of 1.25 mg (0.1 ml) Bevacizumab (Avastin; Genentech Inc., USA) was performed 4 mm posterior to the limbus through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia (Benoxinate HCL 0.4% eye drops; Benox, Eipico, Egypt). After the injection, anterior chamber paracentesis was done and patients were instructed to administer topical antibiotics (Moxifloxacin 0.5% eye drops; Vigamox, Alcon, Egypt) for seven days. Ophthalmic examination including the BCVA was done after one week, two weeks and one month post-treatment. FA, OCT and mfERG were done before and one month post-treatment.

**Statistical Analysis**

All data were analyzed using SPSS software version 13.0 for Windows (SPSS, Inc., Chicago, IL). For statistical analysis, the students t-test and Anova were performed to compare the means of continuous variables before and after treatment. A P value less than 0.05 was considered significant.

**RESULTS**

In this study, 20 cases (66.6%) were males and 10 cases (33.4%) were females with mean age of 61.6 ± 4.58 years (range from 53 to 67 years). Apart from few cases with conjunctival hemorrhage over site of injection, there were no adverse events such as endophthalmitis, cataract or retinal detachment after macular laser treatment or after intravitreal injection.

Table 1 showed the mean Best Corrected Visual Acuity and central macular thickness, mf ERG amplitude and latency at baseline and one month after combined treatment in studied patients.

Before treatment, the mean best corrected visual acuity (BCVA) was 0.49 ± 0.33 (logMAR ± SD). The mean central macular thickness (CMT) measured by OCT at baseline was 446.46 ±73.42µm. One month after combined treatment, the mean BCVA improved significantly to 0.25±0.23 (logMAR ± SD), (p <0.001, student t test) and the CMT decreased significantly to 256.73±46.53µm (p<0.001, student t test), as compared to pretreatment values.

In mfERG, the average of baseline amplitudes of P1 in rings 1 and 2 was 10.15±3.17 (nV/deg²). After combined treatment, it increased significantly to 14.58±5.2 (nV/deg²), (p < 0.001, student t test). From ring 1 and 2, the implicit time of P1 decreased from 38.76±2.30 (msec) to 37.26±1.55 (msec) after combined treatment with statistically non-significant difference, (p = 0.05).

Figure 2 and 3 showed multifocal ERG responses and OCT macular line scan obtained from one of cases before and after treatment.
Table 1: Visual acuity, retinal thickness and mfERG parameters observed in eyes with diabetic macular edema before and after laser photocoagulation and intravitreal Bevacizumab injection.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>1 month after treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA (log MAR) (mean ± SD)</td>
<td>0.49±0.33</td>
<td>0.25±0.23</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Mean foveal thickness by OCT (µm) (mean ± SD)</td>
<td>446.46±73.42</td>
<td>256.73±46.53</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>mf ERG.C P1 amplitude (nv/ deg) (mean ± SD)</td>
<td>10.15±3.17</td>
<td>14.58±5.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>mf ERG.C P1 peak time (µsec) (mean ± SD)</td>
<td>38.76±2.30</td>
<td>37.26±1.55</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

VA; visual acuity, OCT; optical coherent tomography, mfERG; multifocal electroretinogram

*significant P value <0.05, student t test

Fig. 1. mfERG display of a normal eye showing: a) the trace array shows multiple ERG responses from central macula; b) the 3D map represents pseudo color response divided into hexagons. The highest foveal peak in the center is in white color. The black arrow represents the blind spot.

Fig. 2. mf ERG and OCT of a case before treatment showing: a) the trace array and 3D map of mf ERG shows decreased central foveal response and diffuse reduction in amplitude of ERG responses; b) OCT line scan shows lost foveal contour, increased retinal thickness and multiple hyporeflective cystoid spaces of cystoid retinal edema.
DISCUSSION

The transient effect of Bevacizumab in improving the visual acuity and central macular thickness was documented in many studies.[11 & 13] The idea of using a combination therapy of IVB with laser photocoagulation is based on the fast recovery of macular anatomy related to prompt VEGF inhibition (by IVB) associated with the long-term effects of laser, that may decrease the necessity of multiple IVB injections due to the sustained anti-VEGF effects of laser scars.

In the present study, we used combined macular laser photocoagulation followed by single intravitreal Bevacizumab injection. There was significant improvement in visual acuity, macular thickness and the mfERG amplitude values. Our results were in agreement to Yilmaz et al.[13] They found that combined intravitreal Bevacizumab injection after laser treatment showed visual acuity improvement for 6 weeks, but the benefits were no longer present 12 weeks after injection.[13] In Soheilian et al. study, the sequence was reversed i.e. macular laser done after intravitreal Bevacizumab injection, there was improvement in visual acuity and macular thickness in 72% of cases with diffuse and cystoid diabetic macular edema.[14] Solaiman et al, suggested that repeated IVB injection could provide a long-term benefit for the treatment of diffuse diabetic macular edema and performing macular grid photocoagulation once only 3 weeks subsequent to the initial IVB injection might provide a longer disease-free intervals.[15]

Our study was contradictory to work done by Arevalo et al, who provided evidence that injecting Bevacizumab alone showed significant improvement in best-corrected visual acuity in patients with diffuse diabetic macular edema at 24 months as compared to combined laser and IVB.[16] In another three months duration study, combined bevacizumab with subsequent laser photocoagulation had demonstrated significant reductions in central foveal thickness by OCT evaluation and also significant improvements in BCVA with no significant difference from the laser-only treated group.[17]

In our study, we used the mfERG to monitor the functional success of treatment. Multifocal ERG is a technique developed by Sutter and Tran[18], that allows mapping of retinal function in the posterior pole through simultaneous stimulation of different areas of the retina. The mfERG recording can assess many functional abnormalities in the macula including age-related macular degeneration and central serous chorioretinopathy.[19] Bearse et al, found that the mfERG was a good objective indicator for
macular function in patients with DME.\textsuperscript{[20]} Ng and colleagues proposed that the P1 wave of mfERG was generated by inner nuclear layer of retina, specially bipolar cells and the neural dysfunction of retina occured prior to clinical diabetic retinopathy.\textsuperscript{[21]} According to Abdollahi et al, P1 wave amplitude were more important to show tissue changes in diseases affecting bipolar cells, but implicit time of P1 wave could be related to outer retina and photoreceptors.\textsuperscript{[22]} The explanation for the non improving implicit time, in our study, could be attributed to prolonged cystoid edema and neurosensory detachment in our cases which affected the photoreceptors.

Limitations of the current study were the small sample size and the short duration of follow up. A longer follow-up for proper assessment of the response was required because the effect of Bevacizumab was mentioned to last up to 6 months\textsuperscript{[7]}. Also, we did not assess patients who required further injections. Another relevant aspect is that, various patterns of retinal edema as seen by OCT might respond differently to the treatment modalities.\textsuperscript{[23]} These issues needs to be further investigated in larger studies.

The aim of our study was to add the beneficial transient effect over the vascular permeability of Bevacizumab to the long-term macular stabilization provided by grid laser photocoagulation. Our obtained results were promising. In conclusion, the findings suggested that combination treatment comprised of grid laser photocoagulation and subsequent intravitreal Bevacizumab may be a good option for DME patients.

REFERENCES


