Original Article

Intravitreal Bevacizumab with or without Triamcinolone for Refractory Diabetic Macular Edema: Long-term Results of a Clinical Trial

Nasser Shoeibi[1], MD; Hamid Ahmadieh[2], MD; Morteza Entezari[2], MD; Mehdi Yaseri[2,3], PhD

[1]Retina Research Center, Khatam-al-Anbia Eye Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Epidemiology and Biostatistics, Tehran University of Medical Sciences, Tehran, Iran

Purpose: To report the long-term results of intravitreal bevacizumab (IVB) injection alone or combined, at the time of first IVB injection, with intravitreal triamcinolone acetonide (IVT) for treatment of refractory diabetic macular edema (DME).
Methods: In this randomized clinical trial, 115 eyes of 101 patients with refractory DME were enrolled and randomly assigned to one of the three study arms: the IVB group (41 eyes) received three consecutive injections of 1.25 mg IVB at 6-week intervals; the IVB/IVT group (37 eyes) additionally received 2 mg of IVT at the time of first IVB injection; and the control (sham injection) group. Patients in the IVB and IVB/IVT groups were followed for a mean of 13.3 months and received retreatment with IVB alone whenever indicated. Main outcome measures were best corrected visual acuity (BCVA) and central macular thickness (CMT).
Results: At the last follow up, CMT decreased significantly in the IVB group (p=0.013) but it was not significant (p=0.13) in the IVB/IVT group. Mean CMT improvement was 91 (95% CI, 20 to 161) microns and 57 (95% CI, -18 to 133) microns in the IVB and IVB/IVT groups, respectively. Mean BCVA improvement from baseline was 0.28 (95% CI, 0.18 to 0.38) logMAR (P=0.017) in the IVB group and 0.19 (95% CI, 0.08 to 0.30) logMAR (P=0.001) in the IVB/IVT group. There was no difference between the two groups in terms of visual improvment (p=0.42). In generalized linear mixed model, only the time interval between the last injection and CMT measurement was statistically significant (P=0.04). The same results were repeated for visual acuity (P=0.03).
Conclusion: Three loading doses of IVB (added doses if required) have long-term beneficial effects for treatment of refractory DME. Adding triamcinolone to this regimen provides no additional long-term benefit.

Keywords: Refractory Diabetic Macular Edema; Intravitreal Injection; Bevacizumab; Triamcinolone Acetonide

J Ophthalmic Vis Res 2013; 8 (2): 99-106.

Correspondence to: Nasser Shoeibi, MD. Assistant Professor of Ophthalmology, Khatam-al-Anbia Eye Hospital, Mashhad University of Medical Sciences, Abutalib Crossroad, Kolahdouz Blvd., Mashhad 91959-61151, Iran; Tel: +98 511 728 5290, Fax: +98 511 728 9911; email: nshoeibi@gmail.com

Received: October 20, 2012 **Accepted:** February 13, 2013

INTRODUCTION

Macular edema is the predominant cause of visual impairment among diabetics.[1] The Early Treatment for Diabetic Retinopathy Study (ETDRS) revealed that focal laser photocoagulation considerably reduces the risk of visual loss in eyes with clinically significant

macular edema.[2] Laser treatment, however, has limited results in eyes with diffuse diabetic macular edema (DME).[2-4]

Vascular endothelial growth factor (VEGF) levels in ocular tissues of patients with diabetes are greater than that of non-diabetic subjects.[5] Additionally, it has been affirmed that topical or intradermal VEGF administration in rats is followed by a rapid increase in capillary and post-capillary venule permeability.[6] This phenomenon which also disrupts the blood retinal barrier (BRB) can be prevented by anti-VEGF drugs. Accordingly, multiple studies have been conducted to investigate the effects of intravitreal anti-VEGF agents for management of DME,[7-9] which could particularly be promising in the treatment of the laser-refractory type.[10-12]

We have previously reported the 24-week results of a placebo-controlled randomized clinical trial in which we evaluated the effects of IVB alone or combined with IVT, compared to sham injection for refractory DME.[10] The short-term outcomes of this study revealed that adding IVT to the treatment regimen of IVB produced no additional favorable effects. Herein, we present the long-term results of the aforementioned trial.

METHODS

The current study is the extension of a previously reported randomized clinical trial performed at the Ophthalmic Research Center, Labbafinejad Medical Center, Tehran, Iran. Participants are the subjects of the described trial who had completed a 6-month follow-up course. These patients had refractory DME (defined as macular edema not responsive to laser treatment) and were earlier allocated to one of the three treatment arms (IVB, IVB/IVT or sham injection) in that randomized trial. They had received three doses of IVB (alone in the IVB group or combined at the time of first injection with IVT in the IVB/IVT group) at 6-week intervals. As the beneficial effect of IVB at 6 months was established, patients in the sham group also received IVB according to the protocol but were not included in the current study. In bilateral cases, each eye was enrolled individually.

Exclusion criteria consisted of need for cataract surgery during the study period, significant media opacity precluding retinal view, presence of traction on the macula evidenced by optical coherence tomography (OCT), monocularity and pregnancy.

To have a power of 90%, a significance level equal to 0.05, and an assumed standard deviation of 50 μm in central macular thickness, a sample size of 30 eyes for each arm was calculated.

Randomization was performed using the randomly permuted blocks according to a computer generated randomization list. The block lengths varied randomly (3 or 6). A random allocation sequence was performed by a biostatistician. Details of the series were unknown to the investigators. Additionally, the outcome assessors (optometrists responsible for visual acuity and OCT testings) and data analysts were masked to the allocation.

Best corrected visual acuity (BCVA), presence and extent of neovascularization of the iris (NVI), degree of lens opacity in phakic eyes, intraocular pressure (IOP), and severity of diabetic retinopathy were recorded at each visit. Visual acuity assessment was performed by an optometrist who was masked to the groups. BCVA was measured using the Snellen chart and converted to logarithm of the minimum angle of resolution (logMAR) notations. Lens opacity was graded according to the Lens Opacities Classification System III (LOCS III).[13] The extent of NVI and severity of diabetic retinopathy were represented in clock hours and based on ETDRS scale, respectively. OCT was performed prior to the injections and later at each follow-up visit. OCT mapping was carried out using OCT3 (Optical Coherence Tomography 3; Carl Zeiss Meditec, Dublin, CA, USA) which comprised of six radial 6 mm scans of each eye centered on the patient's fixation point. This mapping averaged the six scans to yield central macular thickness in an area of about 1000 microns in diameter.

Patients in the IVB group received 3 loading doses of intravitreal bevacizumab at 6-week intervals. Meanwhile, intravitreal triamcinolone acetonide was added to the first IVB injection in the IVB/IVT group. Patients were followed for 12 weeks after the third injection without

any further intervention. Subsequently, patients were examined every 6 weeks and evaluated in terms of visual acuity and CMT at each visit; subjects with BCVA drop of more than 0.1 logMAR or CMT increase of more than 50 microns compared to the previous visit, or CMT exceeding 350 microns, received an additional IVB injection.

Intravitreal injections were performed under sterile conditions with topical anesthesia and insertion of a lid speculum. For the IVB group, 1.25/0.05 (mg/ml) bevacizumab (Avastin made for F. Hoffmann- La Roche Ltd. Basel, Switzerland by Genentech Inc., San Francisco, CA, USA) was injected intravitreally with a 30-gauge needle into the superotemporal quadrant 3.75 mm and 3.25 mm from the limbus in phakic and pseudophakic eyes, respectively. For the IVB/IVT group, in addition to the intravitreal bevacizumab, 2 mg (0.05 ml) of triamcinolone acetonide (Triamhexal, Hexal AG, Holzkirchen, Germany) was injected intravitreally into the inferotemporal quadrant at the first treatment session.

All eyes underwent ophthalmic examination on days 1 and 7 following each injection evaluating anterior chamber (AC) reaction and IOP rise. The primary outcome measure was changes in CMT as compared to baseline. Secondary outcome measure included changes in BCVA from baseline.

Data was analyzed by treatment as administered (per-protocol analysis). Percentages and mean±standard deviations (SD), or 95% confidence intervals (CI) were employed to describe qualitative and quantitative data, respectively. Chi-square, Fischer's exact and Mann-Whitney tests were used to compare qualitative data. Quantitative data were analyzed employing *t*-test and paired *t*-test to compare changes between and within groups at different time intervals and as compared to baseline, respectively. In order to eliminate any possible correlation between fellow eyes in bilaterally enrolled cases, the marginal regression model (based on generalized estimating equation methods) was applied to compare the outcome measures adjusted for baseline values. Furthermore, we used the generalized linear mixed model (GLMM) to evaluate the simultaneous effect of different factors on BCVA and CMT. The level of significance was set at 0.05. Statistical analysis was performed using SPSS software (Statistical Package for Social Sciences, version 15.0, SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 122 eyes of 106 patients were assessed for eligibility; 7 patients (7 eyes) refused to enter the study, therefore eventually 115 eyes of 101 patients were enrolled (Fig. 1). Eligible eyes were randomized into three groups which included 41, 37 and 37 eyes in the IVB, IVB/IVT and control (sham injection) groups, respectively. Patients within the three study arms were matched for age, sex, baseline visual acuity, hemoglobin A1C levels, systemic hypertension, and history of pan-retinal photocoagulation (PRP) (P>0.05). Baseline demographic and clinical characteristics for participants in the IVB and IVB/IVT groups are presented in table 1. Mean follow-up period was 13.3±3.4 (range, 6–20) months.

At final follow-up, mean CMT was 332±120 microns in the IVB and 354±164 microns in the IVB/IVT group (P=0.21). Compared to baseline, CMT was decreased significantly in the IVB group (P=0.013), while the change was not significant in the IVB/IVT group (P=0.13), (Fig. 2). Mean improvement in CMT was 91 (95% CI, 20 to 161) microns in the IVB group and 57 (95% CI, -18 to 133) microns in IVB/IVT group.

BCVA at final follow-up was 0.61±0.41 versus 0.74±0.27 logMAR in the IVB and IVB/IVT groups, respectively (P=0.24). These data showed significant changes from baseline values in both the IVB (P=0.017) and IVB/IVT (P=0.001) groups (Fig. 3). Mean improvement in BCVA was 0.28 (95% CI, 0.18 to 0.38) logMAR in the IVB group and 0.19 (95% CI, 0.08 to 0.30) logMAR in IVB/IVT group. There was no difference between the two groups in terms of improvement (p=0.42). Sixteen eyes (39.0%) in the IVB group and seven (18.9%) eyes in the IVB/IVT group experienced visual improvement more than 0.2 logMAR (P=0.08). Patients' data at final follow-up are summarized in table 2.

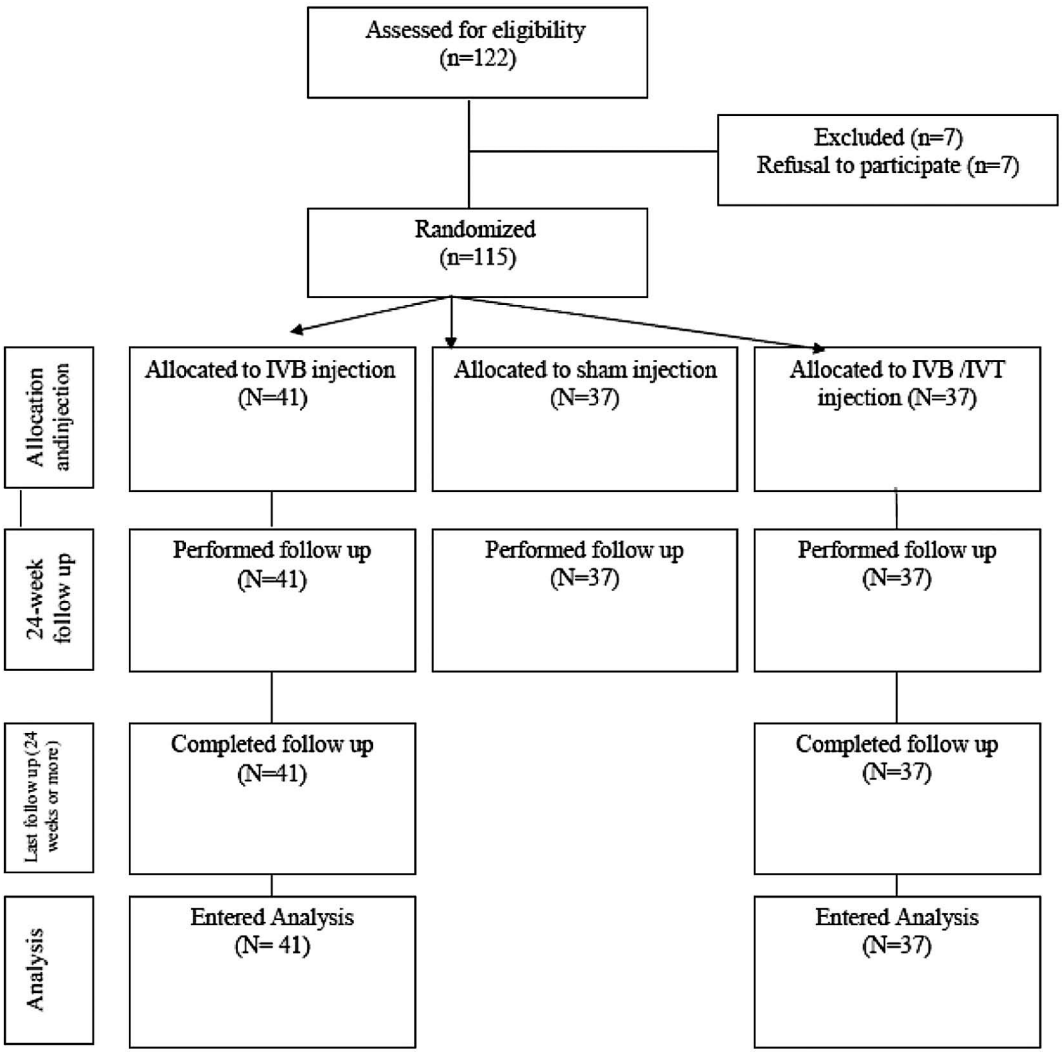

Figure 1. Flow diagram of the study.

The median number of required injections after week 24 was 1 (1 or 2 injections) in the IVB and 1 (1 or 2 injections) in the IVB/IVT groups respectively; no significant difference was observed between the two groups in this regard (P=0.356). Eight patients (20%) in the IVB group and four (11%) in the IVB/IVT group received their first additional injection at a mean of 10.5±1.8 and 9±1.2 months after the first injection, respectively. One patient in each study group required the second additional injection of intravitreal bevacizumab.

Mean interval from the last injection to final follow-up was 37.8±16.6 (range, 8–68; median, 42) weeks and 37.1±14.9 (range, 8–56; median, 40) weeks in the IVB and IVB/IVT groups, respectively.

After adjusting for baseline CMT, group of treatment, number of injections, time from the last injection, creatinine levels, plasma LDL/HDL ratio, hemoglobin A1C levels, compensating for correlation between fellow eyes in bilateral cases and considering the repetition of CMT results at different time points using the GLMM, no statistically significant difference was noted between the two groups in terms of CMT changes over the course of the study. Nevertheless, the effect of the time interval between the last injection and CMT measurement was statistically significant (P=0.04) indicating a gradual increment of CMT over time following the last IVB injection.

BCVA results were also evaluated using the GLMM model which demonstrated similar

Table 1. Demographics and baseline characteristics in the IVB and IVB/IVT groups

Variable	Groups		P-value
	IVB (n=41)	IVB/IVT (n=37)	
Age (years)	60.4±9.3	59.1±8.1	0.544*
Gender (female/male)	8/7	9/7	0.870†
Hypertension (%)	14 (34.1)	11 (29.7)	0.676†
Smoking (%)	5 (12.2)	2 (5.4)	0.436**
Lens Status (%)			
Intraocular lens	10 (24.4)	3 (8.1)	0.054†
Lens Opacity			
NS	30 (96.8)	34 (100)	0.809‡
PSC	28 (90.3)	33 (97.1)	0.051‡
CC	22 (71.0)	31 (91.2)	0.098‡
Retinopathy (%)			
Non-proliferative diabetic retinopathy	30 (73.2)	31 (83.8)	0.571**
Early proliferative diabetic retinopathy	3 (7.3)	0 (0)	
Regressed proliferative diabetic retinopathy	8 (19.5)	6 (16.2)	
Intraocular pressure (mmHg)	15.4±2.8	16.1±2.2	0.310*
Best corrected visual acuity (logMAR)	0.88±0.32	0.92±0.32	0.595*
Central macular thickness (microns)	414.6±62.1	417.7±139.4	0.933*
High density lipoprotein (mg/L)	47.8±10.4	53.1±17.1	0.311*
Low density lipoprotein (mg/L)	135.9±40.3	137.6±41.5	0.920*
Creatinine (mg/L)	1.2±0.4	1.5±0.8	0.185*
Hemoglobin A1C (mg/dL)	9.7±1.6	9.6±1.9	0.799*

IVB, intravitreal bevacizumab; IVT, intravitreal triamcinolone; NS, nuclear sclerosis; PSC, posterior subcapsular; CC, cortical cataract
logMAR, logarithm of minimum angle of resolution
* Based on t-test
** Based on Fisher's exact test
† Based on chi-square test
‡ Based on Mann-Whitney test

findings. Considering repetition of BCVA measurement at different time points, and adjusting for baseline CMT, group of treatment, number of injections, time interval from the last injection, creatinine levels, plasma LDL/HDL

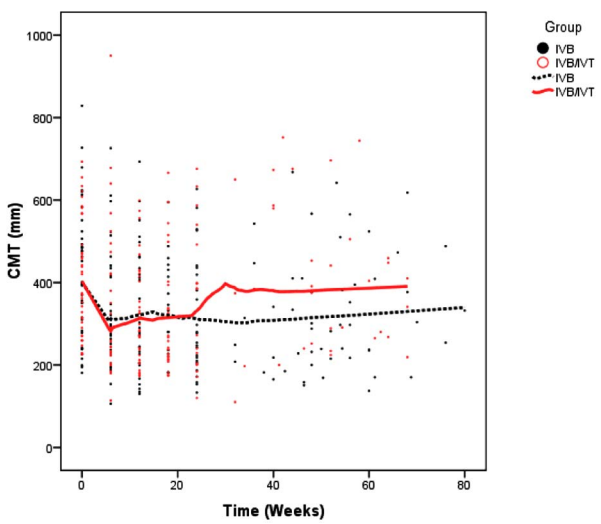

Figure 2. Scatter plot demonstrating mean central macular thickness (CMT, microns) in the IVB and IVB/IVT groups over the study period.

IVB, intravitreal bevacizumab; IVT, intravitreal triamcinolone

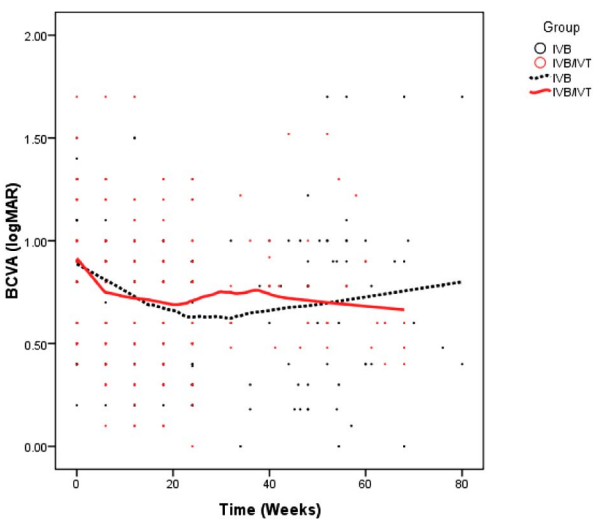

Figure 3. Scatter plot demonstrating mean BCVA in the IVB and IVB/IVT groups during the study period.

BCVA, best corrected visual acuity; IVB, intravitreal bevacizumab; IVT, intravitreal triamcinolone; LogMAR, logarithm of minimum angle of resolution

Table 2. Patient data at final follow-up

	Group		P-value
	IVB (n=41)	IVB/IVT (n=37)	
Time from last injection to last follow-up (weeks)	37.76±16.62	37.09±14.88	0.88
Final central macular thickness (microns)	322±120	354±164	0.21
Final best corrected visual acuity (logMAR)	0.61±0.41	0.74±0.27	0.24

IVB, intravitreal bevacizumab; IVT, intravitreal triamcinolone

ratio and hemoglobin A1C levels, GLMM analysis displayed no significant difference between the two groups in terms of visual acuity changes. Similarly, the effect of the time interval between the last injection and BCVA measurement was statistically significant (P=0.03) implying that BCVA gradually deteriorated after IVB injection.

At the final visit, mean IOP was 15.5±2.4 mmHg in the IVB group and 16.3±4.9 mmHg in the IVB/IVT group. Topical anti-glaucoma medications were administered for only one patient in the IVB/IVT group at final follow-up. There was no new incidence of iris neovascularization within the study period.

DISCUSSION

The long-term outcomes of this clinical trial revealed that IVB is effective for management of refractory DME in terms of decreasing CMT and improving BCVA. Three loading doses of IVB in addition to additional injections as required resulted in visual improvement after mean follow up of 13 months. Yet, IVT provided no further benefit in refractory DME. After 3 loading injections, 6 weeks apart, 20% of eyes in the IVB group and 11% of eyes in the IVB/IVT group required the first additional IVB injection for maintenance at a mean interval of 10.5±1.8 and 9±1.2 months after the first injection, respectively.

Recent studies, including the previous report of the current clinical trial, have shown the beneficial effect of IVB on DME over a short period of time.[14] A randomized clinical trial by Soheilian et al revealed that a single injection of IVB, as a primary treatment for DME, more effectively improves vision in the short term when compared to laser therapy.[9] In the same study 28 percent of the subjects in the IVB group required repeat injections. Their trial however did not include laser-refractory eyes and followed the patients for up to 36 weeks. In our study, the corresponding rate for reinjections was 20% after three loading injections, which is comparable to the results of the aforementioned study.

In a study by the Diabetic Retinopathy Clinical Research Network, it was evident that a single injection of IVB had no advantage over photocoagulation for treatment of DME.[8] In a study by Faghihi et al, the effect of a single IVB injection vanished at week 16,[15] which signified that multiple loading injections are required to attain a sustained result. High concentrations of anti-VEGF agents in the vitreous cavity through booster doses at 6-week intervals may control vascular leakage which is crucial in the pathogenesis of DME. The triple-injection regimen applied in the current study evoked a prolonged effect; meanwhile the mean time from the final injection to last follow-up session was 38 weeks. However, in GLMM analysis, the time interval between the last injection, and measurement of BCVA and CMT were significant implying that the effect of IVB decreases with time and repeat injections may be required in the long term.

Bevacizumab is a pan-VEGF-blocking agent and may impair normal physiologic VEGF-mediated functions,[16] which might be considered as a disadvantage of this agent. There are few reports illustrating that visual loss can be due to disruption of the capillary network and induction of macular ischemia following intravitreal injection of bevacizumab.[17,18] In our study, there was no significant acute visual loss after IVB injection which may indicate that unknown causes other than IVB injection may be responsible for aggravation of ischemia within the macular area.

In our study, we administered triamcinolone as adjunctive treatment to bevacizumab only at the

time of first injection. This single injection solely induced earlier visual improvement; however, it did not establish any significant additional benefit later during follow-up.[10] It also had no effect on the need for repeat injections after the maintenance therapy course. Gillies et al in their 5 year follow-up of subjects who had received IVT for refractory DME demonstrated that IVT treatment does not reduce the risk of recurrent edema for 2 years. Similarly, the rate of visual improvement was not different from the placebo treatment group.[19] Soheilian et al showed no adjunctive effect of IVT when added to IVB.[9] Accordingly, it can be concluded that adding triamcinolone to the bevacizumab regimen has no beneficial long-term effect for refractory DME and hence is not recommended.

One of the drawbacks of the current study was different time points for final follow-up in different patients. We employed the GLMM method to compensate for this mismatch; we also presented the data as the "last visit" results. The other shortcoming was the absence of a control group. After demonstrating the beneficial effect of IVB±IVT in our 24-week results, all patients were offered the option of receiving IVB due to ethical considerations.

In summary, three consecutive injections of IVB, together with additional injections as required, resulted in CMT reduction and visual improvement in eyes with refractory DME in the long-term. To our knowledge, this is the first clinical trial with long-term outcomes of more than 1 year regarding IVB injection for refractory DME in which the rate of reinjection for maintenance therapy was also investigated. Further clinical trials with larger sample sizes, equal follow-up time points and longer follow-up duration are required to confirm the results of this study.

Conflicts of Interest

None.

REFERENCES

1. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy. IV. Diabetic macular edema. *Ophthalmology* 1984;91:1464-1474.
2. Early Treatment Diabetic Retinopathy Study research group. Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. *Arch Ophthalmol* 1985;103:1796-1806.
3. Lee CM, Olk RJ. Modified grid laser photocoagulation for diffuse diabetic macular edema. Long-term visual results. *Ophthalmology* 1991;98:1594-1602.
4. Bainbridge J. Refractory diabetic macular edema. *J Ophthalmic Vis Res* 2010;5:143-144.
5. Lutty GA, McLeod DS, Merges C, Diggs A, Plouet J. Localization of vascular endothelial growth factor in human retina and choroid. *Arch Ophthalmol* 1996;114:971-977.
6. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci* 1995;108:2369-2379.
7. Parravano M, Menchini F, Virgili G. Antiangiogenic therapy with anti-vascular endothelial growth factor modalities for diabetic macular oedema. *Cochrane Database Syst Rev* 2009;7:CD007419.
8. Diabetic Retinopathy Clinical Research Network, Scott IU, Edwards AR, Beck RW, Bressler NM, Chan CK, et al. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. *Ophthalmology* 2007;114:1860-1867.
9. Soheilian M, Ramezani A, Obudi A, Bijanzadeh B, Salehipour M, Yaseri M, et al. Randomized trial of intravitreal bevacizumab alone or combined with triamcinolone versus macular photocoagulation in diabetic macular edema. *Ophthalmology* 2009;116:1142-1150.
10. Ahmadieh H, Ramezani A, Shoeibi N, Bijanzadeh B, Tabatabaei A, Azarmina M, et al. Intravitreal bevacizumab with or without triamcinolone for refractory diabetic macular edema; a placebo-controlled, randomized clinical trial. *Graefes Arch Clin Exp Ophthalmol* 2008;246:483-489.
11. Gulkilik G, Taskapili M, Kocabora S, Muftuoglu G, Demirci G. Intravitreal bevacizumab for persistent macular edema with proliferative diabetic retinopathy. *Int Ophthalmol* 2010;30:697-702.
12. Mehta S, Blinder KJ, Shah GK, Kymes SM, Schlief SL, Grand MG. Intravitreal bevacizumab for the treatment of refractory diabetic macular edema. *Ophthalmic Surg Lasers Imaging* 2010;41:323-329.
13. Chylack LT Jr, Wolfe JK, Singer DM, Leske MC, Bullimore MA, Bailey IL, et al. The Lens Opacities Classification System III. The Longitudinal

Study of Cataract Study Group. *Arch Ophthalmol* 1993;111:831-836.

14. Nicholson BP, Schachat AP. A review of clinical trials of anti-VEGF agents for diabetic retinopathy. *Graefes Arch Clin Exp Ophthalmol* 2010;248:915-930.
15. Faghihi H, Roohipoor R, Mohammadi SF, Hojat-Jalali K, Mirshahi A, Lashay A et al. Intravitreal bevacizumab versus combined bevacizumab-triamcinolone versus macular laser photocoagulation in diabetic macular edema. *Eur J Ophthalmol* 2008;18:941-948.
16. Gillies MC. What we don't know about avastin might hurt us. *Arch Ophthalmol* 2006;124:1478-1479.
17. Chen E, Hsu J, Park CH. Acute visual acuity loss following intravitreal bevacizumab for diabetic macular edema. *Ophthalmic Surg Lasers Imaging* 2009;40:68-70.
18. Goel N, Kumar V, Ghosh B. Ischemic maculopathy following intravitreal bevacizumab for refractory diabetic macular edema. *Int Ophthalmol* 2011;31:39-42.
19. Gillies MC, Simpson JM, Gaston C, Hunt G, Ali H, Zhu M, et al. Five-year results of a randomized trial with open-label extension of triamcinolone acetonide for refractory diabetic macular edema. *Ophthalmology* 2009;116:2182-2187.