

1 **Repeated Intravitreal Bevacizumab Injections versus Single Combined Intravitreal**
2 **Bevacizumab and Posterior Subtenon Triamcinolone Injections for Treatment of**
3 **Diabetic Macular Edema**

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18 **Abstract**

19 **Aim:** To compare efficacy of combined intravitreal bevacizumab and subtenon triamcinolone
20 versus three intravitreal injections of bevacizumab at one month interval for reducing foveal
21 thickness.

22 **Methods:** Sixty eyes of 30 diabetic patients with bilateral diabetic macular edema were
23 randomly enrolled in two groups, group A eyes treated with three intravitreal injections of
24 Bevacizumab of 1 month apart, and group B eyes treated with a single intravitreal injection of
25 Bevacizumab combined with a subtenon triamcinolone in the same operative session. Pre and
26 postoperative clinical data were measured and followed up over 6 months.

27 **Results:** Difference between pre-treatment and post-treatment clinical data for each parameter
28 in the same group was highly statistically significant. However, on comparing results of post
29 treatment BCVA, IOP and CMT in both groups, there was no statistically significant
30 difference.

31 **Conclusion:** Combination of subtenon triamcinolone and intravitreal bevacizumab is a safe
32 and effective choice to treat DME.
33

34 **Keywords:** intravitreal; Subtenon; Bevacizumab; Triamcinolone; macular edema.

35
36 **Introduction**

37 Diabetic macular edema (DME) is the most common cause of vision loss in diabetic
38 patients[1]. Grid laser photocoagulation was proved effective in reducing progressive visual

39 loss in clinically significant macular edema. However, the laser scar may enlarge
40 postoperatively reducing visual acuity afterwards[2,3].

41 Recently, it was found that both intravitreal concentrations of interleukin-6 (IL-6),
42 (an inflammatory cytokine) and vascular endothelial growth factor (VEGF), (a cytokine
43 related with vascular proliferation and hyper-permeability) were increased in DME [4,5] and
44 many reports indicate that intravitreal or posterior subtenon triamcinolone acetate (TA)
45 treatment is effective for reducing macular thickness in DME [6-9]. More recently, an
46 intravitreal injection of bevacizumab, a full-length humanized monoclonal anti-VEGF
47 antibody, has been reported to be also effective in reducing DME [10,11]. As well, variable
48 combinations of the fore mentioned manoeuvres were used to augment the effect of each
49 other and proved effective.

50 The aims of this study were to compare the efficacy of **single** combined intravitreal
51 injection of bevacizumab (IVB) and posterior subtenon triamcinolone (PSTA) versus three
52 intravitreal injection of bevacizumab at one month interval for reducing foveal thickness, and
53 to evaluate the visual prognosis and effect on intraocular pressure over a 6 months follow up
54 period.

55

56 **Patients and methods:**

57 This is a prospective, randomized clinical study that was conducted on 60 eyes of
58 30 diabetic patients (type 2 DM) with bilateral **diffuse** DME, whose foveal thickness was $\geq$
59 250 μm and best corrected visual acuity (BCVA) worse than 0.4 (Snellen's fraction). The
60 eyes were randomly enrolled in two groups, group A eyes that were treated with three
61 intravitreal injections of Bevacizumab of 1 month apart, and group B eyes that were treated
62 with a single intravitreal injection of Bevacizumab combined with a subtenon injection of

63 triamcinolone on the same operative session. **After randomization, the two eyes of the**
64 **same patient received only one type of treatment.**

65 Exclusion criteria included patients who had previous therapies for macular edema,
66 including grid-laser treatment, intravitreal injection of any drugs, and/or vitreous surgery. As
67 well, we excluded patients with diabetic papillopathy, ischemic maculopathy with capillary
68 nonperfusion, and any other ocular diseases such as glaucoma, retinal vessel occlusion,
69 uveitis, or other ocular inflammatory/neovascular diseases. Furthermore, an eye was
70 considered ineligible if OCT suggested that vitreoretinal interface disease (e.g., vitreoretinal
71 traction, epiretinal membrane) was the primary cause of the macular edema.

72 After explaining to the patients the purpose of the study and the possible outcomes,
73 an informed consent was obtained prior to the interventions. This study was approved by the
74 clinical research committee of the Menoufia University Hospital and it followed the tenets of
75 Declaration of Helsinki.

76 All patients received a comprehensive ocular examination before, during and after
77 treatment. BCVA was determined with the Snellen chart (converted to Snellen fraction),
78 intraocular pressure (IOP) was measured with Goldmann applanation tonometer, retinal
79 thickness **including central macular thickness (CMT)**, was measured by optical coherence
80 tomography (OCT) (Zeiss-Humphrey, spectral domain, Dublin, California, USA) before
81 treatment and during the follow-up examinations. A macular thickness map was made from
82 six radial scans that intersected at the fovea using the OCT retinal mapping program (version
83 6.2). This program calculates mean thickness in nine regions: the 1000- μ m central area, and
84 the four quadrants of the inner and outer rings. The diameters of the inner and outer rings
85 were 1000 μ m to 3000 μ m and 3000 μ m to 6000 μ m, respectively.

86 Indirect ophthalmoscopy and slit-lamp biomicroscopy of the posterior segment using
87 a Volk Superfield contact lens (Volk, Mentor, Ohio, USA) were performed by at least two of

the three authors to establish the presence of DME. Fundus photographs were taken at appropriate times. An intravenous fluorescein angiography (IVFA) was performed to detect and assess diffuse leakage around the fovea and to rule out macular ischemia.

Experimental Design: According to the previous reports [11-13] and our clinical experiences, the concentration of each drug was determined as follows. In each patient of group A, 1.25 mg of bevacizumab (Avastin; Genetech, Inc, South San Francisco, California, USA) was injected into the vitreous at each injection setting. Three intravitreal injections were given separated by 1 month. In group B, 4 mg of triamcinolone acetonide (Kenacort; Bristol-Myers Squibb, Tokyo, Japan) was injected in the subtenon space of the affected eye. At the time of the drug injection, topical anesthesia was induced by applying 0.5% proparacaine topical anesthetic drops at least three times. Following disinfection and draping, in group A, in each injection setting, 0.05 ml volume containing 1.25 mg of bevacizumab was injected into the vitreous cavity using a sharp 30 gauge-needle at a distance of 3.5 mm from the limbus. In group B, the same was done as in group A in addition to injecting 1.0 mL of a 40 mg/mL dosage of triamcinolone acetonide in the inferotemporal quadrant using a 27-gauge needle on a 1-mL syringe. In both groups, to avoid an increase of IOP, aqueous humor was removed by paracentesis as appropriate.

Statistical analysis was performed using the SPSS statistical program (SPSS Inc., Chicago, IL, USA). Serial comparison of pre treatment and post treatment main outcome measures was evaluated using the paired Student t test. A p value less than 0.05 was considered statistically significant.

Results

A total of 60 eyes of 30 patients were recruited and randomly enrolled into 2 groups. Group A included 8 males and 7 females ranging in age from 50 to 71 years with a mean of

58.93 ± 6.47 (± SD) years. Group B included 7 males and 8 females ranging in age from 45 to 75 years with a mean of 56.4 ± 8.09 (± SD) years.

Table (1): Pre and post-treatment clinical data of patients of group A.

Group A n=30	Pre-treatment	Post-treatment			P value*
		1 month	3 months	6 months	
BCVA (fraction)	0.20 ± 0.13	0.24 ± 0.15	0.33 ± 0.17	0.36 ± 0.18	0.001
CMT (µm)	466.87 ± 154.07	429.27 ± 137.53	373.47 ± 127.38	329.73 ± 118.79	0.001
IOP (mmHg)	15.73 ± 3.20	17.60 ± 2.82	15.93 ± 2.39	14.87 ± 2.28	0.001

* Friedman test

Table (2): Pre and post-treatment clinical data of patients of group B.

Group B n=30	Pre-treatment	Post-treatment			P value*
		1 month	3 months	6 months	
BCVA (fraction)	0.18 ± 0.11	0.21 ± 0.11	0.29 ± 0.16	0.31 ± 0.15	0.001
CMT (µm)	496.13 ± 137.67	454.87 ± 123.98	386.73 ± 111.62	361.60 ± 103.61	0.001
IOP (mmHg)	15.53 ± 2.29	17.60 ± 1.81	15.93 ± 2.64	15.00 ± 1.78	0.001

* Friedman test

Table 1 demonstrates the pre and post-treatment clinical data of patients of group A which included BCVA (expressed as Snellen's fraction), CMT (in µm) and IOP (in mmHg). The same data for patients of group B are presented in table 2. These data were collected for every patient in both groups before starting treatment as well as in the postoperative follow up period at 1, 3 and 6 month duration. The difference between pre-treatment and post-treatment measurements for each parameter in the same group was highly statistically significant.

Table (3): Comparison between both groups regarding clinical data before and after treatment.

		Group A	Group B	P value*
Pre-treatment	BCVA	0.20 ± 0.13	0.18 ± 0.11	0.488
	CMT	466.87± 154.07	496.13± 137.67	0.857
	IOP	15.73± 3.20	15.53± 2.29	0.391
1 month after treatment	BCVA	0.24 ± 0.15	0.21 ± 0.11	0.627
	CMT	429.27± 137.53	454.87± 123.98	0.458
	IOP	17.60 ± 2.82	17.60 ± 1.81	0.928
3 months after treatment	BCVA	0.33 ± 0.17	0.29 ± 0.16	0.449
	CMT	373.47± 127.38	386.73± 111.62	0.700
	IOP	15.93 ± 2.39	15.93 ± 2.64	0.929
6 months after treatment	BCVA	0.36± 0.18	0.31 ± 0.15	0.224
	CMT	329.73± 118.79	361.60± 103.61	0.225
	IOP	14.87 ± 2.28	15.00 ± 1.78	0.564

* Mann Whitney U test

There was no statistically significant difference between both groups regarding pre-treatment clinical data. As well, on comparing the results of post treatment impact on BCVA, CMT and IOP in both groups, there was no statistically significant difference as shown in table 3.

During the follow up period, there were no significant postoperative complications in both groups apart from one case in group A and 2 cases in group B who had an IOP elevation of more than 5 mmHg than preoperative. No cataract, infection, retinal detachment, vitreous hemorrhage or retinal complications was recorded in any case of the study.

Discussion

DME has been attributed to intravitreal induction of proinflammatory cytokine [4], intraretinal expression of proinflammatory caspases [14] and mediators [15], and therefore, many clinical investigators have found that intravitreal injection of triamcinolone acetonide (IVTA) may reduce macular edema. Although the reduction effect of triamcinolone on macular edema improves visual functions, recurrence of macular edema was often observed within 24 weeks after treatment [7], and IOP was occasionally increased in this therapy.

Therefore, looking for safer and longer-acting therapy for DME was going on. VEGF has been shown to be an endothelial cell-specific mitogen and an angiogenic inducer, and is also known as a vascular permeability factor, which has been demonstrated to increase retinal vessel permeability by increasing the phosphorylation of tight junction proteins [16]. Since it was reported that the vitreous level of VEGF increased and correlated with the severity of macular edema in DME patients [5], anti-VEGF therapy is expected to show a dramatic reduction of DME.

However, the pathogenesis of DME is likely to be more closely related to a corticosteroid sensitive mechanism than a VEGF-dependent one. Corticosteroid affects a number of different cytokine including VEGF [17], thus it may be necessary to reduce more than one cytokine to make an effective reduction in DME. Therefore, at least theoretically, combining bevacizumab with triamcinolone would be expected to induce augmented reduction of CMT and improvement of BCVA than use of bevacizumab alone.

Intravitreal injection of anti-VEGF has a short term effect on DME and multiple injections are required to ensure relatively longer period of improvement. A regimen of three intravitreal injection of bevacizumab one month apart was proved effective in reduction of DME and improvement of visual acuity. We adopted this regimen for treatment of patients of group A in the current study and we observed marked improvement of DME which was

statistically significant as compared by preoperative data without significant increase in the IOP.

Intravitreal injection, although an effective way of drug delivery to the vitreo-retinal complex, has its own dangerous complications such as endophthalmitis, vitreous hemorrhage and retinal detachment. The incidence of these complications increases as the frequency of injections increases. In the current study we compared the effect of three consecutive intravitreal injection of bevacizumab versus a single intravitreal injection of bevacizumab combined with the less dangerous subtenon injection of triamcinolone.

Posterior subtenon triamcinolone (PSTA), as an adjunctive treatment for diffuse DME, can improve the visual outcomes of laser photocoagulation [18-20]. As well, some reports suggest the beneficial effects of subtenon or peribulbar steroid injection therapy for DME [21,22]. In a study done by Bakri and Kaiser, visual acuities remained stable or improved over a 12-month period after PSTA injections for refractory DME in 63 eyes of 50 patients. Complications were rare, with a transient significant rise in intraocular pressure at the 3-months' evaluation as well as occurrence of ptosis in two patients [21].

Many reports compared the results of intravitreal versus subtenon injection of triamcinolone for DME and found no significant difference between both. Özdek et al. [22] showed that both PSTA and IVTA injections caused a significant increase in visual acuity and a decrease in central foveal thickness, especially in the short term. The effect was more pronounced in the IVTA group; however, PSTA injection also seemed to be a safe and effective technique for the treatment of DME [22].

An advantage of posterior subtenon administration is the lower risk of complication. IOP elevation is the most common complication after IVTA [23,24]. Although not statistically significant, IOP after intravitreal injection tended to rise in a study done by Choi et al.[25] At 3 months after injection, the change of IOP in the intravitreal injection group was

greater than that of the posterior subtenon injection group. They concluded that the short-term efficacy of the intravitreal injection and of the posterior subtenon injection of triamcinolone in diffuse diabetic macular edema was similar. The posterior subtenon injection was less invasive and safer than the intravitreal injection [25].

Another study done by Cellini et al., [26] went farther in favouring PSTA to IVTA. They demonstrated that three months after IVTA and PSTA there is a statistically significant improvement in visual acuity and an equally significant reduction in retinal thickness. Six months after IVTA the patients presented a recurrence of macular edema with loss of visual acuity whereas six months after SBT injection, retinal thickness and visual acuity remained stable. After one, three and six months they observed a statistically significant rise of the IOP in the eyes treated with IVT injection whereas in the SBT injection group, no statistically significant variations of the IOP were found. However, none of patients developed cataract or needed anti-glaucoma drugs during the follow-up [26].

Based on the efficacy proposed for subtenon TA in management of DME, we combined it with a single intravitreal injection of bevacizumab for the treatment plane in group B in the current study. Visual acuity and CMT were significantly reduced over the 6 months follow up period. We thought that subtenon TA has a remarkable effect on prolongation of the improvement induced by the intravitreal injection of bevacizumab. There were no statistically significant differences between the results of both groups in the current study at 1, 3 and 6 months during the follow up period. However, the absolute values of VA and CMT may be better in group A.

As well, no significant increase in IOP was observed in patients of group B supporting the weak effect of subtenon TA on IOP compared with intravitreal TA. **Moreover, no cataract has been reported to be induced by subtenon TA in patients of group B**

during the 6 months follow up period. However, longer follow up time may be needed to report occurrence of cataract in those cases.

We conclude that the combination of subtenon TA and intravitreal bevacizumab is a safe and effective choice to treat DME avoiding the multiple intravitreal injections with its potential dangerous complications. Meanwhile, we used TA, which is more effective in reducing DME, in a safer way minimizing the risk of postoperative glaucoma and cataract reported with its intravitreal use. An Additive effect is thus supposed to exist when using both TA and bevacizumab for treatment of DME. However, it is a relatively short term study and more follow up period is needed to confirm the long term effect of both modalities of treatment on the ocular structure.

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