Original Article

Intravitreal Bevacizumab for Treatment of Diabetic Macular Edema

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Purpose: To evaluate the effects of Intra-vitreal Bevacizumab on visual function and macular edema in diabetic patients

Material and Methods: This study was conducted in the Department of Ophthalmology, Madina Teaching Hospital, University Medical and Dental College, Faisalabad. Three months from 1st June 2010 to 31st August 2010. A total of 26 eyes of 26 diabetic patients (mean age 48.92 years) with diabetic macular edema were included in this study. Best – corrected visual acuity, slitlamp biomicroscopic examination of anterior segment, fundus examination and fundus flourescein angiography, were done at baseline and at each follow up visits. All patients were treated with 0.05 ml intravitreal injection containing 1.25 mg of bevacizumab (IVB).

Results: All patients completed 3 months follow up. The mean BCVA at baseline was 0.726 + log MAR. It improved to 0.515, 0.461, and 0.452 + log MAR at 1st week, 1st month, and 3rd months respectively. Final BCVA analysis demonstrated that 17 (65.38%) patients improved ≥ 2 lines of Senellen's visual acuity chart, 7 (26.38%) improved by one line, one (3.84%) remained stable, and one (3.84%) showed deterioration of one line. Macular edema at 1st week resolved completely in 18 (69.23%) and moderately in 7 (26.92%) patients at 1st month and 3rd month follow up resolved moderately in 24 (92.30%). Flourecein leakage stopped in 25 (96.15%) patients. No ocular toxicity or adverse effects to drug were observed.

Conclusions: IVB injection resulted in improvement of visual acuity, resolution of macular edema and stoppage of Flourescein leakage as early as 1st week after injection in patients with DME. The slight reduction in visual improvement at 3rd months suggests wearing of effect of drug. Further clinical trials will be needed to evaluate the long term safety and efficacy of this drug.

Diabetic macular edema (DME) is a manifestation of diabetic retinopathy, which causes visual impairment in working age diabetic population of developed and developing world. Exact pathogenesis of DME is not clearly known, a disruption of inner blood retinal barrier is a reasonable explanation. It causes excessive vascular permeability and leakage of fluid and plasma constituents, such as lipoproteins, in retinal tissues and results in retinal edema. Untreated DME often leads to irreversible visual impairment[1]. Focal laser photocoagulation effectively treats DME and reduces visual loss by 50%[2]. However, some eyes may be refractory to laser treatment. Therefore, failure of laser prompted interest in other treatment modalities, such as intravitreal corticosteroid,[3] pars plana vitrectomy,[4] and oral proteinkinase C inhibitor[5]. Intravitreal corticosteroid is effective in improving vision and reducing edema, both as an initial or as a second line therapy after unsuccessful laser[6]. Hypoxia is primary cause of diabetic retinopathy, which increases expression of vascular endothelial growth factor (VEGF)[7,8]. It contributes to DME[9]. Funastu, proved that VEGF levels and its concentration in ocular fluids has strong correlation with severity of diabetic retinopathy. It is one of the

glycoprotein molecules and is a potent inducer of vascular permeability[10-13]. Introduction of VEGF in normal primate eyes induces the same pathological processes, as is seen in diabetic retinopathy namely microaneurysm formation and increased vascular permeability[14]. Thus a rational approach to treat DME with antibodies targeted VEGF generated considerable interest[15,16]. Bevacizumab, (Avastin, Genentech inc., South San Francisco, CA, USA) is a full length, humanized monoclonal antibody against VEGF, binds and inhibits all the biologically active isoforms of VEGF-A and is approved by food drug Administration as a systemic drug for tumor therapy[17]. Recent studies proved usefulness of bevacizumab injection in the reduction of macular edema secondary to CRVO, proliferative diabetic retinopathy (PDR) and choroidal neovascularization secondary to age related macular degeneration.[18] Although intravitreal use of Bevacizumab is off-label option, its use has risen exponentially in last few years, mainly due to its efficacy and economic consideration. Based on these observations this study was designed to evaluate visual acuity response, stoppage of flourescein leak and resolution of macular edema after IVB injection in patients with diabetic macular edema.

Study Plan

A study was conducted during June to 1st June to 31st August 2010, 26 eyes of 26 diabetic patients were selected for IVB Injection as treatment for DME. There were 14 (53.84%) males and 12 (46.15%) females. The mean age of patients was 48.9 years (range 38 – 60 years). Twenty three (88.5%) of type - 2 and three (11.5%) of type-1 diabetes were patients. Fifteen (58.6%) patients had active proliferative diabetic retinopathy (PDR), and eleven (42%) had severe non proliferative diabetic retinopathy (NPDR) (Table 1).

All patients had diagnosed DME clinically and on fundus flourescein angiography (FFA). All the eyes had received at least one prior alternate therapy for diabetic retinopathy at least six months before IVB injection.

Eyes with following features were excluded from study, (i) Focal macular edema attributed to focal leakages from micro aneurysm (ii) presence of any other macular pathology like vitreo-macular traction and ARMD (iii) optic disc pathology due to chronic glaucoma (iv) and history of treatment of DME with scatter PRP and Grid laser within the prior 6 months. Patients with uncontrolled diabetes, hypertension and chronic renal dysfunction were also excluded.

MATERIAL AND METHODS

Each patient underwent a complete ophthalmic assessment including best corrected visual acuity, anterior segment evaluation by slit lamp biomicroscopy, evaluation of fundus by non contact +90D lens, intraocular pressure (IOP) measurement and fundus fluorescien angiography at the base line and at each follow up visits. Patients were examined at 1st week, 1st month, and then 3rd month.

Vision of each patient was ascertained by using Snellen chart of visual acuity situated at 20 feet (approximately 6 meters) away from the patients, and then all eyes were tested with the same correction throughout follow up period. Each patient's VA was then converted to a logarithm of minimum angle of resolution (Log MAR) scale equivalent for analysis. Written informed consents were taken from all patients and were fully informed about the off label use of the drug and its potential risks and benefits as well as the likelihood that additional treatment might be required.

Injection Procedure

All intravitreal injections were performed under topical anesthesia with Proparacain hydrochloride 0.5% eye drops. The conjunctiva and the fornices were rinsed with povidone-iodine, which was also applied to the eyelid margins and lashes to avoid expression of the Meibomian glands. After application of sterile drape an eyelid speculum was used to stabilize the eye lids. A 30 gauge needle on a 1 cm^3 syringe was used to inject IVB through the supra temporal or supra nasal pars plana 3.5 - 4.00 mm posterior to the Limbus with a dose of 1.25 mg in 0.05 ml. The needle was carefully removed using a sterile cotton applicator to prevent reflex. After injection, antibiotic eye drops were applied four times per day for 5 days.

The study parameters were evaluated at 1st week, 1st month and 3rd month after IVB injection The study parameters included, (1) BCVA improvement, (2) resolution of macular edema and stoppage of flourescein leakage (3) Incidence of ocular sides effects like inflammation, IOP rise (4) systemically monitoring for blood pressure rise, chest pain and thromboembolic events.

Table 1. Degree of Diabetic Retinopathy in different diabetic patients

Gender ratio		Type of Diabetes		Degree of diabetic retinopathy	
Male N (%)	Female n (%)	Type 2 DM n(%)	Type 1 n (%)	NPDR (severe) n (%)	PDR n (%)
14(53.8)	12(46.2)	23 (88.5)	3 (11.5)	11 (42.30)	15(58.7)

Table 2. Pre-injection alternative therapy

Pre treatment type	No. of Patients n (%)
Focal laser	02 (07.69)
Grid +Focal laser	06(38.07)
PRP (scatter)	16(61.53)
IVTA	02(07.69)

Table 3. Showing baseline evaluation

baseline evaluations	
Mean follow up period (months)	Mean follow up period (months)
Mean base line BCVA (logMAR)	Mean base line BCVA (logMAR)
Base line Flourescein Angiography leakage	Base line Flourescein Angiography leakage
Baseline macular edema clinically diagnosed	Baseline macular edema clinically diagnosed

Table 4. Final Visual Activity Analysis

	1st Week	1st Month	3rd Month
	No. of Eyes n (%)	No. of Eyes n (%)	No. of Eyes n (%)
Improvement by > 2 lines of BCVA	18 (69.23)	18 (69.23)	17 (65.35)
Improvement by 1 line of BCVA	07 (26.92)	07 (26.92)	07 (24.92)
Remained unchanged BCVA	01 (0.384)	01 (0.384)	01 ((0.384)
Deterioration of BCVA	00 (00)	00 (00)	01 (3.84)
Chi-square P value	17.15** 0.000	17.15** 0.000	26.31** 0.000

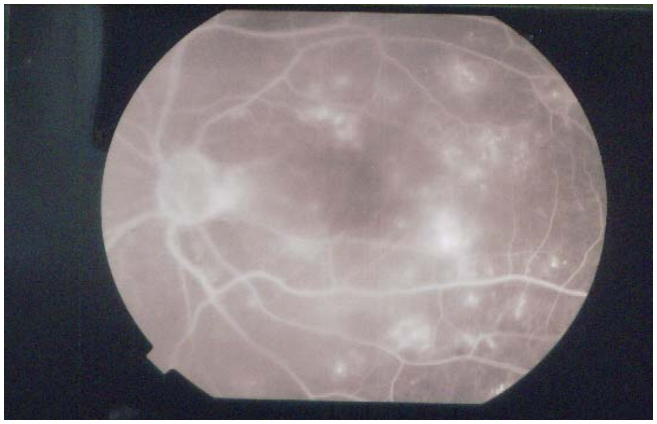

Fig. 1. Fundus flourescein angiography revealed diabetic macular edema with late phase of flourescein leakage from retinal vessels before treatment with IVB injection.

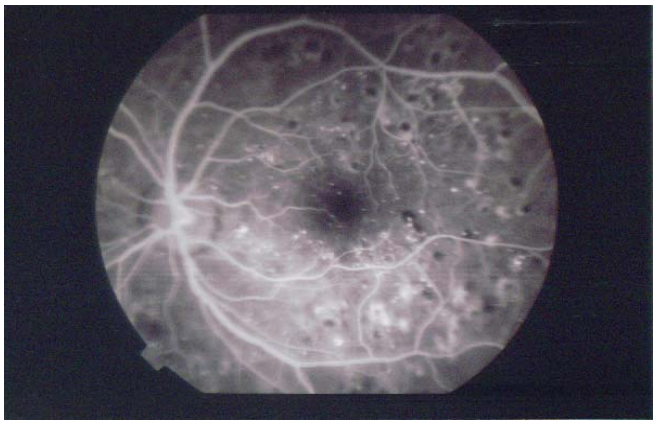

Fig. 2. Same patient after IVB injection showed the stoppage of flourescein leak and resolution of macular edema.

RESULTS

Baseline characteristic

The baseline characteristics included (1) mean BCVA 0.726 +log MAR. (2) clinically diagnosed DME present in all patients (3) flourescein leakage on FFA present in all patients (4) Mean IOP 13.59 mmHg. (Table 3).

1st week outcome

Mean BCVA was 0.515 +log MAR. Eighteen (69.23%) showed improvement of 2 lines of Snellen chart. Seven (26.92%) showed improvement of one line, one (3.84%) showed no improvement of visual acuity. DME resolved completely in 18 (69.23%), moderately in 7 (26.92%) and no resolution of edema in one

(3.84%) patient. Flourescein leakage stopped in 25 (96.15%) patients. Leakage was present in one (3.84%) patient. The mean IOP was 13.39 mmHg. Only one (3.84%) eye had developed anterior segment inflammation with +1 cells in anterior chamber. No other ocular and systemic complications were observed.

1st month outcome

Mean BCVA was 0.461 +log MAR. Eighteen (69.23%) showed improvement of 2 lines of Snellen chart. Seven (26.92%) showed improvement of one line. One (3.84%) showed no improvement of visual acuity. Macular edema resolved in 25 patients. One showed no resolution of edema. Flouroscein dye leakage has stopped in 25 (Fig. 1 and 2) and Mean IOP was 13.25 mmHg. No ocular, or systemic complications were observed.

3rd month outcome

The mean BCVA was 0.452 +log MAR. Seventeen (65.38%) showed improvement of 2 lines of snellen chart. Seven (26.92%) showed improvement of one line. One (3.84%) showed no improvement, and one (3.84%) showed deterioration of VA. Macular edema resolved in 24 patients. Floureoscein leakage stopped in 24 and Mean IOP was 13.25 mmHg. No ocular and systemic complications were seen.

Final BCVA analysis demonstrated seventeen (65.38%) of 26 patients improved ≥2 lines on BCVA, seven (26.92%) improved by one line, one (3.84%) remained stable, and one (3.84%) showed deterioration of BCVA by one line of BCVA (Table 4).

DISCUSSION

DME is the most important cause of visual impairment. Although the exact pathogenesis responsible for DME remain uncertain, the disruption of inner blood – retinal barrier is known to be associated with metabolic alteration affecting the retinal pigment epithelium or retinal vascular endothelium. Several treatment modalities are under investigation like intensive glycemic control,[19] blood pressure control,[20] pharmacologic therapy with oral protein kinase C inhibitors[21]. Intravitreal corticosteroids injection is a promising treatment but It's efficacy is transient and repeated injections may be required[22]. The treatment is not without risks and complications. Complications include intraocular pressure (IOP) elevation, cataract progression, endophthalmitis, vitreous haemorrhage, and retinal detachment[23, 24]. According to ETDRS, [25] DME treated with laser does not exhibit satisfactory visual improvement thus antibodies targeted to VEGF generated considerable interest and are being investigated. VEGF is proved as endothelial cell specific mitogen and angiogenic inducer in a variety of in vitro and in vivo models[26]. Hypoxia up-regulates VEGF, which plays a vital role in pathogenesis of DME in diabetic patients. Therefore anti-VEGF therapy may be promising treatment for DME. Cunningham et al reported that intravitreal pegaptanib sodium injection for treatment of DME has encouraging results[27]. More recently, IVB injection has been shown to have a beneficial effect in prevention and treatment of DME. It decreases VEGF secretion and vascular extravasations thus improves integrity of inner retinal barrier[28,29]. The safety profile of IVB injection has been established by previous animal studies and human trials[30]. Although preclinical experimental data from primates suggested that full length antibody might not penetrate the internal limiting membrane of the retina, but recent studies have shown full thickness penetration of the retina within 24 hours[31]. Recently IVB injection has been reported to be effective in reducing retinal edema and improving visual acuity, in macular edema of various etiologies like CRVO, ARMD and PDR[32-34]. IVB injection (125 mg/0.05 ml) offers, advantages of using a much lower dosage ($1/300^{th}$ to $1/400^{th}$) of systemic dose thus avoiding the rare but significant risk of thromboebmolic events[35]. Recently Haritoglou et al[36]. published a report about patients with persistent DME due to photocoagulation and IVTA treatment, when treated with 1.25 mg IVB injection. The vision improved significantly from base line of 0.86 +log MAR to a value of 0.75 +log MAR at 6 weeks although this effect was not sustained at 12 week. Similarly another study by Gulkilik G,[37] presented, results in which mean vision acuity was significantly better than at baseline at 2 week, diabetic macular edema decreased significantly at week 1, 2 and 4 but at third month macular edema increased, flourescein leak was moderately decreased in all patients at week 1 and 2, there was complete resolution of flourescein dye in 24 (70.5%) and moderately in 10 (29.5%) patients and at third month the flourescein leakage was fully resolved in five (14.7%), moderately in 24 (70.5%) and similar to baseline in 5 (14.7%) patients.

In another study by Sohelian, et al[38]. concluded that IVB injections yield better visual outcome in

DME at three months The results of these studies were confirmed in our study, where 25 (96.15%) out of 26 patients showed improvement in mean VA with a decrease in retinal edema and stoppage of flourescein leak. Mean VA improved from 0.721 +log MAR at baseline to 0.515, 0.461 and 0.452 +log MAR at 1st week, 1st month and last 3rd month respectively. Eighteen (69.23%) of 26 patients showed an improvement of 2 or more Snellen lines, seven (26.92%) showed improvement of one line and one patient (3.84%) showed no improvement. The reduction of macular edema and stoppage of fluorescein leak was seen in all eyes at 1st week and 1st month. Flourescein was completely resolved in 18 (69.23%) and moderately resolved in 7 (26.92%) patients and leak was continued similar to baseline in one (3.84%) patient. At 3rd month fluorescent leakage was increased in only one (3.84%) and remained similar to baseline in one (3.84%) patient. Another report of Pan-American collaborative retina Study Group, showed reduction of DME was achieved after one month of IVB injection and lasted for 6 months[39].

In our study, we found that improvement in both visual acuity, reduction of macular edema and stoppage of flourescein leak was achieved within one week after IVB injection and effect lasted for three months. Twenty five (96.15%) patients have shown an improvement of VA and macular edema resolved completely in 18 (69.23%) and moderately in seven (26.92%) patients. This confirms the findings of previous studies. The duration of action of IVB injection is unknown, recent electrophysiological and retinal penetration studies, have reported that full thickness retinal penetration is present at 24 hours[40]. This may explain the early clinical effect of IVB injection in our study. In our study the slight deterioration of vision, appearance of DME and flourescein leak at 3rd month follow up suggests wearing off effect of IVB injection thus repeat injection might be necessary at third month to maintain its beneficial effect. It is possible that a different dosing schedule, such as a series of IVB injections every 3 month may be superior to the method used in this study. However we chose to retreat the recurrent case only because of drug toxicity concerns. The results of IVB injection on visual acuity and DME was independent of both PDR, NPDR and previous treatments like focal, grid, scatter PRP and IVTA injection. All these did not influence our results.

Our study had some differences from previous studies. First DME was diagnosed clinically and on FFA before and after IVB injection instead of by optical coherence tomography (OCT). Second reduction of edema and stoppage of flourescein leakage indicated as effectiveness of IVB injection.

This study has several limitations. First, the follow-up time was relatively short, but visual improvement and resolution of DME was apparent during this follow-up period. Secondly there is no control group in this study, but it can be argued that the enrolled eyes serve as their own controls because the pre and post treatment VA and macular edema of the same patient were compared. Thirdly VA was measured on a Snellen visual acuity chart, as opposed to the more standardized and accepted ETDRS chart.

CONCLUSION

This study demonstrated that IVB injection appeared to be an effective treatment for DME as it result in significant improvement of visual acuity and resolution of macular edema as early as 1st week after IVB injection and this beneficial effect was shown to persist through out fallow up period of 3 months. However, the slight reduction in improvement in visual acuity and macular edema at 3rd months suggests that repeated IVB injection might be necessary within three months to maintain its effect, The drug is well tolerated and there are no safety concerns. However, to evaluate the long term safety and efficacy of this new treatment, further studies are needed.

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