

Efficacy of Simultaneous Usage of Bevacizumab and Silicone Oil Injection After Vitrectomy in Diabetic Tractional Retinal Detachment

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Abstract

Background: Tractional retinal detachment (TRD), a major vision threatening complication, is one of the most common eye emergencies. The most common cause of TRD is proliferative diabetic retinopathy (PDR). The aim of this study was to evaluate the effect of simultaneous usage of bevacizumab and silicone oil injection after vitrectomy for the treatment of diabetic tractional retinal detachment

Methods: Twenty five patients (25 eyes) with severe proliferative diabetic retinopathy were recruited into the study. All eyes underwent a single intravitreal injection of bevacizumab 1.25 mg in 0.05 mL along with silicone oil injection after vitrectomy for the management of tractional retinal detachment or vitreous hemorrhage due to severe proliferative diabetic retinopathy. Patients were then scheduled for postoperative clinical examinations at one, three and six months. The main outcome measures were regression of neovascularization, intraocular pressure, visual acuity and retinal reattachment.

Results: This study evaluated 25 eyes of 25 patients (12 men, 13 women) with a mean age of 55.68 ± 6.94 years (range 21 to 49 years). In all eyes visual acuity improved and retinal attachment was accomplished. The mean preoperative visual acuity was 2.01 ± 0.03 Log MAR which significantly improved to 1.10 ± 0.50 Log MAR in 6th month after surgery ($P < 0.000$). Active neovascularization regressed significantly ($P < 0.004$) and intraocular pressure was controlled in 88% of patients.

Conclusions: The findings of our study may suggest that simultaneous injection of Bevacizumab and silicone oil after vitrectomy improve early results. We have found it to be particularly useful in diabetic eyes with tractional detachments of short duration.

Keywords: Tractional Retinal Detachment, Proliferative Diabetic Retinopathy, Neovascularization, Vascular Endothelial Growth Factor, Vitreous Hemorrhage, Avastin

1. Background

Vascular endothelial growth factor (VEGF) plays a vital role in several ocular pathologies characterized by neovascularization and increased vascular permeability (1). Diabetic tractional retinal detachment (TRD) is a devastating complication associated with significant intra- and postoperative bleeding due to multiple or thick layers of neovascular proliferative tissue. Risk of vitreous hemorrhage (VH) increases in the early postoperative period that makes it as one of the most challenging procedures during vitreoretinal surgery (2). The postoperative vitreous hemorrhage incidence in patients undergoing pars plana vitrectomy (PPV) for vitreous hemorrhage in proliferative diabetic retinopathy (PDR) varies from 29 to 75% that impairs visual recovery (3-6). Accordingly, neovascular regression is a main concern prior to vitrectomy for PDR. Several authors reported intravitreal bevacizumab (Avastin, Genentech, South San Francisco, CA) injection before surgery for the intention of regressing neovascularization (7-9). Fur-

thermore, several studies reported surgical bleeding reduction (10, 11). Silicone oil plays an outstanding role in the management of complex vitreoretinal surgical procedures (12). During silicone oil tamponade, the concentration and release of injected drugs into the posterior segment is unpredictable, however, Bevacizumab injection was found effective for the management of iris neovascularization associated with TRD by several authors (13). Additionally, to the best of our knowledge, the effect of concomitant treatment of silicone oil and bevacizumab after vitrectomy rarely was reported in the literature (14).

We undertook this study to assess the effect of simultaneous usage of bevacizumab and silicone oil tamponade after vitrectomy for the treatment of diabetic tractional retinal detachment.

2. Methods

This prospective, interventional, non-randomized study consisted of 25 eyes of 25 patients (12 men, 13

women) scheduled to undergo pars plana vitrectomy plus injection of bevacizumab and silicone oil for the management of TRD between February 2013 and August 2013 at Baqiyatallah Hospital, Tehran Iran. After fully explaining the purpose and procedures of the study, all patients were asked to sign an informed consent form before treatment. This study was approved by the institutional review board of the eye research center, Baqiyatallah hospital and followed the tenets of the Declaration of Helsinki. Inclusion criteria were PDR with documented tractional retinal detachment; TRD involving the macula with active neovascularization within the fibro vascular complex, vitreous hemorrhage accompanied with rubeosis precluding pan-retinal photocoagulation (PRP) and high risk features for developing rubeosis following vitrectomy: Severe ischemia on previous fluorescein angiography and fellow eye developing rubeosis following vitrectomy. The exclusion criteria were TRD secondary to other eye diseases such as retinal vessel occlusion or endophthalmitis; subjects with history of previous vitreoretinal surgery and previous intravitreal anti-VEGF injection/s in the past 3 months, the presence of rhegmatogenous retinal detachment (RRD) combined with TRD; the presence of major intraoperative complications, such as suprachoroidal hemorrhage and patients with history of myocardial infarctions ,cerebrovascular accidents or other major thromboembolic diseases. In all cases, a comprehensive ophthalmic examination was performed preoperatively including measurement of corrected distance visual acuity (CDVA), IOP (Goldman tonometry), slit-lamp bio microscopy, fundus evaluation, and indirect ophthalmoscopy. Visual acuity was measured using Snellen notation and then converted to log MAR for statistical analysis. Further investigations such as optical coherence tomography, B-scan and fluorescein angiography were performed as indicated.

All subjects undergone vitreoretinal surgery including standard three-port 20 gauge pars plana vitrectomy with the sclerotomes placed 3.5 - 4.0 mm posterior to the limbus. After removing the core vitreous, the membrane was removed carefully by delamination and segmentation. F-Decalin was injected exactly opposite the optical disc followed by extensive panretinal endolaser photocoagulation. Then, fluid-air exchange was done followed by 1.25 mg (0.05 mL) bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA, USA). Finally, silicone oil was used for end tamponade for the treatment of tractional retinal detachment (TRD). Patients were required to maintain a prone or head-down position. Postoperatively, patients were prescribed betamethasone drops four times a day, and topical antibiotic (ciprofloxacin 0.3%) eye drops three times per day for 7 days. All injections were given by a single surgeon

(A.A). Patients had to have at least 6 months of follow-up after concurrent injection of bevacizumab and silicone oil for inclusion in the study. Patients were then scheduled for postoperative clinical examinations at one, three and six months. Ophthalmic examinations included visual acuity testing, slit lamp bio microscopy, measurement of IOP, regression of retinal neovascularization, dilated fundus copy and recording of adverse events at all visits.

2.1. Statistical Analysis

Statistical analysis were done using the IBM SPSS for version 20 (SPSS, Chicago, IL, USA), with P-value of < 0.05 were considered as significant. Qualitative variables were expressed using percentages, whereas quantitative data were defined using mean \pm SD. The t-test, Mann-Whitney U-test and Chi-square test were used for inferential statistics.

3. Results

Twenty five eyes of 25 patients with a mean age of 55.6 ± 8.3 years (range 42 to 74 years) were studied. [Table 1](#) shows the patient characteristics. The mean preoperative visual acuity was 2.016 ± 0.0374 Log MAR which significantly improved to 1.4 ± 0.4 Log MAR and 1.1 ± 0.5 Log MAR ($P < 0.000$) at 1 and 6 month respectively after surgery. Out of 16 eyes with neovascularization, in 14 cases the iris neovascularization (INV) completely disappeared clinically within 7 days, starting almost 72 hours after injection that was statistically significant ($P = 0.004$). The mean IOP before injections was 19.04 mm Hg (range 9 - 53), while in 22 cases (88%), the IOP decreased within 72 hours and returned to levels of 18.3 mm Hg within 7 days that was significant (0.003). In three patient, intraocular pressure was not controlled post operatively even with timolol but successfully was controlled after silicon oil removal. No inflammation or other complications were observed. Vitreous hemorrhage cleared within 2 weeks after the operation in all eyes (See [Table 2](#)).

4. Discussion

Antiangiogenic agent's administration during diabetic vitrectomies has been shown to decrease postoperative bleeding in the course of regression of neovascularization in diabetic eyes (15, 16). In addition, it was found to be a precious treatment choice in the eyes in which neovascularization was not stopped by panretinal photocoagulation (15-17). However, little studies reported fibrosis and adhesion of the fibro vascular membrane in patients received bevacizumab before surgery (11).

Table 1. Characteristic of Participants (n = 25)^a

Variables	N.
Number of eyes	
OD	11 (44)
OS	14 (56)
Sex	
Male	12 (48)
Female	13 (52)
Age	
Mean (SD)	55.68 (8.3)
Range	42 - 74
Follow-up duration, mo	
Mean (SD)	7.2 (1.75)

^aValues are expressed as mean N. (%) unless otherwise indicated.

Table 2. Comparison Between Preoperative and Postoperative Outcomes^a

Variables	Preoperative	Early (≤ 4 weeks) Postoperative	P Value
BCVA, Log MAR ^b	2.01 ± 0.03	1.4 ± 0.4	0.001
VH ^c	25 (100)	0	0.001
INV ^c	16 (64)	2 (8)	0.004
IOP (mm Hg) ^b	19.04 ± 9.19	18.3 ± 4.7	0.003

Abbreviations: BCVA, Best Corrected Visual Acuity; INV, iris neovascularization; IOP, Intra Ocular Pressure; SD, Standard Deviation; VH, Vitreous Hemorrhage.

^aSignificances are based on Paired T-Test / Wilcoxon signed-rank test.

^bValue are expressed as Mean ± SD.

^cValue are expressed as N. (%).

The aim of this study was to assess the effect of simultaneous usage of bevacizumab and silicone oil injection after vitrectomy on regression of neovascularization, intraocular pressure, visual acuity and retinal reattachment in patients with diabetic tractional retinal detachment. Our study included patients with PDR and TRD. All eyes had preoperative vitreous hemorrhage (VH). After the combined treatment with bevacizumab and silicone oil injection, the VH and rebleeding was cleared completely.

Several authors demonstrated 13 to 25% of vitreous hemorrhage following preoperative intravitreal bevacizumab (18, 19). While, in our study no vitreous hemorrhage was detected postoperatively. The precise mechanism of early postoperative VH cutback and intraoperative bleeding following IVB injection in our series is not clear, however, a new vessel constriction secondary to attenuation of the caliber of the normal retinal vessels (20), reduc-

tion of intraocular vascular endothelial growth factor (21) and lessening of the retinal circulation were demonstrated after IVB injection (22, 23) that may explain our finding.

On the other hand, previous reports demonstrated a rapid resolution of retinal and disc neovascularization soon after IVB injection (17, 24, 25) that may support our suggestion regarding intraoperative injection of avastin for decreasing postoperative VH as well.

Moreover, Yang et al. (2007) in a randomized prospective study, reported early postoperative VH prevention secondary to 10% C3F8 due to mechanical tamponade on the fragile vessels during a 2 - 3 weeks period. We used bevacizumab and silicone oil injection simultaneously after vitrectomy. Silicone oil as an intraocular tamponade in the pars plana vitrectomy has a number of advantages over gas tamponade. Unlike gas tamponades, silicone oil is not absorbed so the injected volume never decreases. This allows prolonged tamponade and improved tamponade of inferior retinal breaks without positioning. On the other side, silicone oil in our study was injected after vitrectomy and IVB injection. We think that entrapment of IVB after silicone oil injection along with mechanical tamponade was provided on the fragile vessels during operation by silicone oil may prolong inhibitory effect of bevacizumab on fibro vascular activity and beneficial antiangiogenic effect and explained better result in our study in comparison to previous studies.

Also, a better visual recovery was shown postoperatively in our study. Our results is in accordance to Randomized clinical trial study by Ahmadiéh et al. (18) demonstrated the efficacy of intravitreal bevacizumab in reducing the overall rate of early post vitrectomy. However, the number of cases was limited in study of Ahmadiéh et al, but in our study the sample size was much higher to draw a nearly definite conclusion regarding safety of the procedure. We believe that decrease in injury to retinal tissue/s during surgery and decrease in extent of hemorrhage after surgery in bevacizumab treated cases may explain our finding.

Furthermore, no cases of uveitis, hypertension, endophthalmitis, and ocular toxicity and/ or thromboembolic events after injection in any of our patients were identified. We have found it to be particularly useful in diabetic eyes with traction detachments.

Despite the some strengths of our study: standardized surgical technique (one center, consistent equipment with similar technique and consistent primary surgeon) and the reliable sample size, our study does have some potential limitations including the non-randomized nature of this study, the lack of a control group, the lack of classification of patients based on severity of fibro vascular proliferation and the relatively short mean postoperative follow-

up time. However, the results in our study shows that intra-operative intravitreal avastin accompanied with silicone oil is associated with good results and no added complications and is a valuable addition to surgery in the management of proliferative diabetic retinopathy.

In conclusion, our results may propose that simultaneous bevacizumab and silicone oil injection after vitrectomy are bonus useful choices for management of patients with TRD. Also, our finding shows inspiring results and appears to be a minimally invasive procedure with no evidence of complications for our described technique. However, to certify our findings on the course of the disease further follow-up and comparative studies should be performed in patients with diabetic tractional retinal detachment.

4.1. Summary

4.1.1. What Was Known Before

Diabetic tractional retinal detachment is associated with severe visual loss. Surgical technique improvement made amazingly improvement in diabetic vitrectomy

4.1.2. What This Study Adds

Bevacizumab injection at the end of vitreoretinal surgery concomitant with silicone oil injection for proliferative vitreoretinopathy may remarkably further the outcome of vitrectomy surgery and prevent development of subsequent proliferative vitreoretinopathy.

Footnotes

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References

- Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev.* 2004;**25**(4):581-611. doi: [10.1210/er.2003-0027](https://doi.org/10.1210/er.2003-0027). [PubMed: [15294883](https://pubmed.ncbi.nlm.nih.gov/15294883/)].
- Thompson JT, de Bustros S, Michels RG, Rice TA. Results and prognostic factors in vitrectomy for diabetic traction retinal detachment of the macula. *Arch Ophthalmol.* 1987;**105**(4):497-502. doi: [10.1001/archophth.1987.01060040073036](https://doi.org/10.1001/archophth.1987.01060040073036). [PubMed: [2436603](https://pubmed.ncbi.nlm.nih.gov/2436603/)].
- Tolentino FI, Cajita VN, Gancayco T, Skates S. Vitreous hemorrhage after closed vitrectomy for proliferative diabetic retinopathy. *Ophthalmology.* 1989;**96**(10):1495-500. doi: [10.1016/S0161-6420\(89\)32700-X](https://doi.org/10.1016/S0161-6420(89)32700-X). [PubMed: [2587044](https://pubmed.ncbi.nlm.nih.gov/2587044/)].
- Schachat AP, Oyakawa RT, Michels RG, Rice TA. Complications of vitreous surgery for diabetic retinopathy. *Ophthalmology.* 1983;**90**(5):522-30. doi: [10.1016/S0161-6420\(83\)34540-1](https://doi.org/10.1016/S0161-6420(83)34540-1).
- Novak MA, Rice TA, Michels RG, Auer C. Vitreous hemorrhage after vitrectomy for diabetic retinopathy. *Ophthalmology.* 1984;**91**(12):1485-9. doi: [10.1016/S0161-6420\(84\)34099-4](https://doi.org/10.1016/S0161-6420(84)34099-4). [PubMed: [6521989](https://pubmed.ncbi.nlm.nih.gov/6521989/)].
- Blankenship GW. Management of vitreous cavity hemorrhage following pars plana vitrectomy for diabetic retinopathy. *Ophthalmology.* 1986;**93**(1):39-44. doi: [10.1016/S0161-6420\(86\)33791-6](https://doi.org/10.1016/S0161-6420(86)33791-6). [PubMed: [2419815](https://pubmed.ncbi.nlm.nih.gov/2419815/)].
- Chen E, Park CH. Use of intravitreal bevacizumab as a preoperative adjunct for tractional retinal detachment repair in severe proliferative diabetic retinopathy. *Retina.* 2006;**26**(6):699-700. doi: [10.1097/OI.iae.0000225351.87205.69](https://doi.org/10.1097/OI.iae.0000225351.87205.69). [PubMed: [16829817](https://pubmed.ncbi.nlm.nih.gov/16829817/)].
- Khafagy A. Role of adjunctive use of intravitreal bevacizumab for severe proliferative diabetic retinopathy before vitrectomy. *Med J Cairo Univ.* 2011;**79**(2).
- Ghasemi Falavarjani K, Hashemi M, Modarres M, Hadavand Khani A. Intrasilicone oil injection of bevacizumab at the end of retinal reattachment surgery for severe proliferative vitreoretinopathy. *Eye (Lond).* 2014;**28**(5):576-80. doi: [10.1038/eye.2014.21](https://doi.org/10.1038/eye.2014.21). [PubMed: [24556886](https://pubmed.ncbi.nlm.nih.gov/24556886/)].
- Yeh PT, Yang CH, Yang CM. Intravitreal bevacizumab injection for recurrent vitreous haemorrhage after diabetic vitrectomy. *Acta Ophthalmol.* 2011;**89**(7):634-40. doi: [10.1111/j.1755-3768.2009.01821.x](https://doi.org/10.1111/j.1755-3768.2009.01821.x). [PubMed: [20064118](https://pubmed.ncbi.nlm.nih.gov/20064118/)].
- Ishikawa K, Honda S, Tsukahara Y, Negi A. Preferable use of intravitreal bevacizumab as a pretreatment of vitrectomy for severe proliferative diabetic retinopathy. *Eye (Lond).* 2009;**23**(1):108-11. doi: [10.1038/sj.eye.6702983](https://doi.org/10.1038/sj.eye.6702983). [PubMed: [17891057](https://pubmed.ncbi.nlm.nih.gov/17891057/)].
- Abu El-Asrar AM, Al-Bishi SM, Kangave D. Outcome of temporary silicone oil tamponade in complex rhegmatogenous retinal detachment. *Eur J Ophthalmol.* 2003;**13**(5):474-81. [PubMed: [12841571](https://pubmed.ncbi.nlm.nih.gov/12841571/)].
- Ghazi NG, Green WR. Pathology and pathogenesis of retinal detachment. *Eye (Lond).* 2002;**16**(4):411-21. doi: [10.1038/sj.eye.6700197](https://doi.org/10.1038/sj.eye.6700197). [PubMed: [12101448](https://pubmed.ncbi.nlm.nih.gov/12101448/)].
- Xu Y, You Y, Du W, Zhao C, Li J, Mao J, et al. Ocular pharmacokinetics of bevacizumab in vitrectomized eyes with silicone oil tamponade. *Invest Ophthalmol Vis Sci.* 2012;**53**(9):5221-6. doi: [10.1167/jovs.12-9702](https://doi.org/10.1167/jovs.12-9702). [PubMed: [22786911](https://pubmed.ncbi.nlm.nih.gov/22786911/)].
- Sivak-Callcott JA, O'Day DM, Gass JD, Tsai JC. Evidence-based recommendations for the diagnosis and treatment of neovascular glaucoma. *Ophthalmology.* 2001;**108**(10):1767-76. doi: [10.1016/S0161-6420\(01\)00775-8](https://doi.org/10.1016/S0161-6420(01)00775-8). [PubMed: [11581047](https://pubmed.ncbi.nlm.nih.gov/11581047/)] quiz1777, 1800.
- Wakabayashi T, Oshima Y, Sakaguchi H, Ikuno Y, Miki A, Gomi F, et al. Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. *Ophthalmology.* 2008;**115**(9):1571-80. doi: [10.1016/j.ophtha.2008.02.026](https://doi.org/10.1016/j.ophtha.2008.02.026). [PubMed: [18440643](https://pubmed.ncbi.nlm.nih.gov/18440643/)] 1580 e1-3.
- Oshima Y, Sakaguchi H, Gomi F, Tano Y. Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. *Am J Ophthalmol.* 2006;**142**(1):155-8. doi: [10.1016/j.ajo.2006.02.015](https://doi.org/10.1016/j.ajo.2006.02.015). [PubMed: [16815267](https://pubmed.ncbi.nlm.nih.gov/16815267/)].
- Ahmadieh H, Shoeibi N, Entezari M, Monshizadeh R. Intravitreal bevacizumab for prevention of early postvitrectomy hemorrhage in diabetic patients: a randomized clinical trial. *Ophthalmology.* 2009;**116**(10):1943-8. doi: [10.1016/j.ophtha.2009.07.001](https://doi.org/10.1016/j.ophtha.2009.07.001). [PubMed: [19699531](https://pubmed.ncbi.nlm.nih.gov/19699531/)].
- Lo WR, Kim SJ, Aaberg TM Sr, Bergstrom C, Srivastava SK, Yan J, et al. Visual outcomes and incidence of recurrent vitreous hemorrhage after vitrectomy in diabetic eyes pretreated with bevacizumab (avastin). *Retina.* 2009;**29**(7):926-31. doi: [10.1097/IAE.0b013e3181a8eb88](https://doi.org/10.1097/IAE.0b013e3181a8eb88). [PubMed: [19584650](https://pubmed.ncbi.nlm.nih.gov/19584650/)].
- Yeh PT, Yang CM, Lin YC, Chen MS, Yang CH. Bevacizumab pretreatment in vitrectomy with silicone oil for severe diabetic retinopathy. *Retina.* 2009;**29**(6):768-74. doi: [10.1097/IAE.0b013e3181a3b7ef](https://doi.org/10.1097/IAE.0b013e3181a3b7ef). [PubMed: [19516117](https://pubmed.ncbi.nlm.nih.gov/19516117/)].
- Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M. Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. *Arch Ophthalmol.* 2007;**125**(10):1363-6. doi: [10.1001/archophth.125.10.1363](https://doi.org/10.1001/archophth.125.10.1363). [PubMed: [17923544](https://pubmed.ncbi.nlm.nih.gov/17923544/)].

22. Arevalo JF, Maia M, Flynn HW Jr, Saravia M, Avery RL, Wu L, et al. Tractional retinal detachment following intravitreal bevacizumab (Avastin) in patients with severe proliferative diabetic retinopathy. *Br J Ophthalmol*. 2008;**92**(2):213–6. doi: [10.1136/bjo.2007.127142](https://doi.org/10.1136/bjo.2007.127142). [PubMed: [17965108](https://pubmed.ncbi.nlm.nih.gov/17965108/)].
23. Jampol LM, Shankle J, Schroeder R, Tornambe P, Spaide RF, Hee MR. Diagnostic and therapeutic challenges. *Retina*. 2006;**26**(9):1072–6. doi: [10.1097/01.iae.0000248819.86737.a5](https://doi.org/10.1097/01.iae.0000248819.86737.a5). [PubMed: [17151497](https://pubmed.ncbi.nlm.nih.gov/17151497/)].
24. Jorge R, Costa RA, Calucci D, Cintra LP, Scott IU. Intravitreal bevacizumab (Avastin) for persistent new vessels in diabetic retinopathy (IBEPE study). *Retina*. 2006;**26**(9):1006–13. doi: [10.1097/01.iae.0000246884.76018.63](https://doi.org/10.1097/01.iae.0000246884.76018.63). [PubMed: [17151487](https://pubmed.ncbi.nlm.nih.gov/17151487/)].
25. Avery RL, Pearlman J, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology*. 2006;**113**(10):1695 et-15. doi: [10.1016/j.ophtha.2006.05.064](https://doi.org/10.1016/j.ophtha.2006.05.064). [PubMed: [17011951](https://pubmed.ncbi.nlm.nih.gov/17011951/)].