Effectiveness of Intravitreal Ranibizumab in Nonvitrectomized and Vitrectomized Eyes with Diabetic Macular Edema: A Two-Year Retrospective Analysis

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Abstract
Background: To compare the effectiveness of intravitreal ranibizumab (IVR) injections for the treatment of diabetic macular edema (DME) in eyes with and without previous vitrectomy. Methods: The medical records of 30 eyes (13 vitrectomized, 17 nonvitrectomized) of 28 patients (mean age, 59.0±9.6 years; male to female ratio 1:1) who were diagnosed with DME and had received IVR treatment were reviewed retrospectively. The best-corrected visual acuity (BCVA), central macular thickness (CMT), and total macular volume (TMV) were measured at baseline and at months 6, 12, 18, and 24 of the follow-up. The number of IVR injections, the duration between diagnosis of DME and IVR injection, and hemoglobin A1c (HbA1c) level at baseline were also recorded. Results: Baseline demographics, HbA1c, BCVA, CMT, and TMV values were similar between the vitrectomized and nonvitrectomized groups (p>0.05). The duration between diagnosis of DME and IVR injections was longer in the nonvitrectomized group than in the vitrectomized group (16±5 years vs. 13±4 years, respectively; p=0.045). IVR injection was performed 6.3 times in vitrectomized eyes and 6.1 times in nonvitrectomized eyes during the 24-month period (p>0.05). BCVA improved significantly during the 24-month period in both groups. The improvement in BCVA was significant at month 6 in nonvitrectomized eyes, while there was no significant improvement in vitrectomized eyes before month 18. Compared to the baseline values, the decrease in both CMT and TMV was significant in months 6, 12, 18, and 24 in the nonvitrectomized group (p<0.05). In the vitrectomized group both CMT and TMV improved significantly only in months 18 and 24 (p<0.05). Conclusion: IVR treatment for DME is equally effective in both vitrectomized and nonvitrectomized eyes. However, the response to treatment is seen later in vitrectomized eyes compared to nonvitrectomized eyes.

Background
Diabetic macular edema (DME) is the most common cause of visual impairment in patients with diabetic retinopathy with a prevalence of 2.7%-11%. [1]

The ophthalmic treatment of DME includes intravitreal antivascular endothelial growth factor (anti-VEGF) drug injections, intravitreal corticosteroid injections, focal/grid argon laser photocoagulation, subthreshold micropulse diode laser photocoagulation, and vitrectomy. Since 2010, anti-VEGF drug
injections have become standard therapy for DME with the proven benefit of improved visual acuity. [1-6]

Vitrectomy as treatment for DME was first introduced for eyes with proliferative diabetic retinopathy (PDR), unresolving vitreous hemorrhage, significant vitreomacular traction commonly associated with shallow traction macular detachment, and persistent DME despite previous focal laser or intravitreal injections. Vitrectomy has recently been studied as potential primary therapy in eyes with more severe edema and greater visual acuity loss at presentation. [7,8]

There is controversy regarding the effects of vitrectomy on the diffusion and clearance of intravitreal anti-VEGF drugs for DME. Some animal studies have shown this clearance to be faster, while others have failed to show any pharmacokinetic changes of intravitreal drugs after vitrectomy. [7,8] In theory, faster clearance of intravitreal drugs could mean decreased effectiveness in vitrectomized eyes. [9]

Intravitreal ranibizumab (IVR), an anti-VEGF drug, has been shown to be an effective treatment for DME, providing a significant improvement in best-corrected visual acuity (BCVA) and in anatomic outcomes. [3,9-11] There are limited data on the comparison of efficacy of IVR in vitrectomized and nonvitrectomized eyes with DME. Chen et al. [3] showed that IVR was effective in both vitrectomized and nonvitrectomized eyes with DME in a 6-month follow-up. They reported that greater anatomical and functional improvements were obtained in nonvitrectomized patients than in vitrectomized cases. However, these findings show the outcome of just the short-term treatment. The aim of this study is to compare the long-term effectiveness of IVR for treatment of DME in vitrectomized and nonvitrectomized eyes.

**Methods**

**Study design and population**

In this retrospective comparative study, we reviewed the medical records of 13 vitrectomized eyes of 11 patients (mean age, 55.0±10.0 years; male to female ratio, 6:5) and 17 nonvitrectomized eyes of 17 patients (mean age, 62.0±9.0 years; male to female ratio 8:9) with severe nonproliferative diabetic retinopathy or proliferative diabetic retinopathy who received IVR injections and were
followed for at least 24 months between April 2013 and December 2017 at Atakoy Dunyagoz Hospital. Exclusion criteria included: alterations that could prevent improvement in BCVA (the presence of apparent retinal pigment epithelium (RPE) atrophy or proliferative diabetic fibrovascular membranes at or near the macula, the presence of diabetic or glaucomatous optic atrophy, etc.), active intraocular inflammation or infection in one or both eyes, uncontrolled or neovascular glaucoma, prior treatment with intravitreal or periocular pharmacologic injections in the studied eye within a 3-month period before the IVR injections, panretinal laser photocoagulation within 6 months or macular focal/grid laser photocoagulation in the studied eye within a 3-month period before the beginning of the study, previous major surgeries like vitrectomy, cataract extraction, or steroid injections within the previous 3 months or during the course of the study, persistence of tractional retinal detachment, vitreomacular traction, epiretinal membrane, and vitreous hemorrhage and systemic diseases that contraindicated IVR injection (history of thromboembolic events including myocardial infarction or cerebral vascular events, uncontrolled hypertension, known coagulation abnormalities or current use of anticoagulative medication other than aspirin).

**Ethical Approval**

This study was approved by the Institutional Ethics Committee of Bahcesehir University (Mar/20th/2019; 2019-06/01) and conducted in accordance with the latest version of the Declaration of Helsinki. According to the Regulation on Clinical Studies of Drugs and Biological Products in Turkey (no: 29474), updated on September 13, 2015, retrospective studies are not subject to the requirement of informed consent of patients. The Institutional Ethics Committee of Bahcesehir University, which operates in accordance with this regulation, waived the requirement of informed consent for this study. All patients with vitrectomized eyes were informed about the risks and benefits of vitrectomy before surgery, and a written consent was obtained after a thorough explanation of the procedure. The potential risks and benefits of IVR injections were also discussed extensively with all the patients. All patients gave written informed consent for IVR injections.

**Study procedures**

All patients underwent a comprehensive clinical assessment and ophthalmologic examination
including measurement of BCVA and indirect and contact lens slit lamp fundoscopic examination. The BCVA was measured with a standard Snellen chart and converted to the logarithm of the minimum angle of resolution (logMAR) units. Spectral domain or swept source optical coherence tomography (OCT) (Topcon 3D OCT-2000, Tokyo, Japan) was used to examine the central macular thickness (CMT) and total macular volume (TMV) of all eyes before surgery at baseline, and at months 6, 12, 18, and 24 of the follow-up. In OCT retinal thickness measurement (the distance between the inner surface of RPE and the inner surface of the neurosensory retina), a 3D model of the retina was computed and retinal volumes (RVs) assessed for each of the nine subfields using the inner, intermediate, and outer rings (with diameters of 1 mm, 2.22 mm, and 3.45 mm, respectively) as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS). [12] CMT was defined as the average thickness of the macula in the central 1 mm ETDRS grid. TMV was calculated by summation of all the volumes obtained in the ETDRS subfields.

The number of intravitreal injections, the duration between the diagnosis of DME and IVR injections, and hemoglobin A1c (HbA1c) levels at baseline were also assessed.

**Vitrectomy surgery**

Vitrectomy was performed at least 12 months prior to the start of IVR treatment. The indications for pars plana vitrectomy (PPV) were intravitreal hemorrhage, tractional retinal detachment, vitreomacular traction, and epiretinal membrane. The internal limiting membrane (ILM) was peeled in patients operated on for epiretinal membrane. Before the appearance of DME, the macula was flat in all vitrectomized patients after surgery.

**Intravitreal ranibizumab treatment**

The indications for anti-VEGF treatment for eyes with DME were CMT of more than 300 μm determined by spectral-domain OCT and/or decimal BCVA less than 0.7. The intravitreal dose of ranibizumab was 0.5 mg/0.05 ml. The nonvitrectomized group of patients initially received three consecutive monthly loading doses of ranibizumab followed by pro re nata (PRN) administration until either an improvement of the central macular edema was confirmed by OCT or the visual acuity was stable. Patients with vitrectomized eyes were treated with PRN regimen from the beginning with monthly
follow-ups. A reduction of >10 μm in CMT was defined as an anatomical improvement considering interexamination measurement bias. Retreatment criteria included persistence of submacular fluid, intraretinal cysts, or CMT of more than 300 μm. Cases without evidence of reduced macular thickness on OCT after IVR injection and a lack of improvement of BCVA were also observed without treatment.

**Statistical analysis**

The study data were summarized using descriptive statistics including the mean, standard deviation, minimum, and maximum for continuous variables, and frequency and percent for categorical variables. The Shapiro-Wilk test was used to test the normality of continuous variables. The Mann-Whitney U test was used to compare independent groups. A paired samples t test and the Wilcoxon signed-rank test were used to compare the mean differences between pre- and post-injection values of all parameters. Frequencies were compared between groups using the Chi-square test. In order to test the significance of the correlations between BCVA, CMT, and TMV, the Pearson correlation test was used.

The Statistical Package for the Social Sciences software (SPSS 20.0; IBM Corporation, Armonk, NY, USA) was used for statistical analysis and Excel 2007 was used for all computations involving descriptive statistics. A p value less than 0.05 was assumed to indicate statistical significance.

**Results**

Baseline age, sex distribution, HbA1c, BCVA, CMT, and TMV values were similar between the vitrectomized and nonvitrectomized groups (p>0.05). However, the duration between diagnosis of DME and treatment with IVR injections was longer in the nonvitrectomized group than in the vitrectomized group (16±5 years vs. 13±4 years, respectively; p=0.045). Baseline demographics and characteristics of the patients are shown in Tables 1 and 2.

IVR injection was performed 6.3 times in vitrectomized eyes and 6.1 times in nonvitrectomized eyes during the 24-month period (p>0.05). The number of IVR injections up to month 6 was significantly higher in nonvitrectomized eyes than vitrectomized eyes (3.2 vs. 1.3, respectively; p=0.04), probably due to the loading dose applied to the nonvitrectomized eyes in the first three months [Figure 1].
The difference in CMT and TMV between the groups was not significant (p>0.05 for all points of measurement). Compared to the baseline values, the decrease in both CMT and TMV was significant in months 6, 12, 18, and 24 in the nonvitrectomized group (p<0.05). In the vitrectomized group both CMT and TMV improved significantly only in months 18 and 24 (p<0.05) [Figures 3,4].

Pearson correlation analysis revealed a weak-to-moderate correlation between the 24-month change in visual acuity and the change in CMT (r=0.428, p=0.021) and TMV (r=0.673, p<0.001) in all eyes.

**Discussion**

Pars plana vitrectomy improves visual acuity by reducing macular thickness in patients with DME. It also reduces retinal ischemia by allowing better oxygenation of the retina [12-14] and has the potential to affect the diffusion and clearance of intravitreal anti-VEGF drugs used for DME. [9] It is therefore clinically important to know whether vitreoretinal surgery alters the anatomical and visual effects of anti-VEGFs in patients with DME. In this study we compared the effectiveness of IVR injections for the treatment of DME in eyes with and without previous vitrectomy and found that although the response to treatment is delayed in vitrectomized eyes, IVR is an equally effective treatment for DME in both vitrectomized and nonvitrectomized eyes.

Ahn et al. [15] compared the concentration and elimination of IVR in vitrectomized and nonvitrectomized rabbit eyes and showed that the concentration of ranibizumab in both groups was not significantly different at 30 days after intravitreal injection. In contrast, Lee et al. [16] showed that the half-life of human recombinant VEGF (hVEGF) in the vitreous cavity was 10 times shorter in vitrectomized eyes, and hVEGF clearance increased after vitrectomy.

IVR is a safe and effective treatment in patients with diabetic macular edema. [17] However, the mechanisms of elimination of ranibizumab and other drugs in the vitreous cavity are not fully understood. [15,18-20] The high molecular weights of the drugs are an important factor in human and rabbit vitreous, which affects the half-life of the drug. For example, the half-life of low molecular weight drugs (molecular weight < 1,000) is between 2 and 10 hours, while the half-life of antibody fragments (molecular weight ≈ 48,000) is 2-3 days. [21,22] Unlike similar molecular weight substances, the half-life of hVEGF (molecular weight 42,000) in vitreous was less than 3 hours. [16]
This suggests that rapid cleaning mechanisms exist to regulate the levels of the hVEGF molecule in the vitreous cavity. Supporting this, in our study no significant difference was found between vitrectomized and nonvitrectomized eyes at the end of the 2 years for BCVA, CMT, TMV, and total number of IVR injections.

In a recent study, Chen et al. [3] retrospectively compared the efficacy of IVR in vitrectomized and nonvitrectomized eyes in 148 patients with DME for up to 6 months. They reported significantly improved BCVA and central foveal thickness in nonvitrectomized eyes than in vitrectomized eyes. [3] Koyanagi et al. [4] compared the efficacy of IVR in 10 vitrectomized and 15 nonvitrectomized eyes and reported no significant differences in the mean changes of BCVA and CMT between both groups at 6 months. Our short-term (6-month) findings in favor of nonvitrectomized eyes were in line with these reports. We found that in the vitrectomized group BCVA, CMT, and TMV significantly improved at the months 18 and 24 visits, similar to the nonvitrectomized group; improvement in all parameters was recorded in all visits starting at month 6. Similar to our findings, Bressler et al. [23] demonstrated that a comparable improvement was achieved in both vitrectomized and nonvitrectomized eyes treated with ranibizumab for DME at the end of one year.

Chen et al. [3] reported that the number of IVR injections was significantly less in nonvitrectomized than in vitrectomized eyes (4.1±0.6 vs. 5.1±0.7, respectively; p<0.001) during a 6-month period. In the study by Koyanagi et al. [4], the number of IVR injections during the 6-month period was similar in both nonvitrectomized and vitrectomized eyes (4.5±1.2 vs. 4.9±1.3, respectively; p=0.484). In our study, the number of IVR injections until month 6 was significantly higher in nonvitrectomized eyes than vitrectomized eyes (3.2 vs. 1.3, respectively; p=0.04), which was probably due to the loading dose applied to the nonvitrectomized eyes in the first three months. Since there was no standardized treatment regimen for IVR injections at the time of the study, vitrectomized eyes were given IVR with PRN regimen without any loading dose.

The main limitation of our study is its small sample size which precludes us from reaching more definitive conclusions on the long-term effectiveness of IVR in vitrectomized and nonvitrectomized eyes in patients with DME. However, this is the first study with up to a 24-month follow-up in the
literature comparing the effectiveness of IVR in vitrectomized and nonvitrectomized eyes. Further large-scale prospective and long-term studies are needed to confirm our findings.

Conclusions
In conclusion, although the response to treatment was obtained later in vitrectomized eyes compared to nonvitrectomized eyes, IVR injection treatment for DME is equally effective in both vitrectomized and nonvitrectomized eyes in the long-term.

Abbreviations
Antivascular endothelial growth factor: anti-VEGF; best-corrected visual acuity: BCVA; central macular thickness: CMT; diabetic macular edema: DME; Early Treatment Diabetic Retinopathy Study: ETDRS; hemoglobin A1c: HbA1c; human recombinant VEGF: hVEGF; internal limiting membrane: ILM; intravitreal ranibizumab: IVR; optical coherence tomography: OCT; pars plana vitrectomy: PPV; proliferative diabetic retinopathy: PDR; pro re nata: PRN; retinal pigment epithelium: RPE; retinal volume: RV; total macular volume: TMV.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Ethics Committee of Bahcesehir University (Mar/20th/2019; 2019-06/01) and conducted in accordance with the latest version of the Declaration of Helsinki. Since our study is ‘retrospectively registered’ consent to participate is not applicable.

Consent for publication: Not applicable

Availability of data and material: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests: The authors declare that they have no competing interests.

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Figures
Figure 1

The mean number of intravitreal ranibizumab (IVR) injections given to the study groups during 24 months of follow-up. P values indicate the outcome of statistical testing for the comparison between groups for the corresponding time of evaluation.
Figure 2

The mean visual acuity (in logMAR) of the study groups during 24 months of follow-up. P values indicate the outcome of statistical testing for the comparison between groups for the corresponding point of time. Accordingly, the difference in visual acuity between the groups was significant only in month 6 (p=0.043). Compared to the baseline values, improvement in visual acuity was significant in months 6, 12, 18, and 24 in the nonvitrectomized group (p=0.004, p=0.019, p=0.011, and p=0.011, respectively). In the vitrectomized group visual acuity improved significantly only in months 18 and 24 (p=0.014 and p=0.047).
Figure 3
The mean central macular thickness (µ) of the study groups during 24 months of follow-up. The difference in central macular thickness between the groups was not significant during the study (p>0.05 for all points of measurement). Compared to the baseline values, improvement in central macular thickness was significant in months 6, 12, 18, and 24 in the nonvitrectomized group (p=0.031, p=0.019, p=0.023, and p=0.008, respectively). However, in the vitrectomized group central macular thickness improved significantly only in months 18 and 24 (p=0.006 and p=0.047).
Figure 4

The mean total macular volume (mm³) of the study groups during 24 months of follow-up. The difference in total macular volume between the groups was not significant during the study (p>0.05 for all points of measurement). Compared to the baseline values, improvement in total macular volume was significant in months 6, 12, 18, and 24 in the nonvitrectomized group (p=0.019, p=0.002, p=0.006, and p=0.001, respectively). In the vitrectomized group the total macular volume improved significantly only in months 18 and 24 (p=0.033 and p=0.013).