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Comparison of the effects of intravitreal ranibizumab and aflibercept on retinal vessel diameters in patients with diabetic macular edema

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ABSTRACT

Purpose: To evaluate and compare the effects of intravitreal ranibizumab and aflibercept treatment on retinal vessel diameters in patients with diabetic macular edema (DME).

Methods: Thirty initial-treatment naïve patients with DME who received three loading doses at monthly intervals of intravitreal ranibizumab or aflibercept were retrospectively reviewed. The diameters of the central retinal artery and vein sections at a distance of 1500 microns from the optical disc boundary were measured and evaluated at baseline and after the first, second, and third month of the treatment, using infrared images from optical coherence tomography (OCT) (Heidelberg Engineering, Heidelberg, Germany).

Results: In the superotemporal artery (STA) measurements, the mean basal vessel diameter decreased from 110.00 ± 17.25 μm to 102.60 ± 16.90 μm ($p = 0.001$) in the third month of the treatment in the ranibizumab group. In the aflibercept group, measurements of the basal STA vessel diameter decreased from 110.20 ± 21.25 μm to 103.93 ± 19.03 μm ($p = 0.001$) at the third month. The mean basal inferotemporal artery (ITA) vessel diameter was significantly decreased at the third month in both ranibizumab ($p = 0.001$) and aflibercept groups ($p = 0.005$). In the superotemporal vein (STV) and inferotemporal vein (ITV) measurements, vessel diameters were found significantly decreased at the end of the third month compared with basal measurements in both the ranibizumab ($p = 0.001$; $p = 0.001$, respectively) and aflibercept ($p = 0.001$; $p = 0.004$, respectively) treatment groups. The retinal vessel measurements were not found to differ significantly between the two intravitreal treatment agents ($p > 0.05$).

Conclusion: Both intravitreal ranibizumab and aflibercept agents cause a significant narrowing in the retinal vessel diameters in patients with DME after three loading doses at monthly intervals.

1. Introduction

Diabetic retinopathy, a common complication of diabetes mellitus, causes damage to the capillary, arterioles, and venules of the retina even in its early stages when patients rarely have any complaints about their vision [1]. However, early diabetic retinopathy progresses to diabetic macular edema (DME) –the primary cause of vision loss in diabetic retinopathy– within 10–20 years of a diabetes diagnosis in up to one-third of patients [2]. The treatment choices for patients with DME are: grid laser, intravitreal anti-vascular endothelial growth factor (anti-VEGF), intravitreal corticosteroids, and different combinations of these treatments [3].

Intravitreal anti-VEGF therapy is effective because VEGF is the most important angiogenic factor in retinal diseases that progress with vascular permeability and neovascularization [4,5]. Decreasing VEGF levels and inflammatory mediators, combined with intravitreal anti-VEGF injections, have been shown to decrease central macular thickness (CMT), re-establish the blood–retina barrier, reduce retinal edema, and consequently improve visual acuity in DME [6,7]. Ranibizumab (Lucentis®; Genentech Inc., San Francisco, CA, USA) and aflibercept (Eylea®; Regeneron Pharmaceuticals, New York, NY, USA) are anti-VEGF agents used to treat DME. Ranibizumab is a short-acting antigen-binding fragment (Fab) without the fragment crystallizable region (Fc) of a recombinant, humanized, anticlonal antibody produced for intraocular use to inhibit isoforms of VEGF-A [8,9]. Aflibercept is a recombinant 115-kDa fusion protein created via the fusion of

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extracellular VEGFR-1 and VEGFR-2, and the Fc of human immunoglobulin G1 [10]. It binds VEGF-A, VEGF-B, and placenta growth factor (PIGF) with a high affinity [11].

Although the intravitreal anti-VEGF treatments have been demonstrated to be beneficial in the treatment of DME, the physiologic functions of VEGF (such as increased production of nitric oxide (NO), a well-known vasodilator) may also be restricted, causing unwanted effects that could disrupt the regulation of retinal vessel diameters and blood flow in addition to other functions [12–14].

The vasoconstrictor effect of intravitreal anti-VEGF treatments on the retinal vessels could primarily lead to ocular blood flow disruption. Hence, we hypothesized that the retinal vessel diameters might be altered after these treatments [15]. This study aimed to compare retinal vessel diameter changes after administering three consecutive monthly loading doses of two different intravitreal anti-VEGF agents in patients with DME.

2. Methods

This study was carried out following the principles of the Helsinki Declaration. Informed consent for inclusion and interventions in the study were obtained from all patients. The institutional medical ethics committee approved the study protocol.

2.1. Study participants and protocol

Patients who were treatment naïve and received three consecutive monthly loading doses intravitreal ranibizumab or aflibercept injection for DME diagnosis between January 2017 and September 2018 at the Ophthalmology Department, Haydarpasa Numune Training and Research Hospital, Health Sciences University, were evaluated retrospectively. All patients had been diagnosed with type 2 diabetes mellitus and were under systemic insulin and oral antidiabetic drugs treatment. The presence and severity of diabetic retinopathy were evaluated using Early Treatment Diabetic Retinopathy Study (ETDRS) protocols, and patients with moderate and severe non-proliferative diabetic retinopathy were included in the study [16]. Exclusion criteria were: any ocular comorbidity or systemic disease, patients who had previously undergone laser photocoagulation therapy, patients who did not attend regular three-month follow-up and optical coherence tomography (OCT) shoots (including pre-treatment), patients who had received anti-VEGF treatment at a different center before being admitted to the ophthalmology department, and patients who had undergone any eye surgery other than cataract surgery.

A total of 30 patients who met the inclusion and exclusion criteria and had received one of the two treatment options were evaluated. The 15 eyes of 15 patients who received consecutive three doses of intravitreal 0.5 mg / 0.05 ml ranibizumab monthly after initial DME diagnosis assigned to one group (the ranibizumab group) and the 15 eyes of 15 patients who received three consecutive doses of intravitreal 2 mg / 0.05 ml aflibercept injection monthly assigned to a second group (the aflibercept group). All patients underwent ocular examinations, including refractive error measurements, best-corrected visual acuity (logMAR), slit-lamp biomicroscopy, dilated fundus examination, Goldmann applanation tonometry and OCT (Spectralis®, Heidelberg, Germany) examination before treatment (baseline), at the first, second, and third month of the intravitreal treatments.

2.2. Imaging protocols and measurements

The OCT images were used for evaluating retinal vessel diameter measurements. An OCT scan protocol was used after using a 3.46 mm circular scan focused on the optical disc center. During these procedures, infrared (IR) reflectance images were simultaneously acquired, and the measurements being done using digital calipers present in the Heidelberg Spectralis (Heidelberg Eye Explorer version 1.7.1.0) OCT software. Vessel diameters were measured from an optic disc diameter distance, 1500 microns from the optic disc boundary (Fig. 1). Superior temporal artery (STA), inferior temporal artery (ITA), superior temporal vein (STV) and inferior temporal vein (ITV) diameters were measured separately. Results were obtained at baseline (pre-treatment) and at the first, second, and third months and compared between the two groups.

The central macular thickness (CMT) was measured using the EUROMED Migration V (EMM5) program and three-dimensional macula scanning programs. The macular foveal thickness (MFT) (μm) and volume (MFV) (mm3) were analyzed and recorded.

All retinal vessel diameter measurements were analyzed twice by two independent researchers, and the inter- and intraobserver reproducibility of measurements was evaluated. The comparison revealed a Spearman correlation coefficient of > 0.90 in all measurements.

2.3. Statistical analysis

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, UT, USA) program was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, and maximum) were used to assign study data. The Shapiro-Wilk test tested the suitability of quantitative data for normal distribution. The Student's *t*-test was used to compare two groups of normally distributed quantitative data, and the Mann Whitney *U* test was used for two-group comparisons of non-normally distributed data. The Pearson chi-square test, Fisher's exact test, and the Fisher-Freeman Halton test were used in the analysis of qualitative data. A repeated measures test (variance analysis in repeated measurements) with the Bonferroni correction was used to evaluate paired variables. The Friedman test was used in variables that were not normally distributed, and the Bonferroni-Dunn test was used to evaluate binary comparisons. Statistical significance was set at a p-value of < 0.05.

3. Results

The study groups' mean age was 61.27 ± 8.81 (48–81) years. Table 1 presents the demographic characteristics of the study groups.

The visual acuity of the two groups was not found statistically different at baseline and during the treatment period ($p > 0.05$) (Table 2). In the aflibercept group, the visual acuity improvement in the second and third months improved was statistically significant compared to baseline values ($p = 0.001$; $p < 0.01$, respectively). In the ranibizumab group, the visual acuity improved significantly in the third month of the treatment compared with baseline values ($p = 0.005$). In the aflibercept group, visual acuity improved significantly more than in the ranibizumab group at the second month compared to baseline ($p = 0.039$).

The CMT measurements did not show a significant difference between the two groups at baseline (pre-treatment) and throughout the treatment period ($p > 0.05$); nor did they show a significant decrease in all the follow-up period when compared with baseline measurements in both groups ($p < 0.05$) (Table 3).

The mean intraocular pressure (IOP) values in the ranibizumab group were: 13.80 ± 1.20 before treatment, 13.8 ± 1.32 in the first month, 13.53 ± 0.99 in the second, and 13.87 in the third month. In the aflibercept group, these values were: 13.86 ± 1.35, 14.0 ± 1.06, 13.66 ± 1.04, and 13.8 ± 1.01, respectively. There were no significant differences in terms of IOP measurements in any follow-up period ($p > 0.05$).

3.1. Retinal vessel diameter measurements

3.1.1. STA and ITA diameter

Significant decreases were found in the STA diameter at the first month, the second month, and the third month compared to pre-treatment measurements ($p = 0.012$, $p = 0.001$, $p = 0.001$,

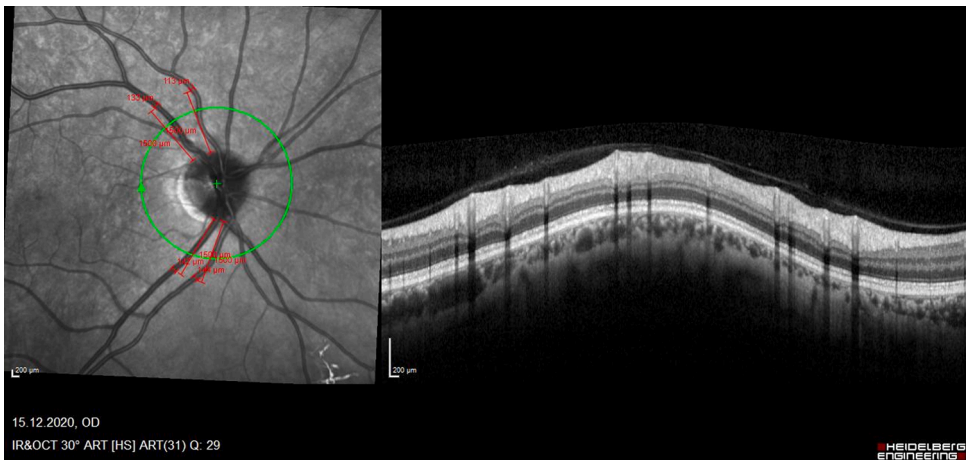

Fig. 1. Infrared image of retinal vessel diameter measurement.

Table 1
Demographic Features of the Groups.

		Total (n = 30)	Agents		*p*
			Aflibercept (n = 15)	Ranibizumab (n = 15)	
Age (year)	*Min/Max (Median)*	48/ 81 (62)	48/ 81 (63)	48/ 75 (62)	[a]0,571
	Mean ± Sd	61,27 ± 8,81	62,20 ± 9,55	60,33 ± 8,23	
Gender	Women	9 (30,0)	5 (33,3)	4 (26,7)	[b]1,000
	Men	21 (70,0)	10 (66,7)	11 (73,3)	
Systolic Blood Pressure	*Min/Max (Median)*	110/ 136 (123,5)	110/ 133 (124)	110/ 136 (123)	[a]0,709
	Mean ± Sd	123,47 ± 6,68	123,00 ± 6,82	123,93 ± 6,75	
Diastolic Blood Pressure	*Min/Max (Median)*	64/ 90 (81)	64/ 88 (81)	69/ 90 (81)	[a]0,404
	Mean ± Sd	80,80 ± 6,00	79,87 ± 7,13	81,73 ± 4,68	
%HbA1c levels	*Min/Max (Median)*	6,1/ 11,7 (7,5)	6,1/ 10,6 (7,4)	6,2/ 11,7 (7,5)	[a]0,976
	Mean ± Sd	8,30 ± 1,77	8,29 ± 1,67	8,31 ± 1,92	
NPDR Severity	Moderate	15 (50,0)	7 (46,7)	8 (53,3)	[c]0,715
	Severe	15 (50,0)	8 (53,3)	7 (46,7)	
Side	Right	16 (53,3)	7 (46,7)	9 (60,0)	[c]0,464
	Left	14 (46,7)	8 (53,3)	6 (40,0)	

[a] Student *t* Test.
[b] Fisher'sExact Test.
[c] Pearson Ki-kare Test.

respectively) in the ranibizumab group. In the aflibercept group, the mean STA diameter before treatment was 110.20 ± 21.25 μm, decreasing to 103.93 ± 19.03 μm ($p = 0.002$) at the third month (Table 4).

Compared to pre-treatment measurements, decreases in the mean ITA diameter at one, two, and three months were found statistically significant ($p = 0.014$, $p = 0.023$, $p = 0.001$, respectively) in the ranibizumab group. In the aflibercept group, the decrease in the mean ITA diameter (compared to pre-treatment) was significant only at third month ($p = 0.009$) (Table 5).

3.1.2. STV and ITV diameter

Decreases in the STV diameter compared to pre-treatment values were significant at the second and third month in both the ranibizumab ($p = 0.006$ and $p = 0.001$, respectively) and aflibercept groups ($p = 0.046$ and $p = 0.001$, respectively) (Table 6).

In the ranibizumab group, the mean ITV diameter decreased from 156.54 ± 20.75 μm to 146.57 ± 17.45 μm ($p = 0.001$) in the third month. Only the third-month measurements of ITV diameters in the aflibercept group were found to be significantly lower than the basal values ($p = 0.018$) (Table 7).

4. Discussion

Decreasing the VEGF level and inflammatory mediators with anti-VEGF injection reduce retinal edema and helps the blood-retinal barrier regenerate. However, the anti-VEGF treatment also impairs the physiological functions of VEGF. Anti-VEGF treatment can inhibit VEGF-induced NO production [12]. The current study aimed to determine vasoconstriction characteristics in various retinal vessels utilizing OCT images following two different intravitreal anti-VEGF treatment in patients with DME.

Previously, Ouyang et al. emphasized that results obtained from infrared images are compatible with previous histopathology studies, and the potential for accuracy of retinal vascular measurement using this technique is high [17]. In the present study, infrared images of 1500 μm diameter to the optical disc were utilized to determine the retinal vessel diameters in patients with DME who received three consecutive monthly loading doses. In the ranibizumab group, the retinal artery vessel diameters showed a significant reduction at the first, second, and third months of the follow-up periods when compared to the baseline values ($p < 0.05$). In the aflibercept group, the STA diameter was reduced significantly at second and third months, as well as the ITA diameter, which was reduced only at the third month compared with baseline measurements. Reported studies have employed various methods of retinal vessel diameter measurements in DME patients who received anti-VEGF treatments. Sabaner et al. administered three doses of intravitreal aflibercept to 29 eyes of 29 patients with DME. They used a software program for fundus photographs as a measurement method, calculating the mean central retinal artery equivalent (CRAE) and

Table 2
Visual Acuity of the Groups.

Visual Acuity (logMAR)		Total (n = 30)	Agents		[a]p
			Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Visual Acuity in Follow-Up					
Before Treatment (BT)	*Min/Max (Median)*	0,1/ 1,5 (0,5)	0,1/ 1,3 (0,5)	0,1/ 1,5 (0,7)	0,983
	Mean ± Sd	0,62 ± 0,40	0,60 ± 0,37	0,63 ± 0,44	
1.Month	*Min/Max (Median)*	0/ 1,5 (0,4)	0/ 1,5 (0,4)	0/ 1,5 (0,5)	0,502
	Mean ± Sd	0,48 ± 0,42	0,45 ± 0,41	0,51 ± 0,43	
2. Month	*Min/Max (Median)*	0/ 1,5 (0,2)	0/ 1,5 (0,2)	0/ 1,3 (0,5)	0,141
	Mean ± Sd	0,39 ± 0,39	0,31 ± 0,41	0,47 ± 0,37	
3. Month	*Min/Max (Median)*	0/ 1,5 (0,2)	0/ 1,5 (0,1)	0/ 1,3 (0,4)	0,105
	Mean ± Sd	0,33 ± 0,39	0,26 ± 0,43	0,40 ± 0,35	
	[b]***p***	0,001**	0,001**	0,001**	
%Change					
BT - 1. Month	*Min/Max (Median)*	−100/ 100 (0)	−100/ 100 (0)	−100/ 0 (0)	0,897
	Mean ± Sd	−22,52 ± 42,98	−21,13 ± 53,49	−23,91 ± 30,99	
	[c]***p***	0,273	1,000	0,463	
BT - 2. Month	*Min/Max (Median)*	−100/ 15,4 (−50)	−100/ 15,4 (−60)	−100/ 0 (−28,6)	0,039*
	Mean ± Sd	−45,38 ± 34,09	−57,95 ± 29,23	−32,80 ± 34,84	
	[c]***p***	0,001**	0,007**	0,142	
BT - 3. Month	*Min/Max (Median)*	−100/ 15,4(−56,7)	−100/ 15,4 (−75)	−100/ 0 (−40)	0,062
	Mean ± Sd	−55,30 ± 37,82	−68,73 ± 32,48	−41,86 ± 38,99	
	[c]***p***	0,001**	0,001**	0,005**	

Bold p values are statistically significant.
[a] MannWhitney *U* test.
[b] Friedman Test.
[c] Bonferroni Dunn Test.
** $p < 0.01$.
* $p < 0.05$.

Table 3
Macular Thickness Measurements of Groups.

Macular Thickness(μm)		Total (n = 30)	Agents		[a]p
			Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Measurements in Follow-Up					
Before Treatment (BT)	*Min/Max (Median)*	277/ 652 (368)	278/589(383)	277/ 652 (359)	0,740
	Mean ± Sd	413,67 ± 113,84	413,33 ± 97,94	414,00 ± 131,35	
1. Month	*Min/Max (Median)*	249/ 485 (324,5)	263/ 480 (329)	249/ 485 (317)	0,967
	Mean ± Sd	341,77 ± 67,76	340,00 ± 62,26	343,53 ± 75,02	
2. Month	*Min/Max (Median)*	246/ 490 (315,5)	257/ 479 (321)	246/ 490 (310)	0,934
	Mean ± Sd	334,87 ± 69,35	334,87 ± 67,62	334,87 ± 73,42	
3. Month	*Min/Max (Median)*	237/ 468 (309)	254/ 422 (324)	237/ 468 (290)	0,836
	Mean ± Sd	321,93 ± 63,24	320,07 ± 52,53	323,80 ± 74,28	
	[b]***p***	0,001**	0,001**	0,001**	
% Change					
BT- 1. Month	*Min/Max (Median)*	−45,9/12,7(−12,4)	−45,9/ −0,3(−12,6)	−35,6/12,7(−10,3)	0,983
	Mean ± Sd	−14,98 ± 13,05	−15,62 ± 13,93	−14,35 ± 12,55	
	[c]***p***	0,001**	0,009**	0,014*	
BT- 2. Month	*Min/Max (Median)*	−48,4/ 15 (−13,6)	−45,7/ 0 (−13,6)	−48,4/ 15 (−12,1)	0,772
	Mean ± Sd	−16,35 ± 15,06	−17,05 ± 14,05	−15,66 ± 16,47	
	[c]***p***	0,001**	0,001**	0,018*	
BT- 3. Month	*Min/Max (Median)*	−46,9/ 29,6 (−15)	−46,9/ −3,4(−14,4)	−45,1/29,6(−16,8)	0,820
	Mean ± Sd	−19,16 ± 16,58	−20,05 ± 14,82	−18,26 ± 18,65	
	[c]***p***	0,001**	0,001**	0,001**	

Bold p values are statistically significant.
[a] MannWhitney *U* test.
[b] Friedman Test.
[c] Bonferroni Dunn Test.
** $p < 0.01$.
* $p < 0.05$.

central retinal vein equivalent (CRVE), and reported a statistically significant difference between the baseline and the third month in the CRAE measurements but no significant difference in the CRVE measurements [18]. In the current study, significant decreases were found in the STV and ITV diameters compared to baseline values with both two treatment agents ($p < 0.05$).

Kurt et al. reported the CRAE and CRVE values significantly decreased at one week and one month after a single dose of ranibizumab and bevacizumab treatment [19]. These results showed that even a single intravitreal anti-VEGF injection could cause a narrowing of the retinal vasculature in patients with DME. Soliman et al. examined changes in the retinal vessel diameter using fluorescein angiographies in recipients of anti-VEGF treatments. They reported that ten patients with DME showed a tendency towards vasoconstriction four months after the first intravitreal bevacizumab injection; however, the comparison with baseline values did not show significant levels of reduction [20].

Table 4
STA Diameter Infrared Measurements According to Groups.

		STA Diameter Measurements (μm)			p
		Total (n = 30)	Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Measurements in Follow-Up					
Before Treatment	*Min/Max (Median)*	59/ 156 (110,5)	80/ 156 (111)	59/ 140 (110)	[a]0,978
	Mean ± Sd	110,10 ± 19,02	110,20 ± 21,25	110,00 ± 17,25	
1. Month	*Min/Max (Median)*	59/ 156 (104)	83/ 156 (102)	59/ 138 (104)	[a]0,892
	Mean ± Sd	106,07 ± 18,38	106,53 ± 20,43	105,60 ± 16,80	
2. Month	*Min/Max (Median)*	58/ 154 (103)	82/ 154 (104)	58/ 136 (103)	[a]0,695
	Mean ± Sd	104,77 ± 17,69	106,07 ± 19,20	103,47 ± 16,61	
3. Month	*Min/Max (Median)*	58/ 148 (101,5)	79/ 148 (101)	58/ 136 (102)	[a]0,841
	Mean ± Sd	103,27 ± 17,70	103,93 ± 19,03	102,60 ± 16,90	
	[d]***p***	0,001**	0,001**	0,001**	
% Change					
BT- 1. Month	*Min/Max (Median)*	−16,9/ 5 (−2,8)	−16,9/ 5 (−2)	−15,5/ 0 (−3,7)	[b]0,442
	Mean ± Sd	−3,46 ± 5,01	−3,03 ± 6,03	−3,89 ± 3,90	
	[c]***p***	0,003**	0,321	0,012*	
BT- 2. Month	*Min/Max (Median)*	−17,2/ 2,5 (−4,8)	−12,7/ 2,5 (−3,4)	−17,2/ −1,7 (−5,6)	[b]0,106
	Mean ± Sd	−4,61 ± 4,31	−3,37 ± 4,56	−5,84 ± 3,80	
	[c]***p***	0,001**	0,048*	0,001**	
BT- 3. Month	*Min/Max (Median)*	−18,1/ 4,4 (−5,5)	−14,4/ 4,4 (−5,4)	−18,1/ −1,7 (−5,7)	[b]0,756
	Mean ± Sd	−6,02 ± 4,28	−5,38 ± 4,37	−6,66 ± 4,23	
	[c]***p***	0,001**	0,002**	0,001**	

Bold p values are statistically significant.
[a] Student *t* Test.
[b] MannWhitney *U* test.
[c] BonferroniDunn Test.
[d] RepeatedMeasures Test.
** $p < 0.01$.
* $p < 0.05$.

Table 5
ITA Diameter Infrared Measurements According to Groups.

		ITA Diameter Measurements (μm)			p
		Total (n = 30)	Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Measurements in Follow-Up					
Before Treatment	*Min/Max (Median)*	83/ 142 (109,5)	93/ 142 (110)	83/ 141 (101)	[a]0,329
	Mean ± Sd	109,47 ± 15,45	112,27 ± 12,27	106,67 ± 18,09	
1. Month	*Min/Max (Median)*	79/ 141 (105)	96/ 141 (107)	79/ 136 (100)	[a]0,263
	Mean ± Sd	106,33 ± 15,10	109,47 ± 12,39	103,20 ± 17,26	
2. Month	*Min/Max (Median)*	77/ 139 (104,5)	96/ 139 (107)	77/ 135 (96)	[a]0,244
	Mean ± Sd	105,77 ± 14,05	108,80 ± 11,31	102,73 ± 16,16	
3. Month	*Min/Max (Median)*	75/ 139 (104)	94/ 139 (107)	75/ 132 (98)	[a]0,178
	Mean ± Sd	104,23 ± 14,88	107,93 ± 11,60	100,53 ± 17,18	
	[d]***p***	0,001**	0,005**	0,001**	
% Change					
BT- 1. Month	*Min/Max (Median)*	−10,9/ 5,6 (−3,2)	−10,9/ 3,2 (−1,9)	−9,7/ 5,6 (−3,4)	[b]0,290
	Mean ± Sd	−2,81 ± 3,32	−2,47 ± 3,39	−3,16 ± 3,33	
	[c]***p***	0,001**	0,064	0,014*	
BT- 2. Month	*Min/Max (Median)*	−10,5/ 5,6 (−3,7)	−8,5/ 4,9 (−2,9)	−10,5/ 5,6 (−4,3)	[b]0,917
	Mean ± Sd	−3,20 ± 3,98	−2,95 ± 3,99	−3,44 ± 4,10	
	[c]***p***	0,001**	0,051	0,023*	
BT- 3. Month	*Min/Max (Median)*	−10,9/ 2 (−5,2)	−9,4/ 2 (−2,9)	−10,9/ 1 (−5,5)	[b]0,171
	Mean ± Sd	−4,73 ± 3,86	−3,77 ± 3,80	−5,7 ± 3,81	
	[c]***p***	0,001**	0,009**	0,001**	

Bold p values are statistically significant.
[a] Student *t* Test.
[b] MannWhitney *U* test.
[c] BonferroniDunn Test.
[d] RepeatedMeasures Test.
** $p < 0.01$.
* $p < 0.05$.

Tatlipinar et al. also evaluated the short-term effects of a single intravitreal bevacizumab injection on the retinal vasculature of eight patients with DME. Although there was a tendency toward vasoconstriction, their results also did not demonstrate statistical significance [21]. Similarly, Terrai et al. showed that intravitreal ranibizumab injection decreased vessel diameters, especially arterial diameters, in the 30 DME patients, but significant differences were not found [22]. The authors noted that this result might be associated with the advanced age of

Table 6
STV Diameter Infrared Measurements According to Groups.

		STV Diameter Measurements (μm)			p
		Total (n = 30)	Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Measurements in Follow-Up					
Before Treatment	*Min/Max (Median)*	105/ 193 (146)	108/ 193 (154)	105/ 181 (139,5)	[a]0,241
	Mean ± Sd	144,83 ± 23,21	149,47 ± 23,98	139,86 ± 22,12	
1. Month	*Min/Max (Median)*	101/ 194 (141)	108/ 194 (143)	101/ 167 (133,5)	[a]0,135
	Mean ± Sd	140,97 ± 22,71	146,93 ± 23,55	134,57 ± 20,69	
2. Month	*Min/Max (Median)*	101/ 193 (137)	107/ 193 (145)	101/ 166 (132,5)	[a]0,163
	Mean ± Sd	139,83 ± 22,41	145,33 ± 24,06	133,93 ± 19,66	
3. Month	*Min/Max (Median)*	101/ 191 (137)	109/ 191 (145)	101/ 167 (130,5)	[a]0,166
	Mean ± Sd	138,72 ± 22,17	144,07 ± 23,77	133,00 ± 19,55	
	[d]***p***	0,001**	0,001**	0,001**	
% Change					
BT- 1. Month	*Min/Max (Median)*	−13/ 8 (−1,6)	−7,2/ 8 (−1,3)	−13/ 3,3 (−3)	[b]0,329
	Mean ± Sd	−2,47 ± 4,04	−1,59 ± 3,76	−3,34 ± 4,24	
	[c]***p***	0,010*	0,556	0,052	
BT- 2. Month	*Min/Max (Median)*	−11,6/ 1,9 (−2,6)	−10,5/ 1,9 (−2,2)	−11,6/ 0 (−3,5)	[b]0,383
	Mean ± Sd	−3,25 ± 3,28	−2,75 ± 3,27	−3,76 ± 3,32	
	[c]***p***	0,001**	0,046*	0,006**	
BT- 3. Month	*Min/Max (Median)*	−11,6/ 1,3 (−4,4)	−7,7/ 1,3 (−4,4)	−11,6/ 0,9 (−4,4)	[b]0,443
	Mean ± Sd	−4,06 ± 2,86	−3,60 ± 2,72	−4,52 ± 3,02	
	[c]***p***	0,001**	0,001**	0,001**	

Bold p values are statistically significant.
[a] Student *t* Test.
[b] MannWhitney *U* test.
[c] BonferroniDunn Test.
[d] Repeated Measures Test.
** $p < 0.01$.
* $p < 0.05$.

Table 7
ITV Diameter Infrared Measurements According to Groups.

		ITV Diameter Measurements (μm)			p
		Total (n = 30)	Aflibercept (n = 15)	Ranibizumab (n = 15)	
Comparison of Measurements in Follow-Up					
Before Treatment	*Min/Max (Median)*	126/ 205 (157)	126/ 194 (161)	131/ 205 (152,5)	[a]0,307
	Mean ± Sd	159,72 ± 19,85	162,60 ± 19,24	156,64 ± 20,75	
1. Month	*Min/Max (Median)*	131/ 199 (153)	131/ 185 (158)	131/ 199 (146,5)	[a]0,174
	Mean ± Sd	154,59 ± 17,12	158,20 ± 15,87	150,71 ± 18,13	
2. Month	*Min/Max (Median)*	123/ 197 (153)	123/ 180 (159)	128/ 197 (143)	[a]0,226
	Mean ± Sd	152,03 ± 16,63	155,07 ± 15,24	148,79 ± 17,99	
3. Month	*Min/Max (Median)*	121/ 193 (148)	121/ 180 (157)	126/ 193 (140,5)	[a]0,150
	Mean ± Sd	150,62 ± 17,09	154,40 ± 16,42	146,57 ± 17,45	
	[d]***p***	0,001**	0,004**	0,001**	
% Change					
BT- 1. Month	*Min/Max (Median)*	−12,6/ 4 (−2,2)	−10,3/ 4 (−1,4)	−12,6/ 1,5 (−2,7)	[b]0,755
	Mean ± Sd	−2,82 ± 4,31	−2,43 ± 4,35	−3,20 ± 4,39	
	[c]***p***	0,005**	0,215	0,071	
BT- 2. Month	*Min/Max (Median)*	−14,4/ 4,6 (−2,6)	−14,4/ 4,6 (−2,1)	−12/ −0,8 (−3)	[b]0,520
	Mean ± Sd	−4,41 ± 4,66	−4,29 ± 5,55	−4,54 ± 3,77	
	[c]***p***	0,001**	0,066	0,004**	
BT- 3. Month	*Min/Max (Median)*	−15,5/ 2,6 (−4,8)	−15,5/ 2,6 (−2,9)	−15,4/ -1,4 (−5,4)	[b]0,351
	Mean ± Sd	−5,43 ± 4,27	−4,82 ± 4,80	−6,04 ± 3,75	
	[c]***p***	0,001**	0,018*	0,001**	

Bold p values are statistically significant.
[a] Student *t* Test.
[b] MannWhitney *U* test.
[c] Bonferroni Dunn Test.
[d] Repeated Measures Test.
** $p < 0.01$.
* $p < 0.05$.

subjects in their study group, which could cause limited changes in vessel diameters due to sclerotic vessels [22].

To the best of our knowledge, the present study compares for the first time the effects of intravitreal ranibizumab and aflibercept agents on retinal vessel diameter measurements, finding that retinal artery diameters were more affected at the first, second, and third months, especially in the ranibizumab group, when compared to the baseline values during the follow-up period. The half-life of anti-VEGF drugs was

measured in human vitreous tissue, and it was estimated that the half-life of ranibizumab in such tissue was 4.75 days, whereas the aflibercept half-life was 7.13 days [23]. Molecules such as aflibercept, which contain Fc fragments, bind to the endothelium's Fc receptors for IgG (FcRn receptors) and protect themselves from destruction by the endosome, thereby prolonging their half-life [24]. Ranibizumab has fast systemic clearance since it does not contain any Fc domains. While ranibizumab is quickly removed from the system, aflibercept remained in systemic circulation for a longer duration [25]. Ranibizumab penetrates the retina via intercellular clefts, whereas aflibercept was taken up by ganglion cells, cells of the inner and outer retinal layers, and the retinal pigment epithelium [26]. Although there are molecular differences between the two agents, it seems that both agents caused the retinal vessel diameters to narrow significantly after three loading doses when compared to basal values. However, no difference between the two agents was found in these measurements. The narrowing in the retinal vessels may cause ischemia, which appears to be a risk secondary to both intravitreal ranibizumab and aflibercept injections. Future studies are needed to investigate the ocular hemodynamic parameters, with a larger number of cases to demonstrate ischemia in these patients.

Previous studies in diabetic patients have shown that veins are often more extensive than in the healthy population, and vessel diameter may increase depending on the severity of diabetes [27,28]. Thus, our study results may reflect a "return" to normal diameter after disease-induced vasodilation, rather than true vasoconstriction. The inability to compare with the healthy control group is one of the other limitations of the study. Only patients with moderate and severe NPDR were included to minimize variations.

The study results showed that intravitreal aflibercept and ranibizumab injection may induce retinal vascular narrowing, these results being largely compatible with those of previous studies. Although this is one of the few studies to report a detailed and longitudinal assessment of vascular effects with these two widely-used agents in DME, the number of patients was limited because of our inclusion/exclusion criteria. Longitudinal studies on larger cohorts validate with OCT angiography are needed to detect the possible progression of retinal vascular alterations on long-term follow-up. Another limiting characteristic is that, although patients with hypertension were excluded, systolic and diastolic blood pressure measurements were not taken.

5. Conclusion

This study shows that both intravitreal ranibizumab and aflibercept agents causes a significant narrowing in the retinal vessel diameters in patients with DME after three loading doses at monthly intervals. The clinical significance of the decrease in the retinal vessel diameter caused by intravitreal agents should be investigated with further prospective studies.

Note

The manuscript has been read and approved by all the authors.

Declaration of Competing Interest

There is no conflict of interest.

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