Original Article

Changes in the Thickness of Different Retinal Layers Following Intravitreal Injection of Bevacizumab in Patients with Diabetic Macular Edema and Its Association with Post Treatment Visual Acuity

Homayoun Nikkhah [1, 2, 3], MD; Masoome Valipoor [*1, 2], MD; Alireza Ramezani [3], MD; Saeed Karimi [1, 2], MD; Morteza Entezari [1, 4, 5], MD; Mohammad Mehdi Hatami [1, 2], MD; Ahmad Shojaei [6], MD

1. Clinical Research Development Unit, Torfeh Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Ophthalmic Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Ophthalmic Epidemiology Research Center, Research Institute for Ophthalmology and Vision Science, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Imam Hossein Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Negah Specialty Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
6. Basir Eye Health Research Center, Iran University of Medical Sciences, Tehran, Iran.

***Corresponding Author:** Masoome Valipoor

E-mail: masoomevalipoor@gmail.com

Abstract

Purpose: To investigate changes in the thickness of different retinal layers following intravitreal injection of bevacizumab in patients with diabetic macular edema (DME) and its association with post treatment visual acuity.

Patients and Methods: Patients with DME were included. Intravitreal Bevacizumab injection was administered three times, four weeks apart, after that injections were continued based on as needed protocol. Automated and manual (if needed) retinal layer segmentation was performed at the beginning of the study and at the final examination (month 6) using the SD-OCT imaging and retinal thickness was recorded in the 1, 3, and 6 mm areas of the central macula and in different quadrants.

Results: A total of 29 eyes from 29 patients were included in the study. The mean retinal thickness in the central macular area decreased from 409 ± 72 microns at the beginning of the study to 315 ± 44 microns at month six ($P < 0.001$). In the inner ring (3 mm), the retinal thickness decreased from 410 ± 44 microns to 359 ± 25 microns ($P < 0.001$). These values in the outer ring (6mm) were 342 ± 42 microns and 320 ± 31 microns, respectively ($P < 0.001$). Among these, the final visual acuity was positively correlated with significant retinal thickness reduction in the nerve fiber layer, inner plexiform layer, and outer nuclear layer as well better baseline visual acuity ($P < 0.001$).

Conclusion: The current study showed that the changes in retinal thickness after intravitreal injection of bevacizumab in DME patients occurs in both the inner and outer retina. Among these, the final visual acuity was positively correlated with significant retinal thickness reduction in the nerve fiber layer, inner plexiform layer, and outer nuclear layer as well better baseline visual acuity.

Keywords: Thickness; Retina; Bevacizumab; Diabetic Macular Edema Vision.

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Introduction

Macular edema is the most significant cause of vision loss in patients with diabetes [1]. It appears that the cause of macular edema in these patients is the breakdown of the blood-retinal barrier and fluid leakage into the intraretinal space [2]. In the last decade, the use of optical coherence tomography (OCT) in the diagnosis and treatment of exudative macular diseases, such as diabetic macular edema (DME), has been rapidly advancing [3,4]. The central macular thickness (CMT), measured by the OCT device, is the primary diagnostic tool for monitoring treatment success among these patients. However, in some studies, a weak correlation has been found between CMT and visual function in DME patients [5].

Recent advancements have increased the image resolution in OCT and enabled segmentation of different retinal layers through spectral domain OCT (SD-OCT). Studies have shown that by using imaging of the ganglion cell inner plexiform layer (GC-IPL) in the macular region, a wealth of information can be obtained regarding glaucoma and other neurodegenerative diseases [6]. Similarly diabetes is a neurodegenerative disease. The impact of intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) drugs, used in the treatment of DME, on various retinal layers has not yet been thoroughly investigated and requires further detailed study. Bevacizumab, is widely used for the treatment of DME [7]. Although it has been shown that this drug is effective in reducing retinal swelling and improving patients' vision, to the best of our knowledge, its effect on different retinal layers has not yet been thoroughly examined.

The aim of the present study was to investigate the changes in the thickness of different retinal layers following intravitreal injection of bevacizumab in patients with DME and its association with post treatment visual acuity.

Patients and Methods

This was a prospective and interventional study. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and all patients entering the study gave written consent before entering the study. Patients were selected from those attending the retina clinic at Torfeh and Imam Hossein Hospitals, Tehran, Iran between 2016 and 2017. Patients were included in the study if they met the following criteria: they had center-involved DME, defined as CMT ≥ 300 μm, and the presence of subretinal fluid or intraretinal fluid detected on OCT imaging, accompanied by reduced vision (20/400 ≤ VA ≤ 20/30). Additionally, patients had to be 18 years or older.

Patients were excluded from the study if the severity of diabetic retinopathy (DR) was more than the non-proliferative diabetic retinopathy (NPDR), Hemoglobin A1c levels of 12 or higher, required laser treatment or intraocular surgery during the study, had a history of macular laser treatment at any time in the past, or had any intravitreal injection within the past 4 months. Other exclusion criteria included the presence of macular pathology unrelated to diabetic retinopathy, such as age-related macular degeneration; any vitreomacular interface disorder, such as vitreomacular traction or epiretinal membrane; glaucoma; any history of intraocular surgery (except for patients with a history of uncomplicated cataract surgery, who could be included if 6 months had passed from their surgery). Patients with a history of deep vitrectomy at any time in the past, evidence of macular ischemia on fluorescein angiography, or a follow-up duration of less than 6 months

were also excluded.

All patients with DME who passed the inclusion and exclusion criteria underwent a full examination during their initial visit. Visual acuity was assessed using the Snellen chart, and the type and stage of retinal involvement due to diabetes were recorded. The initial examination included determining the best-corrected visual acuity (BCVA), slit-lamp examination, intraocular pressure measurement, slit-lamp biomicroscopy, and fundoscopy.

Upon enrollment, baseline SD-OCT imaging (SPECTRALIS SD-OCT; Heidelberg Engineering, Heidelberg, Germany) of the macula and fluorescein angiography were performed for all patients. If the CMT was greater than 300 microns and subretinal fluid or intraretinal fluid was present in the central macula without macular ischemia, intravitreal bevacizumab injection was administered three times, four weeks apart. Four weeks after the third injection, patients were re-examined, and the BCVA was measured. Additionally, slit-lamp examination, slit-lamp biomicroscopy, and fundoscopy were repeated. Clinical examinations were repeated at weeks 18 and 24, and SD-OCT imaging was repeated at weeks 12, 18, and 24. If center-involved DME persisted, and visual acuity was $20/400 \leq VA \leq 20/30$, the bevacizumab injection was repeated. Automated and manual (if needed) retinal layer segmentation using SD-OCT was performed at the beginning of the study and at the final examination (month 6). If any patient received fewer than three injections during the study, they were excluded from the study. The minimum follow-up duration from the start of the study was 6 months.

In the SD-OCT imaging, retinal thickness was recorded in the 1, 3, and 6 mm areas of the central macula and in different quadrants. Following retinal segmentation, the thicknesses of the retinal nerve fiber layer (RNFL), retinal ganglion cell layer (RGCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), and the retinal pigment epithelium photoreceptor (RPE-PR) complex were recorded and measured separately in each retinal quadrant. Inner retinal thickness (sum of RNFL + GCL + IPL + INL) and Outer retinal thickness (sum of OPL + ONL + RPE-PR) were also calculated.

Statistical methods

To describe the data, mean, standard deviation, median, range, frequency, and percentage were used. All analyses were conducted using SPSS 21 (Armonk, NY: IBM Corp) statistical software.

Results

A total of 29 eyes from 29 patients with center-involving DME who received intravitreal bevacizumab injections were included in the study. The mean age of the patients at the time of entry into the study was 64.92 ± 9.4 years. Twelve patients (41.4 %) were male. Nine patients (31 %) were in the mild to moderate nonproliferative diabetic retinopathy (NPDR) stage, 18 patients (62.1 %) were in the severe NPDR stage, and 2 patients (6.9 %) had regressed NPDR.

Six patients (20.7 %) had a history of previous bevacizumab injections, while 23 patients (79.3 %) had no prior injection history. None of the patients had a history of MPC laser treatment. Three patients (10.3 %) received three injections, 16 patients (55.2 %) received four injections, and 10 patients (34.4 %)

Table 1: Baseline Clinical Characteristics of Patients Included in the Study

Variable	Values
Age (Mean ± SD)	4.9 ± 92.64
Gender Male	12 (41.4 %)
Female	17 (58.6 %)
Mild to Moderate NPDR	9 (31 %)
Severe NPDR	18 (62.1 %)
Regressed NPDR	2 (6.9 %)
Prior History of Injection	
Yes	6 (20.7 %)
No	23 (79.3 %)
Number of Injection	
3 injections	3 (10.3 %)
4 injections	16 (55.2 %)
5 injections	10 (34.4 %)
Number of Injection (Mean ± SD)	0.7 ± 4.27
BCVA (Mean log Mar ± SD)	0.13 ± 0.47
CMT (Mean ± SD)	72 ± 409

NPDR: Non-Proliferative Diabetic Retinopathy; BCVA: Best Corrected Visual Acuity; CMT: Central Macular Thickness

received five injections. The average number of bevacizumab injections per patient was 4.27 ± 0.7.

As shown in table 2, the mean BCVA of the patients, measured in LogMAR units, improved from 0.47 ± 0.13 at the beginning of the study to 0.38 ± 0.12 at week 12, 0.42 ± 0.12 at week 18, and 0.32 ± 0.14 at week 24. The improvement of visual acuity at week 12 and 24 compared to the baseline was statistically significant ($P < 0.001$).

Considering changes based on early treatment diabetic retinopathy study (ETDRS) letters, at the beginning of the study, patients could read 61 ± 6 letters, which increased to 69 ± 7 letters at week 24 ($P < 0.001$) (Table 3).

The mean CMT before injection in patients was 409 ± 72 microns (ranging from 308 to 541 microns). After the injection, the CMT decreased to 355 ± 44 microns at week 12, 342 ± 35 microns at week 18, and 315 ± 44 microns by the end of week 24. As shown in table 4, the reduction in CMT at week 24 was significant compared to baseline levels ($P < 0.001$). Additionally, the reduction at week 18 compared to week 12, and at week 24 compared to week 18, was also statistically significant, indicating a continued decrease in thickness throughout the study.

As shown in table 5, the retinal thickness in the central macular area decreased from 409 ± 72 microns at the beginning of the study to 315 ± 44 microns at month six ($P < 0.001$). In the inner ring (3 mm), the retinal thickness decreased from 410 ± 44 microns to 359 ± 25 microns, which was also a significant change ($P < 0.001$). These values in the outer ring (6 mm). were 342 ± 42 microns and 320 ± 31 microns, respectively ($P < 0.001$). The average percentage reduction in retinal thickness at week 24 was 22 % in the central macular area, 12 % in the inner ring, and 6 % in the outer ring compared to baseline values. The average percentage reduction in retinal thickness in the central macular area was significantly greater than in the inner ring and outer ring ($P < 0.001$).

As mentioned in the methods section, the inner retina refers to the sum of the NFL, GCL, IPL, and INL layers, while the outer retina refers to the sum of the OPL, ONL, and RPE-PR layers. Considering these two parts and dividing each according to the ETDRS circles into central, inner ring, and outer ring, the following results were obtained, as shown in table 6. At the beginning of the study, there was a significant difference in thickness between the inner retina and the outer retina. The inner retina

Table 2: Best corrected visual acuity and its changes at baseline, week 12, week 18, and week 24 based on LogMAR

Variable			Mean ± SD	Median (range)
BCVA	Baseline	Value	0.47 ± 0.13	0.52 (0.22 to 0.7)
	wk 12	Value	0.38 ± 0.12	0.4 (0.22 to 0.7)
		Change from Base	0.09 ± 0.11	0.1 (- 0.12 to 0.4)
		P value from Base†	< 0.001	
	wk 18	Value	0.42 ± 0.12	0.4 (0.15 to 0.7)
		Change	0.09 ± 0.11	0.1 (- 0.12 to 0.4)
		P value from Base†	0.059	
		P value from wk 12†	0.216	
	wk 24	Value	0.32 ± 0.14	0.3 (0.1 to 0.7)
		Change from Base	0.15 ± 0.14	0.18 (- 0.18 to 0.4)
		P value from Base†	< 0.001	
		P value from wk 12†	0.138	
		P value from wk 18†	< 0.001	

LogMAR: Logarithm of the Minimum Angle of Resolution

† Based on paired t test

wk: week

had significantly less thickness compared to the outer retina in the central subfield, inner ring, and outer ring at the start of the study. This trend continued at week 24, where the inner retina remained significantly thinner than the outer retina in the central, inner ring, and outer ring. Additionally, the thickness of the inner retina significantly decreased at week 24 compared to baseline ($P < 0.001$). This was also observed in the outer retina ($P < 0.001$). Furthermore, the reduction in retinal thickness in the inner retina was significantly greater than in the outer retina ($P = 0.029$) in the central subfield. However, in the inner ring and outer ring, the reduction in thickness between the inner retina and outer retina did not show a significant difference.

As shown in table 7, the changes in the NFL, IPL, and ONL layers were significant in all three regions: central, inner ring, and outer ring.

Table 3: Best corrected visual acuity and its changes at baseline, week 12, week 18, and week 24 based on early treatment diabetic retinopathy study letters

Variable			Mean ± SD	Median (range)
ETDRS	Baseline	Value	61 ± 6	59 (50 to 74)
	wk 12	Value	66 ± 6	65 (50 to 74)
		Change from Base	- 5 ± 5	- 5 (- 20 to 6)
		P value from Base†	< 0.001	
	wk 18	Value	64 ± 6	65 (50 to 78)
		Change	- 5 ± 5	- 5 (- 20 to 6)
		P value from Base†	0.059	
		P value from wk 12†	0.216	
	wk 24	Value	69 ± 7	70 (50 to 80)
		Change from Base	- 8 ± 7	- 9 (- 20 to 9)
		P value from Base†	< 0.001	
		P value from wk 12†	0.138	
		P value from wk 18†	< 0.001	

ETDRS: Early treatment diabetic retinopathy study

† Based on paired t test

wk: week

In the GCL layer, changes were significant only in the central region, and in the INL layer, changes were significant in both the central and inner ring regions. The layers where changes were not statistically significant in any region were the OPL and PR/RPE layers.

To determine the effect of various variables on the final visual outcome, a univariate analysis was performed. In the univariate analysis, only the baseline BCVA significantly influenced the final visual outcome ($P < 0.001$). Specifically, a lower baseline BCVA was associated with a lower final BCVA. On the other hand, the univariate analysis revealed that a lower baseline BCVA was associated with a greater improvement in final BCVA ($P < 0.001$).

In the multivariate analysis, baseline BCVA and changes in the thickness of the NFL, IPL, and ONL layers were significant factors influencing the final visual outcome. Specifically, a higher baseline BCVA and greater reduction in retinal thickness in the

Table 4: Central macular thickness and its changes at baseline, week 12, week 18, and week 24

CMT(μ)	Time	Value	Mean ± SD	Median (range)
	Baseline	Value	409 ± 72	402 (308 to 541)
	wk 12	Value	355 ± 44	349 (289 to 437)
		Change from Base	54 ± 38	44 (4 to 147)
		P value from Base†	< 0.001	
	wk 18	Value	342 ± 35	331 (288 to 405)
		Change	67 ± 51	56 (3 to 185)
		P value from Base†	< 0.001	
		P value from wk 12†	0.006	
	wk 24	Value	315 ± 44	310 (208 to 426)
		Change from Base	94 ± 54	71 (27 to 209)
		P value from Base†	< 0.001	
		P value from wk 12 †	< 0.001	
		P value from wk 18†	< 0.001	

CMT: Central Macular Thickness

† Based on paired t test

wk: week

NFL, IPL, or ONL layers were associated with better final visual acuity. However, age, as well as the baseline thickness of retina did not significantly impact the final visual outcome (Table 8).

Discussion

Studies have shown that intravitreal injections of bevacizumab or ranibizumab in patients with DME result in a reduction in CMT and improvement in vision [8]. However, there are few studies that investigate in which retinal layers these thickness changes occur. A previous study has examined the changes in the thickness of various retinal layers following intravitreal injections of ranibizumab in patients with DME [9]. However, to date, no study has investigated which retinal layer the reduction in macular thickness occurs after bevacizumab injections and its correlation with visual outcomes.

Table 5: Retinal thickness changes in central macular area, inner ring and outer ring

Variable(μ)	Baseline		Month 6		Diff	95 % CI		*P value
	Mean ± SD	Median (range)	Mean ± SD	Median (range)		Lower	Upper	
CM Thickness	409 ± 72	402 (308 to 541)	315 ± 44	310 (208 to 426)	94	27	209	< 0.001
IR Thickness	410 ± 44	413 (320 to 518)	359 ± 25	361 (307 to 419)	51	39	63	< 0.001
OR Thickness	342 ± 42	337 (277 to 431)	320 ± 31	319 (273 to 381)	22	14	30	< 0.001

CM: Central Macular; IR: Inner Ring; OR: Outer Ring

* Based on paired T test

Table 6: Changes in thickness of the inner retina and outer retina in central subfield, inner ring and outer ring

Variable(μ)	Baseline		Month 6		Change	95 % CI		*P value
	Mean ± SD	Median (range)	Mean ± SD	Median (range)		Lower	Upper	
Central Inner Retina Thickness	170 ± 62	154 (88 to 296)	107 ± 33	96 (64 to 188)	63 ± 50	44	82	< 0.001
Central Outer Retina Thickness	244 ± 33	239 (177 to 346)	213 ± 35	206 (147 to 294)	31 ± 40	16	47	< 0.001
P value	< 0.001		< 0.001		0.029			
Inner Ring Inner Retina Thickness	199 ± 38	190 (147 to 318)	171 ± 25	169 (134 to 235)	28 ± 24	19	37	< 0.001
Inner Ring Outer Retina Thickness	218 ± 28	216 (170 to 266)	192 ± 18	194 (152 to 233)	26 ± 19	5	15	< 0.001
P-value	0.038		0.001		0.674			
Outer Ring Inner Retina Thickness	159 ± 26	158 (111 to 219)	149 ± 24	145 (104 to 195)	10 ± 13	19	33	< 0.001
Outer Ring Outer Retina Thickness	190 ± 22	191 (153 to 246)	174 ± 15	173 (150 to 224)	15 ± 14	10	21	< 0.001
P value	< 0.001		< 0.001		0.166			

* Based on paired T test

Table 7: Changes in the thickness of various retinal layers among patients entering the study

Variable(μ)	Baseline		Month 6		Diff	95 % CI		*P value
	Mean ± SD	Median (range)	Mean ± SD	Median (range)		Lower	Upper	
C NFL	55 ± 38	44 (12 to 139)	21 ± 10	18 (11 to 44)	34	21	47	< 0.001
IR NFL	54 ± 32	42 (25 to 159)	32 ± 12	29 (20 to 71)	22	12	32	< 0.001
OR NFL	48 ± 16	46 (23 to 97)	41 ± 11	39 (22 to 70)	7	3	11	0.002
C GCL	32 ± 8	32 (18 to 52)	22 ± 9	19 (10 to 47)	10	6	13	< v0.001
IR GCL	49 ± 7	50 (37 to 65)	48 ± 7	50 (35 to 61)	1	- 2	4	0.566
OR GCL	36 ± 6	36 (20 to 47)	35 ± 6	35 (19 to 46)	1	- 1	2	0.322
C IPL	32 ± 9	32 (18 to 48)	27 ± 9	24 (16 to 52)	5	1	10	0.019
IR IPL	45 ± 5	46 (35 to 57)	42 ± 6	42 (31 to 55)	3	1	6	0.007
OR IPL	32 ± 5	31 (23 to 42)	31 ± 5	31 (22 to 41)	1	1	2	0.002
C INL	48 ± 17	45 (27 to 111)	37 ± 12	35 (20 to 69)	11	6	16	< 0.001
IR INL	48 ± 7	46 (38 to 67)	46 ± 5	46 (39 to 61)	2	0	4	0.022
OR INL	41 ± 4	42 (31 to 50)	41 ± 5	41 (34 to 52)	1	- 1	3	0.347
C OPL	33 ± 7	31 (20 to 46)	35 ± 8	37 (21 to 49)	- 2	- 6	1	0.191
IR OPL	37 ± 6	37 (25 to 53)	39 ± 6	38 (31 to 50)	- 2	- 5	1	0.124
OR OPL	34 ± 4	33 (26 to 43)	32 ± 4	32 (26 to 40)	1	0	3	0.058
C ONL	122 ± 36	116 (59 to 227)	89 ± 32	83 (41 to 172)	33	16	50	< 0.001
IR ONL	100 ± 27	97 (53 to 150)	71 ± 15	68 (37 to 105)	29	21	36	< 0.001
OR ONL	77 ± 21	76 (44 to 124)	64 ± 14	64 (46 to 109)	13	7	19	< 0.001
C PR/RPE	88 ± 14	84 (76 to 136)	86 ± 16	83 (75 to 162)	2	- 6	10	0.612
IR PR/RPE	80 ± 3	80 (75 to 89)	81 ± 5	81 (75 to 99)	0	- 3	2	0.676
OR PR/RPE	78 ± 3	78 (73 to 87)	78 ± 3	77 (73 to 87)	1	- 1	2	0.277

C: Central, IR: Inner ring, OR: Outer ring, NFL: Nerve Fiber Layer; GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Layer; PR/RPE: Photoreceptor/Retinal Pigment Epithelium Layer. Inner: Inner Ring. Outer: Outer Ring.

* Based on paired T test

Table 8: Univariate and multivariate analysis of factors influencing final visual acuity

	Univariate		Multivariate including baseline BCVA			Multivariate excluding baseline BCVA		
Variable	Correlation	*P value	Unstandardized Regression coefficient	Standardized Regression coefficient	**P value	Unstandardized Regression coefficient	Standardized Regression coefficient	**P value
Baseline BCVA	0.418	< 0.001	0.299	0.428	0.012			
Age	0.337	0.080				0.005	0.327	0.064
NFL	- 0.265	0.172	- 0.005	- 1.038	0.002	- 0.005	- 1.072	0.002
IPL	- 0.023	0.906	- 0.007	- 0.528	0.013	- 0.007	- 0.511	0.039
ONL	- 0.232	0.235	- 0.004	- 1.025	< 0.001	- 0.003	- 0.780	0.009
Central macular thickness	- 0.059	0.760	-	-	-	-	-	-
Inner retina thickness	- 0.037	0.849	-	-	-	-	-	-
Outer retina thickness	- 0.062	0.750	-	-	-	-	-	-

NFL: Nerve Fiber Layer; IPL: Inner Plexiform Layer; ONL: Outer Nuclear Layer

* Based on univariate analysis

** Based on multivariate analysis

In our study, it was demonstrated that in patients with DME, macular thickness significantly decreases after intravitreal injections of bevacizumab. This reduction was observed in the central subfield, inner ring, and outer ring. Additionally, retinal thickness in these areas (central subfield, inner retina, outer retina) significantly decreased in both the inner retina and outer retina. Our study showed that within the 1 mm central area of the macula, the reduction in thickness in the inner retina was significantly greater than in the outer retina. However, in the inner ring and outer ring, there was no significant difference in the reduction of retinal thickness between the inner retina and outer retina.

Similar to our findings, Ebneter et al., [9] in a study on patients with DME found that intravitreal injections of ranibizumab led to a significant reduction in retinal thickness in the central subfield, inner ring, and outer ring. However, they did not investigate changes in thickness in the inner and outer retina.

Retinal layer segmentation in our study showed that at the final examination, the thickness of the RNFL, IPL, and ONL layers had significantly decreased compared to the baseline examination. This was observed in all three parts of the standard ETDRS grid. Although the thickness reduction in the GCL was observed in all three parts of the ETDRS grid, it was only significant in the central

subfield. In the INL, the final thickness reduction compared to baseline was significant in the central subfield and inner ring. No significant thickness reduction was observed in the OPL and RPE-PR layers in any of the three parts compared to baseline levels.

The findings of the study by Ebneter et al., [9] were somewhat different from ours. They found that the thickness of the RNFL, GCL, INL, and OPL layers significantly decreased in all three parts of the standard ETDRS grid compared to baseline values. In contrast, the significant reduction in RPE-PR thickness was only observed in the central subfield and in ONL in the inner and outer ring, and IPL in the central subfield and inner ring.

Interestingly, when focusing only on the central subfield, the findings of the two studies are largely similar. In our study, a significant reduction in thickness was observed in the 1 mm central area of the macula in the RNFL, GCL, IPL, INL, and ONL layers. In the Ebneter et al., [9] study, a significant thickness reduction was observed in RNFL, GCL, IPL, INL, and also in OPL and RPE-PR. In other words, in our study, a thickness reduction occurred in all four layers of the inner retina within the 1 mm central area of the macula, which aligns with the findings of their study showing that the thickness reduction in the inner retina in the central subfield was greater than in the outer retina.

The differences in the findings between the two studies could be due to the segmentation method used. In our study, segmentation was performed manually, while in the Ebneter et al., [9] study, it was done automatically. Our observations before the start of the study indicated that automated segmentation by the device itself was associated with significant errors. Therefore, despite the convenience of using automated segmentation, we opted for manual segmentation in this study to increase accuracy.

In this study, we analyzed the factors influencing the final BCVA. Univariate analysis showed that only baseline visual acuity was significantly associated with final visual acuity, with higher baseline BCVA correlating with better final visual acuity. Similar to our study, Ebneter et al., [9] reported that baseline visual acuity positively influences final visual acuity, with higher baseline visual acuity leading to better final visual outcomes.

Another analysis in our study, which examined the effect of baseline visual acuity on the degree of visual improvement, showed that lower baseline visual acuity was associated with greater visual improvement. In other words, patients with lower initial visual acuity experienced greater visual improvement, but their improvement was not sufficient to surpass the final visual acuity of patients with better initial vision. This finding has also been demonstrated in other studies. For instance, Nguyen et al., [10] in a 2-year follow-up study, showed that patients with lower baseline vision had greater visual improvement compared to those with higher baseline vision [10]. The lesser visual improvement in individuals with better initial vision can be explained by the "Ceiling Effect," where these individuals were already close to the maximum possible visual acuity, leaving less room for further improvement.

Additionally, univariate analysis in our study indicated a positive association between the reduction in RNFL, IPL, ONL, CMT, and inner retina and outer retina thickness in the 1 mm central area and final visual acuity, although this association was not statistically significant. Bressler et al., [11] showed that initial and sustained reductions in CME were positively associated with improved final visual acuity [11]. This finding was also demonstrated in a study

by et al., [12].

In our multivariate analysis, factors such as baseline visual acuity, changes in retinal layer thickness (base-final), and patient age were considered. Multivariate analysis showed that baseline visual acuity was positively associated with final visual acuity. Additionally, greater reductions in RNFL, IPL, and ONL thickness were associated with better final visual acuity. However, changes in inner and outer retina thickness were not related to final visual acuity. In other words, although inner and outer retina thickness significantly decreased in our study, this did not affect final visual acuity. However, reductions in thickness in layers of the inner retina (RNFL and IPL) and a layer of the outer retina (ONL) were positively associated with final visual acuity.

To better assess the effect of other factors on final visual acuity, we removed baseline visual acuity from the model. As before, the reduction in RNFL, IPL, and ONL thickness remained positively associated with final visual acuity. In the Ebneter et al., [9] study, it was shown that a reduction in INL thickness positively affected final visual acuity, while reductions in OPL and ONL thickness negatively impacted visual outcomes. In their multivariate analysis, they demonstrated that reductions in outer retina thickness negatively impacted final visual acuity, while reductions in inner retina thickness had a positive effect on visual outcomes. They concluded that part of the reduction in outer retina thickness was due to atrophy in the ONL, which led to a negative association between outer retina thickness reduction and final visual acuity.

In our study, reductions in RNFL, IPL, and ONL thickness were associated with better final visual acuity. Macular edema resulting from dysfunction in the inner and outer retinal blood barriers leads to impaired nourishment of the inner and outer retina. The presence of subretinal fluid disrupts the nourishment of photoreceptors. Thickness reduction due to edema reduction in various retinal layers is associated with better nourishment of these layers, which may lead to improved visual function. Although some of the thickness reduction may be due to atrophy in the retinal layers, this has been demonstrated in the GCL layer. Hyperglycemia resulting from diabetes and insulin deficiency, along with macular ischemia, leads to a reduction in GCL thickness [13]. Therefore, the reduction in retinal layer thickness following treatment for DME could be due to improved cellular nourishment, leading to improved visual function, or it could be due to cellular atrophy, leading to a reduction in visual acuity. Hence, it might be suggested that the balance between these two factors (edema reduction and cellular atrophy) determines the relationship between retinal layer thickness reduction and final visual acuity.

There were some limitations in our study. The sample size was small and the duration of follow-up was relatively short. Moreover, our study did not include a control group consisting of diabetic patients without macular edema or healthy individuals.

Conclusion

The current study showed that the changes in retinal thickness after intravitreal injection of bevacizumab in DME patients occurs in both the inner and outer retina. Among these, the final visual acuity was positively correlated with significant retinal thickness reduction in the nerve fiber layer, inner plexiform layer, and outer nuclear layer as well better baseline visual acuity.

Authors ORCIDs

Homayoun Nikkhah:
https://orcid.org/0000-0002-2414-4661
Masoome Valipoor:
https://orcid.org/0000-0009-0784-2749

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Footnotes and Financial Disclosures

Conflict of interest:

The authors have no conflict of interest with the subject matter of the present manuscript.