Change of Retinal Nerve Fiber Layer Thickness in Various Retinal Diseases Treated With Multiple Intravitreal Antivascular Endothelial Growth Factor

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Purpose. To investigate the effect of multiple intravitreal injection of anti-VEGF on the retinal nerve fiber layer (RNFL) in AMD, diabetes mellitus retinopathy (DMR), and retinal vein occlusion (RVO).

Methods. In this retrospective controlled case series, we reviewed the AMD, DMR, and RVO patients who received more than three anti-VEGF injections (injection group: 148 eyes). Patients without treatment were included as a control group (noninjection group: 183 eyes). RNFL thickness was measured by SD-OCT. Also, correlation between RNFL change and associated factors, including intraocular pressure (IOP), injection times, and severity of retinal ischemia, were analyzed using multivariate logistic regression.

Results. RNFL thickness (μm) had not changed in AMD, but it decreased from 100.0 to 97.1, and from 101.1 to 98.0 in injection groups of DMR and RVO, respectively, as well as the noninjection group. However, decreased RNFL thickness of the injection groups was not significantly different from those of the noninjection groups. Severity of retinal ischemia was associated with decreased RNFL thickness (odds ratio: 4.667). However, number of injections and IOP-related variables had no association with RNFL change.

Conclusions. Multiple intravitreal injections of anti-VEGF did not lead to significant change in RNFL thickness in wet AMD, DMR, and RVO patients. Furthermore, IOP fluctuations and number of injections did not appear to adversely affect RNFL thickness. Decreased RNFL thickness associated with severity of retinal ischemia in the DMR and RVO patients suggests that inner retinal ischemia itself could be a cause of RNFL loss rather than anti-VEGF effect.

Keywords: antivascular endothelial growth factor, retinal nerve fiber layer thickness, age-related macular degeneration, diabetes mellitus retinopathy, retinal vein occlusion, retinal ischemia

Age-related macular degeneration (AMD), diabetes mellitus retinopathy (DMR), and retinal vein occlusion (RVO) are major causes of visual impairment in the elderly worldwide. Increase of intraocular vascular endothelial growth factor (VEGF) has been reported among these retinal diseases.[1–5] VEGF plays a crucial role in the development of choroidal neovascular membrane in AMD[6,7] and in the increased retinal vascular permeability associated with macular edema (ME) in DMR[8] and RVO.[9] Anti-VEGF has beneficial effects on the progression of retinopathy including regression of retinal neovascularization and improved ME. Recently, the use of intravitreal anti-VEGF agents has become the standard therapy for patients with exudative AMD[10] and is commonly used for the treatment of ME, secondary to DMR[11] and RVO.[12]

While the use of anti-VEGF in clinical practice has increased, the literature lacks studies that have assessed the long-term safety of repeated anti-VEGF injections on the retinal nerve fiber layer (RNFL). Horsley et al.[13] reported that repeated intravitreal anti-VEGF injections did not lead to significant change in RNFL thickness in wet AMD patients. On the contrary, Martinez-de-la-Casa et al.[14] reported that repeated intravitreal anti-VEGF injections resulted in deterioration of the RNFL due to its direct drug toxicity and intraocular pressure (IOP) fluctuations in wet AMD patients. Similarly, Zayit-Soudry et al.[15] conducted an experimental animal study, in which rabbits were tested using nine intravitreal injections of bevacizumab or ranibizumab administered at 14-day intervals under the assumption that IOP spikes induced RNFL damage. Therefore, it is important to determine whether repeated intravitreal injections of anti-VEGF may influence RNFL thickness. However, previous studies have focused mainly on AMD. Information on RNFL change in other retinal diseases, such as DMR and RVO, is limited.

In this study, we investigated the change of RNFL thickness in patients receiving repeated intravitreal anti-VEGF injection among three representative retinal diseases (AMD, DMR, and RVO) using spectral domain optical coherence tomography (SD-OCT). We also investigated the correlation between change of RNFL thickness and associated factors that could potentially affect the RNFL, such as injection times, type of anti-VEGF, IOP-related factors, cup-disc (C/D) ratio, center foveal thickness (CFT), and severity of retinal ischemia using multivariate logistic regression analyses.

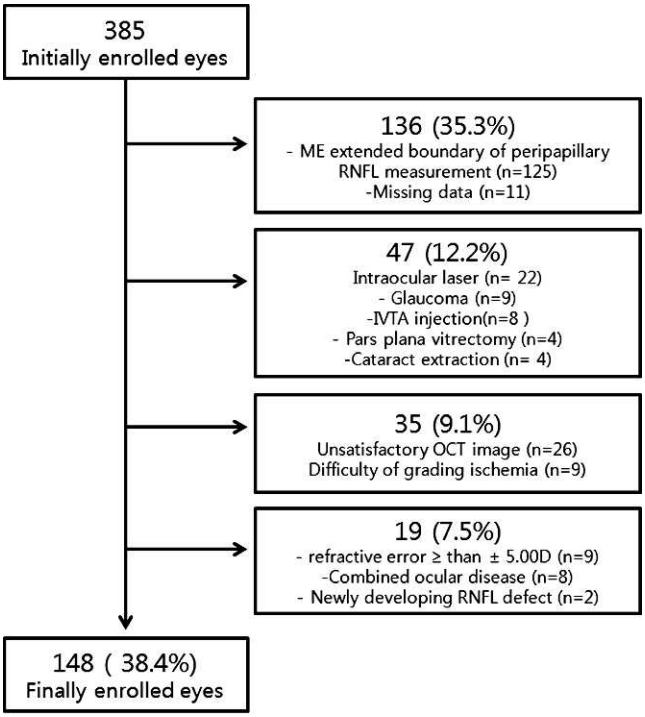

FIGURE 1. Flow diagram of patient selection.

SUBJECTS AND METHODS

Subjects (Injection Group)

We conducted a retrospective chart review of 135 consecutive patients (148 eyes) who had received more than three intravitreal anti-VEGF injections for ME secondary to AMD or DMR or RVO, and who were followed-up for at least 12 months between March 2010 and August 2013 at Konkuk University Medical Center. The retrospective chart review was approved by the Institutional Review Board/Ethics Committee at Konkuk University Medical Center in Korea and was performed in accordance with the tenets of the 1964 Declaration of Helsinki. The exclusion criteria were: (1) eyes with ME extended boundary of peripapillary RNFL measurement; (2) eyes with history of treatment that could potentially affect the RNFL, including intraocular laser,[16] intravitreal triamcinolone acetonide injection (IVTA), and vitrectomy; (3) eyes with any unsatisfactory SD-OCT image and different signal strength between initial and last follow up; (4) difficulty of ischemia grading in RVO due to severe retinal hemorrhage on fluorescein angiography (FA); (5) eyes with a history of IOP $\geq$ 22 mm Hg or treatment for glaucoma, optic disc not following ISNT rule or showing RNFL defect; (6) combined other ocular problems (e.g., uveitis, optic neuritis, severe peripapillary atrophy; and (7) eyes with a refractive error exceeding $\pm$5.00 diopters (D). Details of patient selection are shown in Figure 1. Each subject underwent a complete ophthalmic examination, including best corrected visual acuity (BCVA) measured with Snellen chart, IOP, slit-lamp biomicroscopy, fundus photography, FA, and indocyanine green angiography (ICGA; performed only in AMD patients). SD-OCT examination was performed at the initial and final visits, and several times during the follow-up period to evaluate RNFL thickness and macular thickness. Intravitreal anti-VEGF injection was performed by two retinal specialists (HC, HCK) with bevacizumab (0.125 mg/0.05 mL) or ranibizumab (0.5 mg/0.05 mL). Patients with AMD received bevacizumab and/or ranibizumab, while patients with RVO and DMR received only bevacizumab.

Control Group (Noninjection Group)

To compare the pre– and post–anti-VEGF RNFL changes in injection groups with those of the noninjection groups, age-matched AMD, DMR, and RVO patients without significant ME who were observed without anti-VEGF injection served as the control group (183 patients, 183 eyes). The exclusion criteria were the same as for the injection group.

SD-OCT Measurement

RNFL thickness and macular thickness were measured by SD-OCT (Spectralis SD-OCT; Heidelberg Engineering, Heidelberg, Germany). Measurements were made by a well-trained technician who was blinded to information about the eyes. Following pupil dilatation, RNFL thickness measurements (diameter 3.5 mm, 768 A-scans) were obtained. The device's eye tracking system compensated for eye movement. The auto-rescan function using a reference point was activated to minimize variation in allocating the acquisition protocols to the follow-up sessions. RNFL thickness from the inner margin of the internal limiting membrane to the outer margin of the RNFL layer was automatically segmented using SD-OCT software (Spectralis 4.1.1; Heidelberg Engineering). Quality criteria included sharp scan beam and definition of vessels, scan beam centered on optic disc, even illumination, automatic real-time (ART) score of 16, and signal-to-noise ratio $\geq$ 15 dB. Peripapillary RNFL thickness measurements of global (G) average thickness of RNFL, temporal superior (TS), temporal (T), temporal inferior (TI), nasal inferior (NI), nasal (N), and nasal superior (NS) were analyzed. Macular thickness measurements were obtained from a 9- $\times$ 6-mm area of the macular region centered on the fovea, which were determined by fundus photography (1536 $\times$ 1536 pixels). We selected the retinal thickness map analysis to display extension of ME and numeric average of the measurement for each of the nine subfields as defined by the Early Treatment Diabetic Retinopathy Study (ETDRS). The inner, intermediate, and outer ring having a diameter 1, 3, and 6 mm, respectively, was analyzed. The average of all points within the inner 1-mm diameter circle was defined as the central foveal thickness (CFT). The intermediated ring was divided into four zones: inner superior (IS), inner nasal (IN), inner inferior (II), and inner temporal (IT). The outer ring was divided into outer superior (OS), outer nasal (ON), outer inferior (OI), and outer temporal (OT) zones. The numerical values recorded for each of the nine zones were used in the analyses of correlation with RNFL change. In the thickness map, if there was any abnormal color change extended from ME within the boundary of peripapillary RNFL measurement, ME was considered to have affected RNFL thickness and was excluded (Fig. 2).

Main Outcome Measures

Main outcome measures included change of RNFL thickness in each group and correlation between the change of RNFL thickness and associated factors that could potentially affect the RNFL after anti-VEGF injection. The factors included: (1) number of injections; (2) IOP-related factors (baseline IOP, average IOP under treatment, IOP spike); (3) severity of retinal ischemia in the DME and RVO groups; (4) CFT-related factors including baseline CFT and change of CFT; (5) kind of retinal disease which were classified into two groups with the outer retina group comprising AMD and the inner retina group comprising DME and RVO; (6) kind of anti-VEGF agent

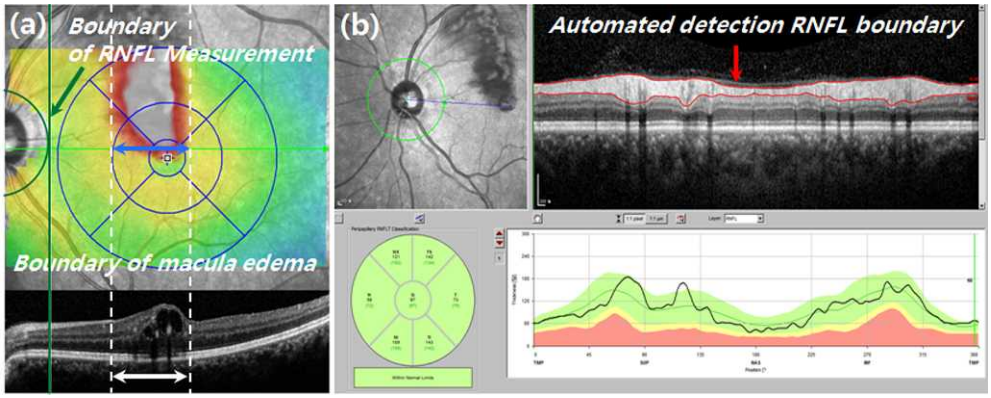

FIGURE 2. Representative case of an enrolled patient. Image obtained from a 62-year-old man with a 1-month history of blurred vision (BCVA 20/40) in the left eye with macular edema secondary to superotemporal BRVO. (**a**) SD-OCT showed that boundary of macular edema (*white dotted line*) did not extend to the boundary of the peripapillary RNFL (*green line*). Also, thickness color map of SD-OCT showed that there was no abnormal color change within RNFL thickness measurement area (*top green circle*). Nine subfields circled defined by the Early Treatment Diabetic Retinopathy Study. The *inner*, *intermediate*, and *outer* ring had a diameter of 1, 3, and 6 mm, respectively. The average of thickness within the inner 1-mm radius circle was defined as CFT. The patient's CFT was 367 μm. (**b**) SD-OCT-generated RNFL map. The RNFL thickness was automatically segmented (*red line*) using the SD-OCT software. In the *bottom left*, Peripapillary RNFL thickness measurements of global average thickness of RNFL (G), temporal superior (TS) temporal (T), temporal inferior (TI), nasal inferior (NI), nasal (N), and nasal superior (NS) were analyzed. The patient of global average thickness of RNFL (*center circle*) was 97 μm.

(bevacizumab and/or ranibizumab); (7) baseline C/D ratio; and (8) patient age and follow-up period. Correlations between the change of RNFL thickness and the associated factors were assessed by odds ratio (OR). To determine OR, patients were divided into two groups according to the absence or presence of a significant decrease of RNFL thickness. Considering test-retest variability, ≥6-μm change in global average RNFL thickness from a baseline was defined as an acceptable change in RNFL measurements.[17] The IOP spike was defined IOP elevation, ≥6 mm Hg from baseline during the follow-up period. IOP was measured in each patient before each injection, 1 hour after the injection procedure, and every time of follow-up. C/D ratio of optic disc photograph was graded by the same person (HJS) blinded to the identity of the patients. Severity of retinal ischemia was graded by ETDRS grading in the DMR group. More than very severe nonproliferative diabetic retinopathy was considered as the standard ischemic type. All patients with DMR and RVO included in this study had at least one fluorescein angiogram available for review. In each angiogram, the area of venous outflow obstruction and the area of retinal nonperfusion were estimated in disc diameter (DD) in branch retinal vein occlusion (BRVO) and disc area (DA) in central retinal vein occlusion (CRVO). In the RVO group, a nonperfusion area greater than 5 DD in BRVO and a 10 DA in CRVO on FA image were considered as the standard for the ischemic type.

Statistical Analyses

Statistical analyses were performed using statistical software (SPSS 17.0 for Windows; SPSS, Inc., Chicago, IL, USA). Results are expressed as mean ± SD. Continuous variables (e.g., RNFL and foveal thickness, patient age, injection times) were analyzed with a normality test (Shapiro-Wilk test). Baseline characteristics among groups (AMD, DMR, and RVO) were evaluated by ANOVA test with Bonferroni's method for multiple comparisons. χ^2 test compared discrete variables (e.g., sex) among the groups. Change of RNFL thickness from baseline to last follow-up was analyzed with paired *t*-test. Change of RNFL thickness according to the nonperfusion area within the DMR and RVO groups and between the injection and noninjection group were evaluated by Student's *t*-test. Bivariate relationships between change of RNFL thickness and number of injections were analyzed using Pearson's correlation coefficient. For the associated factor analysis, logistic regression was constructed to determine the OR and 95% confidence interval (CI). Then, multivariate analysis was performed with linear logistic regression over the specific predictor. A P value < 0.05 was considered statistically significant.

RESULTS

The baseline clinical characteristics including follow-up period and injection times were not significantly different among the AMD, DMR, and RVO injection groups, except for patient age. The average follow-up period was 21.3 ± 4.1 months in all patients. Considering that decrease of RNFL thickness by increasing age was 2 to 3 μm per 10 years, the effect of decreased RNFL during the follow-up period was negligible.[18,19] There were no differences in baseline characteristics between injection and noninjection group, except for CFT (Table 1). A total of 125 cases (32.4%) were excluded due to extending ME to the boundary of peripapillary RNFL measurement. Nine cases (2.3%) with difficulty of grading ischemia in RVO with severe retinal hemorrhage on FA were excluded. Nine cases among the enrolled 148 eyes displayed a detection error of the automated SD-OCT algorithm delineating the anterior and posterior margins of RNFL. In these cases, manual correction of the RNFL boundary was done using a manual segmentation function built into the SD-OCT software. There was no case of serious complication, such as sustained IOP elevation and endophthalmitis, during the follow-up period.

Change of RNFL Thickness in the AMD Group

In AMD, there was no significant RNFL thickness change in injection group (from 98.0 ± 11.7 μm to 97.5 ± 12.1 μm after injection, $P = 0.577$) as well as noninjection groups (from 98.8 ± 13.2 μm to 98.2 ± 12.5 μm, $P = 0.621$). In sector assessment, there was no change of RNFL thickness in injection group of AMD in any sector (Fig. 3a). There were no differences of RNFL change according to type of injection (Table 2). ANOVA test with Bonferroni's method for multiple comparisons showed that there were no significant differences in change of RNFL thickness when comparing among patients

Table 1. Clinical Characteristics of All Study Eyes With AMD, DMR, and RVO

Parameters	AMD Injection/Control (*P**)	DMR Injection/Control (*P**)	RVO Injection/Control (*P**)	*P* Value†
Patients, *n*	82/98	34/48	32/37	
Mean age, y	69.95/70.39 (0.78)	62.30/63.1 (0.54)	60.15/61.7 (0.25)	0.001‡
Sex, male/female	59–23/68–30 (0.61)	21–13/30–18 (0.55)	17–15/20–17 (0.31)	0.437§
Eye, right/left	38–44/45–53 (0.47)	18–16/23–25 (0.26)	16–16/17–20 (0.28)	0.108§
Follow-up period, mo	21.5/22.8 (0.34)	20.9/21.2 (0.41)	19.88/20.8 (0.39)	0.256‡
Number of injections, SD	5.69 ± 2.7	4.77 ± 1.42	5.07 ± 2.25	0.157‡
Baseline VA, logMAR	0.40/0.31 (0.07)	0.37/0.30 (0.11)	0.38/0.29 (0.07)	0.087‡
Refractive error, SE	+0.16/+0.11 (0.12)	−0.33/−0.12 (0.15)	−0.45/−0.21 (0.18)	0.058‡
Baseline IOP, mm Hg	14.7/14.9 (0.54)	15.4 /15.1 (0.49)	15.4/15.2 (0.62)	0.538‡
Average IOP, mm Hg	14.1/14.8 (0.32)	14.7/15.0 (0.57)	14.3/14.9 (0.26)	0.857‡
IOP spike, *n* (%)	14 (17.0)	5 (14.7)	4 (12.5)	0.084§
Baseline C/D ratio	0.39/0.41 (0.47)	0.40/0.42 (0.26)	0.44/0.42 (0.35)	0.136‡
CFT				
Initial CFT, μm	385.0/301.0 **(0.02)**	376.7/298.5 **(0.02)**	393.7/297.8 **(0.01)**	0.072‡
Final CFT, μm	331.3/292.7 **(0.03)**	332.4/291.1 **(0.02)**	344.2/289.8 **(0.01)**	0.129‡
CFT change, μm	53.7/8.3 **(0.01)**	44.3/7.4 **(0.01)**	49.5/8.0 **(0.01)**	0.073‡
Average RNFL thickness				
Initial RNFL, μm	98.0/98.8 (0.09)	100.0/100.2 (0.64)	101.1/101.5 (0.71)	0.162‡
Final RNFL, μm	97.5/98.2 (0.24)	97.1/98.1 (0.14)	98.0/99.2 (0.11)	0.216‡
RNFL change, μm	0.47/0.62 (0.22)	2.90/2.11 (0.08)	3.11/2.30 (0.09)	0.011‡

Number of injections and IOP spike: measurement of injection group was listed. Significant *P* values are listed in bold. SE, spherical equivalent; VA, visual acuity.

* *P* value between the injection and control group in the same disease.

† *P* value of three injection groups (AMD, DMR, RVO).

‡ *P* value related to ANOVA test.

§ *P* value related to χ^2 test.

treated with bevacizumab alone, ranibizumab alone, or with both drugs.

Change of RNFL Thickness in the DMR and RVO Groups

The average RNFL thickness decreased from 100.0 to 97.1 μm, and from 101.1 to 98.0 μm in the injection group of DMR and RVO patients, respectively, as well as the noninjection group (from 100.2 to 98.1 μm in DMR, and from 101.5 to 99.2 μm in RVO). Specifically, RNFL thickness by sectoral measurement displayed a significant reduction in the NS (7.14 ± 3.87 μm, $P = 0.024$) sector in the DMR group; TS (4.41 ± 3.12 μm, $P = 0.037$); T (4.63 ± 2.37 μm, $P = 0.031$); and NI (3.10 ± 2.05 μm, $P = 0.047$) sectors in the RVO group (Figs. 3b, 3c). However, decreased average RNFL thickness of injection groups (2.9 ± 3.1 μm in DMR, 3.1 ± 2.9 μm in RVO) were not significantly different from that of the noninjection group (2.1 ± 2.8 μm, $P = 0.081$ in DMR, 2.3 ± 2.7 μm, $P = 0.093$ in RVO).

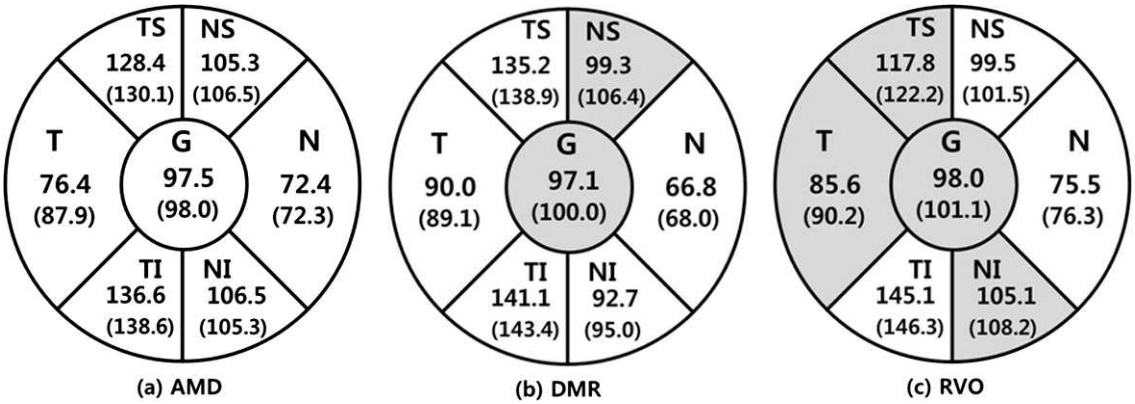

Figure 3. Summary of RNFL thickness (μm) of three groups (AMD, DMR, and RVO) for each sector. Data in *brackets* are mean initial RNFL thickness and *without brackets*, mean final RNFL thickness. *Gray-colored* sector is mean significant decreased RNFL sector. Global average RNFL thickness (μm) decreased in the (**b**) DMR group (2.9 ± 3.1) and (**c**) RVO group (3.1 ± 2.9; $P = 0.026$ and 0.008, respectively), but not in the (**a**) AMD group (0.49 ± 3.16, $P = 0.577$). Also the NS sector (7.14 ± 3.87) in the (**b**) DMR group ($P = 0.024$), and the TS (4.41 ± 3.12), T (4.63 ± 2.37), and NI (3.10 ± 2.0 5) sectors in the (**c**) RVO group ($P = 0.037$, $P = 0.031$, and $P = 0.047$, respectively) displayed decreased RNFL thickness, while no differences were found in (**a**) AMD group in any sector.

Table 2. Change of RNFL Thickness After Various Anti-VEGF Injections in AMD

	Eyes, *n*	Agent(s) Used	Injection, *n*	Mean F/U, mo	Change of RNFL Thickness, μm	*P* Values*
Group 1	34	B	5.6	16.5	−0.41 ± 3.49	>0.05
Group 2	25	R	4.1	10.1	−1.04 ± 3.11	>0.05
Group 3	23	B and R	7.9	17.2	+0.30 ± 2.81	>0.05

Change of RNFL thickness was calculated by average change between preinjection and final RNFL thickness. B, bevacizumab; F/U, follow-up; group 1, bevacizumab alone; group 2, ranibizumab alone; group 3, combination of bevacizumab and ranibizumab; R, ranibizumab.

* Change of RNFL thickness in the three groups was compared using the ANOVA test followed by pairwise multiple comparison using Bonferroni's method.

Figure 4 summarizes the findings concerning change of RNFL thickness both injection and noninjection group of RVO and DMR according to the severity of ischemia. Ischemic type in the injection and noninjection group comprised 15 and 22 patients, respectively, in DMR and 9 and 12 patients, respectively, in RVO. In the DMR group, decreased RNFL thickness of the nonischemic and ischemic group was 1.96 ± 3.94 μm and 3.87 ± 3.34 μm, respectively, and was 1.91 ± 3.42 μm and 6.87 ± 6.82 μm, respectively, in the RVO group. There were significant differences of change of RNFL thickness between nonischemic and ischemic groups in the DMR and RVO groups ($P = 0.041$ and $P = 0.015$, respectively). Ischemic type of DMR and RVO patients showed a tendency to more decreased RNFL thickness than nonischemic type.

Correlation Between RNFL Change and Macular Thickness

To assess the effect of macular thickness on the change of RNFL thickness in the DMR and RVO groups, macular thickness was determined during follow-up. Figure 5 displays the thickness of each macular zone at the baseline and last follow-up. Significant differences were detected in the central zone (CFT 44.3 ± 58.69 μm, $P = 0.027$) and temporal zone (38.7 ± 23.47 μm, $P = 0.038$) of the outer ring in the DMR group, and the central zone (49.5 ± 58.9 μm, $P = 0.017$) and two of the four zones of the inner ring (temporal 52.6 ± 32.41 μm, $P = 0.023$; inferior 53.4 ± 22.94 μm, $P = 0.025$) in the RVO group.

Decreased RNFL thickness in DMR and RVO patients had no association with change of macular thickness after anti-VEGF injection. In the DMR group, no significant correlation was evident between thickness change produced in the temporal region of the outer macular ring and those produced in the NS sector of the RNFL ($r = 0.07$, $P = 0.806$). In the RVO group, no significant correlation was evident between thickness changes produced in the temporal and inferior zone of the inner macular ring and those produced in the TS sector of RNFL ($r = 3.14$, $P = 0.118$ and $r = 0.02$, $P = 0.761$). Similarly, no significance was apparent in the T ($r = 0.389$, $P = 0.072$ and $r = 0.282$, $P = 0.162$) and NI sectors of RNFL ($r = 0.124$, $P = 0.546$ and $r = 0.118$, $P = 0.565$).

Correlation Between RNFL Change and Associated Factors

Table 3 summarizes the correlation between change of RNFL thickness and associated factors that could potentially affect the RNFL. Significant decrease of RNFL thickness was evident in 7 (8%) AMD, 11 (32%) DMR, and 10 (32%) RVO patients.

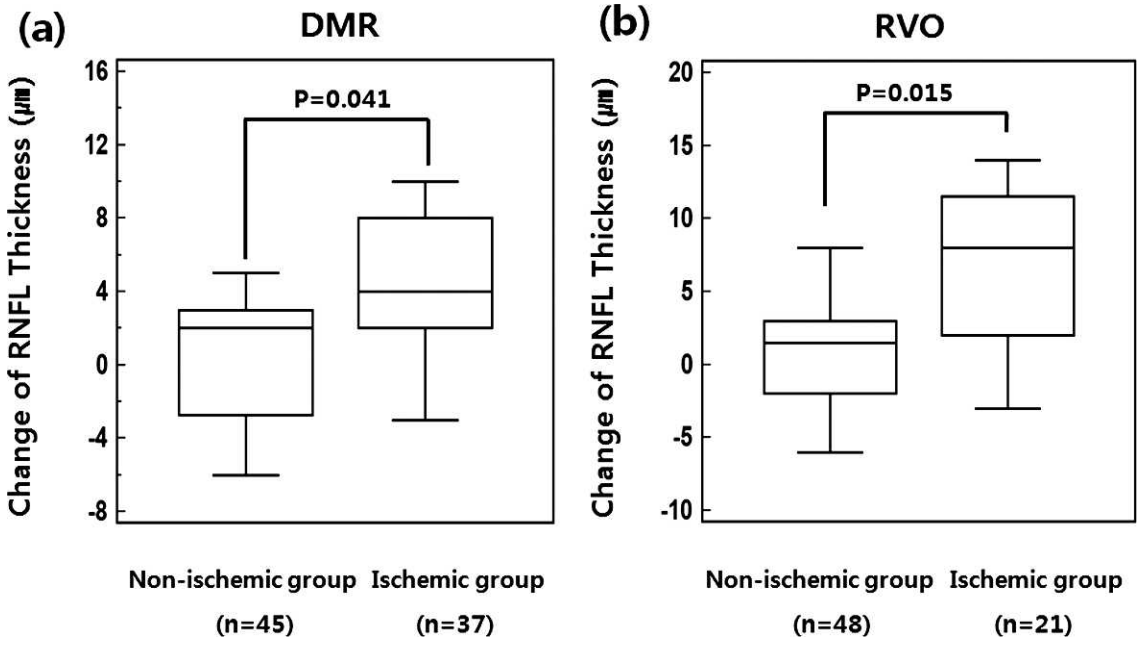

Figure 4. Change of RNFL thickness in RVO and DMR according to the severity of ischemia. Change of RNFL thickness in both injection and noninjection groups of RVO and DMR according to the severity of ischemia. (**a**) RNFL thickness was decreased more in the ischemic group (3.87 ± 3.34) than in the nonischemic group (1.96 ± 3.94) in DMR ($P = 0.041$). (**b**) RNFL thickness was more decreased in the ischemic group (6.87 ± 6.82) than in the nonischemic group (1.91 ± 3.42) in RVO ($P = 0.015$).

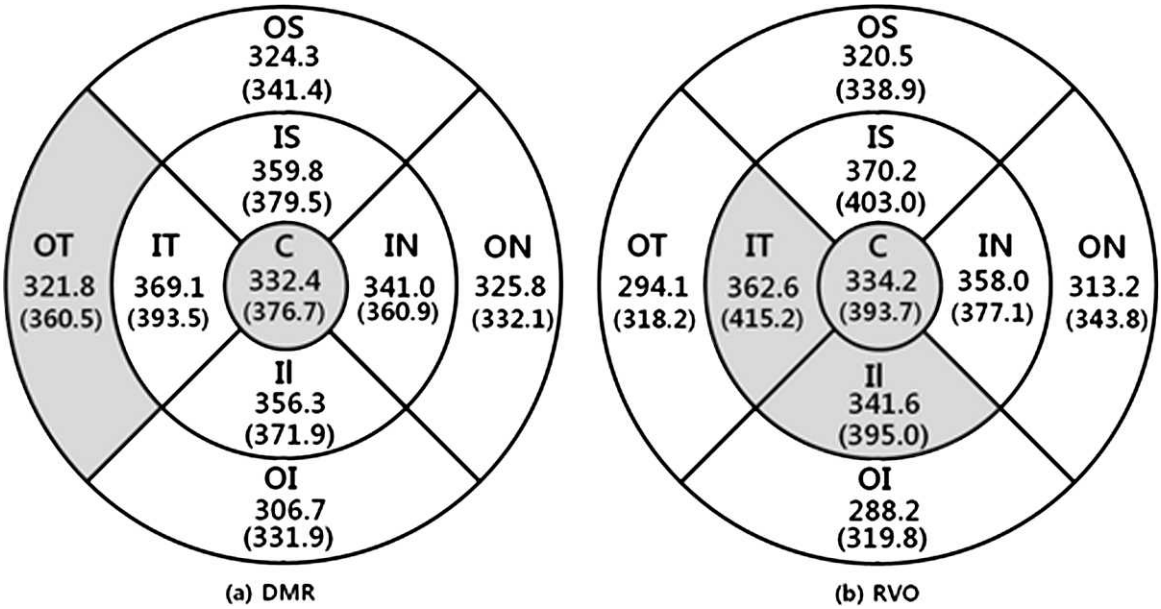

Figure 5. Change of macular thickness of each macular zone at the baseline and end of follow-up. Macular thickness significantly decreased in central (44.3 ± 58.69) and temporal zone (38.7 ± 23.47) of the *outer ring* in the (**a**) DMR group ($P = 0.027$, $P = 0.038$, respectively) and central (49.5 ± 58.9) and two of the four zones of the *inner ring* (temporal, 52.6 ± 32.41; inferior, 53.4 ± 22.94) in (**b**) RVO ($P = 0.017$, $P = 0.023$, and $P = 0.025$, respectively).

Among the associated factors, injection times, type of anti-VEGF agents, IOP-related variables (baseline IOP, average IOP under treatment, IOP spike), and the C/D ratio had no association with change of RNFL thickness. Figure 6 summarizes data concerning the change of RNFL thickness (global average thickness) according to the number of injections in the AMD, DMR, and RVO groups. There was no significant correlation between change of RNFL thickness and number of injections in AMD ($r = -0.08$, $P = 0.592$); DMR ($r = -0.11$, $P = 0.543$); and RVO ($r = -0.15$, $P = 0.417$). The difference in retinal diseases, patients with DMR and RVO were more likely to decrease RNFL thickness (OR, 4.018; 95% CI, 1.514–10.60; $P = 0.025$) than AMD. Also, the difference in severity of retinal ischemia, patients with the ischemic type of DMR, RVO were more likely to exhibit decreased RNFL thickness (OR, 4.667; 95% CI, 1.312–16.597; $P = 0.017$) than those with the nonischemic type.

Discussion

This is a controlled case series with three representative retinal diseases (AMD, DMR, and RVO) and anti-VEGF therapy. There was no apparent decline of RNFL thickness after intravitreal anti-VEGF injection in the wet AMD group, but there was a decrease in the DMR and RVO groups in both injection and noninjection groups. However, decreased RNFL thickness of injection groups was not statistically different from that of the noninjection group. Neovascular (wet) AMD in outer retinal disease is not associated with ischemia, whereas DMR and RVO are inner retinal diseases associated with retinal ischemia. In this study, eyes with greater ischemia as measured on FA had greater decreases in RNFL than those more perfused. Considering that the frequency of the injections and IOP-related variables had no association with RNFL change, it would be reasonable to conclude that it is not anti-VEGF therapy but actually the inner retinal ischemia itself that attributes to RNFL thinning in DMR and RVO groups.

To our knowledge, this is the first study to investigate the change of RNFL thickness in various retinal diseases including DMR and RVO after intravitreal anti-VEGF injection. There was no significant thickness change in the AMD group. Also, there were no significant differences in RNFL thickness according to the type of injected agents (bevacizumab and/or ranibizumab). The present results correspond with a prior study that reported that long-term treatment with anti-VEGF (bevacizumab, ranibizumab) did not lead to significant changes in RNFL thickness in AMD patients.[13] Similarly, another study reported

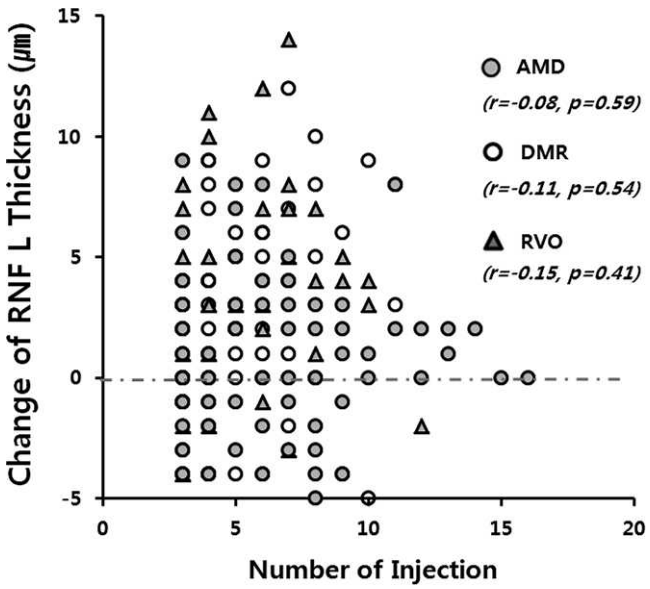

Figure 6. Change of RNFL thickness (global average thickness) according to the number of injections in the AMD, DMR, and RVO groups (marked with *gray circle*, *white circle*, and *gray triangle*, respectively, and there were some overlapping points). There was no significant correlation between change of RNFL thickness and number of injections in AMD ($r = -0.08$, $P = 0.592$), DMR ($r = -0.11$, $P = 0.543$), and RVO ($r = -0.15$, $P = 0.417$).

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TABLE 3. Multivariate Logistic Regression Analysis of Variables Associated With RNFL Thickness in Enrolled Eyes

Risk Factors	Age, y	Disease*	Injection Times†	Baseline IOP, mm Hg	Average IOP, mm Hg	Spike of IOP	Baseline C/D Ratio	Retinal Ischemia‡	SE	Baseline CFT, μm	Change of CFT, μm§
Odds ratio	0.959	**4.018**	0.943	1.006	1.035	1.658	1.159	**4.667**	0.935	1	1.001
95% CI	0.912–1.008	**1.514–10.66**	0.782–1.137	0.866–1.168	0.823–1.255	0.417–6.588	0.046–29.065	**1.312–16.597**	0.762–1.147	0.995–1.004	0.997–1.004
P value‖	0.099	**0.025**	0.536	0.941	0.728	0.473	0.929	**0.017**	0.519	0.828	0.691

CI, confidence interval.
* Disease classified into two groups: the AMD (neovascular) group and the DME and RVO (ischemic) group.
† Number of anti-VEGF injections received.
‡ Patients with RVO and DME categorized as nonischemic group and ischemia group.
§ Change of CFT was obtained by subtracting the final FT from baseline FT.
‖ Multivariate *P* values over the specific variable.

that there was no adverse affects on the optic nerve, resulting in increased C/D ratio, with AMD patients who received multiple intravitreal anti-VEGF injections.[20] Contrary to the AMD group, there were significant decreases of RNFL thickness in the DMR and RVO groups in the present study. These groups showed a 4.6-times greater decrease in RNFL thickness than the AMD group. Severity of retinal ischemia on FA was associated with decreased RNFL thickness, while injection times and IOP related variables had no association.

VEGF is a well-known angiogenic and neurotrophic factor.[21,22] Theoretically, long-term suppression of neurotrophic cytokine in chronic anti-VEGF treated eyes may result in deleterious downstream effect on the RNFL and could potentially lead to RNFL thinning.[23,24] Nevertheless, many experimental studies with repeated intravitreal anti-VEGF injections showed no toxic effect on the retina. For example, no significant toxic effect on the photoreceptor was detected after intravitreal bevacizumab therapy in AMD in an electrophysiological study.[25] In our study, there was no RNFL change in AMD patients after anti-VEFG injections and injection times had no association with a decrease of RNFL thickness in DMR and RVO patients.

Intravitreal anti-VEGF injection is reported to cause both transient and sustained IOP elevation with volume increase or intraocular inflammation related to trabecular meshwork obstruction due to anti VEFG agents itself or impurities within the injected fluid.[26,27] However, previous studies have shown transient IOP elevation after injection, with a return to baseline by 30 to 60 minutes in most patients.[28–30] Sharei et al.[31] reported an increase in mean IOP of 25 mm Hg immediately after injection, with a return to normal IOPs without the need for treatment by 10 minutes. In our study, none of the patients required IOP-lowering medication after injection and no instance of sustained IOP rise or inflammatory reaction that requiring intervention were observed. The general consensus is that IOP elevations are limited to within the first few minutes of the injection procedure, and that prophylactic IOP-lowering treatment does little to prevent their appearance. In practically all cases, the pressure returns to normal values without the need for additional treatment.

Inner retinal ischemia can result in RNFL defect, and the severity of RNFL defect was closely related to that of ischemia. In our study, decreased RNFL thickness associated with severity of retinal ischemia in the DMR and RVO patients suggests that it is inner retinal ischemia, not anti-VEGF effect, which causes RNFL loss. Earlier studies have shown that RNFL thickness may be reduced because of the retinal ganglion cell death and axonal degeneration in diabetic retinopathy.[32,33] RNFL thickness in DMR is related to vascular abnormalities of the retina.[34] RNFL defects are significantly more severe in ischemic eyes than in nonischemic eyes in RVO.[35]

In order to investigate possible association of ME with the decreased RNFL thickness in DMR and RVO patients, we compared sectoral RNFL and macular thickness change in the injection group. Eyes with ME extending within the boundary of peripapillary RNFL measurement were excluded. During the follow-up, macular and RNFL thickness was continuously measured to assess whether the changes produced in the RNFL could be due to ME possibly reaching the peripapillary lesion. There was no association between RNFL change and macular thickness thinning (inner, outer ring, and CFT) by multivariated logistic regressing analysis. It thus seems unlikely that the decrease in observed RNFL thickness in RVO and DMR may be secondary to changes in the amount of macula layer hydration.

This study included control patients without significant ME who were observed without anti-VEGF injection. Therefore, the macular thickness in the injection groups was 84.5 ± 46.7

μm higher than that in the noninjection (control) group. The ideal control group is the patients without any treatment although they had macular edema secondary to AMD, DMR, and RVO. However, most of patients who were presented to the ophthalmic department showed vision problems due to ME. Clinically, it is not feasible to follow-up and observe patients in need of anti-VEGF therapy without providing treatment; failing to do so would raise ethical issues. To overcome this distinction of macular thickness between the injection and noninjection group, we excluded eyes with ME extended boundary of peripapillary RNFL measurement. We demonstrated that there was no association between ME and RNFL change in our study group.

Although only images with high quality were selected and SD-OCT provides better visualization than conventional time domain–OCT (TD-OCT), it is still unclear why the detection error of auto-segmented RNFL boundary occurred in the algorithm. In this study, error of auto-segmented RNFL boundary happened in nine cases (6.1%) among the enrolled 148 eyes. Han et al.[36] reported artifacts in SD-OCT in various retinal diseases despite lower frequencies compared with those of conventional TD-OCT. RNFL thickness measurement algorithms on the OCT machines were designed to work in the setting of normal eyes or eyes with glaucoma. Diseases that disrupt the retina, particularly those with exudation, can affect the reflectivity of the retinal layers and can confound these algorithms. In those cases, RNFL should be assessed with manual segmentation function built into the SD-OCT software.

The current study has several limitations that include its retrospective nature, variable follow-up period, and relatively small number of patients in the DMR and RVO groups. We also did not standardized injection intervals due to various courses of retinal disease in each patient.

Although we did not find an association between IOP and RNFL change, theoretically, chronic IOP fluctuations are a potential risk factor for glaucomatous optic nerve damage.[15,37] Therefore, more long-term follow-up of patients following anti-VEGF injections should be assessed. On the other hand, it has been suggested that RVO and glaucoma may share systemic risk factors reflecting a common pathogenic mechanism. Therefore, reduction of RNFL could develop during the natural course of disease in RVO.[38] In this study, we excluded glaucoma patients, but future studies should address whether certain patient populations, such as those with open-angle glaucoma, are more susceptible to optic nerve damage from chronic intravitreal injections of anti-VEGF agents.

In conclusion, multiple intravitreal injection of anti-VEGF did not lead to significant change in RNFL thickness in wet AMD, DMR, and RVO patients. IOP fluctuations and the frequency of the injections do not appear to adversely affect RNFL thickness. Decreased RNFL thickness associated with severity of retinal ischemia in the DMR and RVO patients suggest that inner retinal ischemia itself could be a cause of RNFL loss rather than anti-VEFG effect.

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References

1. Funk M, Karl D, Georgopoulos M, et al. Neovascular age-related macular degeneration: intraocular cytokines and growth factors and the influence of therapy with ranibizumab. *Ophthalmology*. 2009;116:2393–2399.
2. Funatsu H, Yamashita H, Ikeda T, et al. Vitreous levels of interleukin-6 and vascular endothelial growth factor are related to diabetic macular edema. *Ophthalmology*. 2003; 110:1690–1696.
3. Churchill AJ, Carter JG, Ramsden C, et al. VEGF polymorphisms are associated with severity of diabetic retinopathy. *Invest Ophthalmol Vis Sci*. 2008;49:3611–3616.
4. Ehlken C, Rennel ES, Michels D, et al. Levels of VEGF but not VEGF(165b) are increased in the vitreous of patients with retinal vein occlusion. *Am J Ophthalmol*. 2011;152:298–303.
5. Campochiaro PA, Bhisitkul RB, Shapiro H, et al. Vascular endothelial growth factor promotes progressive retinal nonperfusion in patients with retinal vein occlusion. *Ophthalmology*. 2013;120:795–802.
6. Aiello LP, Pierce EA, Foley ED, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc Natl Acad Sci U S A*. 1995;92:10457–10461.
7. You JY, Chung H, Kim HC. Evaluation of changes in choroidal neovascularization secondary to age-related macular degeneration after anti-VEGF therapy using spectral domain optical coherence tomography. *Curr Eye Res*. 2012;37:438–445.
8. Zhang X, Bao S, Hambly BD, et al. Vascular endothelial growth factor-A: a multifunctional molecular player in diabetic retinopathy. *Int J Biochem Cell Biol*. 2009;41:2368–2371.
9. Campochiaro PA. Anti-vascular endothelial growth factor treatment for retinal vein occlusions. *Ophthalmologica*. 2012;227:30–35.
10. Eyetech Study Group. Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. *Ophthalmology*. 2003;110:979–986.
11. Stewart M. Anti-vascular endothelial growth factor drug treatment of diabetic macular edema: the evolution continues. *Curr Diabetes Rev*. 2012;8:237–246.
12. Singer MA, Bell DJ, Woods P, et al. Effect of combination therapy with bevacizumab and dexamethasone intravitreal implant in patients with retinal vein occlusion. *Retina*. 2012; 32:1289–1294.
13. Horsley MB, Mandava N, Maycotte MA, et al. Retinal nerve fiber layer thickness in patients receiving chronic anti-vascular endothelial growth factor therapy. *Am J Ophthalmol*. 2010; 150:558–561.
14. Martinez-de-la-Casa JM, Ruiz-Calvo A, Saenz-Frances F, et al. Retinal nerve fiber layer thickness changes in patients with age-related macular degeneration treated with intravitreal ranibizumab. *Invest Ophthalmol Vis Sci*. 2012;53:6214–6218.
15. Zayit-Soudry S, Zemel E, Loewenstein A, Perlman I. Safety evaluation of repeated intravitreal injections of bevacizumab and ranibizumab in rabbit eyes. *Retina*. 2010;30:671–681.
16. Lee SB, Kwag JY, Lee HJ, et al. The longitudinal changes of retinal nerve fiber layer thickness after panretinal photocoagulation in diabetic retinopathy patients. *Retina*. 2010;33:188–193.
17. Tan BB, Natividad M, Chua KC, et al. Comparison of retinal nerve fiber layer measurement between 2 spectral domain OCT instruments. *J Glaucoma*. 2012;21:266–273.
18. Sung KR, Wollstein G, Bilonick RA, et al. Effects of age on optical coherence tomography measurements of healthy retinal nerve fiber layer, macula, and optic nerve head. *Ophthalmology*. 2009;116:1119–1124.
19. Ha SW, Rho SH. Age-related differences of optical coherence tomography data in Koreans. *J Korean Ophthalmol Soc*. 2005; 46:2037–2044.
20. Seth RK, Salim S, Shields MB, et al. Assessment of optic nerve cup-to-disk ratio changes in patients receiving multiple intravitreal injections of antivascular endothelial growth factor agents. *Retina*. 2009;29:956–959.

21. Foxton RH, Finkelstein A, Vijay S, et al. VEGF-A is necessary and sufficient for retinal neuroprotection in models of experimental glaucoma. *Am J Pathol*. 2013;182:1379–1390.
22. Zachary I. Neuroprotective role of vascular endothelial growth factor: signaling mechanisms, biologic function, and therapeutic potential. *Neurosignals*. 2005;14:207–221.
23. Storkebaum E, Lambrechts D, Carmeliet P. VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection. *Bioessays*. 2004;26:943–954.
24. Nishijima K, Ng YS, Zhong L, et al. Vascular endothelial growth factor-A is a survival factor for retinal neurons and a critical neuroprotectant during the adaptive response to ischemic injury. *Am J Pathol*. 2007;171:53–67.
25. Maturi RK, Bleau LA, Wilson DL. Electrophysiologic finding after intravitreal bevacizumab (Avastin) treatment. *Retina*. 2006;26:270–274.
26. Frenkel MP, Haji SA, Frenkel RE. Effect of prophylactic intraocular pressure-lowering medication on intraocular pressure spikes after intravitreal injections. *Arch Ophthalmol*. 2010;128:1523–1527.
27. Kahook MY, Kimura AE, Wong LJ, et al. Sustained elevation in intraocular pressure associated with intravitreal bevacizumab injections. *Ophthalmic Surg Lasers Imaging*. 2009;40:293–295.
28. Falkenstein IA, Cheng L, Freeman WR. Changes of intraocular pressure after intravitreal injection of bevacizumab (Avastin). *Retina*. 2007;27:1044–1047.
29. Bakri SJ, McCannel CA, Edwards AO, et al. Persistent ocular hypertension following intravitreal ranibizumab. *Graefes Arch Clin Exp Ophthalmol*. 2008;246:955–958.
30. Hollands H, Wong J, Bruen R, et al. Short-term intraocular pressure changes after intravitreal injection of bevacizumab. *Can J Ophthalmol*. 2007;42:807–811.
31. Sharei V, Höhn F, Köhler T, et al. Course of intraocular pressure after intravitreal injection of 0.05 mL ranibizumab (Lucentis). *Eur J Ophthalmol*. 2010;20:174–179.
32. van Dijk HW, Verbraak FD, Kok PH, et al. Decreased retinal ganglion cell layer thickness in patients with type 1 diabetes. *Invest Ophthalmol Vis Sci*. 2010;51:3660–3665.
33. Shahidi AM, Sampson GP, Pritchard N, et al. Retinal nerve fiber layer thinning associated with diabetic peripheral neuropathy. *Diabet Med*. 2012;29:e106–e111.
34. Oshitari T, Hanawa, K, Adachi-Usami E. Change of macular and RNFL thickness measured by stratus OCT in patients with early stage diabetes. *Eye*. 2009;23:884–889.
35. Lu H, Zang Q. The effects of retinal ischemia on retinal nerve fiber layers of patients with retinal vein occlusion. *Yan Ke Xue Bao*. 1997;13:21–24.
36. Han IC, Jaffe GJ. Evaluation of artifacts associated with macular spectral-domain optical coherence tomography. *Ophthalmology*. 2010;117:1177–1189.
37. Caprioli J. Intraocular pressure fluctuation: an independent risk factor for glaucoma? *Arch Ophthalmol*. 2007;125:1124–1125.
38. Kim MJ, Woo SJ, Park KH, et al. Retinal nerve fiber layer thickness is decreased in the fellow eyes of patients with unilateral retinal vein occlusion. *Ophthalmology*. 2011;118:706–710.

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