The Relationship Between Disorganitazion of The Retinal Inner Layers and Anti-VEGF Response in Branch Retinal Vein Occlusion

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ABSTRACT

Purpose: To determine whether the disorganization of retinal inner layers (DRIL) on spectral-domain (SD) OCT is associated with visual outcomes in eyes with treatment-naive branch retinal vein occlusion (BRVO).

Materials and Methods: Thirty-two eyes of 32 treatment-naive patients with macular edema (ME) due to BRVO receiving three initial consecutive monthly intravitreal ranibizumab were enrolled in this study. SD-OCT images from baseline, 3rd-month, 6th-month visits were observed and the presence and extent of DRIL was examined in the central 1-mm-wide foveal area. The efficacy of the ranibizumab treatment, assessed by best-corrected visual acuity (BCVA), was evaluated at baseline, 3-month and 6-month visits.

Results: A total of 32 eyes were included and DRIL was present in 14 (43.7%) eyes at baseline and 9 eyes (28.1%) at 6th month (*P*:0.01). Baseline DRIL was found to be associated with lower baseline BCVA (0.46 LogMAR for without DRIL vs. 0.65 LogMAR for DRIL, *P*: 0.013) and higher central retinal thickness (405.7 vs. 442.8, *P*: 0.016). Decrease in DRIL intensity at 3-month follow-up was associated with increased visual acuity (VA) at 6 months (*P*:0.001) and baseline DRIL extent was a predictive parameter for 6 months improvement in VA (p:0.01). Six-month DRIL change was related to 6-month VA improvement (Pearson's correlation test, r=0.726, *P*<0.001).

Conclusion: In the present study, it was observed that the improvement of BCVA was found to be related to the decrease of the DRIL intensity. Therefore, the presence of DRIL at baseline and extent of DRIL following treatment might serve as a potential SD-OCT biomarker for patients with ME secondary to treatment-naive-BRVO.

Key Words: Branch Retinal Vein Occlusion; Disorganization of Retinal Inner Layers; Optical Coherence Tomography; Ranibizumab.

INTRODUCTION

Retinal vein occlusion (RVO) is the second most common retinal vascular pathology following diabetes and may cause severe loss of vision.[1,2] Increased pressure in the vein may cause increased permeability with the help of upregulation of vascular endothelial growth factor (VEGF), breakdown of the blood-retinal barrier and thus leads to intraretinal/subretinal fluid accumulation. The chronic fluid accumulation may lead to severe visual impairment along with cystoid edema, which occurs in about 5 to 15 % of the eyes with branch retinal vein occlusion[3] (BRVO) and may cause disorganization in the ellipsoid zone in the long term period.[4]

Anti-VEGF agents are considered to be effective and safe for ME secondary to RVO. Spectral-domain optic coherence tomography (SD-OCT) biomarkers such as disorganization of retinal inner layers (DRIL) may predict future best-corrected visual acuity (BCVA) in eyes with BRVO. Foveal DRIL is defined as a failure in identification of the demarcations between the ganglion cell–inner plexiform layer complex, inner nuclear, and outer plexiform layer[5] in more than half of the 1-mm central foveal zone and it is reported that the presence of DRIL was related to worse BCVA at baseline, and its resolution was found to be associated with improved visual outcomes.[6] DRIL may indicate specific anatomical damage to the structure that transmits the visual information and may reflect capillary

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non-perfusion in the macular area in patients with diabetic retinopathy and RVO.

The main study objective was to investigate the association of DRIL and VA before and following treatment with anti–VEGF agents in patients with macular edema (ME) secondary to BRVO.

MATERIALS AND METHODS

The medical charts of patients for this retrospective study were collected from Kayseri City Hospital, department of ophthalmology, Kayseri, Turkey. Ethics committee approval was obtained from Kayseri City Hospital medical ethics committee (76397871).

Study Design

Thirty-two eyes of 32 patients with a new diagnosed with ME secondary to BRVO in Kayseri City Hospital from January 2017 to December 2018 were included in this study. Patients who were followed for at least 6 months from diagnosis and received 3 initial consecutive months of ranibizumab treatment with followed by pro-re-nata (PRN) regimen were included.

Study Subjects

Medical records of the patients enrolled in the present study were reviewed and baseline, 3rd and 6th-month BCVA, presence, and extent of DRIL, central retinal thickness (CRT), and the number of ranibizumab interventions were recorded. BCVA measured with Snellen chart converted to logarithm of the minimum angle of resolution (logMAR).

All of the patients were treatment-naive and ME due to BRVO was diagnosed by fundoscopic examination, SD-OCT and fundus fluorescein angiography (FFA). SD-OCT testing was performed by Heidelberg spectralis with the posterior pole protocol (Heidelberg Engineering, Franklin, USA). Seven B-scans were selected at each visit, one from the center of the fovea, 3 scans just above and below the fovea at each visit and the same scannes were used by the follow-up feature of the SD-OCT.

Exclusion Criteria

Patients with previous history of pars plana vitrectomy, macular edema secondary to diabetes mellitus, wet-type macular degeneration, macular hole, use of prostaglandin analogues, amblyopia, previous laser photocoagulation and steroid/anti- VEGF treatment, corneal disease, cataract or posterior capsule opacification affecting BCVA and decreasing OCT image quality in the study eye were excluded from the study.

Treatment protocol and measurements

All the patients were examined in detail including BCVA, biomicroscopic examination, FFA, and SD-OCT at baseline. SD-OCT was captured by a certified ophthalmic nurse. For this study, a total of 7 scans as mentioned above were analyzed for DRIL and evaluated by two experienced clinicians (CO, ND) and in case of disagreement, a third clinician was consulted. DRIL can be defined as the failure to recognize any of the boundaries between the ganglion cell-inner plexiform layer complex (since these two-layer interfaces cannot be easily distinguished), the inner nuclear layer or the outer plexiform layer independent of ME or other structural pathologies.[5] Among the studies, there has not been a uniform methodology for calculating the severity of DRIL. While 1000 μm centered 7 scans were being examined, scan accepted as DRIL (+) if the detected DRIL was grater than 500 μm horizontal extent in the same scan.[7] Finally, the mean DRIL value was calculated by summing the number of DRIL (+) scans from 7 horizontal scans. All scores ranged from 0 (no DRIL present in all scans) to 7 (DRIL present in all scans).

We included patients who had no previous treatment, had ME due to BRVO and had received 3 consecutive months of Ranibizumab 0.5 mg/0.05 ml (Lucentis, Novartis pharmaceuticals) injections, followed by a PRN regimen with monthly intervals. During the six-month follow-up, changes in BCVA measurement and OCT scan parameters were recorded. The responses at the end of the 3rd and 6th months, the relationship between DRIL extent with baseline BCVA and BCVA improvement and whether there is a reduction in DRIL extent with anti-VEGF treatment during follow-up were evaluated.

Despite the short follow-up period, no serious ocular and systemic adverse events occurred in any of the patients during and after the ranibizumab treatment. All injections were performed in the operating room under sterile conditions by using topical anesthesia, 0.5% proparacaine hydrochloride ophthalmic solution (Alcaine®). All of the patients dropped topical % 0.5 moxifloxacin four times a day for four days after the procedure.

STATISTICAL ANALYSIS

All analyses were performed using the SPSS for Windows V.22.0 software package (SPSS Inc., Chicago, IL). The variables were presented as mean±standard deviation (SD). The normal distribution of all variables was determined with the Kolmogorov-Smirnov test and homogeneity of variances with a one-way ANOVA test. The change in parameters before, at 3rd month and 6th month was analyzed with the *t*-test. Bivariate linear regression was

Table 1. *Patient baseline and demographic characteristics.*	
Parameter	**Mean ± SD or %**
Age (y)	58.64±12.7
Male gender, n (%)	56.2%
Baseline visual acuity (logMAR)	0.54 ± 0.16
Central retinal thickness (µm)	421.9 ± 56.1
Mean number of injections	3.53±.71
Previous laser/anti-VEGF treatment (%)	0/0
Abbreviations; VEGF, vascular endothelial growth factor	

used for cross-sectional analyses between measurements and BCVA, univariate analyses were used for predictive analyses in visual outcomes at 3rd and 6th months and for this analysis p values <0.05 were considered statistically significant.

RESULTS

Thirty-two patients with treatment-naive ME secondary to BRVO were included in the present study. The demographic and clinical characteristics of the patients such as sex distributions, baseline CRT and BCVA are presented in table 1. The mean age of the patients was 58.64±12.7 with 56.2% of the male gender. The mean BCVA was 0.54 ± 0.16 logMAR at baseline, 0.43 ± 0.10 at 3rd month and 0.41± 0.09 at 6th month (*P*<0.001 for both, paired sample *t*-test). The CRT was 421.9 ± 56.1 µm at baseline, 346.5 ± 27.5 at 3rd month and 328.1± 21.1 at the 6th month (*P*:0.001 and p:0.019 respectively, paired sample *t*-test). The mean number of injections at the end of the 6th month was 3.53±.71 and there was no significant difference in the number of injections according to the presence of DRIL (*P*:0.624, Chi-square test) but significant difference was seen in baseline BCVA according to the presence of DRIL (0.46 ± 0.11 logMAR vs 0.65 ± 0.15 logMAR, *P*:0.013). Also, baseline DRIL was found to be associated with higher CRT (405.7 vs. 442.8, *P*: 0.016). None of the eyes received laser or anti-VEGF treatment before. At baseline, 43.7% of the patients had DRIL of different extent, 37.5% at 3rd month and 28.1% at 6th month and the decrease in the DRIL rate during follow-up was significant (*P*: 0.001 and p:0.01, respectively, paired sample *t*-test). Neither neovascularization nor significant peripheral ischemia requiring laser photocoagulation was observed in any eye in FFA.

In the bivariate analysis, there was a negative correlation between baseline BCVA and baseline DRIL (Pearson's correlation test, *r*=-0.846, *P*<0.001) and baseline BCVA with baseline CRT (Pearson's correlation test, *r*=-0.818, *P*<0.001). Additionally, there was a positive correlation between baseline DRIL extent and higher CRT (Pearson's correlation test, *r*=0.694, *P*:0.006).

In the univariate analyses, as demonstrated in table 2, higher DRIL extent per B-scan (*P*:0.001) and higher CRT at baseline (*P*:0.004) were found to be predictive of greater VA improvement over 6 month. In addition, univariate analyses showed that VA improvement over 6 month was predicted by decrease in DRIL extent (*P*:0.001) and CRT values at 3rd month (*P*:0.032). As in baseline, there was a negative correlation between BCVA and DRIL extent (Pearson's correlation test, *r*=-0.726, *P*<0.001) and BCVA and CRT (Pearson's correlation test, *r*=-0.526, *P*:0.002) at 6th month. We have not experienced any ocular and/or systemic serious adverse events during the follow-up period.

DISCUSSION

In the present study, patients with center-involved ME secondary to BRVO and treated with ranibizumab were investigated and tried to answer whether the inner retinal layer was important for VA. Recently, DRIL is considered to be an important OCT biomarker in the evaluation of DME, because many studies have reported a significant relationship between VA and DRIL in DME patients[5,6-8], but the situation for RVO was not so clear. Firstly, an anatomical interruption in the pathway of visual transmission secondary to the disruption of the cells in

Table 2. *Association of baseline SD-OCT parameters and change in SD-OCT parameters at 3rd month with 6 month logMAR BCVA*				
Variable	**Association of baseline SD-OCT parameters with 6 month logMAR BCVA**		**Association between change at 3 months and BCVA at 6 month logMAR BCVA**	
	Point Estimate (95% CI)	**P*-Value**	**Point Estimate (95% CI)**	**P*-Value**
Central retinal thickness per 100 µm	0.12 (0.07 to 0.30)	0.004	0.37 (0.06 to 0.70)	0.032
Average DRIL extent per B-scan	0.03 (0.01 to 0.06)	0.001	0.03 (0.02 to 0.04)	0.001
Abbreviations; DRIL; Disorganization of retinal inner layers, BCVA; Best Corrected Visual acuity , * P<0.05 considered to be statistically significant.				

the inner retinal layers may impair neural transmission from outer retina to inner retina.[5] Secondly, ischemia may compromise internal retinal circulation and can cause DRIL development in BRVO.[9,10] In addition, inflammation and secondary vascular leakage have been associated with chronic inner retinal neurodegeneration.[11] Mimouni et al.[12] reported a significant relationship between DRIL and vision in RVO patients however, Babiuch et al. reported stronger association in the central RVO than BRVO.[13] Besides, Nakano et al. reported that DRIL had a minor role in determining the vision for ME due to BRVO.[14] In our study, about up to half of the patients presented with varying extent of DRIL and baseline BCVA was significantly lower when any DRIL was present and we observed that, VA improvement was higher in eyes with lower DRIL extent. As baseline, 6th-month DRIL correlated with 6th-month BCVA and moreover, decrease in DRIL extent at 3rd month predicted 6th month BCVA, therefore changes in DRIL burden with ranibizumab demonstrated potential OCT biomarker of VA improvement. We think that the degree of acute ischemic damage affects the survival of retinal neuronal structures.

Acute edema and subsequent atrophy of the inner retinal structures have been demonstrated by experimental models. Increased venous pressure after acute venous obstruction, decreased the inner retinal micro-vascular perfusion[15-17] so, DRIL may indicate at least partially reversible ischemia of inner retinal cells that responsible for relaying information and disorganization in their synapses.[8] This may explain why an SD-OCT biomarker, such as DRIL, correlates with BCVA in pathology such as RVO, which is more concerned with the circulation and function of primary internal retinal structures. Thus, recovery of DRIL in the early period may represent reperfusion, without concomitant development of irreversible neuronal degeneration. It has been reported that anatomical improvement may be less associated with functional improvement as the disease becomes more chronic; in our study with treatment-naive eyes, it has been shown that DRIL continued to decrease with 6 months follow-up with treatment. This, in contrast to chronic disease, reflects that a certain proportion of treatment-naive eyes may be reversible in anatomical and functional damage to the inner retinal layers. Radwan et al. reported that damage to the inner retinal layers may be irreversible in patients with high DRIL extent.[6] In ME, which can accompany DRIL, it is assumed that neural conduction defect is caused by neuronal deformation.[18,19] The pathophysiologic mechanisms of DRIL in ME due to BRVO are not fully understood and finally, further studies are required to confirm the anatomic basis of DRIL in BRVO.

In addition to the DRIL extent, higher CRT values were also cross-sectionally associated with BCVA in bivariate analyses. The greater extent of the presence of DRIL in eyes with a thicker CRT shows that DRIL tends to develop in eyes with prolonged exudative changes. Therefore, chronic edema of the inner retina may damage retinal cells and DRIL may be associated with a long-term vision in eyes with recurrent or chronic ME. [20, 21] However, treatment of ME secondary to BRVO should not only reduce ME but also aim to preserve the integrity of the inner retinal layer.

The present study has some limitations. First, it has a retrospective and cross-sectional structure, and the number of cases is insufficient to interpret the results. Therefore, the results of our study should be confirmed by further studies that include prospective, larger sample size and longer observations. Second, we did not investigate angiographic results and focused especially on SD-OCT parameters. However, several studies suggest that the foveal avascular zone (FAZ) is associated with vision in eyes with RVO.[22, 23] Given that DRIL is frequently found with capillary nonperfusion areas in diabetic retinopathy, simultaneous analysis of DRIL and FAZ can be considered reasonable. Shortly, we believe that macular ischemia assessment based on OCT angiography will be important in elucidating the vascular pathophysiological features of DRIL in BRVO. Third, there is no common consensus among studies on internal retinal markers used in DRIL measurement and may be affected by errors of personal origin.[5,6,9,24] Perhaps, this can be more practical with objective measurements provided by the automated software. Therefore, automated DRIL identification and measurement may be useful OCT biomarker for clinicians for VA prediction, evaluating treatment effects and adjustment of treatment methods for each patient.

In conclusion, in the present study, we observed that DRIL in a 1 mm foveal area investigated on 7 B scans was associated with baseline BCVA and outcome BCVA in BRVO-ME over 6 months follow-up. The change of the DRIL extent following treatment may identify eyes with a high likelihood of subsequent VA improvement. Therefore, the extent of DRIL before, during and after treatment is a useful OCT biomarker that can help specialists in the treatment of patients with ME secondary to BRVO.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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