

Evaluation of the Relationship Between the Number of Hyperreflective Spots and Ischemia Parameters Detected by Optical Coherence Tomography in Eyes with Retinal Vein Occlusion

Yucel Yigit¹, Kivanc Kasal², Yurdagul Girgin³, Eyyup Karahan⁴

ABSTRACT

Purpose: This study aimed to investigate the associations between hyperreflective dots (HRDs) and ischemia-related OCT biomarkers—specifically paracentral acute middle maculopathy (PAMM) and the prominent middle limiting membrane (p-MLM)—and to evaluate the relationship between HRDs and inflammatory markers in patients with retinal vein occlusion (RVO).

Methods: This retrospective study included 40 eyes from 40 patients diagnosed with RVO. Comprehensive ophthalmologic examinations and spectral-domain optical coherence tomography (SD-OCT) were performed at baseline and after three intravitreal injections of bevacizumab. HRDs, PAMM, p-MLM, central macular thickness (CMT), external limiting membrane (ELM), and the integrity of the ellipsoid zone (EZ) were independently evaluated by two masked retina specialists. Statistical analyses were performed.

Results: Inter-rater reliability was high for the assessment of HRDs, PAMM, and p-MLM. HRD counts showed a significant correlation with CMT and foveal depression. Higher HRD counts were observed in cases with serous macular detachment (SMD) compared to those without SMD. No significant association was found between HRDs and PAMM, p-MLM, or ELM defects.

Conclusion: The findings of this study suggest that HRDs in RVO may be more closely linked to inflammatory processes rather than ischemia. While structural ischemia-related OCT biomarkers, such as PAMM and p-MLM, were not associated with HRD presence, their association with CMT and subretinal fluid may indicate an indirect relationship with inflammatory mechanisms. However, due to the study's retrospective design and the relatively small sample size, these results should be interpreted cautiously. Larger, prospective studies are needed to clarify the underlying mechanisms and better define the relationship between HRDs, ischemia-related biomarkers, and inflammation.

Keywords: Hyperreflective Dots, Inflammation, Paracentral Acute Middle Maculopathy, Retinal Ischemia, Retinal Vein Occlusion

INTRODUCTION

Retinal vascular diseases, such as diabetic retinopathy and retinal vein occlusion (RVO), are among the most common causes of severe vision loss worldwide. The key pathophysiological mechanisms underlying these conditions include endothelial dysfunction, inflammation, capillary leakage,

and ischemia.⁽¹⁾ Spectral-domain optical coherence tomography (SD-OCT) is a non-invasive imaging modality that is widely used in the diagnosis and follow-up of macular diseases. In eyes affected by RVO, SD-OCT allows the detection of various biomarkers related to ischemia and inflammation.

1 Amavutkoy State Hospital, Ophthalmology, Istanbul, Türkiye

2 Bigadic State Hospital, Ophthalmology, Balikesir, Türkiye

3 Corlu State Hospital, Ophthalmology, Tekirdag, Türkiye

4 University of Balikesir of Medicine, Ophthalmology, Balikesir, Türkiye

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Correspondence author:

Yucel Yigit

Email: yucel.yigit1995@gmail.com

Coscas et al. identified a novel hyperreflective feature on SD-OCT, referred to as hyperreflective dots (HRDs), which revealed previously unrecognized intraretinal changes.⁽²⁾ Several studies have hypothesized that HRDs may correspond to activated microglial or Müller glial cells—the primary immune cells of the retina—that migrate and release proinflammatory mediators during retinal injury.⁽³⁾ Although the relationship between HRDs and inflammation has been well described, it is theoretically plausible that the severity of ischemia is also associated with microglial activation. Activated microglial cells primarily secrete cytokines and other proinflammatory molecules, which facilitate phagocytosis and the elimination of damaged cells associated with neurodegeneration.⁽⁴⁾ In areas with severe ischemia, increased cell damage is expected, which may, in turn, lead to enhanced microglial activity. However, to date, no study has specifically investigated the relationship between HRDs and microglia presumed to be in an activated state.

Paracentral acute middle maculopathy (PAMM), first described by Sarraf et al., is considered a variant of acute macular neuroretinopathy.⁽⁵⁾ It is characterized by a hyperreflective band involving the inner nuclear layer (INL) on SD-OCT and is thought to result from ischemia of the deep retinal capillary plexus.⁽⁶⁾ This localized ischemia is believed to reflect sublethal hypoxia affecting the middle retinal layers, predominantly the INL.⁽⁷⁾ Chu et al. introduced the “prominent middle limiting membrane (p-MLM) sign,” described as a thin hyperreflective line within the outer plexiform layer (OPL) on SD-OCT B-scan images.⁽⁸⁾ This sign has been associated with acute ischemic injury in retinal artery occlusion (RAO) and is proposed to help differentiate ischemic from non-ischemic forms of RVO. Although the underlying pathophysiology of the p-MLM sign is thought to be similar to that of PAMM, its relationship with other ischemia-related biomarkers has not yet been fully elucidated.⁽⁸⁾

In this study, we aimed to evaluate the relationship between the number of HRDs detected by OCT and both inflammatory and ischemia-related parameters in eyes with retinal vein occlusion. Clarifying this association may enhance our understanding of RVO pathophysiology and support the development of more targeted diagnostic and therapeutic strategies.

MATERIAL AND METHOD

In this retrospective study, the medical records of 40 patients diagnosed with RVO at Balikesir University were reviewed. All patients met predefined inclusion and exclusion criteria. The study was conducted in accordance with the principles of the Declaration of Helsinki, and ethical approval was obtained from the Institutional Review Board/Ethics Committee on 02/09/2025 with approval number 2025/6-19.

A total of 40 eyes from 40 patients diagnosed with RVO were included. Each patient underwent a comprehensive ophthalmic examination, including best-corrected visual acuity (BCVA) assessment, anterior segment evaluation, intraocular pressure (IOP) measurement, and spectral-domain OCT imaging (SD-OCT, Optovue, Fremont, CA, USA).

The following parameters of SD-OCT evaluations at baseline and after the third intravitreal injection of bevacizumab were recorded: the presence and horizontal length of PAMM, the presence and horizontal length of the p-MLM sign, central macular thickness (CMT), and the integrity of the ellipsoid zone (EZ) and that of the external limiting membrane (ELM).

The number of HRDs, as well as the presence and lengths of PAMM and p-MLM, were independently evaluated by two masked retina specialists (Y.G. and K.K.). Horizontal macular OCT scans from all participants were reviewed, and the image corresponding to the minimum foveal thickness was selected. This scan was exported as a “.tiff” file and analyzed using ImageJ software (<http://imagej.nih.gov/ij/>; National Institutes of Health, Bethesda, MD, USA). A standardized region of interest (ROI) measuring 3000 × 1500 μm was delineated and centered on the foveal center. Within this predefined ROI, HRDs were independently quantified by two masked observers for each image. HRDs were defined as discrete, punctate lesions ≤ 30 μm in diameter, exhibiting moderate reflectivity without intervening hyporefective spaces, resembling the reflectivity pattern of the RNFL. Counting of HRD was restricted to the retinal layers extending from the RNFL to the outer plexiform layer. Measurements were averaged across the same scan to minimize variability. Inter-rater reliability was assessed using Cohen’s kappa (κ) coefficient.

Statistical analyses were performed using SPSS version 25.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables are presented as means \pm standard deviation (SD), and categorical variables as frequencies and percentages. The Kolmogorov-Smirnov test was used to assess the normality of data distribution. A p -value of ≤ 0.05 was considered statistically significant. To assess interobserver agreement in HRD quantification, the intraclass correlation coefficient (ICC) was calculated. Non-parametric comparisons between groups were conducted using the Mann-Whitney U test, and correlations were evaluated using Spearman's rank correlation test.

RESULTS

The mean age of the patients was 62.9 ± 12.0 years. Of the 40 patients, 21 (52.5%) were female, and 19 (47.5%) were male. Based on clinical diagnosis, 25 patients were followed for branch retinal vein occlusion (BRVO), 6 for central retinal vein occlusion (CRVO), and 9 for hemiretinal vein occlusion (HRVO).

Inter-rater reliability between the two retina specialists for the evaluation of HRDs (Figure 1), PAMM (Figure 2), and

p-MLM (Figure 3) was high, with κ values of 0.841, 0.861, and 0.917, respectively (all $p < 0.001$).

Correlation analyses were conducted to assess the relationship between initial and final HRD counts and OCT parameters. There was no statistically significant correlation between the number of HRDs and the presence of PAMM or p-MLM. Similarly, no significant association was observed between HRD counts and the presence of ELM defects or subretinal fluid.

On the other hand, significant correlations were identified between both initial and final HRD numbers and CMT ($p < 0.05$ and $p = 0.05$, respectively).

Furthermore, the final HRD counts were significantly associated with the presence of foveal depression ($p = 0.03$).

Although not statistically significant, both initial and final HRD counts were found to be higher in patients with serous macular detachment (SMD) compared to those without SMD.

Detailed correlation data between the initial and final HRDs counts and OCT findings are presented in Table 1.

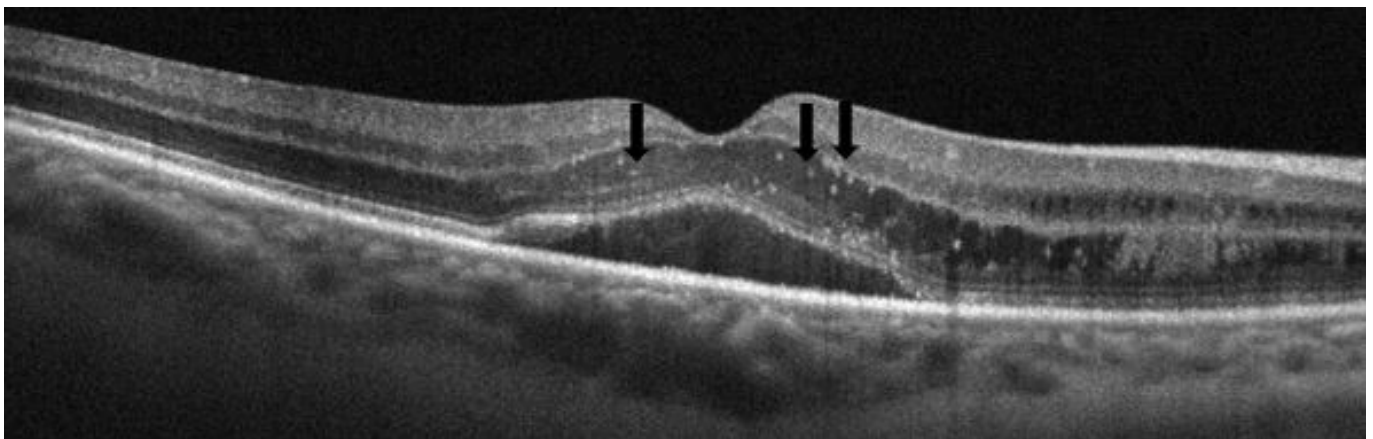


Figure 1. SD-OCT image of a patient with right branch retinal vein occlusion (BRVO). Hyperreflective Dots (HRD) within a 1500- μm region extending nasally and temporally from the foveola were identified on the horizontal macular OCT section, where the fovea was thinnest, by two masked investigators. The hyperreflective dots is indicated by the black arrow.

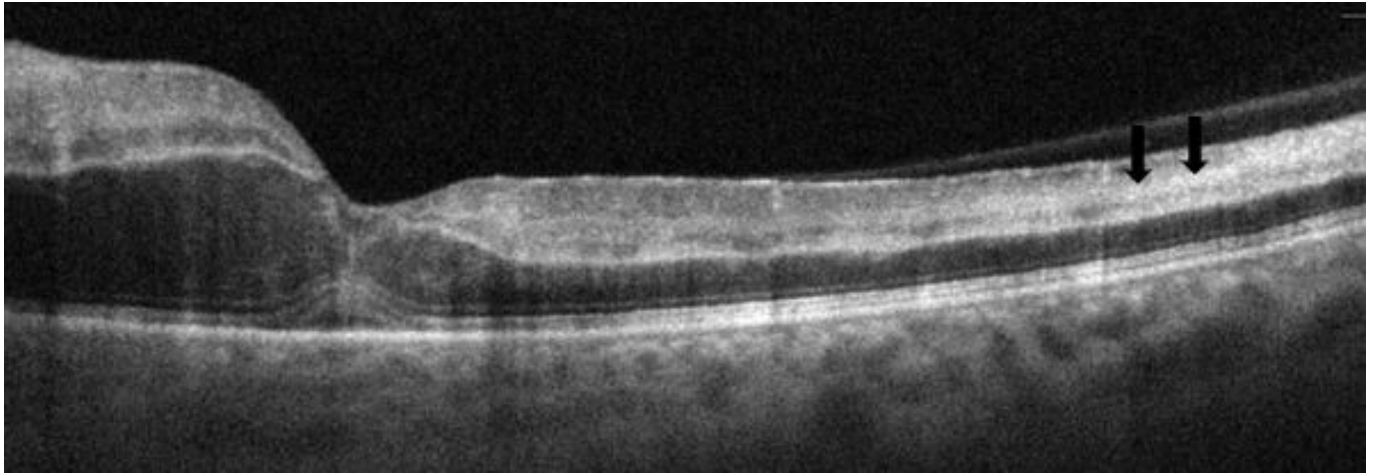


Figure 2. SD-OCT image of a patient with left central retinal vein occlusion (CRVO). A hyperreflective band at the level of the inner nuclear layer, consistent with paracentral acute middle maculopathy (PAMM), was identified on the horizontal macular OCT section within the predefined region of interest by two masked investigators. The black arrow indicates the hyperreflective band.

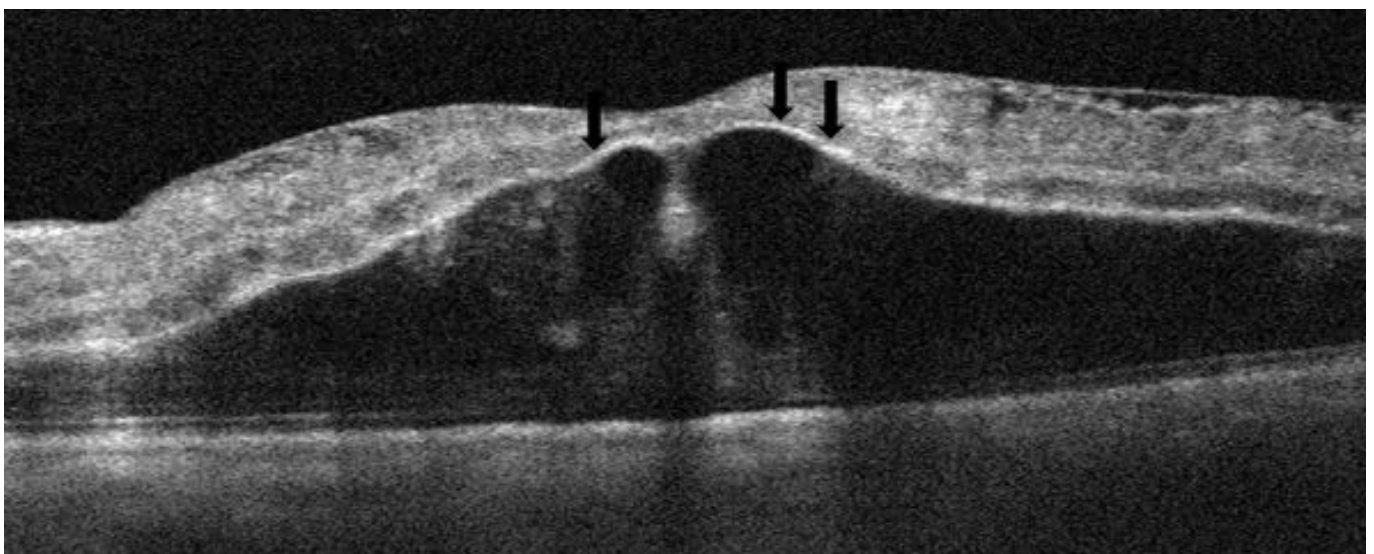


Figure 3. SD-OCT image of a patient with left hemiretinal vein occlusion (HRVO). A thin, linear hyperreflective band at the level of the middle limiting membrane, consistent with perifoveal middle limiting membrane (p-MLM) involvement, was identified on the horizontal macular OCT section by two masked investigators. The black arrow indicates the p-MLM finding.

Table 1 *The correlation between initial and final HRD counts and OCT findings*

	Initial Mean HRD	Final Mean HRD
Average PAMM Length	0.858	0.544
Presence of PAMM (Y.G)	0.881	0.253
Presence of PAMM (K.K)	0.684	0.464
Average p-MLM Length	0.259	0.582
Presence of p-MLM (Y.G)	0.136	0.094
Presence of p-MLM (K.K)	0.417	0.318
CMT	≤ 0.05	0.051
Presence of ELM Defect	0.152	0.478
Presence of EZ Defect	0.212	0.744
Presence of Foveal Depression	0.137	0.030
Presence of Subretinal Fluid	0.308	0.360
Vision	0.218	0.277

DISCUSSION

In this study, we investigated the relationship between ischemic biomarkers—PAMM and p-MLM—and inflammatory markers in order to better understand the pathophysiology of HRDs observed in patients with RVO.

HRDs are abnormal, punctate, and scattered hyperreflective elements observed in OCT scans. They are typically located in the outer retinal layers or adjacent to intraretinal or subretinal fluid, and unlike hard exudates, they usually do not coalesce.⁽⁹⁾

Although the exact etiology of HRDs remains unclear, several theories have been proposed. After being described as a novel OCT biomarker by Coscas et al., HRDs have since been identified in various retinal pathologies, including age-related macular degeneration (AMD), diabetic macular edema (DME), RVO, central serous chorioretinopathy, uveitis, and macular telangiectasia.^(2,10) It has been suggested that HRDs may represent activated microglial cells or inflammatory cells, as they can migrate across retinal layers and respond to local cytokine activity. Notably, HRDs tend to regress rapidly following anti-VEGF or anti-inflam-

matory treatment, supporting the notion that inflammation plays a central role in their formation.⁽²⁾

Zeng et al. reported that in human donor eyes, activated microglia increasingly infiltrate deeper retinal layers, particularly the outer retina, as diabetic retinopathy progresses.⁽¹¹⁾ Similarly, Karalezli et al. observed significant structural alterations in HRDs, ELM, and EZ on OCT in patients treated with intravitreal ranibizumab for RVO. They recorded associations between HRD density, outer retinal layer disruption, and reduced visual acuity, suggesting that HRDs may serve as clinical prognostic indicators for photoreceptor dysfunction. Furthermore, they linked persistent HRDs to poor visual recovery despite CMT.⁽¹²⁾

Consistent with these findings, our study revealed a significant correlation between HRD counts and CMT, SMD, and foveal depression. However, in contrast to some previous reports, we did not observe a significant correlation between HRDs and ELM or EZ defects. Similarly, no association was found between HRDs and baseline or final best-corrected visual acuity. This discrepancy may be attributed to differences in sample size, treatment protocols, or imaging resolution.

Uji et al. also found that HRDs—particularly those located in the outer retinal layers—were associated with disruption of the ELM and IS/OS junction and correlated with reduced visual acuity in patients with DME.⁽¹³⁾ However, our findings did not support this association in the context of RVO.

PAMM, first described by Sarraf et al., results from ischemic hypoxia affecting the inner nuclear layer and appears as a hyperreflective band on SD-OCT.⁽⁵⁾ The p-MLM sign, defined by Chu et al., is a hyperreflective line in the outer plexiform layer and indicates acute ischemic damage in retinal artery occlusion; it has also been proposed as a tool for distinguishing between ischemic and nonischemic RVO.⁽⁸⁾ In our study, to better understand the potential ischemic contribution to HRD formation, we evaluated the presence and horizontal extent of PAMM and p-MLM in patients with RVO. Independent evaluations by two retina specialists revealed no statistically significant correlation between the presence or extent of these ischemic markers and HRD counts.

To the best of our knowledge, this is the first study to systematically investigate the relationship between PAMM, p-MLM, and HRDs in patients with RVO. Our findings suggest that while HRDs are present in ischemic retinal conditions, their formation may not be directly driven by ischemia but rather may be more closely related to inflammatory mechanisms.

In eyes with RVO, the number of HRDs was not significantly associated with structural ischemia-related biomarkers such as PAMM and p-MLM. However, significant correlations were found with CMT, serous macular detachment, and foveal depression. These findings suggest that inflammation, rather than ischemia-induced apoptosis, may play a more prominent role in the pathogenesis of HRDs in RVO.

This study has several limitations. First, an a priori power analysis was not performed. Due to the retrospective design and the relatively small sample size, the statistical power—especially for subgroup analyses of CRVO and HRVO—may have been limited. As a result, the study might not have been sufficiently powered to identify subtle associations between HRDs and ischemia-related biomarkers.

Second, ischemia was evaluated solely using structural OCT biomarkers, specifically PAMM and pMLM. Al-

though these findings suggest ischemic injury, they do not directly indicate retinal perfusion status. The lack of OCT angiography or fluorescein angiography data, such as capillary nonperfusion area, ischemic index, or vascular density, is a significant limitation and prevents definitive conclusions about vascular ischemia.

Further prospective studies with larger cohorts and longitudinal follow-up are warranted to confirm these findings and to elucidate the underlying mechanisms contributing to HRDs formation in retinal vascular diseases.

DISCLOSURE OF INTEREST

No potential conflict of interest was reported by the authors.

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