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Altered platelet morphological parameters in patients with retinal vein occlusion

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Abstract. – OBJECTIVE: The purpose of this work was to investigate whether platelet morphology or functions are altered in retinal vein occlusion (RVO) patients.

PATIENTS AND METHODS: Eighty-three patients with a RVO and eighty-five healthy subjects were included in this prospective study. RVO was diagnosed by ophthalmic fundus examination, and complete ophthalmic evaluations of both eyes were performed. The platelet large cell ratio (PLCR), platelet distribution width (PDW), mean platelet volume (MPV), platelet crit (PCT), and platelet count were determined for each participant.

RESULTS: The MPV, PDW and PLCR were significantly higher in the RVO group than in the control group (MPV: 8.26 ± 1.22 fL *vs*. 7.41 ± 0.69 fL, respectively, $p = 0.006$; PDW: 13.43 ± 1.75% *vs*. 12.19 ± 1.51%, respectively, $p = 0.0022$; and PLCR: 30.62 ± 4.65% *vs*. 28.59 ± 4.18%, respectively, $p = 0.003$). There were no significant differences in the PCT or platelet count between the two groups (253.76 ± 70.87 x 10^3/µl *vs*. 248.96 ± 62.44 x 10^3/µl, respectively $p > 0.05$; and PCT: 0.24 ± 0.07 % *vs*. 0.27 ± 0.06%, respectively, $p > 0.05$).

CONCLUSIONS: We observed that the platelets RVO patients exhibit morphological evidence of hyperreactivity (e.g., a higher MPV, PDW and PLCR). Also, larger platelets are hemostatically more active than small ones and an increased proportion of large platelets is a risk factor for developing RVO.

Key Words:
Retina vein occlusion, Platelet activation, Mean platelet volume, Platelet large cell ratio, Platelet distribution width.

Introduction

Retinal vein occlusion (RVO), which is most commonly observed in the elderly, can result in loss of vision and neovascular glaucoma; and it is the second most frequently observed retinal vascular disease after diabetic retinopathy[1]. It is classified as either branch retinal vein occlusion (BRVO) or central retinal vein occlusion (CRVO) according to the location of the vessel occlusion. The prevalence of CRVO is 0.08%, and that of BRVO is 0.44%[2]. The pathogenesis of RVO is not clearly understood. High intra-ocular pressure, or glaucoma, and short axial distance are believed to be local risk factors[3]. Hypertension, arteriosclerosis, diabetes mellitus, hypercholesterolemia, systemic inflammatory diseases, pregnancy, oral contraceptive use, smoking, hereditary thrombophilia, and increased coagulability may also be risk factors for RVO[4].

Platelets play an important role in vaso-occlusive diseases[5]. The mean platelet volume (MPV) provides an estimate of platelet dimension of and is a marker of platelet activation[6]. Large platelets are relatively more active than small ones; they produce more glycoprotein Ib and glycoprotein IIb/IIIa receptors, release more thromboxane A2 and rapidly aggregate[7]. A high MPV has been shown to be a risk factor for cardiovascular disease[6]. This parameter has been investigated in RVO, Behcet's disease, hypertension, rheumatoid arthritis, pseudo-exfoliation syndrome, and diabetes mellitus[8-13]. In addition to the MPV, other markers of platelet morphology, such as the platelet distribution width (PDW), platelet large cell ratio (PLCR) and platelet crit (PCT), may play important roles in vascular diseases, including atherosclerosis and thrombosis[14,15].

To the best of our knowledge, the morphologic parameters that act as indicators of subclinical platelet activation, including the PDW, PLCR and PCT, have not yet been investigated in RVO patients. Therefore, the aim of this study was to investigate indicators of platelet morphology, including the MPV, PLCR, PDW, PCT, and platelet count in patients with RVO.

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Patients and Methods

All participants underwent complete ocular examinations, including evaluations of best corrected visual acuity, pupillary reactions, slit lamp biomicroscopic and funduscopic examinations, and measurements of intraocular pressure at the beginning of the study. Patients who were diagnosed with any type of RVO between January 2015 and December 2015 were enrolled at Kocaeli Derince Education and Research Hospital. These patients underwent careful fundus fluorescein angiography and optical coherence tomography analyses.

Patients with diabetes mellitus, uncontrolled hypertension, anemia (hemoglobin of below 12%), an infectious disease, malignancy, liver or renal insufficiency, glaucoma, cerebrovascular disease or any cardiovascular disease such as congestive heart failure and, heart valve disease, as well as smokers and those who were being treated with an anticoagulant, were excluded. The healthy control group was age- and sex-matched to the study group.

To avoid the confounding effects of diurnal variation on the hemostatic system, sampling procedures were performed in the morning while the participants were in a fasting state. Blood was collected with minimal stasis into EDTA Vacutainer tubes and analyzed within one hour using a commercially available analyzer (Sysmex XT 1800i Automated Cell Counter, Japan). This study was approved by the Institutional Ethics Committee of the Kocaeli University School of Medicine and was carried out in compliance with the Declaration of Helsinki.

Statistical Analysis

Statistical analyses were performed with using SSPS statistical software (SPSS for Windows 18.0, Chicago, IL, USA). The values are presented as the mean± standard deviation (SD). The significance level was set at a $p < 0.05$. The Kolmogorov-Smirnov test was applied to test the distribution pattern of each value, and the data were compared between groups using Student's *t*-test. Receiver operating characteristic (ROC) analyses were conducted to identify the cutoff values and specificity/sensitivity of the platelet indices. Further logistic regression analysis was performed to identify associations between RVO and platelet parameters.

Results

A total of 168 participants were recruited, including 83 patients with any type of RVO and 85 healthy subjects. The average age of the subjects in the RVO group was 64.1 ± 16.4 years, and that of those in the control group was 63.3 ± 13.8 years. The overall female: male ratios were 31/52 in the RVO group and 33/52 in the control group. There were no significant differences in age or sex between the groups ($p > 0.05$).

The mean MPV values were 8.26 ± 1.22 fL in RVO patients, and 7.41 ± 0.69 fL in the controls. A significant difference in the MPV was observed between the groups ($p = 0.006$) (Table I). Logistic regression analysis revealed that MPV was an independent predictor of RVO (odds ratio (OR) = 2.69, 95% confidence interval = 1.53-4.73; $p = 0.01$). In addition, in ROC analysis comparing the RVO patients and controls, cutoff value of 7.65 (area under the curve (AUC): 0.685), specificity of 67.1% and sensitivity of 71.1% were calculated for the RVO patients ($p < 0.001$) (Figure 1).

The mean PDW values were 13.43 ± 1.75% in the RVO patients and 12.19 ± 1.51 % in the controls. A significant difference in the PDW was detected between the groups ($p = 0.0022$) (Table I). Logistic regression analysis revealed that the

Table I. The platelet morphological indices and demographic data of the groups.

	RVO group	Control group	*p*-value
Age	64.1 ± 16.4	63.3±13.8	0.68
Gender	52/31	52/33	0.29
MPV	8.26 ± 1.22	7.41 ± 0.69	0.006
PDW	13.43 ± 1.75	12.19 ± 1.51	0.0022
PLCR	30.62 ± 4.65	28.59 ± 4.18	0.003
PCT	0.24 ± 0.07	0.27±0.06	0.28
Platelet count	253.76 ± 70.87	248.96 ± 62.44	0.16

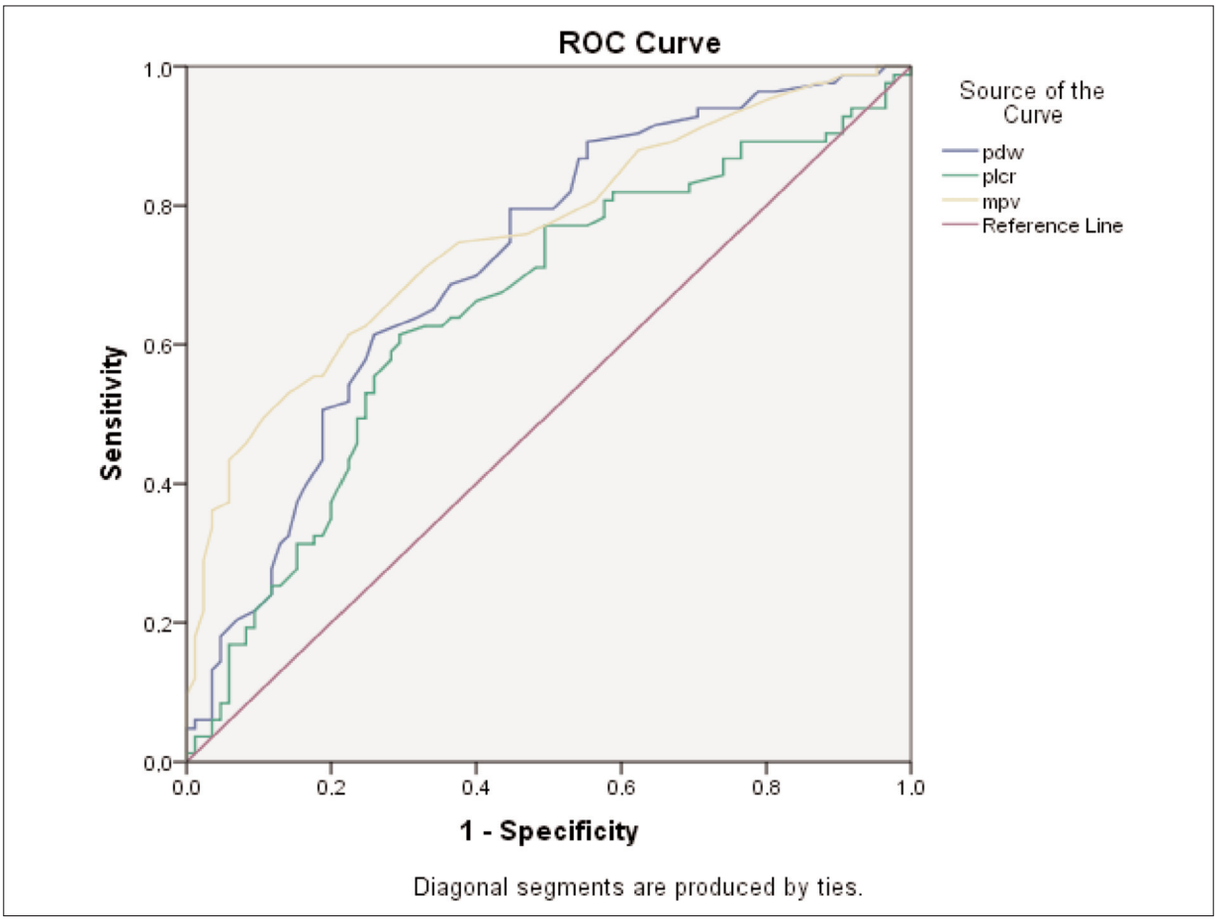

Figure 1. Receiver operating characteristic curves for predictors of retinal vein occlusion. Area under the curve for the MPVs = 0.685, yellow line; area under the curve for the PDWs = 0.719, blue line; and area under the curve for the PLCRs = 0.653, green line.

PDW was also an independent predictor of RVO (OR = 8.68, 95 % confidence interval 23.5-3.20; $p < 0.001$). Further, in ROC analysis comparing the RVO patients and controls, a cutoff value of 12.65 (AUC: 0.719), specificity of 66.8% and sensitivity of 65.1% were calculated for the RVO patients ($p < 0.001$) (Figure 1).

The mean PLCR values were 30.62 ± 4.65% in the RVO patients and 28.59 ± 4.18% in the controls. A significant difference in the PLCR was detected between the groups ($p = 0.003$) (Table I). Logistic regression analysis revealed that the PLCR was also an independent predictor of RVO (OR = 0.46, 95% confidence interval = 0.66-0.32; $p < 0.001$). In addition, in ROC analysis comparing the RVO patients and controls, a cutoff value of 29.04 (AUC: 0.653), specificity of 62.4% and sensitivity of 63.9% were calculated for the RVO patients ($p = 0.001$) (Figure 1).

The mean PCT values were 0.24 ± 0.07 % in the RVO group and 0.27 ± 0.06% in the control group. There was no significant difference in the PCT between the groups ($p < 0.05$). The mean platelet counts were 253.76 ± 70.87 × 10^3/ L in the RVO group and 248.96 ± 62.44 × 10^3/ L in the control group. There was no significant difference in the mean platelet count between the groups ($p < 0.05$).

Discussion

The results of this study showed that the MPV, PDW and PLCR were higher in the RVO patients than in the healthy controls. However, no significant differences in the platelet count or PCT were observed between these two groups. To the best of our knowledge, this is the first study to determine the PDW, PCT and PLCR in RVO patients.

Platelets play an important role in the pathogenesis of vascular diseases[5]. The MPV is an indicator of platelet dimension and activation. Associations between an increased MPV and deep vein thrombosis[16], myocardial infarction[17] and acute ischemic cerebrovascular syndrome[6] have been previously reported. Also, several studies examining the relationship between the MPV and RVO have been published. For example, Sahin et al[12] have reported that the MPV is significantly higher in RVO patients than in controls, and these authors have suggested that an abundance of large platelets may contribute to RVO pathogenesis. Further, Onder et al[18] has shown that the MPV is significantly increased in patients with hypertensive BRVO, and they have suggested that this parameter may be, therefore, a potentially useful biomarker for monitoring disease progression. However, Ornek et al[19] have found that the MPV is significantly lower in RVO patients and have indicated that this parameter may thus not be a useful biomarker of RVO. We found that the MPV was significantly higher in the RVO patients than in the controls. Logistic regression analysis revealed that the risk of developing RVO was increased by 2.69-fold in the subjects with an increased MPV (OR = 2.69, $p = 0.01$).

Vagdatli et al[20] have shown that the MPV and PDW are both increased in disorders related to platelet activation. These findings support the notion that the PDW may be a more specific marker of platelet activation. These authors have also indicated that high PDW is associated with platelet anisocytosis and have suggested that this anisocytosis may be related to the formation of pseudopodia. Khandekar et al[21] have found that the PDW is higher in patients with acute ST-segment elevation than in those with stable coronary artery disease. Celik et al[22] have suggested that the PDW is an independent correlate of in-hospital major adverse cardiovascular events. In addition, Rechkinski et al[23] have reported that the PDW is an independent risk factor for cardiac mortality and either death, recurrent MI or the need for an additional revascularization procedure. Further, Jindal et al[24] have reported that the PDW is significantly increased in diabetic patients, and these authors have stressed that it may be even higher in patients who have developed microvascular complications. In our study, a significantly higher PDW was observed in the RVO patients than in the controls. Logistic regression analysis showed that the risk of developing RVO was increased by 8.68-fold in the subjects with an increased PDW (OR = 8.68, $p < 0.001$). As far as we know, this is the first study to demonstrate an association between the PDW and RVO.

The PLCR is another marker of platelet volume that represents the proportion of large platelets. A high PLCR generally indicates the presence of a high density of new platelets (young platelets tend to be larger than old platelets). Babu et al[25] have shown that the PLCR has an inverse relationship with the platelet count but that it is directly related to the MPV and PDW. In addition, Khandekar et al[21] have found that the PLCR is higher in patients with acute coronary syndrome than in those with stable coronary artery disease and in controls. Further, Rechkinski et al[23] have argued that the PDW and PLCR may be useful prognostic markers after myocardial infarction and that they may actually be stronger markers than the MPV. Malachovska et al[26] have shown that the PLCR is significantly increased in patients with type 1 diabetes. Further evaluation of the PLCR is necessary in future studies. We did not find any studies examining the relationship between RVO and the PLCR in the medical literature. In our study, the PLCR was significantly higher in the RVO patients, and logistic regression analysis showed that it was an independent predictor of RVO (OR = 0.46, $p < 0.001$).

In this report, no significant differences in the PCT or platelet count were observed between the RVO and control groups. Onder et al have observed that the platelet count is significantly lower in BRVO patients[18]. However, Ornek et al[19] have indicated that the platelet count is significantly higher in BRVO patients and that it is significantly lower in CRVO patients. The PCT is considered a marker of circulating platelets per unit volume of blood (calculated as the platelet count x MPV/10^7), and it is used as an effective screening tool to detect abnormalities in the platelet count[27]. Akpınar et al[28] supported the notion that the PCT has important predictive value in patients with a saphenous vein graft, and they stressed the importance of using antiplatelet treatments in patients who developed graft atherosclerosis after bypass surgery. Further investigation is needed to support these findings.

Thrombosis and thrombolysis occur in nearly all RVO patients and elucidation of the underlying biochemical pathways are, therefore, important for understanding the pathogenesis of this disease[29]. Young platelets are larger and more active than old platelets, and they produce high lev-

els of thromboxane A1, serotonin and beta-thromboglobulin[30]. Mean platelet indices (MPIs, *e.g.* MPV, PDW and PLCR) provide clues as to the proportion of these large, young platelets. The detection of increases in not only the MPV but also the PDW and PLCR support the notion that increased platelet aggregation plays a role in RVO pathogenesis.

RVO patients are known to be at an increased risk of developing cardiovascular disease[31]. Kaderli et al[32] have found that myocardial performance is impaired in BRVO patients. Further, Martin et al[33] have shown that RVO patients have a significantly higher rate of generalized cardiovascular disease than expected according to analysis of age-matched individuals and that their coronary heart disease risk is also much higher than the normal 10-year occurrence. Patients with cardiac disease may have higher MPI values, similar to those with RVO. These data suggest that a relationship exists between cardiovascular disease and RVO, and that the development of these conditions are associated with the MPI values.

Conclusions

The results of the present study revealed that the MPI values (MPV, PDW, and PLCR) were significantly higher in the RVO patients than in the healthy controls. No significant differences in the platelet count or PCT were observed between the groups. Thus, MPI values may be important in the diagnosis and treatment of RVO patients, and it is possible that higher MPI values are correlated with the rates of cardiovascular disease and cardiac mortality in these patients. Platelet parameters can be easily assessed by conducting a routine complete blood count, which is simple and inexpensive to perform.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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