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ORIGINAL ARTICLE

Predictive Role of Hematologic and Inflammatory Biomarkers in Proliferative Diabetic Retinopathy: A Comparative Analysis

Proliferatif Diyabetik Retinopatide Hematolojik ve İnflamatuar Biyobelirteçlerin Öngörüsel Rolü: Karşılaştırmalı Bir Analiz

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ABSTRACT

Aim: Diabetic retinopathy is a common microvascular complication of diabetes mellitus, and proliferative diabetic retinopathy (PDR) represents its most advanced form, often leading to vision loss. Early identification of high-risk patients through accessible biomarkers may enable more effective monitoring and timely intervention. This study aimed to evaluate the association between systemic hematologic and biochemical biomarkers and retinopathy severity.

Methods: This retrospective study included 111 patients with diabetes mellitus, categorized into three groups based on fundoscopic findings: non-retinopathic diabetes mellitus, non-proliferative diabetic retinopathy (NPDR), and PDR. Hematologic biomarkers including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), monocyte-to-lymphocyte ratio (MLR), and eosinophil-to-monocyte ratio were evaluated along with biochemical markers such as urea, creatinine, albumin, and bilirubin. Receiver operating characteristic (ROC) curves and logistic regression were used to assess predictive ability for PDR.

Results: Neutrophil count, NLR, PLR, SII, SIRI, and MLR significantly increased with advancing retinopathy, while lymphocyte count, hemoglobin, hematocrit, and albumin decreased. ROC analysis showed that NLR (AUC: 0.782), PLR (AUC: 0.758), SII (AUC: 0.760), SIRI (AUC: 0.771), MLR (AUC: 0.748), and neutrophil count (AUC: 0.743) were effective in distinguishing PDR. Logistic regression identified SIRI (OR: 3.475), NLR (OR: 3.369), MLR (OR: 1.971), PLR (OR: 1.024), SII (OR: 1.005), and neutrophil count (OR: 1.838) as independent predictors. These markers followed consistent trends across diabetic retinopathy stages and showed significant correlation with proliferative changes. Among them, SIRI and NLR showed the strongest association with PDR, exceeding traditional biochemical parameters in predictive performance.

Conclusions: Systemic inflammatory biomarkers—particularly SIRI, NLR, PLR, MLR, and SII—are independently associated with PDR. These widely available and cost-effective markers may aid in early risk stratification and help guide ophthalmologic referrals in clinical practice.

Keywords: Biomarkers, diabetes mellitus, diabetic retinopathy, inflammation, lymphocyte count, neutrophils, platelet count.

ÖZ

Amaç: Diyabetik retinopati, diyabetes mellitusun sık görülen mikrovasküler komplikasyonlarından biridir ve proliferatif diyabetik retinopati (PDR), hastalığın en ileri evresini temsil eder. PDR, sıklıkla kalıcı görme kaybına yol açabilir. Kolay erişilebilir biyobelirteçler ile yüksek riskli hastaların erken dönemde belirlenmesi, etkin takip ve zamanında müdahale açısından kritik öneme sahiptir. Bu çalışmada, sistemik hematolojik ve biyokimyasal biyobelirteçlerin retinopati şiddeti ile ilişkisi değerlendirilmiştir.

Gereç ve Yöntemler: Bu retrospektif çalışmaya funduskopik bulgulara göre üç gruba ayrılmış 111 diyabet hastası dahil edilmiştir: retinopatisiz diyabet, non-proliferatif diyabetik retinopati (NPDR) ve PDR. Nötrofil/lenfosit oranı (NLR), trombosit/lenfosit oranı (PLR), sistemik immün-inflamasyon indeksi (SII), sistemik inflamatuar yanıt indeksi (SIRI), monosit/lenfosit oranı (MLR) ve eozinofil/monosit oranı gibi hematolojik belirteçlerin yanı sıra üre, kreatinin, albümin ve bilirubin gibi biyokimyasal değişkenler analiz edilmiştir. PDR öngörüsündeki performans, ROC eğrileri ve lojistik regresyon analizleriyle değerlendirilmiştir.

Bulgular: Retinopati şiddeti arttıkça nötrofil sayısı, NLR, PLR, SII, SIRI ve MLR anlamlı şekilde artarken; lenfosit sayısı, hemoglobin, hematokrit ve albümin düzeylerinde azalma saptanmıştır. ROC analizinde NLR (AUC: 0,782), PLR (AUC: 0,758), SII (AUC: 0,760), SIRI (AUC: 0,771), MLR (AUC: 0,748) ve nötrofil sayısı (AUC: 0,743) PDR'yi ayırt etmede etkili bulunmuştur. Lojistik regresyon analizine göre SIRI (OR: 3,475), NLR (OR: 3,369), MLR (OR: 1,971), PLR (OR: 1,024), SII (OR: 1,005) ve nötrofil sayısı (OR: 1,838) PDR için bağımsız öngörücülerdir. Bu belirteçler, diyabetik retinopati evreleri boyunca tutarlı bir artış göstermiş ve proliferatif değişikliklerle anlamlı korelasyon sergilemiştir. Özellikle SIRI ve NLR, prediktif gücü açısından klasik biyokimyasal belirteçlerin önüne geçmiştir.

Sonuçlar: SIRI, NLR, PLR, MLR ve SII gibi sistemik inflamatuar biyobelirteçler, PDR varlığıyla bağımsız ve güçlü ilişki göstermektedir. Bu düşük maliyetli ve yaygın kullanılabilir parametreler, klinik uygulamada erken risk sınıflaması ve göz hastalıkları yönlendirmesi açısından değerli araçlar olabilir.

Anahtar Kelimeler: Biyobelirteçler, diyabet mellitus, diyabetik retinopati, inflamasyon, lenfosit Sayısı, nötrofiller, trombosit sayısı.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia, with its pathophysiology influenced by a variety of factors. Despite advancements in treatment, a definitive cure remains unavailable due to the complex interplay of genetic, environmental, and lifestyle factors. According to current estimates, approximately 529 million individuals worldwide are affected by diabetes, and this number continues to rise annually (1). The increasing incidence of diabetes, coupled with declining rates of glycemic control, contributes to the growing prevalence of diabetic microvascular complications. Diabetic nephropathy (DN), diabetic peripheral neuropathy (DPN), and diabetic retinopathy (DR) are among the most prevalent and clinically significant microvascular complications associated with the disease (2,3).

Among the microvascular complications of DM, DR is estimated to affect 27.0% of individuals with diabetes and cause approximately 0.4 million cases of blindness worldwide. DR is also recognized as a priority eye disease in the 2030 IN SIGHT strategy (4,5). However, effective screening remains a challenge due to the limited availability of retinal specialists. Identifying novel predictive biomarkers could offer a promising approach to improve the management of patients with diabetes by enabling early detection of those at increased risk for vision-threatening complications.

Therefore, many hematologic and biochemical parameters have been investigated in the literature as possible biomarkers in DR. These include urea, creatinine, cholesterol, triglycerides, and hemogram sub-parameters (6). Ratios derived from hemogram sub-parameters—such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), eosinophil-to-monocyte ratio (EMR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), and systemic inflammatory response index (SIRI)—have also been evaluated individually as inflammatory markers in previous studies involving DM and DR (6–9).

However, to our knowledge, no study has investigated the efficacy of all these biomarkers in DR subtypes. Therefore, this study aims to investigate the efficacy of these hematologic and biochemical biomarkers in DR subtypes.

MATERIALS and METHODS

This retrospective comparative case study was conducted with 111 patients with DM. Patients were divided into three groups according to their fundoscopic examination. Patients with no evidence of DR in either eye were classified as the non-retinopathic diabetes mellitus group (NRDM), patients with DR in at least one eye but no proliferative diabetic retinopathy (PDR) in any eye were classified as the non-proliferative diabetic retinopathy (NPDR) group, and patients with PDR in at least one eye were classified as the PDR group. The diagnosis of PDR was made based on diabetes-induced vitreous or retrohyaloid hemorrhage, neovascularization on the optic disc or retina, or neovascular vessels seen on fluorescein fundus angiography (Canon CX-1, Canon INC., Tokyo, Japan).

Demographic data such as age, gender, height, weight, duration of DM diagnosis, complete blood count, fasting blood glucose (Fbg), hemoglobin A1c (HbA1c),

urea, creatinine, albumin, glomerular filtration rate (GFR), total cholesterol (Tchol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), direct bilirubin (Dbil), indirect bilirubin (iDbil), total bilirubin (Tbil) values, presence of diabetic polyneuropathy (DPN), and presence of diabetic nephropathy (DN) were obtained through patients' medical records.

Body mass index (BMI) was obtained by dividing weight in kilograms by the square of height in meters in all patients. The iDbil value was obtained by subtracting the Dbil value from the Tbil value.

NLR was obtained by dividing the neutrophil count by the lymphocyte count, PLR by dividing the platelet count by the lymphocyte count, EMR by dividing the eosinophil count by the monocyte count, and MLR by dividing the monocyte count by the lymphocyte count. SII was calculated as (platelet count × neutrophil count) / lymphocyte count, and SIRI was calculated as (neutrophil count × monocyte count) / lymphocyte count (6–9).

Exclusion Criteria

Patients with ocular inflammation, hematological or other malignancies, patients with iron deficiency anemia or undergoing treatment for it, and patients on immunosuppressive or immunomodulatory therapy were excluded from the study.

Statistical Analyses

Statistical analyses were performed using IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Numerical descriptive statistics are presented as means and standard deviations for normally distributed variables, while categorical variables are presented as frequencies and percentages. The Kolmogorov–Smirnov test was used to assess the normality of the data. Analysis of variance (ANOVA) with Bonferroni correction was used to compare more than two independent groups. The Chi-square test was used to compare categorical data.

Receiver operating characteristic (ROC) curves were used to analyze the sensitivity and specificity of the parameters for predicting PDR. An AUC value ≥ 0.6 was considered indicative of an acceptable level of discrimination (10). Logistic regression analysis was performed to assess associations between biological parameters and PDR. The predictive performance of the regression models was compared using ROC AUC scores. A P value < 0.05 was considered statistically significant.

Ethical Approval

The principles of the Declaration of Helsinki were followed throughout this study. Informed consent was obtained from the patients after the study design and the possible outcomes were explained. The study was approved by the ethics committee of a university training and research hospital on 02.12.2024 with the approval number TÜTF-GOBAEK 2024/252.

RESULTS

This retrospective study was conducted with 111 patients. Patients were divided into three groups according to DR severity: NRDM group (37 patients), NPDR group (37 patients), and PDR group (37 patients). When comparing the groups in terms of demographic characteristics and biochemical parameters, no significant difference was found in terms of age, gender, duration of DM, BMI, Fbg, HbA1c, GFR, Tchol, LDL, HDL, TG, iDbil, Tbil, DPN, and DN. However, urea and creatinine increased

with DR progression, whereas albumin and Dbil showed a significant decrease (P = 0.047 for urea, P = 0.017 for creatinine, P < 0.001 for albumin, and P = 0.045 for Dbil, respectively). Details are presented in Table1.

Table 1: Statistical comparison of mean values of demographic features and biochemical values between groups.

Parameter		NRDM	NPDR	PDR	Total	P Value
Numbers		37	37	37	111	
Age (years)	mean±SD	63.4±8.0	63.1±7.6	61.9±6.8	62.8±7.5	0.665
	min-max	45-76	49-81	47-83	45-83	
Gender	Male N (%)	21 (56.8%)	19 (51.4%)	22 (59.5%)	62 (55.9%)	0.774
	Female N (%)	16 (43.2%)	18 (48.6%)	15 (40.5%)	49 (44.1%)	
Duration of DM (months)	mean±SD	202.0±93.3	205.2±91.4	208.5±91.7	205.3±91.3	0.955
	min-max	4-420	24-372	48-528	4-528	
BMI (kg/m²)	mean±SD	29.2±5.7	29.6±4.1	30.7±4.0	29.8±4.7	0.376
	min-max	20.6-49.0	21.5-40.1	22.1-43.1	20.6-49.0	
Fbg (mg/dL)	mean±SD	124.9±36.4	144.1±54.2	153.4±62.0	140.8±52.9	0.060
	min-max	49-229	51-292	54-414	49-414	
HbA1c (%)	mean±SD	8.1±1.5	8.7±2.1	8.7±2.0	8.5±1.9	0.265
	min-max	5.8-14.5	5.6-13.8	5.2-14.4	5.2-14.5	
Urea (mg/dL)	mean±SD	39.4±15.0	45.5±20.2	53.1±32.1	46.0±24.0	*0.047
	min-max	18-95	17-101	19-142	17-142	
Creatinine (mg/dL)	mean±SD	0.9±0.3	1.1±0.7	1.6±0.3	1.2±0.1	*0.017
	min-max	0.6-2.0	0.5-4.2	0.5-6.9	0.5-6.9	
Albumin (g/dL)	mean±SD	4.5±0.3	4.3±0.3	4.1±0.6	4.3±0.4	*<0.001
	min-max	3.4-5	3.6-5	2.8-5.1	2.8-5.1	
GFR (ml/minute/1.73 m²)	mean±SD	80.7±21.3	74.5±26.6	66.3±31.9	73.8±27.4	0.074
	min-max	26.8-110.0	15.0-114.8	7.6-111.7	7.6-114.8	
Tchol (mg/dL)	mean±SD	173.1±45.3	169.8±49.5	182.1±56.7	175.0±50.6	0.560
	min-max	109.0-297.0	91.0-278.0	89.0-358.0	89.0-358.0	
LDL (mg/dL)	mean±SD	102.9±32.4	99.5±40.3	104.2±43.2	102.2±38.6	0.866
	min-max	61.0-178.0	33.0-189.0	27.0-227.0	27.0-227.0	
HDL (mg/dL)	mean±SD	48.9±13.1	46.1±12.6	45.0±13.7	46.6±13.2	0.428
	min-max	24.0-77.0	29.0-83.0	13.0-72.0	13.0-83.0	
TG (mg/dL)	mean±SD	163.0±125.7	161.1±100.4	151.8±88.4	158.6±105.1	0.888
	min-max	51.0-597.0	35.0-610.0	47.0-440.0	35.0-610.0	
Dbil (mg/dL)	mean±SD	0.19±0.09	0.18±0.07	0.15±0.07	0.17±0.08	*0.045
	min-max	0.1-0.8	0-0.6	0.1-0.6	0-0.8	
iDbil (mg/dL)	mean±SD	0.26±0.14	0.27±0.14	0.23±0.14	0.25±0.14	0.428
	min-max	0.1-0.8	0-0.6	0.1-0.6	0-0.8	
Tbil (mg/dL)	mean±SD	0.44±0.21	0.46-0.17	0.38±0.19	0.43±0.19	0.180
	min-max	0.2-1.3	0.2-0.8	0.1-0.8	0.1-1.3	
Presence of DPN	N (%)	4 (10.8%)	3 (8.1%)	5 (13.5%)	12 (10.8%)	0.756
Presence of DN	N (%)	9 (24.3%)	7 (18.9%)	15 (40.5%)	31 (27.9%)	0.098

NRDM = non-retinopathic diabetes mellitus; NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; SD = standard deviation; N = number; DM = diabetes mellitus; BMI = body mass index; Fbg = fasting blood glucose; GFR = glomerular filtration rate; Tchol = total cholesterol; LDL = low-density lipoprotein; HDL = high-density lipoprotein; TG = triglyceride; Dbil = direct bilirubin; iDbil = indirect bilirubin; Tbil = total bilirubin; DPN = diabetic polyneuropathy; DN = diabetic nephropathy.

* Statistically significant values were identified using the Analysis of Variance test with Bonferroni correction.

When hematological parameters and inflammatory markers were compared between the groups, no significant difference was found in terms of white blood cell (WBC), platelet, monocyte, eosinophil, basophil counts; red cell distribution width-coefficient of variation (RDW-CV), mean platelet volume (MPV), and EMR. In contrast to these parameters, lymphocyte and red blood cell (RBC) counts, hemoglobin (HGB), and hematocrit (HCT) decreased significantly with DR progression ($P < 0.001$, $P = 0.034$, $P = 0.003$, $P = 0.002$), while the levels of neutrophils, NLR, PLR, SII, SIRI, and MLR significantly increased ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively). Details are presented in Table 2.

In the pairwise comparisons between groups, urea and creatinine levels increased progressively from NRDM to NPDR and from NPDR to PDR. However, a statistically significant difference was observed only between the NRDM and PDR groups ($P = 0.042$ for urea and $P = 0.020$ for creatinine). No significant differences were found between NRDM–NPDR or NPDR–PDR for either parameter. Albumin and Dbil levels decreased stepwise from NRDM to NPDR and from NPDR to PDR. For albumin, a statistically significant difference was found only between the NRDM and PDR groups ($P < 0.001$), while the differences between NRDM–NPDR and NPDR–PDR were not statistically significant. For Dbil, no statistically significant differences were found in any of the pairwise group comparisons.

The pairwise comparison of hematological and inflammatory markers between groups showed a significant increase in neutrophil, NLR, PLR, SII, and SIRI values from NRDM to NPDR and NPDR to PDR as DR severity increased. Lymphocyte count showed a gradual decrease between the groups as DR severity increased; however, this decrease was significant between NRDM-NPDR ($P = 0.006$) and NRDM-PDR ($P < 0.001$) but not between NPDR-PDR. There was also an increase in MLR with DR progression; however, this increase was not significant between NRDM-NPDR, but it was significant between NRDM-PDR ($P < 0.001$) and between NPDR-PDR ($P = 0.035$). Although HGB and HCT values also showed a relative decrease between the groups from NRDM to PDR, this difference was significant only between NRDM-PDR ($P = 0.002$ for HGB and $P = 0.001$ for HCT) but not between NRDM-NPDR and NPDR-PDR. RBC count also decreased as the severity of DR increased, but this decrease was not significant in pairwise comparisons. Details are presented in Table 3.

Assessment of Inflammatory Biomarkers for Predicting PDR Using ROC Curve Analysis

The predictive power of the biomarkers, which showed statistically significant differences in distribution among the study groups after post hoc analysis, was further assessed using ROC curves for PDR. The urea, creatinine, RBC, and HCT analyses were not statistically significant. However, although the analysis for albumin, Dbil, lymphocyte count, and HGB was statistically significant, none of these biomarkers met the minimum AUC ROC criterion of 0.6 to predict PDR in the study group. NLR, PLR, SII, SIRI, MLR, and neutrophil count values showed good discrimination power with high specificity and sensitivity. Details are presented in Table 4.

Table 3. Pairwise comparative subgroup analysis of statistically significant parameters

Parameter	Pairwise subgroup comparison		P Value
Neutrophil	NRDM	NPDR	*<0.001
	NPDR	PDR	*0.013
	NRDM	PDR	*<0.001
Lymphocyte	NRDM	NPDR	*0.006
	NPDR	PDR	0.748
	NRDM	PDR	*<0.001
RBC	NRDM	NPDR	0.073
	NPDR	PDR	1.000
	NRDM	PDR	0.070
HGB	NRDM	NPDR	0.330
	NPDR	PDR	0.165
	NRDM	PDR	*0.002
HCT	NRDM	NPDR	0.236
	NPDR	PDR	0.217
	NRDM	PDR	*0.001
NLR	NRDM	NPDR	*<0.001
	NPDR	PDR	*0.013
	NRDM	PDR	*<0.001
PLR	NRDM	NPDR	*0.022
	NPDR	PDR	*0.014
	NRDM	PDR	*<0.001
SII	NRDM	NPDR	*0.003
	NPDR	PDR	*0.001
	NRDM	PDR	*<0.001
SIRI	NRDM	NPDR	*0.024
	NPDR	PDR	*0.005
	NRDM	PDR	*<0.001
MLR	NRDM	NPDR	0.097
	NPDR	PDR	*0.035
	NRDM	PDR	*<0.001
Urea	NRDM	NPDR	0.802
	NPDR	PDR	0.506
	NRDM	PDR	*0.042
Albumin	NRDM	NPDR	0.533
	NPDR	PDR	0.015
	NRDM	PDR	*<0.001
Creatinine	NRDM	NPDR	1.000
	NPDR	PDR	0.095
	NRDM	PDR	*0.020
Dbil	NRDM	NPDR	1.000
	NPDR	PDR	0.113
	NRDM	PDR	0.078

NRDM = non-retinopathic diabetes mellitus; NPDR = non-proliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit; NLR = neutrophil to lymphocyte ratio; PLR = platelet to lymphocyte ratio; SII = systemic immuno-inflammation index; SIRI = systemic inflammatory response index; MLR = monocyte to lymphocyte ratio; Dbil = direct bilirubin.

* Statistically significant differences based on Analysis of Variance test post hoc comparisons

Logistic Regression Model

A binary logistic regression analysis examined the relationship between PDR (1 = present, 0 = absent) and each statistically significant independent variable listed in Tables 1 and 2. Variables with a P value < 0.05 and a lower confidence interval bound for each odds ratio (OR) greater than 1 were considered potential risk factors for PDR, ensuring each factor provides a statistically significant additional risk with 95% confidence.

Among the values investigated, RBC was not significantly correlated with PDR. The required OR values for urea, albumin, Dbil, lymphocyte count, HGB, and HCT could not be obtained. A higher correlation with PDR was encountered for NLR (OR: 3.369), PLR (OR: 1.024), SII (OR: 1.005), SIRI (OR: 3.475), MLRx10 (OR: 1.971), and neutrophil count (OR: 1.838) models. Details are presented in Table 5.

DISCUSSION

The incidence and prevalence of DR, a chronic complication of DM, are increasing gradually (11). PDR is one of the leading pathological changes that adversely affect patients' vision (12). Neovascularization and subsequent preretinal or vitreous hemorrhage, fibrous tissue formation, and

Table 4. Sensitivity and specificity at the "cut-off" value predicting PDR

	PDR Sensitivity (%)	PDR Specificity (%)	Cut-Off Value	AUC	95% CI		P Value
					Lower Limit	Upper Limit	
NLR	73.0	70.3	>2.40	0.812	0.735	0.889	*<0.001
PLR	73.0	71.6	>135.57	0.773	0.685	0.862	*<0.001
SII	78.4	78.4	>648.46	0.831	0.756	0.906	*<0.001
SIRI	64.9	64.9	>1.23	0.760	0.665	0.856	*<0.001
MLR	73.0	71.6	>0.271	0.725	0.622	0.827	*<0.001
Urea	54.1	54.1	>41.5	0.586	0.468	0.704	0.142
Creatinine	56.8	58.1	>99.5	0.601	0.483	0.720	0.083
Albumin	32.4	45.9	<4.35	0.320	0.208	0.433	*0.002
Dbil	43.2	35.1	<0.15	0.370	0.260	0.480	*0.026
Neutrophil	62.2	63.5	>4.67	0.709	0.612	0.807	*<0.001
Lymphocyte	37.8	39.2	<1.99	0.343	0.239	0.447	*0.007
RBC	48.6	51.4	<4.51	0.453	0.335	0.570	0.416
HGB	45.9	45.9	<12.55	0.341	0.233	0.448	*0.006
HCT	43.2	43.2	<37.75	0.359	0.016	0.248	0.470

PDR = proliferative diabetic retinopathy; CI = Confidence interval; AUC = area under the curve; NLR = neutrophil to lymphocyte ratio; PLR = platelet to lymphocyte ratio; SII = systemic immuno-inflammation index; SIRI = systemic inflammatory response index; MLR = monocyte to lymphocyte ratio; Dbil = direct bilirubin; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit.
* Statistically significant values

retinal detachment during the advanced stage are also vision-threatening complications that may occur in PDR patients (2). Considering that there are more than half a billion DM patients, whose numbers are likely to increase in the coming years, it is evident that the public health burden attributable to DR-related complications alone is projected to be substantial (1). For this reason, many investigators are searching for easily testable biomarkers to predict these complications and perhaps prevent them through early intervention (6,7,13–15).

NLR is one of the most extensively investigated biomarkers in this context. Especially in cardiovascular diseases, malignancies, and chronic inflammatory diseases, increased NLR values have been associated with adverse outcomes (16–19). Dascalu et al. (6) divided DM patients into three groups: NRDM, NPDR, and PDR, similar to our study, and found a significant increase in NLR with DR progression in parallel with our study. In the ROC Curve analysis, they determined the cut-off value for NLR was > 3.18 to predict PDR. However, they found a low AUC value of 0.662 and a low test sensitivity of 40.0%. In this study, ROC analysis for predicting PDR showed a cut-off value > 2.40, an AUC value of 0.812, a sensitivity of 73%, and a specificity of 70.3%, which are quite acceptable. In the mentioned study, the risk for NLR was 1.645-fold in logistic regression analysis, while this rate was found to be 3.369 in the current study. The most crucial difference between the two studies is that while the duration of DM was quite similar between the groups in our study, the duration of DM increased significantly as DR progression increased between the groups in the aforementioned

Table 5. Logistic regression model for the dependent variable of PDR.

Risk	Estimated Co-Efficient	Standard Error	Wald	DF	P Value	OR	Lower	Upper
NLR	1.215	0.294	17.106	1	*<0.001	3.369	1.895	5.990
PLR	0.024	0.006	15.462	1	*<0.001	1.024	1.012	1.036
SII	0.005	0.001	19.658	1	*<0.001	1.005	1.003	1.007
SIRI	1.246	0.315	15.673	1	*<0.001	3.475	1.876	6.439
MLRx10	0.679	0.206	10.861	1	*0.001	1.971	1.317	2.952
Urea	-1.544	0.459	11.328	1	*0.001	0.213	1.001	1.036
Creatinine	0.567	0.247	5.261	1	*0.022	1.764	1.086	2.864
Albumin	-1.938	0.564	11.831	1	*0.001	0.144	0.048	0.434
Dbil	-7.279	3.004	5.871	1	*0.015	0.001	0	0.249
Neutrophil	0.609	0.173	12.352	1	*<0.001	1.838	1.309	2.581
Lymphocyte	-1.105	0.388	8.124	1	*0.004	0.331	0.155	0.708
RBC	-4.22	0.325	1.689	1	0.194	0.656	0.347	1.239
HGB	-0.286	0.133	8.428	1	*0.004	0.680	0.524	0.882
HCT	-0.136	0.048	8.120	1	*0.004	0.873	0.796	0.959

PDR = proliferative diabetic retinopathy; DF = Degrees of Freedom; OR = estimated odds ratio; CI = confidence interval; NLR = neutrophil to lymphocyte ratio; PLR = platelet to lymphocyte ratio; SII = systemic immuno-inflammation index; SIRI = systemic inflammatory response index; MLR = monocyte to lymphocyte ratio; Dbil = direct bilirubin; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit.
Proliferative diabetic retinopathy variable is binary (1 = present/0 = absent), and all risks are binary (1 = yes/0 = no).
* Statistically significant values

study. Despite these differences, these two studies showed similar results regarding the role of NLR ratio in the pathogenesis of DR. Swathi et al. (14) also compared NLR values between groups with different HbA1c values and reported that higher values were associated with poor glycemic control. Although HbA1c values did not differ statistically between the groups in the present study, similar conclusions were reached regarding NLR values. In parallel with our results, Rajendrakumar et al. (20) also suggest that NLR has a promising potential in predicting the incidence of DR, especially in individuals under 65 years of age and those with well-controlled glycemic status, in their comprehensive study conducted in the Scottish population. El-Tawab et al. (21) divided DM patients without DR into two groups, normal patients and preclinical-DR patients, according to visual evoked potentials (VEP) results, and reported significantly increased NLR in preclinical-DR patients. They also reported a cut-off point ≥ 1.97, which can predict preclinical-DR with 89.3% sensitivity and 84.3% specificity. In a linear regression model, they revealed that NLR is the only independent factor that predicts preclinical-DR (OR: 3.312; 95% confidence interval 1.262–8.696, P = 0.015). Despite some differences in details, our study is in line with the studies mentioned above regarding the effectiveness and role of NLR.

PLR is another critical parameter investigated in this regard, but results across studies are inconsistent (6,9,13,14). Dascalu et al. (6) reported increasing mean PLR values with DR severity among NRDM, NPDR, and PDR groups; however, this

increase was not statistically significant (P = 0.127). Similarly, in another study by Dascalu et al. (13), three similar groups were compared, and it was observed that PLR increased between the groups, but no statistical difference was found; however, the P value was reported as 0.059, which is just above the threshold. The fact that the P values for PLR in the 2 studies designed by the same lead author were different, and especially that the P value in the 2nd study was reported as 0.059, which is just above the significance threshold, made us think that possible changes in sample size or other factors may reduce the P value below 0.05. Contrary to the results of the mentioned studies, Swathi et al. (14) detected statistically significantly higher PLR values in patients with HbA1c levels greater than 7%. In their study investigating microvascular complications of DM, Li et al. (9) reported a significantly higher proportion of participants with PLR values in the DR group than in the group without microvascular complications. In this study, PLR values increased with DR progression in the groups. Unlike the two studies of Dascalu et al. (6,13) mentioned above, the increase in PLR was significant between groups in the present study. Despite the similar results of the studies, it is thought that this difference in statistical significance may be due to differences in sample size between the studies and differences in demographic data, such as duration of DM and HbA1c values in the study groups.

SII and SIRI are other biomarkers investigated in DR patients in this study. Wang et al. (8) investigated SII and SIRI in DM patients and found significantly higher values in DR patients compared to the group without DR. In binary logistic regression analysis for DR, both SII and SIRI were significant (OR: 1.002, 95% confidence interval 1.000–1.004, P = 0.045 for SII; and OR: 25.954, 95% confidence interval 7.382–91.253, P < 0.001 for SIRI). In our current study, patients were divided into three groups: NRDM, NPDR, and PDR, but in the mentioned study, the groups were designed as two groups with and without DR. In the binary regression analysis, the risk of SII and SIRI in terms of PDR was examined in our study. In contrast, the mentioned study evaluated the risk in terms of DR. Although both studies give similar results, it is believed that the difference in odds ratio, especially for SIRI, is due to these design differences. Dascalu et al. (6) examined SII with a design similar to the present study and observed a significant increase in SII between the groups with DR progression. In binary regression analysis regarding SII, they also detected a considerable risk similar to the present study (OR: 1.001, 95% confidence interval 1–1.003, P = 0.007). In support of the results of our research, Wang et al. (8) reported that the DR group showed significantly higher SII and SIRI values compared to the non-DR group. In their study, ROC curve analysis demonstrated that the combined use of SII and SIRI achieved the highest diagnostic accuracy for DR, with an AUC of 0.782; sensitivity of 74.6%, and specificity of 69.9%. As a result, they reported that binary logistic regression analysis identified SII and SIRI as independent risk factors for DR. In the present study, SII and SIRI also showed high sensitivity and specificity in ROC analysis, emerged as significant independent predictors for PDR in the regression analysis.

The ratio between monocytes and lymphocytes has also been investigated as a parameter in various studies. Lei et al. (22) found no significant difference in the

lymphocyte-to-monocyte ratio between groups with and without diabetic macular edema. In contrast, Dascalu et al. (6) found a considerable increase in MLR with DR progression. The cut-off for predicting PDR was set as > 0.364, but the sensitivity of this value remained at 35.6%. Logistic regression analysis revealed a significant increase in risk for MLR x 10 (odds ratio 1.662, 95% confidence interval 1.209–2.284, P = 0.0017). In the present study, there was also a significant increase in MLR with DR progression. However, in predicting PDR, highly satisfactory values of 73% sensitivity and 71.6% specificity at a cut-off > 0.271 have been detected (P < 0.001). Also, logistic regression analysis showed a significantly increased risk for MLR x10 (odds ratio 1.971, 95% confidence interval 1.317-2.952, P = 0.001).

EMR is another parameter that has already been investigated in the literature. Usalp (7) compared DR patients with non-DM and found significantly higher rates of eosinophilia and EMR in DR patients. In this study, no significant difference was found between the groups for EMR. This difference in results between the studies is thought to be due to the control group in the study above included patients without a diagnosis of DM. In contrast, all patients in this study were diagnosed with DM.

In the present study, anemia-related RBC, HGB, and HCT values significantly decreased with DR progression. Literature also supports these results. A Korean cross-sectional study involving 2,123 patients with DM demonstrated that elevated HGB levels significantly reduced the risk of DR prevalence (23). Additionally, other researchers reported a significant negative correlation between HGB levels and the severity and indices of retinal ischemia in DR (24). Furthermore, previous studies have established that anemia is an independent risk factor for DR and DN. These findings suggest that higher HGB levels play a protective role in delaying the progression of DR (25). Considering this information in this study, it is unsurprising that RBC, HGB, and HCT values decrease with DR progression. However, despite this significant decrease, significant cut-off values for RBC and HCT to predict PDR could not be obtained. For HGB, although the cut-off value obtained was significant, the AUC remained at a low value of 0.341.

Urea and creatinine values have been previously investigated in DR. Dascalu et al. (6) found no significant difference in urea values between the NRDM, NPDR, and PDR groups. Still, they observed a substantial increase in patients with urea values above 60 mg/dL with DR progression. Regarding creatinine, they found significant values increasing with DR progression in logistic regression analysis and a significantly increased risk for PDR. Still, they did not share the cut-off value necessary to predict PDR. In another study by Dascalu et al. (13), no significant difference was found between three similar groups regarding urea values, while creatinine values showed a significant increase with DR progression. However, in the same study, no significantly increased risk value for PDR was obtained in logistic regression analysis regarding creatinine. Wang et al. (8) found a significant increase in creatinine in the DR group compared to the non-DR group, but did not present a regression analysis for creatinine. This study detected increased creatinine values as DR progressed, a significant risk factor in logistic regression analysis. However,

the cut-off value to predict PDR in ROC analysis exceeded the significant P value of 0.05. Although there was a difference only between NRDM and PDR groups for urea, no significant cut-off value was obtained to predict PDR in ROC analysis, and the expected risk significance was not achieved in logistic regression analysis.

Reports on albumin values in DR differ in the literature. Wang et al. (8) did not report any difference in albumin values between the groups with and without DR. On the other hand, Chen et al. (26) reported that albumin levels were lower in the group with DR compared to the group without DR. In parallel with the study by Chen et al. (26), the present study found a significant decrease in albumin only between the NRDM and PDR groups. Also, no significant albumin effect for PDR was observed in ROC and logistic regression analysis.

This study aimed to observe the changes in some hematologic and biochemical parameters at different DR stages and to explore how we can use the parameters with significant modifications to predict PDR. While our results support the literature, some detailed differences that emerged due to design differences in our study also contribute to the literature in this sense.

Most studies with similar designs in the literature, especially HbA1c levels, may differ between NRDM/NPDR/PDR groups or non-DR/DR groups (14,26). Some studies compared the DR group with patients without DM (7). Also, in most studies, the DM duration parameter either differs between the groups or information about DM duration is not provided (6,8,9,13,14,26).

It has been emphasized in similar studies that DM duration is associated with PDR or DR (6,15). In light of this information, it should be considered that prolonged DM duration can lead to serious pathologies such as DR and even PDR. It may also affect hematological and biochemical parameters during the same period. For this reason, we believe that standardization of the duration of DM between the groups may provide more accurate results to differentiate whether the changes in the mentioned parameters are related to PDR or DR, or whether they are related to the duration of DM. Therefore, one of the strengths of our study is the lack of significant differences between the groups in terms of age, gender, HbA1c, BMI values, and especially duration of DM. Moreover, our study is one of the few to classify DR patients into three distinct groups—NRDM, NPDR, and PDR—while simultaneously evaluating biomarkers such as NLR, PLR, SII, SIRI, MLR, and EMR, which have been analyzed individually in previous studies, within a single comprehensive framework. The most important limitation of our study is undoubtedly the sample size. Studies with a larger sample size are required to obtain precise and effective cut-off values, especially for the aforementioned biomarkers.

CONCLUSION

This study highlights the impact of chronic systemic inflammation on DR progression. Specific biomarkers of systemic inflammation are more effective in predicting DR than individual white blood cell counts. However, it is undoubtedly essential to determine which biomarker will be more effective in this context. In our study, SIRI, NLR, MLR, PLR, and SII values are significantly associated with PDR. We

believe that the widespread use of such easily accessible, cost-effective, and objective parameters in clinical practice will substantially and positively impact the follow-up and management of DR.

Conflict of Interest

No conflict of interest was declared by the authors.

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