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Original Article

The Optimal Cutoff Value of Neutrophil/Lymphocyte Ratio for Severe Grades of Diabetic Retinopathy

Cagri Ilhan,[1] Mehmet Citirik,[2] Mehmet Murat Uzel,[3] Kemal Tekin[4]

[1]Department of Ophthalmology, Hatay State Hospital, Hatay, Turkey
[2]Department of Ophthalmology, Ulucanlar Eye Training and Research Hospital, Ankara, Turkey
[3]Department of Ophthalmology, Balikesir University, Balikesir, Turkey
[4]Department of Ophthalmology, Ercis State Hospital, Van, Turkey

Abstract

Objectives: The aim of this study was to define the optimal cutoff value of the neutrophil/lymphocyte ratio (NLR) to predict severe grades of diabetic retinopathy (DR).

Methods: A total of 40 patients with proliferative DR (PDR) and 40 patients with severe non-proliferative DR (NPDR) were included this prospective, case control study, and 35 age- and sex-matched healthy subjects were recruited as a control group. White blood cell (WBC) count mean values and ratios were compared between the groups.

Results: The groups were statistically similar in terms of age and sex. The neutrophil, lymphocyte, monocyte, platelet counts, and main platelet volume (MPV) values were similar in all 3 groups (all p values >0.05). The mean NLR was 2.67±1.02 in the PDR cases, 2.16±0.58 in severe NPDR, and 1.85±0.49 in the control group, which represented a statistically significant difference between the 3 groups (p=0.003). In post-hoc analysis, the NLR of the PDR and severe NPDR groups was statistically significantly greater than that of the control group (p=0.002 and p=0.048, respectively), but there was no statistically significant difference between the PDR and severe NPDR groups (p=0.083). The monocyte/lymphocyte, platelet/lymphocyte, and MPV/lymphocyte ratios were also similar in all 3 groups (all p values >0.05).

Conclusion: An NLR value of 2.11 or more predicted DR (PDR or severe NPDR) with a sensitivity of 76% and a specificity of 80%.

Keywords: Neutrophil/lymphocyte ratio, proliferative diabetic retinopathy, severe nonproliferative diabetic retinopathy.

Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by chronic hyperglycemia. In type 1 DM, chronic hyperglycemia is a result of pancreatic beta cell destruction. Type 2 DM is a result of insulin resistance and subsequent pancreatic beta cell dysfunction (1). DM can lead to various microvascular and macrovascular complications. Diabetic retinopathy (DR) is the most common microvascular complication of DM, and can be responsible for severe visual loss (2). Approximately one-third of the diabetic population has some grade of DR (3). Low-grade systemic inflammation plays a role in the development of DM complications, and particularly DR. Prostaglandins and thromboxane are generated through induction of the cyclooxygenase-2 (COX-2) pathway. These dysfunctional products trigger chronic inflammation and result in the local secretion of vascular endothelial growth factor (VEGF) in the retina (1). VEGF is the primary agent responsible for the development of diabetic macular edema and retinal neovascularization.

Address for correspondence: Cagri Ilhan, MD. Hatay Devlet Hastanesi, Oftalmoloji Bolumu, Hatay, Turkey
Phone: +90 533 133 97 09 **E-mail:** cagrilhan@msn.com

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The white blood cell (WBC) count and analysis of WBC subtypes, and the calculation of various ratios of these components can be useful markers of systemic low-grade inflammation (4). The value of the neutrophil/lymphocyte ratio (NLR) in comparison with the total leukocyte count has been demonstrated in previous studies (5, 6). The role of the NLR in some systemic diseases has been examined, but its relationship with ocular diseases has not yet been clearly defined (7–10).

To the best of our knowledge, there is only 1 report that has evaluated the relationship between NLR and DR. That study was well-designed and analyzed NLR in detail in terms of the severity of DR, but it did not suggest a cutoff value for predicting DR (11). The objective of the present study was to define a value for NLR that indicates an increased risk of severe grades of DR. While periodic ophthalmological examinations are recognized as necessary for all diabetic patients, this research may lead to earlier ophthalmology consultations, which could be very important.

Methods

This prospective, case-control study was conducted in the retina department of a single tertiary hospital from July 2018 through November 2018. The study followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee. All of the participants were verbally informed about the study and subsequently provided written informed consent.

Study Subjects

Patients from the retina department with PDR or severe NPDR who were diagnosed with type 2 DM and underwent insulin therapy were identified for this study. The presence of diabetes had been confirmed by the corresponding internal medicine department and values for body mass index (BMI), blood pressure, fasting glucose, and glycated hemoglobin (HbA1c), as well as smoking history were obtained. Patients who had connective tissue diseases, inflammatory bowel diseases, hematological disorders, malignancy, acute or chronic infection, other inflammatory ocular and systemic diseases, a history of steroid use, or any ocular medication were excluded. The coexistence of additional ocular pathologies, such as retinal vascular diseases, retinal break, intraocular tumor, or a history of uveitis, retinal surgery, or ocular trauma were exclusion criteria. The status of existing retinopathy and macular edema were assessed using fundus photography and confirmed with fluorescein angiography and optical coherence tomography. In all, 40 patients with PDR (one or both of the following: neovascularization, vitreous or preretinal hemorrhage) and 40 patients with severe NPDR (any of the following: more than 20 intraretinal hemorrhages in each of 4 quadrants, definite venous beading in 2 or more quadrants, prominent intraretinal microvascular abnormalities in 1 or more quadrants, and no signs of PDR) diagnosed according to the International Clinical Diabetic Retinopathy Disease Severity Scale, were included the study (12). In addition, 35 completely healthy age- and sex-matched subjects were recruited from the general ophthalmology clinic and consulted to the same internist to verify the absence of DM and other diseases before inclusion in the study.

Calculation of Neutrophil/Lymphocyte Ratio

The neutrophil, lymphocyte, monocyte, and platelet counts, as well as the main platelet volume (MPV) values were evaluated with a Horiba ABX Pentra 120 (Horiba Ltd., Kyoto, Japan). The NLR and monocyte/lymphocyte, platelet/lymphocyte, and MPV/lymphocyte ratios were calculated by dividing the count of neutrophils, monocytes, platelets, and the MPV by the lymphocyte count.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp, Armonk, NY, USA). The mean age and female/male ratio of the groups are provided as descriptive data. Normality of the distribution of the numerical data was evaluated using the Kolmogorov-Smirnov test. The nonparametric Kruskal-Wallis H test was used to compare 3 independent samples of numerical data that was not normally distributed. The Bonferroni test was used as a post hoc test after the Kruskal-Wallis H test. The Mann-Whitney U test was used for post-hoc analysis of 2 independent samples. A p value of ≤ 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was performed to assess the sensitivity and specificity of admission NLR values and determine the optimal cutoff value to predict severe grades of DR.

Results

The mean age of the patients was 59.63±7.07 years in the PDR group, 61.14±9.33 years in the NPDR group, and 62.68±10.40 years in the control group (p=0.371). There were 22 female and 18 male patients in the PDR group, 21 female and 19 male patients in the NPDR group, and 18 female and 17 male patients in the control group (p=0.801). There were statistically significant differences between groups in baseline characteristics, including BMI, blood pressure, fasting glucose, HbA1c, and smoking history (p<0.017). Table 1 summarizes the demographic and baseline characteristics of the groups.

Neutrophil, lymphocyte, monocyte, and platelet counts,

Table 1. Demographic and baseline characteristics of the PDR, severe NPDR, and control groups

	PDR (n=40) (Mean±SD)	Severe NPDR (n=40) (Mean±SD)	Control (n=35) (Mean±SD)	p
Age (years)	59.63±7.07	61.14±9.33	62.68±10.40	0.371
Gender (male/female)	22/18	21/19	18/17	0.801
Body mass index	31.08±3.68	30.40±3.62	23.28±29.08	<0.001*
Blood pressure (mm/Hg)	143/92±21/14	142/89±19/15	114/78±14/8	<0.001*
Fasting glucose (mg/dL)	240.65±79.80	208.1±68.58	86.25±23.49	<0.001*
HbA1c (%)	8.32±1.07	8.12±1.09	5.26±0.44	<0.001*
Smoking (package/year)	10.01±4.43	10.51±4.32	7.18±3.02	0.044*

HbA1c: glycated hemoglobin; NPDR: non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; *p<0.05 in PDR vs. control; p<0.05 in severe NPDR vs. control; p>0.05 in PDR vs. severe NPDR.

and the MPV values were similar in the 3 groups (p>0.05). The mean NLR was 2.67±1.02 in the PDR group, while it was 2.16±0.58 in the NPDR group, and 1.85±0.49 in the control group (Fig. 1). Analysis of the NLR values yielded a statistically significant difference between the 3 groups (p=0.003). In post-hoc analysis, the NLR of the PDR and severe NPDR groups was significantly higher than that of the control group (p=0.002 and p=0.048, respectively), but there was no statistically significant difference between the PDR and severe NPDR groups (p=0.083). The monocyte/lymphocyte, platelet/lymphocyte, and MPV/lymphocyte ratios were also similar in the 3 groups (p>0.05). The mean counts of WBC and calculations of defined variables are compared in Table 2. The area under the ROC curve for NLR was 0.716, and an NLR of 2.11 or higher predicted DR (PDR or severe NPDR) with a sensitivity of 76% and a specificity 80% (Fig. 2 and Table 3).

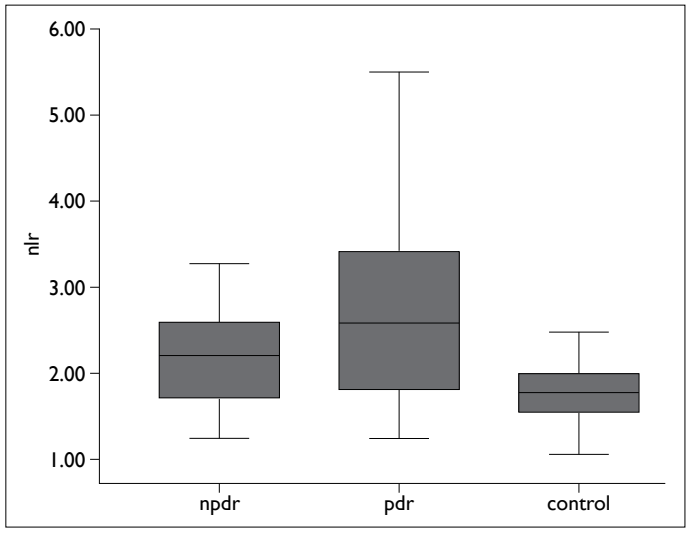

Figure 1. The mean neutrophil/lymphocyte ratio values in the groups.

Discussion

The NLR is a new and reliable indicator for many diseases with a pathophysiology of systemic inflammation. Celik et al. (13) reported that patients with acute appendicitis who have an elevated NLR level may be more likely to develop a complication. Pektezel et al. (14) found that the NLR increased in the first 24 hours after patients experienced acute ischemic stroke. It has also been shown that an elevated NLR is an independent risk factor for coronary artery diseases in asymptomatic patients (15). Furthermore, it has been demonstrated that the NLR can be a potential prognostic indicator for many cancers, such as digestive cancer or malignant melanoma (16, 17). Numerous studies in the literature have examined the relationship between the NLR and systemic inflammation-related diseases.

The NLR is also a new indicator for several inflammation-related ocular diseases. The NLR can be used as a novel biomarker in primary open-angle glaucoma, and WBC counts have diagnostic value in patients with primary angle-closure glaucoma (18, 19). Kurtul et al. (20) reported that NLR is a simple, inexpensive, and reliable prognostic biomarker for age-related macular degeneration. Ilhan et al. (21) reported that an elevated NLR can be a potential indicator of vitreomacular traction syndrome, regardless of the etiology. Dursun et al. (22) suggested that the optimal cut-off value of NLR to predict retinal vein occlusion with 72.5% sensitivity and 100% specificity was 1.89. These reports revealed the critical role of inflammatory cascades in the pathophysiology of these diseases and the diagnostic value of NLR. In future, studying the association between the NLR and ophthalmological diseases will likely provide more information about the pathophysiology of ophthalmological diseases and may lead to the development of new therapies.

The key role of chronic systemic low-grade inflammation

Table 2. Comparison of the mean white blood cell count and calculated variables between the PDR, NPDR, and control groups

	PDR (n=40) (Mean±SD)	Severe NPDR (n=40) (Mean±SD)	Control (n=35) (Mean±SD)	p
Neutrophil count (x10^3µL)	4.96±1.49	4.56±1.15	4.27±1.13	0.216
Lymphocyte count (x10^3µL)	2.02±0.65	2.21±0.60	2.39±0.64	0.129
Monocyte count (x10^3µL)	0.30±0.14	0.35±0.16	0.43±0.18	0.020
Platelet count (x10^3µL)	218.80±71.68	240.50±69.62	233.28±49.08	0.440
MPV (fL)	8.43±0.95	8.04±0.70	8.33±1.00	0.354
NLR	2.67±1.02	2.16±0.58	1.85±0.49	0.003*
Monocyte/Lymphocyte ratio	0.15±0.07	0.17±0.09	0.23±0.14	0.374
Platelet/Lymphocyte ratio	121.01±60.43	113.01±32.32	103.00±30.48	0.525
MPV/Lymphocyte ratio	4.70±1.84	3.90±1.11	3.73±1.14	0.105

MPV: main platelet volume; NLR: neutrophil/lymphocyte ratio; NPDR: non-proliferative diabetic retinopathy; PDR: proliferative diabetic retinopathy; *p<0.05 in PDR vs. control; p<0.05 in severe NPDR vs. control; p>0.05 in PDR vs. severe NPDR.

Table 3. Analysis of the area under the receiver operating characteristic curve for NLR

Cutoff	2.11
Sensitivity	76%
Specificity	80%
AUC	0.716
95% CI	0.599–0.832
p	0.002

AUC: area under the curve; CI: confidence interval; NLR: neutrophil/lymphocyte ratio.

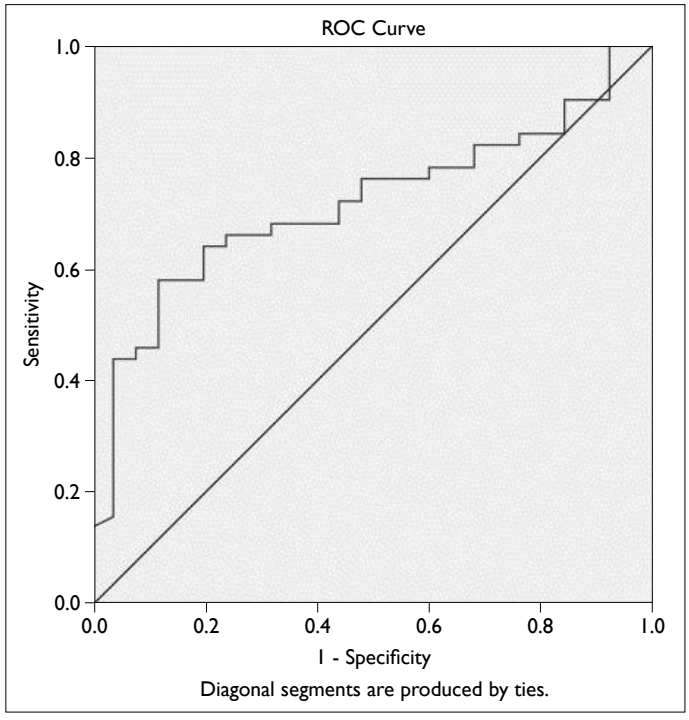

Figure 2. The area under the receiver operating characteristic curve for the neutrophil/lymphocyte ratio.

in the development of DR has been documented in recent studies (1–3). The release of inflammatory mediators by immune cells, such as macrophages, lymphocytes, and leukocytes, causes a breakdown of the blood-retina-barrier (23). An increase in vascular permeability and angiogenesis, which are associated with inflammatory mediators, are primary reasons for the development of DR. Therefore, while many patients are referred by endocrinologists, periodic ophthalmological examinations to detect the presence of DR are necessary for patients with DM. Many clinicians are investigating new diagnostic techniques to predict DM-related complications such as DR. New imaging modalities and circulating biomarkers are the subject of special attention and the focus of clinical trials. Interleukin 6, tumor necrosis factor alpha, and C-reactive protein have all been revealed to be associated with DR (24). Vujosevic et al. (25) reported an increase in glial fibrillary acidic protein in the aqueous humor of patients with low-grade DR. Intercellular adhesion molecule 1 and basic fibroblast growth factor are associated with the development of retinal hard exudates and diabetic macular edema (26, 27). Ulu et al. (11) reported an elevated NLR in patients with DR and found a correlation between NLR and grades of DR. That study included patients with all grades of DR. Our study, however, was designed to define a cutoff value of NLR that indicated increased risk for only severe grades of DR. The NLR in the PDR and severe NPDR groups was higher than that of the control group. Our findings suggest that the NLR, which can be easily calculated using peripheral blood samples and interpreted by any clinician, can be used as a predictive test for severe DR.

There are several limitations to our study. First, there is a relatively small sample size. Second, the sensitivity and specificity of the ROC curve analysis results for NLR are

somewhat low to draw meaningful conclusions for a diagnosis. Third, the relevance of blood cell parameters for clinical monitoring or individual judgment on the presence of severe grades of DR is limited. Finally, the diabetic patients were not separated based on the presence of macular edema and we do not know the potential effect on NLR.

In conclusion, the most important finding of this study is the demonstration of the importance of chronic, systemic, low-grade inflammation in the development of severe DR. We determined that a cutoff for NLR of 2.11 or higher predicted severe DR (PDR or severe NPDR). A periodic ophthalmological examination is important for all diabetic patients; however, these results may assist the internist in recommending what could be a critical ophthalmology consultation.

Disclosures

Ethics Committee Approval: Health Sciences University Dışkapı Yıldırım Beyazıt Training and Research Hospital Clinical Research Ethics Committee, April 2, 2018, no: 48/11.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Involved in design and conduct of the study (CI, MC, MMU, KT); preparation and review of the study (CI, MC, MMU); data collection (MC); and statistical analysis (CI, MMU, KT).

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