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Is Neutrophil Lymphocyte Ratio an Indicator for Proteinuria in Chronic Kidney Disease?

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Background: Recent studies have shown that neutrophil lymphocyte ratio (NLR) is a strong indicator in determining inflammation in cardiac and non-cardiac diseases. We aimed to evaluate the relationship between proteinuria and NLR in chronic kidney disease (CKD) patients without diabetes mellitus (DM). *Methods:* Between 2011 and 2012 files of a total of 1000 CKD patients attending outpatient clinic were retrospectively scanned. Patients with DM, chronic disease, malignancy or stage 5 CKD were excluded. After these patients were excluded, a total of 69 patients with stage 3 and 4 CKD were evaluated. *Results:* The study comprised 27 patients with CKD without proteinuria (Group 1), 42 patients with CKD and proteinuria (Group 2) and 30 healthy volunteers (Group 3). NLR was highest in Group 2 and this was statistically significant compared with the control group ($p = 0.012$). The platelet lymphocyte ratio (PLR) in Group 2 was higher than the control group at a significant level ($p = 0.004$). There was a moderate positive correlation found between proteinuria and NLR ($p = 0.013$, $r = 0.3$). There was a positive correlation found between proteinuria and PLR ($p = 0.002$, $r = 0.306$). *Conclusion:* In conclusion, NLR, a parameter easily found in routine blood counts of CKD patients, is a marker with prognostic value for the presence and degree of proteinuria. J. Clin. Lab. Anal. 28:487–492, 2014.

Key words: chronic kidney disease; neutrophil lymphocyte ratio; proteinuria

INTRODUCTION

In the world population, 5–7% of people have chronic kidney disease (CKD) and it is known that they have a high risk for recurrent hospital stays and heart diseases (1). In recent years, in spite of developments in prevention and treatment of kidney disease, studies have shown that progression rates for end-stage renal disease (ESRD) are still very high (2). Proteinuria in both patients with and without diabetes mellitus (DM) shows strong association to risk of progression to chronic renal failure (3).

Clinical studies have shown that reducing proteinuria can delay the progression of renal disease with a renoprotective effect. Apart from the progress of kidney disease, proteinuria is an important indicator of arteriosclerotic cardiovascular diseases that increase the risk of cardiovascular incidents and mortality in patients both with and without DM (4).

The presence of proteinuria generally reflects impaired glomerular filtration barrier or impaired tubular function, in which advanced immunoinflammatory activity has been found to play an important role (5, 6). Peripheral white cell count is a well-known marker of systemic immunoinflammatory activity. Recent studies have shown that neutrophil lymphocyte ratio (NLR) is a strong indicator in determining inflammation in cardiac and noncardiac diseases. Especially, in patients with CKD, the strong association of NLR with progression of ESRD is supported by these studies. For this reason in CKD patients, proteinuria monitoring has an important role. While there are studies relating NLR and CKD, to date, the information on the relationship between different peripheral

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leukocyte counts and proteinuria is limited (7, 8). Studies have generally involved patients with DM. We aimed to evaluate the relationship between proteinuria and NLR in CKD patients without DM.

PATIENTS AND METHODS

Subjects

Between January 2011 and December 2012, files of a total of 1,000 CKD patients attending Kocaeli Derince Education and Research Hospital internal medicine and nephrology clinic were retrospectively scanned. This study was approved by the local ethical committee of Çanakkale Onsekiz Mart University. Demographic characteristics of the patients (such as stage of CKD, amount of proteinuria, cigarette and alcohol use, DM, or presence of infection) were obtained from medical records. Patients with DM, positive indicators of infection in blood tests (high sedimentation and CRP), with malignancy, smoking, alcohol intake, or Stage V CKD patients were excluded. After these patients were excluded, a total of 69 patients with Stage III and IV CKD were evaluated. CKD staging was determined according to the National Kidney Foundation (NKF) guidelines. Stage I is defined by a normal glomerular filtration rate (GFR); greater than 90 ml/min per 1.73 m^2, Stage II by a GFR between 60 and 89 ml/min per 1.73 m^2, Stage III by a GFR between 30 and 59 ml/min per 1.73 m^2, Stage IV by a GFR between 15 and 29 ml/min per 1.73 m^2, and Stage V by a GFR of less than 15 ml/min per 1.73 m^2 or ESRD requiring renal replacement therapy (RRT). Proteinuria was defined as more than 150 mg protein lost in urine more than 24 h. At the end of scanning, the patients were divided into three different groups. Group 1 was composed of CKD patients without proteinuria over 24 h, Group 2 with CKD and proteinuria patients, and Group 3 was composed of healthy cases (without proteinuria). Patients were informed about 24-h urine collection. Total and differential leukocyte counts were determined with the CELL-DYN 3700 (Abbott 2010, USA) device in the hematology laboratory of Kocaeli Derince Education and Research Hospital. Other biochemical measurements and electrolyte levels were determined with the ARCHITECT c16000 (Abbott 2009, Japan) device in the central laboratory of Kocaeli Derince Education and Research Hospital.

Statistical Analyses

All statistical analyses were performed using SPSS 20.0 statistical package. Normally distributed variables were expressed as mean ± SD. A P-value <0.05 was considered to be statistically significant. Between-group comparisons for nominal variables were assessed with the Chi-square test, and the rest of the variables with Kruskal–Wallis test (ANOVA). Spearman's rank correlation was used to determine correlations between paired variables. Data are presented in the form of β-coefficient and 95% confidence intervals (CIs). A receiver operator characteristic (ROC) curve analysis was performed to identify the sensitivity and specificity of NLR cutoff value in prediction of proteinuria. Power and sample size calculations were performed according to Schesselman (9). The power of the study is 99%. A multiple linear regression model was used to identify independent predictors of proteinuria. A 5% type-1 error level was used to infer statistical significance.

RESULTS

The study comprised 27 patients with CKD without proteinuria (Group 1), 42 patients with CKD and proteinuria (Group 2), and 30 healthy volunteers (Group 3). In Group 1, the most common cause of CKD was hypertension (HT), while in Group 2 the most common cause was glomerulonephritis (GN). The etiologies of CKD are given in Table 1.

The average age of the control group was significantly lower than the other two groups ($P = 0.002$). Systolic blood pressure in Groups 1 and 2 with CKD was significantly higher than in the control group ($P = 0.001$). The general demographic characteristics of the patients and drugs are given in Table 2. Values for glucose, calcium, and phosphorus in plasma showed no significant differences between the groups. The basic laboratory results for the patients are shown in Table 3. Comparing the groups based on hematologic parameters, as expected, hemoglobin (Hb) and hemotocrit (Htc) values in the groups with CKD were lower than the control group at a significant level ($P = 0.02$ and 0.008). Platelet count in the CKD and proteinuria group was significantly higher than the group with CKD without proteinuria; however on comparison with the control group, this was not significant. Mean platelet volume in the control group was higher when compared to the other groups, however this was not statistically significant (Table 4).

TABLE 1. Etiology of Chronic Kidney Disease

	Group 1 ($n = 27$)	Group 2 ($n = 42$)
Glomerulonephritis	3	14
Stone	2	6
Pyelonephritis	4	1
Reflux nephropathy	1	4
Alport syndrome	0	1
Hypertension	10	10
Polycystic kidney disease	3	5
Unknown	4	1

TABLE 2. General Demographic Characteristics of the Patients

	Group 1 ($n = 27$)	Group 2 ($n = 42$)	Group 3 ($n = 30$)	P
Age (years)	55.5 ± 14.6	47.7 ± 13.2	43.6 ± 8.9	**0.002**
Height (cm)	164.8 ± 8.1	163.6 ± 9.9	165.9 ± 7.1	0.566
Weight (kg)	75.1 ± 10.01	78.2 ± 19.4	75.4 ± 14.7	0.675
BMI (kg/m^2)	27.6 ± 3.2	29.3 ± 7.7	27.3 ± 4.8	0.330
Gender (M/F)	10/17	18/24	9/21	0.539
SBP (mmHg)	129.6 ± 14	124.6 ± 14.4	115.1 ± 13.2	**0.001**
DBP (mmHg)	79.6 ± 8.9	80.3 ± 7.6	76 ± 9	0.089
ACE inhibitors	7	4	0	**0.000**
CaCB	5	12	0	**0.000**

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACE, angiotensin-converting enzyme; CaCB, calcium channel blocker.

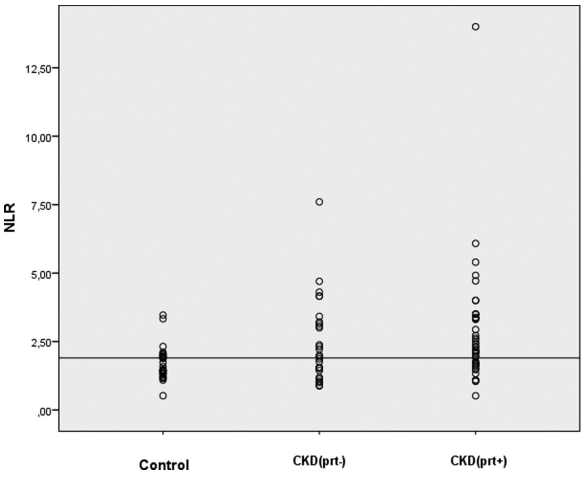

Fig. 1. Neutrophil/lymphocyte ratio between groups.

The neutrophil count in Group 2 was significantly higher when compared to the control group ($P = 0.031$). Lymphocyte counts showed no significant difference between the groups. NLR was highest in Group 2 and this was statistically significant compared with the control group ($P = 0.012$; Fig. 1). There was no significant difference between the NLR in Groups 1 and 2. Platelet values in Group 2 were significantly high compared to Group 1 ($P = 0.026$). The platelet lymphocyte ratio (PLR) in Group 2 was higher than the control group at a significant level ($P = 0.004$). There was a significant positive correlation between proteinuria and neutrophil count

TABLE 3. Baseline Characteristics of Patients and Control Groups

	Group 1 ($n = 27$)	Group 2 ($n = 42$)	Group 3 ($n = 30$)	P
Glucose (mg/dl)	96.5 ± 5.7	93.8 ± 9.1	96 ± 6.5	0.284
Urea (mg/dl)	64.2 ± 33.4	73.6 ± 31.4	25.3 ± 6.3	<0.001
Creatinine (mg/dl)	1.7 ± 0.47	2.4 ± 1.25	0.73 ± 0.1	<0.001
Uric acid (mg/dl)	6.6 ± 1.3	6.8 ± 1.6	4.5 ± 1.3	<0.001
Albumin (g/dl)	4.01 ± 0.3	3.9 ± 0.36	4.03 ± 0.21	0.397
Proteinuria (mg/day)	79.3 ± 33.7	1,685.4 ± 1,786	0	<0.001
Calcium (mg/dl)	9.3 ± 0.67	9.1 ± 0.8	9.1 ± 0.37	0.488
Phosphate (mg/dl)	3.54 ± 0.67	3.74 ± 0.86	3.59 ± 0.75	0.225
Parathyroid hormone (pg/ml)	168.94 ± 162.08	198 ± 145.24	64.66 ± 15.18	<0.001
Sodium (mmol/l)	139.3 ± 3.04	141.5 ± 2.28	139.9 ± 2.27	0.001
Potassium (mmol)	4.64 ± 0.53	4.7 ± 0.57	4.2 ± 0.21	<0.001
eGFR (ml/min/1.73 m^2)	42.79 ± 10.72	37.45 ± 14.41	96.3 ± 12.21	<0.001

eGFR, estimated glomerular filtration rate.

TABLE 4. Complete Blood Count Results

	Group 1 ($n = 27$)	Group 2 ($n = 42$)	Group 3 ($n = 30$)	P
Hemoglobin (g/dl)	12.4 ± 1.6	12.6 ± 1.6	13.5 ± 1.5	**0.02**
Hematocrit (%)	36.7 ± 4.92	37.5 ± 4.91	40.45 ± 4.25	**0.008**
Platelet (mm^3)	231.63 ± 48.82	272.14 ± 72.43[a]	248.2 ± 56.75	**0.029**
Mean platelet volume (fl)	8.24 ± 1.07	8.5 ± 1.31	10.62 ± 13.71	0.408
White blood count (mm^3)	7,337.04 ± 2,000.17	7,702.38 ± 2,363.07	6,883.33 ± 1,674.36	0.262
Neutrophil (mm^3)	4,448.15 ± 1,968.67	4,895.24 ± 2,189.54	3,730 ± 853.04	**0.031**
Lymphocyte (mm^3)	2,033.33 ± 690.59	2,038.10 ± 820.45	2,390 ± 1,037.68	0.177
Neutrophil/lymphocyte	2.49 ± 1.52	2.85 ± 2.14[b]	1.70 ± 0.6	**0.016**
Platelet/lymphocyte	0.12 ± 0.03	0.14 ± 0.05[c]	0.11 ± 0.03	**0.004**

[a] $P = 0.026$ vs. Group 1.
[b] $P = 0.012$ vs. Group 3.
[c] $P = 0.04$ vs. Group 3.

($P = 0.001$, $r = 0.343$); however, there was no significant correlation between proteinuria and lymphocyte count ($P = 0.176$). There was a moderate positive correlation found between proteinuria and NLR ($P = 0.013$, $r = 0.3$; Fig. 2A). There was a positive correlation found between proteinuria lymphocyte ratio and PLR ($P = 0.002$, $r = 0.306$; Fig. 2B). To investigate the relationship between NLR and proteinuria in CKD, ROC analysis was completed. A value of 1.94 for NLR gave 69% sensitivity and 60% specificity, and was the effective cutoff point to indicate proteinuria (area under the curve (AUC): 0.66; 95% CI: 0.55–0.77, $P = 0.005$; Fig. 3A). For NLR in CKD without proteinuria, the ROC analysis had an AUC of 0.518, with a 95% CI between 0.39 and 0.66, $P = 0.78$ (Fig. 3B). The patients in the study were divided into two groups according to NLR ratio of $\geq$1.94 and <1.94. When investigating these two groups based on proteinuria values, there was a significant difference found (1,123.63 $\pm$ 1,737.59 mg/day vs. 290.8 $\pm$ 707.202 mg/day, $P < 0.001$).

When an ROC analysis was completed for the relationship between PLR and proteinuria with CKD, the results were AUC: 0.648, 95% CI: 0.53–0.76, $P = 0.012$. Comparing PLR and NLR as indicators of proteinuria, there was no significant difference between the two parameters (Fig. 3C). We used multivariate regression analysis to determine the predictors of proteinuria. We entered age, gender, blood pressure, ACE inhibitors, or calcium channel blocker intake and NLR as an independent variable. NLR was shown to effect proteinuria independently from the other risk factors (Table 5).

DISCUSSION

Our study showed the relationship between NLR, PLR, and the presence and degree of proteinuria in CKD patients. Between PLR and NLR as indicators of proteinuria, there was no significant difference. Both may be useful markers to indicate proteinuria. Furthermore, our

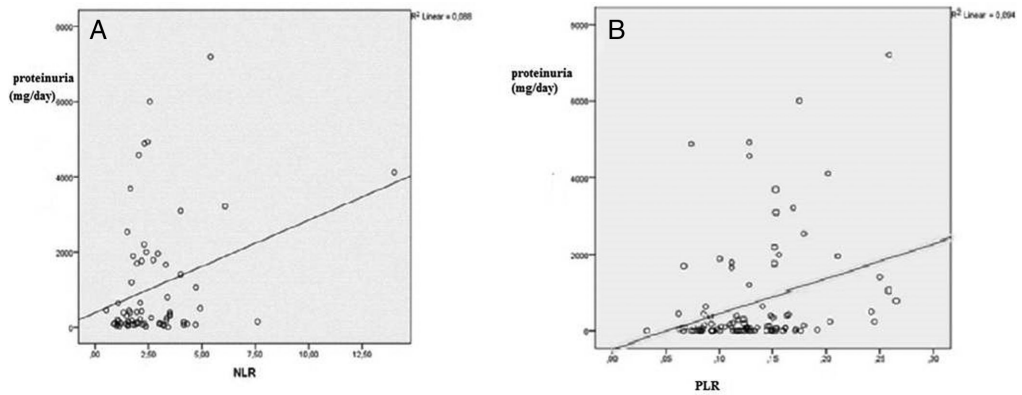

Fig. 2. (A) The scatter plot graphic between logarithmically converted proteinuria and neutrophil/lymphocyte ratio ($P = 0.013$, $r = 0.3$). (B) The scatter plot graphic between logarithmically converted proteinuria and platelet/lymphocyte ratio ($P = 0.002$, $r = 0.306$).

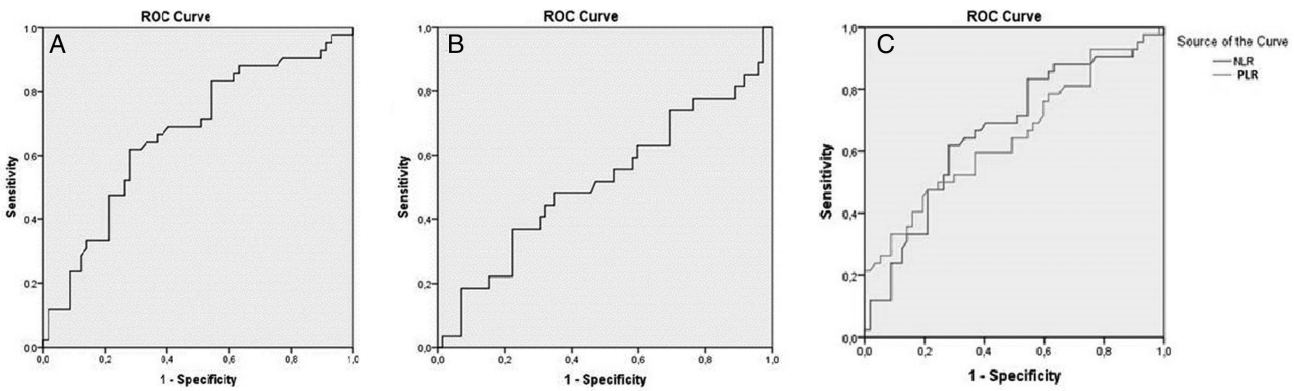

Fig. 3. (A) Receiver operator curve (ROC) plot showing the sensitivity and specificity of neutrophil/lymphocyte ratio in CKD with proteinuria (AUC: 0.66, 95% CI: 0.55–0.77, $P = 0.005$; a value of 1.94 for NLR gave 69% sensitivity and 60% specificity). (B) ROC plot showing the sensitivity and specificity of neutrophil/lymphocyte ratio in CKD without proteinuria (AUC: 0.518, CI: 0.39–0.66, $P = 0.78$). (C) Comparing platelet lymphocyte ratio (PLR) and NLR as indicators of proteinuria (PLR and proteinuria with CKD, the results were AUC: 0.648, 95% CI: 0.53–0.76, $P = 0.012$).

TABLE 5. Multivariate Regression Analysis of Proteinuria With Adjustments of NLR, Age, SBP, Gender, ACE Inhibitors, and CaCB

	β	*P*-value	CI
NLR	221.384	**0.038**	12.330–430.437
Age	− 20.609	0.180	−50.994–9.776
SBP	6.812	0.653	−23.348–36.971
Gender	− 8.614	0.983	−829.760–812.538
ACE inhibitors	872.429	0.093	−148.763–1,893.622
CaCB	− 470.783	0.297	−1,365.827–424.26

NLR, neutrophil to lymphocyte ratio; SBP, systolic blood pressure; ACE inhibitors, angiotensin-converting enzyme inhibitor; CaCB, calcium channel blocker.

results strongly suggested that NLR is an independent predictor of proteinuria in patients with CKD.

In the progression of CKD, there are factors that cannot be modified (age, gender, race, genetics) and factors that can be modified (proteinuria, HT, blood glucose, blood lipid levels, obesity, hyperuricemia, cigarettes, alcohol, caffeine, etc.; 10). Proteinuria is the basic finding of renal damage and is an important indicator of development of fibrogenesis and glomerulosclerosis linked to the progression of several kidney diseases (11). Proteinuria may cause an increase in morbidity and mortality in the general population. Glomerulopathies that accompany proteinuria cause abnormal protein traffic through the glomerular capillary barrier, which is not only a part of the disease but also causes intrinsic toxicity and is accepted as directly affecting the progression of the disease. This effect of proteinuria is essentially due to an increase in inflammation that is already present. Evidence for this includes the amount of increased protein in the urine and is associated with increased amount of tubulointerstitial inflammatory cells, especially, T lymphocytes. Especially, T-lymphocyte amount is accepted as a marker for decrease in kidney functions (12). These suggest that there is more than one mechanism for the increase in tubulointerstitial damage caused by proteinuria. One of these mechanisms is that inflammation accompanying proteinuria causes receptors for T-lymphocyte CD 40 in proximal cells normally found on the basal wall to move to the tubular walls (13). Proximal cells connected to T lymphocytes produce more inflammatory cytokine. Taking into account all this information, it is believed that there should be a relationship between proteinuria and neutrophil and lymphocyte ratios. Our study confirms this.

There is only one study in the literature that evaluates the relationship between proteinuria and NLR. In a study by Baris et al., the relationship between proteinuria and NLR ratio in newly diagnosed type-2 DM patients was evaluated and a significant correlation was found between proteinuria and neutrophil, lymphocyte, and NLR (14). It was emphasized that the NLR ratio was a marker for proteinuria independent of other risk factors in newly diagnosed type-2 DM patients. Our study is the first to evaluate the relationship between proteinuria and NLR in CKD in the absence of DM. We found a significant correlation between NLR and proteinuria in our CKD patients. The results of our study prove that increased NLR and PLR are effective markers for proteinuria.

Turkmen et al. in a study found a correlation between PLR and NLR in ESRD with tumor necrosis factor (TNF)-alpha and interleukin (IL)-6 among patients undergoing dialysis and showed this was a part of inflammation (15). Additionally, comparing PLR and NLR, they determined that PLR in ESRD patients was a better marker for inflammation than NLR. In our study comparing PLR and NLR, there was no significant difference as a marker for proteinuria. Huang et al. studied 12,225 normal healthy individuals who were called to hospital for checkup and found a significant relationship between total leukocytes, neutrophil, and monocyte counts and the presence of proteinuria but found no relation with lymphocyte counts (16).

These results support the view that proteinuria pathogenesis has an immunoinflammatory basis. However, they emphasized that the lack of significant relationship between lymphocyte count and proteinuria may indicate that the development of proteinuria may be related more to inflammatory activity than immune activity. In our study, neutrophil count was highest in the CKD group with proteinuria and this increase was statistically significant when compared to the control group. Additionally, while there was a significant positive relationship between neutrophil count and proteinuria, there was no relationship between proteinuria and lymphocyte count. These results support the hypothesis that inflammatory processes are the basis for proteinuria.

In conclusion, our study demonstrated that NLR and PLR, which are easily measured parameters in routine blood counts of CKD patients, are markers with prognostic value for the presence and severity of proteinuria. Increased NLR is independently related to 24-h urinary protein excretion (UPE) in CKD patients without DM. A value of 1.94 for NLR gave 69% sensitivity and 60% specificity, and was the effective cutoff point to predict proteinuria. This study is unique, which specifically shows the relationship between NLR and proteinuria in CKD patients.

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