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Association between Neutrophil-To-Lymphocyte and Platelet-To-Lymphocyte Ratios with Chronic Allograft Nephropathy

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ABSTRACT

Background: In several diseases, both neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are reliable indicators of chronic inflammation. Endothelial dysfunction is widely distributed in renal transplant patients and is caused by inflammation and can lead to the malfunction of the graft. **Objective:** To detect the correlations of NLR and PLR with chronic allograft nephropathy (CAN) in recipients of kidney transplants and determine the cutoff values for the prediction of CAN. **Patients and Methods:** 68 kidney transplant recipients shared in the study between January 2017 and December 2019. They were two groups. Group 1 (44 subjects) had estimated glomerular filtration rate (eGFR) ≥ 60 ml/m/1.73 m^2 and group 2 (24 subjects) had eGFR less than 60 ml/m/1.73 m^2. **Results:** The two groups had similar age and sex distributions. eGFR was shown to be adversely linked to NLR and PLR. The optimal cutoff level of NLR for predicting chronic allograft nephropathy was ≥1.58 and the optimal cutoff level of PLR was ≥109.13. **Conclusion:** Significant correlations were detected between kidney function tests and each of NLR and PLR. PLR is a more sensitive inflammatory marker to predict chronic allograft nephropathy than NLR with a sensitivity of 83.33 % versus 66.67 %.

Keywords: Glomerular Filtration Rate, Graft Rejection, Inflammation, Kidney Transplantation

INTRODUCTION

At present, transplantation of the kidney is the ideal choice for treating end stage kidney disease (ESKD). The main benefits are better survival estimates and better quality of life than long term dialysis [(1)]. However endothelial dysfunction is widely distributed in recipients of renal transplants. Additionally, it is related to the higher prevalence of inflammation. Inflammation enhances the stiffness of the blood vessel wall. Chronic inflammation leads to atherosclerosis in individuals with end-stage kidney disease (ESKD) [(2)]. In several diseases, such as ischemic heart disease, heart failure, atrial arrhythmia, malignancy, and ESKD, the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are reliable indicators of chronic inflammation and poorer outcomes [(3)]. Thus, the objectives of this work were to detect the correlations of NLR and PLR with chronic allograft nephropathy (CAN) and determine the cutoff values for the prediction of CAN.

PATIENTS AND METHODS

68 kidney transplant recipients between January 2017 and December 2019 were recruited for this retrospective cross-sectional research. Exclusion criteria included active infection, acute kidney injury, active malignancy, hematological disorders, and patients requiring maintenance dialysis. The research was composed of two groups. Group 1 consisted of 44 subjects who had eGFR ≥ 60 ml/m/1.73 m^2. They were 22 men and 22 women with a median age of 45.5 years. Group 2 contained 24 subjects who had eGFR less than 60 ml/m/1.73 m^2. They were 12 men and 12 women with a median age of 42.5 years. Demographic and clinical features of each patient were recorded. Investigations included complete blood count, kidney function test, urinary protein creatinine ratio (PCR), fasting lipid profile, bone profile, and other routine metabolic screening tests. The eGFR was assessed using the Modification of Diet in Renal Disease Study formula [(4)].

Ethical approval:

The protocol of the research was authorized by the Institutional Review Board of the Ethical Committee of Zagazig University and followed the Helsinki ethical guidelines.

Statistical analysis

Analysis of data was conducted using the MedCalc 20 for windows and the Statistical Package for the Social Sciences version 26. The Shapiro Wilk test was utilized to assess the distribution of continuous variables. Means and standard deviations for normally distributed continuous variables and medians and interquartile ranges (25^{th} percentile to 75^{th} percentile) for skewed continuous variables were calculated. Categorical data are presented as numbers and relative frequencies. Normally distributed continuous variables were compared using the Student's t-test, while the Mann-Whitney U test was employed for skewed continuous data. Ordinal data were compared utilizing the Chi-square test. Correlations between NLR and PLR and selected study parameters were calculated using Spearman's rank correlation test. Receiver operating characteristic (ROC) curves were plotted to calculate the sensitivity and specificity of NLR and PLR in the prediction of CAN. P-value below 0.05 was deemed significant.

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RESULTS

Age and sex distributions did not differ between the two groups, while group (2) had higher systolic blood pressure than group (1) as introduced in table (1).

Table (1): Demographic and clinical features of the studied patients

Variable	**Group 1** (n=44)	**Group 2** (n=24)	**Test**	***p***
Age (years), Median (IQR)	45.5 (38-54)	42.5 (34-61.25)	-0.488	0.625
Male sex, No (%)	22 (50%)	12 (50%)	0.000	1.000
History of diabetes mellitus, No (%)	8 (18.2%)	6 (25%)	0.442	0.506
History of hypertension, No (%)	30 (68.2%)	12 (50%)	2.174	0.14
History of IHD, No (%)	4 (9.1%)	2 (8.3)	0.011	0.916
Weight (Kg), Median (IQR)	70 (54.4-83.5)	67 (48.2-84.1)	-0.308	0.758
Body mass index (kg/m^2), Median (IQR)	25.5 (19-32)	26 (20-34.25)	-0.334	0.738
SBP (mm Hg), Mean ± SD	129.6±16.13	139.7±15.5	-2.496	0.015*
DBP (mm Hg), Median (IQR)	81.45±9	85.6±10.8	-1.681	0.098
Transplantation duration (years), Median (IQR)	10.5 (4-15)	13.5 (8.3-17.8)	-1.803	0.071

(*): Significant, (IQR): Interquartile range, (SD): Standard deviation, (IHD): Ischemic heart disease, (SBP): Systolic blood pressure, (DBP): Diastolic blood pressure. Serum phosphorus, serum creatinine, blood urea, neutrophil count, serum uric acid, urine PCR, NLR, and PLR were greater in group (2) than in group (1). On the other hand, serum albumin, eGFR, hemoglobin, and lymphocyte count were lesser in group (2) than in group (1) as demonstrated in table (2).

Table (2): Laboratory features of the studied patients

Variable	**Group 1** (n=44)	**Group 2** (n=24)	**Test**	***p***
FBS (mg/dL), Median (IQR)	107 (93 -126)	109.8 (92.7-119)	-0.616	0.538
ALT (IU/L), Median (IQR)	19.7 (15-27)	19.2 (11.2-24.25)	-1.079	0.281
Alkaline phosphatase (U/L), Median (IQR)	68.1 (57-84)	98.5 (45.25-131)	-1.669	0.095
Serum total protein (g/dL), Mean±SD	7.14±0.49	7.11±0.6	0.181	0.857
Serum albumin (g/dL), Median (IQR)	4.2 (4-4.5)	4.07 (3.4-4.2)	-2.495	0.013*
Serum calcium (mg/dL), Median (IQR)	9.28 (9-9.74)	9.32 (9.1-9.7)	-0.36	0.719
Serum phosphorus (mg/dL), Median (IQR)	3.16 (2.9-3.38)	3.34 (2.78-3.8)	-2.005	0.045*
iPTH (pg/mL), Median (IQR)	52.34 (37.7-64)	62 (31.12-292.3)	-0.972	0.331
Serum creatinine (mg/dL), Median (IQR)	0.94 (0.84-1.07)	1.8 (1.4-2.9)	-6.316	< 0.001**
Serum cholesterol (mg/dL), Mean±SD	192±30.36	175±5.8	1.806	0.076
Serum TG (mg/dL), Median (IQR)	152 (100-180)	124 (92-178)	-0.796	0.426
LDL (mg/dL), Median (IQR)	107 (86-124)	81 (69-116)	-1.926	0.054
HDL (mg/dL), Median (IQR)	54 (46.8-63)	51 (38.6-58.9)	-1.207	0.227
Urea (mg/dL), Median (IQR)	26.13 (22.8-33.6)	53.46 (35.9-75.23)	-4.111	< 0.001**
eGFR (ml/m/1.73m^2), Median (IQR)	79 (70-86)	40.5 (21.25-48)	-6.784	< 0.001**
Hemoglobin (g/dL), Median (IQR)	13.1 (11.72-14.3)	10.49 (8.96-13.2)	-3.851	< 0.001**
Neutrophil (x10^3/mm^3), Median (IQR)	2.82 (2.31-4.17)	4.22 (2.53-7.3)	-2.26	0.024*
Lymphocytes (x10^3/mm^3), Median (IQR)	2.3 (1.6-2.8)	1.73 (0.73-2.17)	-2.439	0.015*
Serum uric acid (mg/dL), Median (IQR)	6.06 (5.16-7.48)	7.46 (6.24-8.94)	-3.338	0.001**
WBC (x10^3/mm^3), Median (IQR)	5.92 (4.52-7.2)	6.49 (5.11-10.2)	-1.13	0.259
Platelets (x10^3/mm^3), Median (IQR)	235.5 (191-264)	235.5 (202.75-297)	-1.156	0.248
Serum iron (µg/dL), Median (IQR)	69.3 (53.5-86.4)	76.4 (54-92)	-0.377	0.706
TIBC (µg/dL), Mean±SD	206.7±5.55	211.87±5.77	-0.311	0.757
Transferrin saturation (%), Median (IQR)	32 (25.5-39.88)	35.4 (24.6-43.75)	-0.472	0.637
Ferritin (ng/mL), Median (IQR)	434 (257-490)	756 (451-839)	-1.882	0.06
Urine PCR (mg/g), Median (IQR)	203 (132-375)	527 (232-2084)	-2.961	0.003**
NLR (%), Median (IQR)	1.29 (1-1.58)	2.9 (1.41-5.62)	-3.621	< 0.001**
PLR (%), Median (IQR)	100.8 (84-135)	126 (114-315)	-3.594	< 0.001**

(SD): Standard deviation, (IQR): Interquartile range, (*): Significant, (**): Highly significant, (FBS): Fasting blood sugar, (ALT): Alanine transaminase, (WBC): White blood cells, (eGFR): Estimated glomerular filtration rate, (iPTH): Intact parathyroid hormone, (HDL): High density lipoproteins, (TG): Triglyceride, (LDL): Low density lipoproteins, (TIBC): Total iron binding capacity, (PCR): Protein creatinine ratio.

NLR was shown to be positively associated with diastolic blood pressure (DBP), alkaline phosphatase, intact parathyroid hormone (iPTH), serum creatinine, blood urea, neutrophil count, white blood cell count, and PLR, while it was negatively associated with eGFR and lymphocyte count. PLR was shown to be positively associated with iPTH, serum creatinine, and NLR. On the other hand, it was negatively correlated with lymphocyte count, hemoglobin, white blood cell count, high density lipoproteins (HDL), and eGFR as shown in table (3).

Table (3): Correlation between NLR and PLR and selected study parameters

Variable	NLR		PLR	
	r	***p***	**r**	***p***
Age (years)	-0.074	0.549	0.023	0.85
Male sex	0.153	0.213	-0.171	0.164
Weight (Kg)	0.013	0.916	-0.108	0.381
Body mass index (kg/m2)	0.057	0.646	-0.029	0.814
SBP (mm Hg)	0.033	0.79	0.1	0.417
DBP (mm Hg)	0.250	0.04*	0.155	0.207
Transplantation duration (years)	-0.023	0.854	-0.108	0.38
FBS (mg/dL)	0.000	0.998	-0.012	0.92
ALT (IU/L)	0.179	0.145	0.185	0.13
Alkaline phosphatase (U/L)	0.244	0.045*	0.15	0.223
Serum total protein (g/dL)	-0.199	0.103	-0.043	0.731
Serum albumin (g/dL)	-0.162	0.188	-0.074	0.547
Serum calcium (mg/dL)	0.152	0.217	0.162	0.186
Serum phosphorus (mg/dL)	-0.087	0.481	-0.186	0.129
iPTH (pg/mL)	0.340	0.007**	0.368	0.003**
Serum creatinine (mg/dL)	0.458	< 0.001**	0.268	0.027*
Serum cholesterol (mg/dL)	-0.041	0.742	0.104	0.4
Serum triglycerides (mg/dL)	0.089	0.468	0.143	0.246
LDL (mg/dL)	-0.114	0.356	0.212	0.082
HDL (mg/dL)	0.144	0.241	-0.269	0.027*
Urea (mg/dL)	0.323	0.007**	0.196	0.109
eGFR (ml/m/1.73 m^2)	-0.425	< 0.001**	-0.354	0.003**
Hemoglobin (g/dL)	-0.195	0.112	-0.545	< 0.001**
Neutrophil (g/dL)	0.620	< 0.001**	-0.018	0.883
Lymphocytes ($x10^3/mm^3$)	-0.602	< 0.001**	-0.796	< 0.001**
Serum uric acid (mg/dL)	0.118	0.337	-0.135	0.272
White blood cells ($x10^3/mm^3$)	0.260	0.032*	-0.305	0.012*
Platelets ($x10^3/mm^3$)	0.046	0.708	0.177	0.149
Serum iron (mg/dL)	-0.22	0.152	-0.151	0.329
TIBC (µg/dL)	-0.008	0.959	-0.223	0.146
Transferrin saturation (%)	-0.165	0.285	0.05	0.746
Ferritin (ng/mL)	-0.087	0.593	0.066	0.685
Urine PCR (mg/g)	0.214	0.085	0.205	0.098
PLR (%)	0.592	< 0.001**	---	---
NLR (%)	---	---	0.592	< 0.001**

(r): Correlation coefficient, (*): Significant, (**): Highly significant

In kidney transplant recipients, the optimal cutoff values of NLR and PLR for predicting CAN were ≥1.58 and ≥109.13, respectively as demonstrated in figure (1) and figure (2).

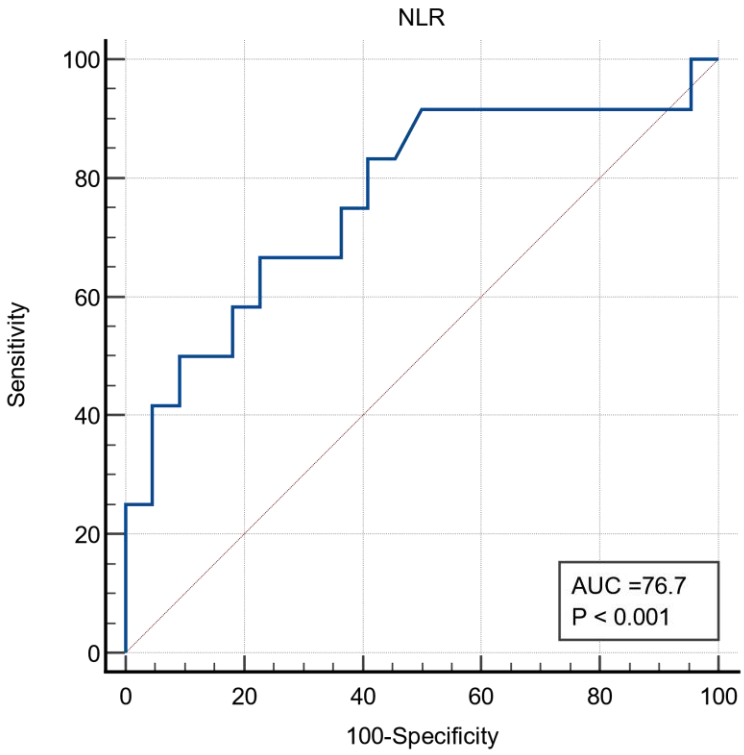

Figure (1) ROC curve demonstrating the performance of NLR in the prediction of chronic allograft nephropathy among the studied renal transplant patients

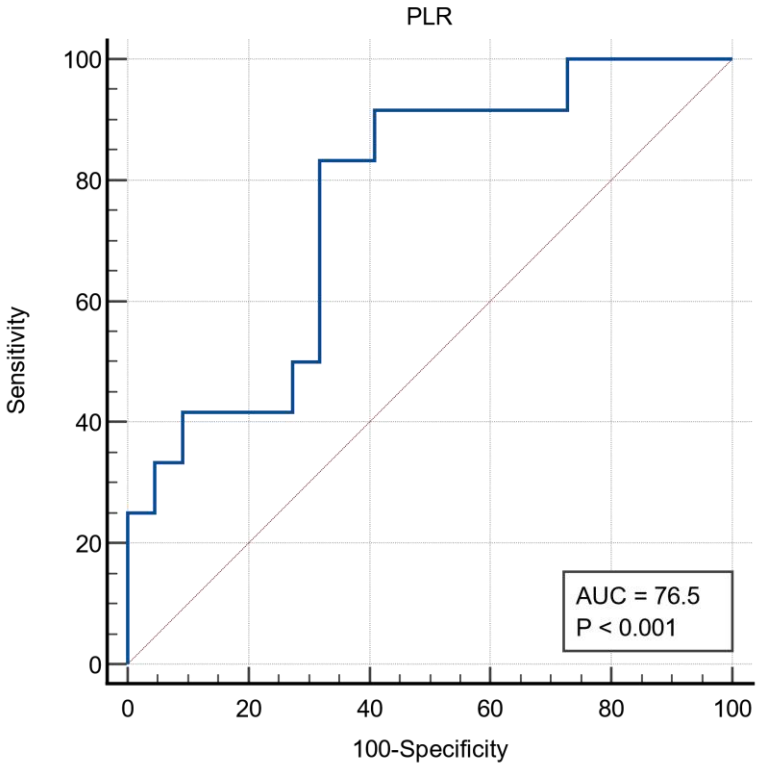

Figure (2) ROC curve demonstrating the performance of PLR in the prediction of chronic allograft nephropathy among the studied renal transplant patients

DISCUSSION

Although renal biopsy is more secure throughout the years to identify loss of renal transplant, it is an invasive approach [5]. Other simple noninvasive methods are needed, so the NLR and PLR were assessed as simple, repeatable, and ready-to-use to assess renal allograft function.

This study included two age and sex matched groups. Group (2) had greater systolic blood pressure than group (1). This finding is in agreement with the findings of **Lee *et al*** [6]. CAN could result in hypertension due to hypervolemia, transplant renal artery stenosis, accumulation of phosphatonin, and side effects of treatment with calcineurin inhibitors and steroids [7].

In the comparison of laboratory features between the two groups, serum albumin was lower in the group (2). This is consistent with the results stated by **Zhang *et al*** [8]. Hypoalbuminemia in these patients results from malnutrition, systemic inflammation, proteinuria, and gastrointestinal loss of protein [9]. Additionally, serum phosphorus levels were greater in group (2). This is like the results obtained by **Jeon *et al*** [10]. This is caused by loss of renal graft function leading to the accumulation of fibroblast growth factor 23, depletion of klotho, and dysregulation of calcium phosphate homeostasis [11]. Moreover, group (2) had a higher neutrophil count, higher NLR, higher PLR, lower hemoglobin, and lower lymphocyte count than group (1). These results agree with those reported by **Zhang *et al*** [12]. In fact, the reason for these results may be the active process of inflammation that occurs in patients with CAN. Triggered neutrophils could boost the release of myeloperoxidase, metalloproteinases, and reactive oxygen species [13]. Additionally, apoptotic neutrophils release mediators in the circulation, which increase the risk of inflammation and even mortality [14]. Furthermore, a high PLR may be due to platelet overstimulation and lymphocyte depletion leading to tissue injury. This prothrombotic condition causes further progress of chronic kidney disease [15].

Serum levels of uric acid were more elevated in the group (2). This is like the results reported by **Weng *et al*** [16]. Hyperuricemia results in the accumulation of uric acid crystals in collecting ducts, thickening of arteriolar walls, and subsequent hypoxia and tubulointerstitial fibrosis. Additionally, hyperuricemia boosts local chemokine expression and inflammatory process in renal tissues [17]. Furthermore, urine PCR was markedly higher in group 2. This agrees with the results stated by **Mertens *et al*** [18] and **Naesens *et al*** [19]. This is because CAN is characterized by basement membrane injury and glomerulopathy resulting in proteinuria [20].

eGFR was shown to be negatively associated with NLR and PLR. Earlier studies demonstrated that both NLR and PLR are associated with indicators of inflammation. A variety of cytokines are secreted by neutrophils inducing inflammation. On the contrary, activated platelets can stimulate leukocytic infiltration of the arterial wall and induce inflammation [21]. **Turkmen *et al*** [22] discovered a link between NLR and tumor necrosis factor alpha, C-reactive protein, interleukin 6, and pentraxin 3. Additionally, PLR was linked to NLR, interleukin 6, and tumor necrosis factor alpha, according to **Balta *et al*** [23].

On plotting the ROC curve, the optimal cutoff value of NLR to predict CAN in renal transplant patients was 1.58 with 66.67 % sensitivity, while PLR had better sensitivity at the cutoff value of 109.13 with 83.33 % sensitivity.

CONCLUSION

NLR and PLR were shown to be linked to eGFR and can be utilized as noninvasive markers to detect CAN. PLR had a better sensitivity to predict CAN in renal transplant patients.

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