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The Predictive Value of Platelet/Lymphocyte Ratio in Hemodialysis Patients With Erythropoietin Resistance

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Abstract: The most important cause of anemia in CKD is relative deficiency of erythropoietin (EPO) secretion from the diseased kidney and EPO therapy has become the standard treatment for anemia of CKD. However, some patients do not respond well to erythropoiesis stimulating agent (ESA), so-called ESA resistance. One of the most important causes of ESA resistance is chronic inflammation in hemodialysis (HD) patients. ESA hyporesponsiveness index (EHRI), calculated as the weekly dose of EPO divided by kilograms of body weight divided by the hemoglobin level, and has been considered useful to assess the EPO resistance. Neutrophil/lymphocyte (NLR) ratio and platelet/lymphocyte ratio (PLR) were also found to be associated with inflammation in HD patients. However, the relationship between NLR, PLR and EHRI has not been investigated before. HD patients underwent medical history taking, physical examination, calculation of dialysis adequacy and biochemical analysis and calculation of EHRI. Logarithmically converted EHRI (logEHRI) was correlated only with hemoglobin (r –0.381, $P < 0.0001$) and PLR (r = 0.227, $P = 0.021$) but not with NLR. Comparison of PLR among 25th, 50th and 75th percentile of EHRI showed that PLR levels increased going from the 25th to 75th percentile ($P = 0.032$). Posthoc analysis revealed that 25–75th percentile ($P = 0.014$) and 50–75th percentile ($P = 0.033$) were different with respect to PLR. In linear regression analysis, PLR (standardized β = 0.296, confidence interval: 0.000–0.001, $P = 0.003$) was independently associated with logEHRI. We found that PLR was independently associated with EHRI in HD patients. PLR, which is quite a simple and cheap method, may guide clinicians for detecting EPO resistance. **Key Words:** Erythropoietin resistance, Hemodialysis, Inflammation, Platelet/lymphocyte ratio.

Anemia is a common complication in patients with chronic kidney disease (CKD) and is associated with increased risks of hospitalization and death. Iron deficiency, occult blood loss, vitamin B12 and folate deficiency, hemoglobinopathies, inadequate dialysis, hyperparathyroidism and chronic inflammation may cause anemia and erythropoietin (EPO) resistance (1). However, the most important cause of anemia in CKD is relative deficiency of EPO secretion from the diseased kidney relative to the degree of anemia. Therefore, EPO therapy has become the standard treatment for the anemia of CKD (2). Although most patients respond adequately to erythropoiesis stimulating agents (ESA), about 10% of them did not respond well to ESA, so-called ESA resistance (3). ESA resistance is defined as a failure to achieve target hemoglobin/hematocrit levels despite a higher than usual dose of ESA, or a continuous need for this higher dose to maintain these hemoglobin/hematocrit levels. Specifically, EPO resistance is defined as the failure to attain the target hemoglobin concentration in patients who receive more than 300 IU/kg per week (20 000 IU/week) of erythropoietin or 1.5 mg/kg of darbepoetin alfa (100 mg/week), or who have a

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continued need for such high dosages to maintain the target (4). In recent studies, the ESA hyporesponsiveness index (EHRI), calculated as the weekly dose of EPO divided by per kilogram of body weight divided by the hemoglobin level (g/dL), has been considered useful to assess EPO resistance. The EHRI can be easily calculated in the clinic and directly related with co-morbidity and mortality in patients on hemodialysis (HD) (5,6).

Total leukocyte count provides a crude but sensitive assessment of inflammatory status, with low cost and wide availability. White blood cell count is positively associated with increased cardiovascular mortality, mainly from coronary heart disease and ischemic stroke. Additionally, recent data demonstrated that some specific subtypes of leukocytes have higher predictive value in assessing cardiovascular risk than total WBC count. Such risk is even higher when neutrophil/lymphocyte ratio (NLR) is used (7,8). In HD and PD patients NLR was closely associated with increased inflammation (9). Recently platelet/lymphocyte ratio (PLR) was also found to be associated with inflammation even more than NLR in HD patients (10).

Thus in light of the aforementioned data; we hypothesized that EHRI (as a measure of ESA resistance), which can be related with inflammation can be associated with NLR and/or PLR as a measure of increased inflammation in HD patients.

PATIENTS AND METHODS

The subjects of this cross-sectional investigation were clinically stable regular HD patients receiving ESA treatment. This study was performed in accordance with the Declaration of Helsinki (http://www.wma.net/e/policy/b3.htm), and written, informed consent was obtained from all patients before enrollment. Necmettin Erbakan University ethics committee approved the study with a number of 2013/394. The exclusion criteria of the patients were as follows: iron deficiency (serum ferritin values ≤30 ng/mL indicate iron deficiency according to KDIGO anemia guidelines available at: http://www.kdigo.org/clinical_practice_guidelines/pdf/KDIGO-Anemia%20GL.pdf) overt infection/inflammation, hospital admission within the preceding 3 months, history of blood transfusion in last 3 months, having hematologic malignancy and patients receiving steroid treatment. The dialysis prescription was 4–5 h of HD thrice weekly for all patients with blood flow rates of 300–400 mL/min, using a standard bicarbonate dialysis solution. Urea kinetic modeling was performed to assess the delivered equilibrated dose of dialysis. Demographic characteristics including age, sex, body mass index (BMI), smoking status (smoker or nonsmoker), and etiologies of ESRD were recorded. At the baseline laboratory parameters including ferritin, serum iron, albumin, calcium, phosphorus, total cholesterol, trigylcerides, intact parathyroid hormone (intact-PTH), high sensitive C reactive protein (hs-CRP) were recorded. The EHRI was calculated as the weekly ESA dose per kilogram of body weight divided by the hemoglobin level (g/dL). For this calculation means of 3 months erythropoetin dose body weight and hemoglobin was used.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 15.0 (SPSS, Evanston, IL, USA). Results were considered statistically significant if two-tailed *P*-value was less than 0.05. Data were checked for normality. Pearson's correlation coefficients were used for correlations. For the comparison of PLR among EHRI percentiles Kruskal–Wallis test was used. For the post hoc analysis of PLR among EHRI percentiles, Bonferroni corrected Mann–Whitney *U*-test was used. Stepwise linear regression analysis was also performed to analyze the independent factors including (age, gender, HD duration, smoking status, use of ACEi/ARB, Kt/V, serum albumin, intact PTH, hs-CRP, ferritin and iron related with logarithmically converted EHRI (as a dependent variable).

RESULTS

Initially, 214 HD patients were enrolled. 92 patients who did not receive ESA, three patients with active malignancy, one patient with tuberculosis, four patients receiving steroids, and 10 patients with active infection were excluded. The study was conducted in the remaining 104 patients. The etiologies of the ESRD were as follows: Diabetes mellitus ($N=30$), hypertension ($N=47$), glomerulonephritis ($N=9$), urolithiasis ($N=2$), vesicouretheral reflux/pyelonephritis ($N=6$), amyloidosis ($N=1$), polycystic kidney disease ($N=4$), renal cell carcinoma ($N=1$), and unknown ($N=4$). The demographic, clinical and laboratory parameters are shown in Table 1.

In the whole group, logarithmically converted EHRI (logEHRI) was correlated only with hemoglobin (r = –0.381, $P<0.0001$) and PLR (r = 0.227, $P=0.021$) (Fig. 1), but not with NLR. Comparison of PLR among 25th, 50th and 75th percentile of EHRI showed that PLR levels increased as going from 25th to 75th percentile ($P=0.032$). Post hoc analysis revealed that 25 and 75 percentile

TABLE 1. *Demographic, clinical and laboratory parameters of 104 hemodialysis patients*

Parameters	Mean ± SD
Age (years)†	62.9±12.2
Male/female (*N*)	41/63
Hemodialysis duration (months) †	56.4±43.0
Body mass index† (kg/m^2) †	25.4±5.1
Smoker/non-smoker (*N*)	6/98
Kt/V	1.51±0.27
ACEi/ARB use (present/absent) (*N*)	43/61
Hemoglobin (g/L) †	110.5±11.5
Albumin (g/L) †	40.7±3.0
Calcium (mmol/L) †	2.17±0.16
Phosphorus (mmol/L) †	1.59±0.41
Total cholesterol (mmol/L)†	4.29±1.07
Triglycerides (mmol/L)†	1.95±1.34
Intact parathyroid hormone (pg/mL) †	400.7±340.3
Serum iron (μmol/L) †	13.1±4.99
Ferritin (ng/mL)†	943.3±405.3
hs-CRP (mg/dL) †	10.3±11.8
Mean erythropoietin dosage (IU/week)	7342.8±2417.5
EHRI†	9.94±3.32
Total white blood cell count (×10^3/μL)	5974±1932
Neutrophil count (×10^3/μL)	3602±1367
Lymphocyte count (×10^3/μL)	1466±533
Platelet count (10^3/mm^3) (×10^3/μL)	184.1±63.6
Neutrophil/lymphocyte ratio	2.68±1.34
Platelet/lymphocyte ratio	135.6±56.8

†Mean ± Standard Deviation. EHRI, erythropoiesis stimulating agent hyporesponsiveness index; HDL, high density lipoprotein; hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein.

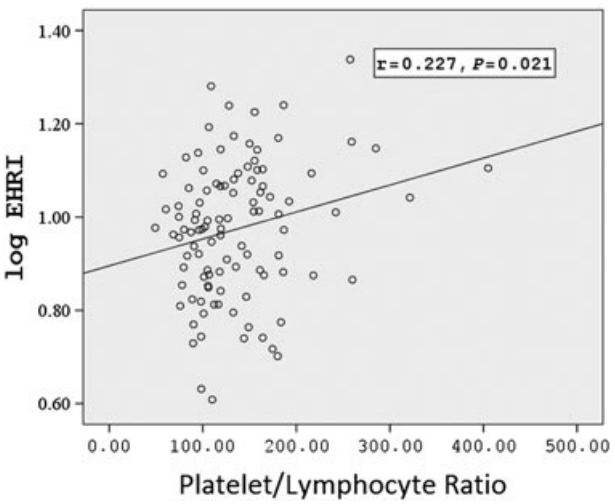

FIG. 1. Scatter plot graphic between logarithmically converted erythropoietin hyporesponsiveness index (logEHRI) and platelet/lymphocyte ratio (PLR).

($P = 0.014$) and 50th and 75th percentile ($P = 0.033$) but not that of the 25th and 50th percentile ($P = 0.795$) were different with respect to PLR (Fig. 2).

Lastly, linear regression analysis of independent factors (mentioned above) related with logarithmically converted EHRI (as a dependent variable) showed

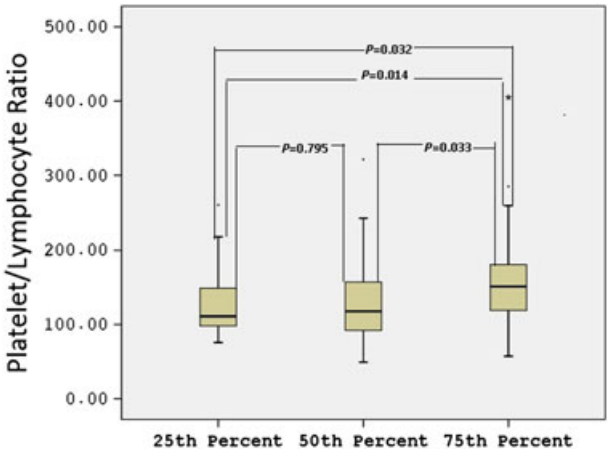

FIG. 2. Comparison of platelet/lymphocyte ratio (PLR) among 25th, 50th and 75th percentiles erythropoietin hyporesponsiveness index (EHRI).

that only ACEi/ARB usage (standardized β = 0.231, CI: 0.012–0.123, $P = 0.018$) and PLR (standardized β = 0.296, Confidence Interval: 0.000–0.001, $P = 0.003$) were found to be independent predictors of logarithmically converted EHRI.

DISCUSSION

In the current study, we sought to determine whether erythropoietin resistance as measured by EHRI was associated with NLR and/or PLR as a measure of inflammation. As a result we demonstrated that PLR but not NLR was independently associated with EHRI. To the best of our knowledge, the relationship between PLR and EHRI is the first in the literature and has not been demonstrated before.

Platelets beyond their thrombotic effects are involved in the atherosclerosis process by actively secreting proinflammatory cytokines (11,12). Besides, binding of platelets to endothelial cells might trigger leukocyte transmigration and adhesion especially in the presence of shear stress (13). Last but not least, activated platelets could be an important part of increased atherogenesis, especially in the area of inflammation (14,15). Platelets can interact with a variety of different cell types including endothelial cells, dendritic cells, T-lymphocytes, neutrophils, and mononuclear phagocytes. Recent studies showed that the interactions of platelets with these cells mentioned above might initiate and exacerbate the inflammation in the arterial wall. There has been increasing evidence demonstrating that activated platelets could incite leukocyte recruitment to the vessel wall and trigger inflammation that can mainly be seen in the pathogenetic mechanisms of atherosclerosis (13,16). Thus,

one of the mechanisms regarding PLR with EHRI may be inflammation. A second explanation may be the role of lymphocytes. Indeed, lymphocytes represent a more appropriate and controlled immune response (17).

Additionally, low lymphocyte numbers may be another explanation. Indeed, the diagnostic and prognostic utilities of a low lymphocyte count were demonstrated in patients with myocardial infarction and chronic coronary artery disease (18,19). It has been proposed that in response to physiologic stress during myocardial ischemia/infarction, there is a release of cortisol. High cortisol leads to lymphopenia. Thus, high levels of physiologic stress mean high levels of cortisol, which can be translated into a lower lymphocyte count (20). Hence, in the face of low lymphocytes exaggerated immune reaction occurs, resulting in EPO resistance.

The advantage of the PLR is that it reflects both hyperactive coagulation and inflammatory pathways, and it may be superior to either the individual platelet or the lymphocyte counts in the prediction of inflammation which is related to EPO resistance.

Interestingly there was no relationship between NLR with EHRI in this study. We do not know the exact causes of this finding. As PLR is a better inflammatory marker as compared to NLR, this may be one of the explanations (10).

We also found that ACE/ARB usage was associated with higher EHRI. Although the mechanism related to this finding is beyond the scope of this paper, various previous studies have demonstrated these relationships (21,22).

The study has some limitations that deserve mention. First, as the study is cross-sectional, a cause and effect relationship cannot be suggested. Second, the patient population is relatively small. However, the patients are iron replete and clinically stable diminishing the effect of co-morbidity. Third, not all of the potential confounders of EPO resistance (e.g., hepcidin) were evaluated. Last, although patients did not complain of any blood loss, endoscopy and colonoscopy were not performed.

CONCLUSION

We found that PLR but not NLR was independently associated with EHRI in stable HD patients. Calculation of PLR, which is quite a simple and cheap method, may guide clinicians in detecting EPO resistance.

Conflict of interest: none declared.

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