Associations of platelet indices with proteinuria and chronic kidney disease

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Abstract

Objectives: Platelet (PLT) indices are predictive in many diseases and conditions. The relationships of these indices with proteinuria and progression of renal disease are not well known. This study aimed to assess PLT indices in patients with primary glomerular nephrotic range proteinuria (PGNRP), with and without chronic kidney disease (CKD), and to compare these indices with those of healthy individuals (His).

Methods: This cross-sectional study was performed from January 2015 to May 2015. HIs (n = 57) and patients with PGNRP (n = 41) were enrolled. PLT indices and blood biochemistry parameters were compared between HIs and patients with PGNRP, as well as between subgroups of patients with PGNRP who had CKD (n = 23) and those who did not have CKD (n = 18).

Results: There were no statistically significant differences in any PLT indices (i.e., platelet number, mean platelet volume, plateletcrit, and platelet distribution width) between HIs and patients with PGNRP, or between the subgroups of patients with PGNRP. However, patients with PGNRP who had CKD exhibited higher median C-reactive protein and mean albumin levels, compared with patients who did not have CKD.

Conclusions: Pathological processes in proteinuria and CKD are not associated with PLT indices.
Keywords
Platelet indices, primary glomerular disease, nephrotic range proteinuria, chronic kidney disease, platelet count, mean platelet volume, plateletcrit, platelet distribution width, C-reactive protein, serum albumin

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Introduction
Proteinuria is associated with platelet (PLT) activation, inflammation, and endothelial dysfunction. When the endothelium is intact, circulating PLTs remain in the inactive form. Collagen is released from subendothelial tissue upon damage to the endothelium, and PLTs adhere to collagen. This adhesion and subsequent aggregation result in PLT activation, which is followed by degranulation, swelling, and increased mass and volume.

PLT activation plays an important role in the development of thrombotic events and PLT indices can serve as markers of this activation. Common complications of the metabolic consequences of proteinuria include venous and pulmonary embolism. Moreover, proteinuria is associated with the progression of kidney disease. Importantly, chronic kidney disease (CKD) is associated with coagulation disorders, which lead to prolonged bleeding and defects in PLT aggregation.

Alterations of PLT indices occur as a result of endothelial dysfunction, which contributes to the pathophysiology of proteinuria and CKD. PLT indices (e.g., plateletcrit [PCT], mean platelet volume [MPV], and platelet distribution width [PDW]) are easily determined by means of complete blood counts. This study was performed to compare PLT indices, as well as C-reactive protein (CRP), lipid values, albumin, and urinary protein, between healthy individuals (HIs) and patients with primary glomerular nephrotic range proteinuria (PGNRP). In addition, subgroups of patients with PGNRP were compared, according to CKD status.

Patients and methods
This cross-sectional study was performed in accordance with the Declaration of Helsinki, with permission from the local ethics committee (T.C. Sağlık Bakanlığı Batman Bölge Devlet Hastanesi Etik Kurulu). Verbal informed consent was obtained from all participants.

Study groups
Between January 2015 and May 2015, HIs were selected from among healthcare professionals in our hospital who volunteered to participate in this study. Demographic characteristics, as well as clinical and biochemical data, were recorded on patient admission. Patients with hypertension, CKD, cardiovascular diseases, neoplastic diseases, hematologic diseases, endocrine diseases, acute or chronic active inflammatory disorders, antiaggregant or anticoagulant use were excluded from the HI group.

Patients with PGNRP were also recruited between January 2015 and May 2015 from among patients admitted to Firat University Medical Faculty Nephrology Clinic (Elazığ, Turkey). Demographic characteristics, as well as clinical and biochemical data, were recorded on patient admission. Patients
with PGNRP were also divided into two subgroups according to the presence of CKD. The inclusion criterion for patients with PGNRP was urinary protein excretion of $>3$ g/day. Exclusion criteria were the presence of chronic or systemic diseases (e.g., hypertension and diabetes mellitus), as well as a history of thrombosis. Little is known regarding the effects of various drugs on PLT size. Antihypertensive, anti-aggregant, and immunosuppressive drugs are frequently used in patients with proteinuria or nephrotic syndrome; these drugs affect the PLT surface. Clopidogrel significantly inhibits adenosine diphosphate and induces an increase in MPV in vitro; in addition, aspirin is associated with high MPV. Therefore, patients using PLT inhibitors and any other immunosuppressants were excluded from the study. Because PLTs also are affected by smoking, smokers were also excluded.

Furthermore, there is a significant relationship between antihypertensive drug use and MPV. Amlodipine has been shown to exhibit no effect, whereas doxazosin treatment reduces MPV. Conversely, selective or non-selective B-blockers do not affect MPV during the acute period. While angiotensin II increases MPV, various types of angiotensin receptor blockers and angiotensin-converting enzyme inhibitors have different effects. For example, losartan increases MPV, whereas candesartan and perindopril do not. Because of these complex relationships, antihypertensive users were excluded from this study.

**Definitions**

The glomerular filtration rate was evaluated with the Modification of Diet in Renal Disease formula. Proteinuria was defined as a 24-hour urinary protein excretion $\geq 150$ mg. Pathological proteinuria was defined as urinary protein excretion $>4$ mg/m$^2$/hour. Nephrotic range proteinuria is the loss of $\geq 3$ g per day of protein into the urine, or the presence of 2 g protein per 1 g urinary creatinine in a single spot urine collection. Nephrotic range proteinuria has many causes, including primary glomerular diseases such as minimal-change disease, focal segmental glomerulosclerosis, membranous glomerulonephritis, immunoglobulin A nephropathy, and membranoproliferative glomerulonephritis. CKD was defined as either kidney damage or a glomerular filtration rate of $<60$ mL/minute/1.73 m$^2$ for $\geq 3$ months, regardless of the underlying etiology, which leads to a progressive decline in the glomerular filtration rate.
Statistical analysis

Continuous variables were reported as mean ± standard deviation or median (range); categorical variables were reported as numbers alone. Differences between two groups were evaluated by the t-test or Mann–Whitney U test (continuous variables) or by the chi-squared test (categorical variables). Differences with P < 0.05 were considered statistically significant. All analyses were performed using SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA).

Results

As shown in Table 1, HIs included 57 participants (seven men, 50 women), aged 18 to 77 years (mean age, 43.73 ± 16.65 years). Patients with PGNRP included 41 participants (24 men, 17 women), aged 19 to 70 years (mean age, 43.58 ± 14.27 years). Fifteen patients underwent renal biopsy; biopsy findings were either inconclusive or unavailable for the remaining 26 patients that clinical, physical examination and laboratory findings supported the characteristics of primary glomerular disease or nephrotic syndrome. Renal biopsy results of the 15 patients were as follows: focal segmental glomerulosclerosis (n = 8), membranous glomerulonephritis (n = 4), membranoproliferative glomerulonephritis (n = 2), and immunoglobulin A nephropathy (n = 1). Comparison of PLT indices revealed no significant differences in PLT, MPV, PCT, or PDW between PGNRP and HI groups (Table 1).

As shown in Table 2, patients with PGNRP who did not have CKD included 23 participants (12 men, 11 women), aged 22 to 67 years (mean age, 43.35 ± 13.94 years). Patients with PGNRP who had CKD included 18 participants (12 men, six women), aged 19 to 70 years (mean age, 43.89 ± 15.08 years). There were significant differences in serum albumin and CRP levels between the two subgroups (P = 0.005 and P = 0.038, respectively). However, there were no significant differences in lipid values, urinary protein, or PLT indices (Table 2).

Discussion

Proteinuria may worsen because of increased incidence of CKD and complications secondary to nephrotic syndrome (e.g., thrombotic episodes and infection), or infectious complications of immunosuppressive drug therapy that is necessary for treatment. Microangiopathic complications, such as microalbuminuria and retinopathy, are significantly correlated with PLT activation. Early prognostic predictors of proteinuria have not yet been identified. The efficacy of PLT indices in assessment of the pathological stages of proteinuria remains unknown. In this

| Table 1. Characteristics of participants in main groups. |
|-----------------------------------|-----------------|-----------------|---|
| **Characteristic**                | Healthy individuals (n = 57) | Patients with PGNRP (n = 41) | P  |
| Age, years (mean ± SD)           | 43.73 ± 16.65    | 43.58 ± 14.27   | 0.964 |
| Sex, n (M/F)                     | 7/50             | 24/17           | 0.000 |
| PLT, 10^9/mm³ (mean ± SD)        | 288.42 ± 84.03   | 305.65 ± 94.59  | 0.347 |
| MPV, fl (mean ± SD)              | 8.44 ± 0.90      | 8.34 ± 0.73     | 0.416 |
| PCT, % (median [min–max])        | 0.23 (0.12–0.39) | 0.26 (0.11–0.40) | 0.425 |
| PDW, % (median [min–max])        | 40.40 (15.20–61.90) | 39.30 (28.90–55.90) | 0.516 |

Abbreviations: PGNRP, primary glomerular nephrotic range proteinuria; SD, standard deviation; PLT, platelet; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width.
study, we compared HIs and patients with PGNRP in terms of PLT indices; our results revealed no statistically significant differences. Therefore, we presume that the pathological processes in proteinuria are not associated with PLT indices. To the best of our knowledge, this is the first such investigation of the relationship of PLT indices with proteinuria and progression of renal disease.

In a prior study, the PLT indices PCT, PDW, and MPV were found to be significantly higher in patients with proteinuria, compared with individuals who did not exhibit proteinuria. Furthermore, Kocyigit et al. reported that MPV was associated with nephrotic syndrome prognosis; in particular, high MPV levels on admission were observed in patients with recalcitrant disease during 1 year of follow-up, while MPV levels were significantly correlated with the level of proteinuria. Proteinuria has been associated with kidney disease progression, increased atherosclerosis, and left ventricular abnormalities that indirectly contribute to cardiovascular morbidity and mortality. In the present study, we divided patients with PGNRP into two subgroups, according to CKD status. We did not find statistically significant differences between the two groups in terms of PLT indices, or in terms of CRP, triglycerides, total cholesterol, urinary protein, and albumin. MPV has been shown to reflect inflammatory burden in various chronic diseases. In a previous study, we compared healthy control, ischemic heart disease, peritoneal dialysis, and hemodialysis groups in terms of MPV level; we found no statistically significant changes in MPV levels among the four groups. Bilen et al. performed a study of 200 patients with CKD (50 kidney transplantation, 50 hemodialysis, 50 peritoneal dialysis, and 50 Stage 3 to 4 CKD); they found no differences among the groups in terms of MPV. Taken together, the past and present findings suggest that PLT indices, lipid values, and urinary protein levels do not exhibit robust associations with kidney disease progression. However, the present findings suggest that hypoalbuminemia and CRP elevation may be associated with progression of renal failure.

There were some unique features in the present study. Patients in our study had proteinuria; the amount of urinary protein

| Characteristic | Patients with PGNRP, without CKD (n = 23) | Patients with PGNRP, with CKD (n = 18) | P |
|---------------|------------------------------------------|----------------------------------------|---|
| Age, years (mean ± SD) | 43.35 ± 13.94 | 43.89 ± 15.08 | 0.97 |
| Sex, n (M/F) | 12/11 | 12/6 | 0.35 |
| CRP median, mg/L (min–max) | 3.66 (3.0–30.7) | 5.20 (3.27–46.9) | 0.038 |
| Albumin, g/dL (mean ± SD) | 2.79 ± 0.73 | 3.52 ± 0.84 | 0.005 |
| TG, mg/dL (mean ± SD) | 238.39 ± 152.47 | 276.44 ± 220.19 | 0.517 |
| TCL, mg/dL (mean ± SD) | 268.56 ± 77.83 | 223.94 ± 92.19 | 0.101 |
| Urinary protein, g/day (mean ± SD) | 7.59 ± 3.39 | 6.23 ± 3.28 | 0.206 |
| PLT, 10³/mm³ (mean ± SD) | 304.26 ± 91.87 | 307.44 ± 100.62 | 0.916 |
| MPV, fl (mean ± SD) | 8.37 ± 0.78 | 8.31 ± 0.69 | 0.808 |
| PCT, % (median [min–max]) | 0.27 (0.14–0.40) | 0.24 (0.11–0.38) | 1.0 |
| PDW, % (median [min–max]) | 39.3 (30.6–55.9) | 39.2 (28.9–44.2) | 0.47 |

Abbreviations: PGNRP, primary glomerular nephrotic range proteinuria; CKD, chronic kidney disease; SD, standard deviation; CRP, C-reactive protein; TG, triglycerides; TCL, total cholesterol; PLT, platelet; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width.
was within the nephrotic range (>3.57 g/day). Causative diseases included were primary glomerular diseases, while secondary or systemic causes were excluded. Therefore, the patients in this study had pure glomerular diseases. Subgroups of patients with and without CKD were compared within this group, to identify the causes of primary glomerular diseases and the pathological processes involved in those diseases. Elucidation of these processes will greatly aid in diagnosis and treatment.

This study had a few notable limitations. First, blood samples were stored in EDTA tubes, which may have influenced assessment of PLT indices; PLT indices measured from samples in citrate tubes are lower than those measured from EDTA tubes. Notably, an optimal measurement time of 120 minutes was recommended after venous puncture for both types of samples. Second, the number of patients in the PGNRP group was small, which may have limited the generalizability of the findings in terms of sex and age.

Conclusion

PLTs play important roles in pathological processes. Thus far, the efficacies of indices associated with PLT function in determining the prognoses of proteinuria and renal failure in patients with primary glomerular diseases remain unknown. Prospective randomized controlled trials involving larger numbers of patients with primary glomerular diseases are needed to determine the associations of these two pathologic processes with PLT indices.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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