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Periton Diyaliz Hastalarında Trombosit Agregasyonu Üzerinde Periferik Trombosit ve Lökosit Sayılarının Etkileri

The Effects of Peripheral Platelet and Leukocyte Counts on Platelet Aggregation in Peritoneal Dialysis Patients

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ÖZ

GİRİŞ ve AMAÇ: *Bugüne kadar yapılan çalışmalar, sağlıklı kişiler ve koroner arter hastalığı olan hastaların kanındaki trombosit ve lökosit sayısının trombosit agregasyonunu etkilediğini ortaya koymuştur. Çalışmamızın amacı, periton diyalizi uygulayan son dönem böbrek yetmezliği hastalarında trombosit agregasyonu ile lökosit ve trombosit sayısı arasında bir ilişki bulunup bulunmadığını araştırmaktı.*

YÖNTEM ve GEREÇLER: *Kırk üç periton diyalizi hastası çalışmaya dahil edildi. Tam kandan trombosit agregasyonu ölçümü yapabilen multiple elektrot agregometri yöntemi kullanıldı.*

BULGULAR: *Trombosit agregasyonu ile tam kan sayımındaki trombosit ve lökosit sayısı arasında istatistiksel anlamlı pozitif yönde korelasyon saptandı. Bu pozitif korelasyon trombositlerde, lökositlerden daha güçlüydü.*

TARTIŞMA ve SONUÇ: *Periton diyaliz hastalarında trombosit agregasyonu üzerinde tam kan sayımındaki trombosit ve lökosit sayısının önemi olabilir. Bu durum, hastaların artmış tromboz riskine katkı yapan ilave bir faktör olabilir.*

Anahtar Kelimeler: *tam kan sayımı, çoklu elektrot agregometri, trombosit agregasyonu, böbrek yetmezliği*

ABSTRACT

INTRODUCTION: *Studies in healthy individuals and patients with coronary artery disease have demonstrated that circulating platelet and leukocyte counts affect platelet aggregation. The aim of our study was to investigate whether there is such a relationship between leukocyte/platelet counts and platelet aggregation in end-stage renal disease patients on peritoneal dialysis (PD).*

METHODS: *Forty-three PD patients were included in the study. Multiple electrode aggregometry method capable of measuring platelet aggregation in whole blood was used.*

RESULTS: *A statistically significant positive correlation between platelet aggregation and leukocyte and platelet counts in complete blood count (CBC) was seen. This positive correlation was stronger for platelets than leukocytes.*

DISCUSSION and CONCLUSION: *Platelet and leukocyte counts in CBC in PD patients might be important on platelet aggregation. This might be another risk factor contributing to already increased thrombosis risk in these patients.*

Keywords: *complete blood count, multiple electrode aggregometry, platelet aggregation, renal failure*

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INTRODUCTION

Cardiovascular diseases and related adverse events are the most important causes of morbidity and mortality in end-stage renal disease (ESRD) patients (1). Chronic kidney disease (CKD) is a low-grade inflammatory status and this contributes to the progression of many chronic diseases, especially atherosclerosis (2). Inflammation, by itself, is characterized by the interaction between platelets, leukocytes and endothelial cells. In the pathogenesis of thrombotic disorders and hemostasis, platelets have a very important role (3).

Platelet function tests enable to detect the residual increase in aggregation (4). Since it includes all three series, whole blood constitutes a more physiological environment than other methods for platelet aggregation measurement (5). The studies done so far on healthy people and patients with coronary artery disease in stable condition had revealed that the number of peripheral platelets (PLT) and leukocytes (WBC) effect platelet aggregation (4,6,7,8,9).

Platelet dysfunction in uremia is caused by many factors and it depends on both aggregation and secretion defect of the platelets (10). Increased platelet-monocyte aggregates in circulation is associated with increased risk of cardiovascular events in ESRD patients on dialysis similar to non-uremic individuals (11,12). The aim of our study was to investigate whether there is a correlation between the disfunctional platelet aggregation and the number of peripheral WBC and PLT in peritoneal dialysis (PD) patients.

METHODS

Patients

Forty-three ESRD patients on continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD) were included in the study. All patients have been followed in Nephrology Clinic of Kocaeli University between October 2011-April 2012 and they were randomly selected from residents of district of Kocaeli.

Study inclusion criteria of PD patients were to be on dialysis for at least 3 months, to be volunteer for research and aged between 18-80 years. Exclusion criteria: Previous diagnosis of type 1 or type 2 diabetes mellitus, having an inherited thrombasthenias like von Willebrand disease, Bernard-Soulier syndrome or a Glanzmann's disease, afibrinogenemia; history of an infectious disease in the last 10 days; and use of at least one of the following medications within the last two weeks: acetylsalicylic acid, clopidogrel, ticlopidine, beta lactam antibiotics, corticosteroids and non-steroidal anti-inflammatory drugs. After the approval of local ethics committee for the study written informed consent was obtained from all participating patients.

Blood samples for routine biochemical markers and complete blood count (CBC) were obtained following at least 8 hours of fasting in all patients and sent to central laboratory. The samples collected in tubes containing EDTA from each patient for CBC were analyzed using CELL-DYN 3700 device (Abbott Laboratories, Philippines) that works with combined impedance and Multi-Angle Polarized Scatter Separation (M.A.P.S.S) flow cytometry methods.

Adenosine diphosphate (ADP) solution as platelet agonist was used in our study (Multiplate company/Siemens PFA collagen /ADP Test Cartridge, Marburg / Germany) and it was added to the test cell to get a final concentration of 10 µmol/L.

Multiple Electrode Aggregometry (MEA)

It is a whole-blood impedance aggregometry method and allows the assessment of platelet function. MEA was practised on the Multiplate analyser (Dynabyte medical, Munich, Germany).Three criteria were calculated after analysis of the device.

These were:

a) Aggregation: Unit is the aggregation unit (AU)
b) Velocity: AU/minute
c) Area Under the aggregation Curve (AUC): AU x minute

Study Design

Blood samples were studied in Multiplate device within 30-120 minutes after venipuncture. Platelet aggregation measurement was made as follows: 300 µL of blood in hirudinated tube was placed into a test cell and 300 µL of normal saline was added and after 3 minutes10 µmole/L ADP was added. After six minutes platelet aggregation measurement was made.

Statistics

The data analysis was performed using the SPSS version 19.0 software. Compliance of variables with normal distribution was examined using the Kolmogorov-Smirnov test. The relationship between continuous variables in the patient group was measured using Pearson correlation test for parametric variables and Spearman's correlation test for nonparametric variables. P values<0.05 were considered statistically significant.

RESULTS

A total of 43 ESRD patients (22 female) on peritoneal dialysis were enrolled in the study. The PD modality was mainly CAPD (41 patients) only two patients were doing APD. The most common cause of ESRD in patients was hypertension (53.5%) followed by chronic glomerulonephritis (25.6%), autosomal dominant polycystic kidney disease (7%), amyloidosis (2.3%) and urolithiasis (2.3%) respectively. In 9.3% of patients the etiology was unknown. The clinical features and biochemical findings of the patients are shown in table 1.

Table 1.Clinical characteristics and biochemical findings of the PD patients

	Mean ± Std. deviation	Median (min-max)
Age (year)	48±11,2	48 (20-78)
Dialysis duration (month)	40,7±22,8	39 (4-96)
Total Kt/V (week)	2,37±0,9	2,23 (1-4,97)
Serum BUN (mg/dl)	53,3±15	54 (29-101)
Serum albumin (g/dl)	3,5±0,4	3,58 (2,61-4,26)
Hemoglobin (g/dl)	10,5±1,9	10,7 (7,4-16,2)
WBC (mm^3)	7112±1779	7050 (3670-10400)
PLT (mm^3)	254332±72779	245.10^3 (92,3.10^3-439.10^3)
AUC (AUxminute)	708±254,3	755 (243-1344)
Aggregation (AU)	130,6±49,6	138 (42-291,4)
Velocity (AU/minute)	15,7±5,9	15,4 (5,5-30,7)

BUN: Blood Urea Nitrogen, WBC: Leukocytes,PLT: Platelets, AUC: Area Under the aggregation Curve

In patients, AUC showed a strong positive correlation with PLT and a moderate one with WBC (p<0.001, r =0.627; p<0.001, r =0.462 respectively). A moderate positive correlation between aggregation and PLT and WBC was detected (p =0.001, r =0.487; p =0.003, r =0.437 respectively). A strong positive correlation of velocity with PLT, and a moderate one with WBC was detected (p<0.001, r =0.745; p =0.013, r =0.376 respectively). The relationship between platelet aggregation values and platelet and leukocyte counts is given in table 2.

Table 2. Comparison of platelets, leukocytes and aggregation values of PD patients

		PLT	WBC
AUC	p	<0,001*	<0,001
	r	0,627	0,462
Aggregation	p	0,001*	0,003
	r	0,487	0,437
Velocity	p	<0,001*	0,013
	r	0,745	0,376

*P: Pearson correlation test, r: correlation coefficient *: Spearman's correlation test, WBC: Leukocytes, PLT: Platelets*
AUC: Area Under the aggregation Curve

DISCUSSION

The work done so far showed that platelet and leukocyte counts in healthy individuals and patients with coronary artery disease in stable condition affect platelet aggregation (4,6,7,8,9). The clinical research on this issue in dialysis patients is limited. One similar study is the report of Kilickesmez et al, where they measured platelet aggregation with MEA method in aspirin using dialysis patients. Their study demonstrated that hemodialysis patients compared with normal renal function patients had a nearly two-fold increased risk of aspirin non-response (13).

Adenosine triphosphate (ATP) released by exocytosis from erythrocytes, platelets and damaged cells increases platelet aggregation in blood through conversion to ADP by ecto-ATPases located in leukocytes (14,15). Cathepsin G and elastase that are released from polymorphonuclear leukocytes (PMNs) stimulated by N-formyl-Met-Leu-Phe (fMLP) are responsible for the activation of platelets (16). There are some contradictory reports in the literature as well. In a study by Schattner et al it was reported that platelet aggregation was inhibited by PMNs (17). Unstimulated neutrophils inhibit reactivity of platelets stimulated with collagen or thrombin (18).

The study of Würtz et al, included both patients and controls and they used two different techniques for platelet aggregation (MEA and turbidimetric optical detection) and collagen and arachidonic acid as platelet agonists. Their study did not include thrombocytopenic patients and the authors found that

whole blood platelet aggregometry was dependent on the number of platelets even within the normal limit. In the same study, it was seen that leukocytes also could influence whole blood platelet aggregometry depending on the platelet aggregation method and agonist used (4) In our study, we made platelet aggregation measurement in PD patients with MEA method and used ADP as agonist.

Lack of evaluation of leukocyte subtypes, soluble P-selectin levels and a control group of healthy individuals are the limitations of our study including relatively small number of study population.

Based on our results platelet and leukocyte counts migth be another contributory factor for hypercoagulable state seen in ESRD patients. The clinical or therapeutic implications of this finding remains to be clarified. Due to above mentioned limitations of our study it is not possible to withdraw a certain conclusion regarding this issue. Further studies with larger sample size and healthy controls in prospective setting are obviously needed to prove the clinical significance of platelet and leukocyte counts on platelet aggregation in PD patients.

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