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Association of Platelet Count and Mean Platelet Volume with Serum C-reactive Protein in Regular Hemodialysis Patients

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Abstract: The aim of the present study was to elucidate whether and how in patients with uremia on hemodialysis the level of C-reactive protein (CRP) affects the Mean Platelet volume (MPV) and count. The total patients were 36 (f = 15, m = 21). The mean patient's age was 46±6 years. The median length of the time patients had received hemodialysis was 19 months. The mean PLT count was 165±70 [$\times 10^3 \mu\text{L}^{-1}$]. The mean of MPV was 9±1 fl. The mean serum CRP was 8.7±6.6 mEq L⁻¹. In this study a significant inverse correlation of PLT count with MPV and a significant inverse correlation of MPV with serum CRP were found. No significant correlation PLT count and serum CRP was found ($p > 0.05$), while their association was positive. We concluded that using biocompatible polysulfone membrane during hemodialysis may have a lower complement cascade activation and results in diminution of inflammation during the hemodialysis procedure. Inverse association of MPV with serum CRP, needs to more attention and investigation in hemodialysis patients.

Key words: Platelet count, hemodialysis, end-stage renal failure, Mean Platelet Volume (MPV), C-reactive protein (CRP)

Introduction

C-reactive Protein (CRP) is an acute phase protein whose synthesis in the liver is regulated by different cytokines, particularly interleukin 6 (IL-6). Plasma levels of CRP in the absence of active disease are low, but can rise up to 1000-fold in patients with an inflammatory reaction. Besides being a marker of inflammation, CRP itself may have proinflammatory properties since it can activate the complement system (Wolbink *et al.*, 1996; Morrow and Ridker, 2000). Thus elevated plasma concentrations of C-reactive protein (CRP), a sensitive marker of underlying systemic inflammation (Clyne and Olshaker, 1999; McIntyre *et al.*, 1997; Haubitz *et al.*, 1996). Serum CRP concentrations have also been found to be significantly elevated in hemodialysis patients (Stenvinkel *et al.*, 2000; Panichi *et al.*, 2000) and reflects chronic inflammation and as an acute-phase reactant, is a sensitive and independent marker of malnutrition (Ortega *et al.*, 2002). An elevated serum C-reactive protein has been shown to be strongly predictive of morbidity and mortality in dialysis patients, specially is a strong predictor of cardiovascular mortality in hemodialysis patients (Kim *et al.*, 2005; Caglar *et al.*, 2002). Patients with end-stage renal disease suffer from complex homeostatic disorders. Uremic

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patients show a bleeding diathesis that is mainly due to abnormalities of primary hemostasis (Boccardo *et al.*, 2004; Moal *et al.*, 2003). The increased bleeding tendency of chronic renal failure patients has been attributed to platelet dysfunction (Boccardo *et al.*, 2004; Moal *et al.*, 2003; Krawczyk *et al.*, 1994).

The most common abnormalities are defective platelet aggregation, decreased platelet adhesiveness, decreased platelet factor-3 availability and prolongation of the bleeding time (Jubelirer, 1985). Some of the pathophysiologic mechanisms which have been implicated include platelet inhibition by plasma metabolites, e.g., urea, guanidinosuccinic acid, phenolic acid; increased vessel wall prostacyclin; abnormal platelet arachidonic acid metabolism and increased levels of parathyroid hormone (Wiwanitkit, 2004). Recently, an indice related to platelet count has been provided by hematologic analyzers. Concerning the platelet parameter, MPV has been described (Bancroft *et al.*, 2000). Platelet volume is a marker and possibly a determinant of platelet function such a way large platelets are more active than normal sized platelets. MPV, a measure of platelet size, reflects changes in either the level of platelet stimulation or the rate of platelet production (Ozdemir *et al.*, 2004). Increased MPV may reflect increased platelet activation or increased numbers of large, hyper aggregable platelets and is accepted as an independent coronary risk factor (Henning *et al.*, 2002) and mean platelet volume could be an independent risk factor for myocardial infarction in the general population and also CHD in hemodialysis (HD) patients (Henning *et al.*, 2002). Regarding the present data, studies concerning the association of CRP, as an acute phase protein with MPV and platelet count to better found the association of systemic inflammation with MPV and platelet count in HD patients are quite scarce. Therefore, the aim of the present study was to elucidate whether and how in patients with uremia on hemodialysis the level of CRP as the marker of inflammation affects the mean PLT volume and count.

Materials and Methods

Patients

This cross-sectional study was conducted on patients with End-Stage Renal Disease (ESRD), who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. The study was done in hemodialysis section of Hajar Medical Educational and Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord of Iran. The study carried out from July to August of 2005. According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 (Rocaltrol), calcium carbonate and Rena-Gel capsules at various doses. According to the severity of anemia, patients were under IV iron therapy with Iron sucrose (venofer) at various doses after each dialysis session, all patients were under treatments of 6 mg folic acid daily, 500 mg L-Carnitine daily, oral Vitamin B-complex tablet daily and also 2000U IV Eprex (recombinant human erythropoietin (rHuEPO) after each dialysis session routinely. Exclusion criteria were active or chronic infection and using NSAID or ACE inhibitor drugs and also using the other drugs had adverse effects on platelet production or function .

Laboratory Methods

Blood samples were collected after an overnight fast for patients, complete blood count containing hemoglobin (Hgb), hematocrit (Hct), platelet (PLT) count and also Mean Platelet Volume (MPV) (Ref. Range 7.5-11.5 fl) were measured using Sysmex-KX-21N cell counter. Levels of serum C-reactive protein (CRP), calcium (Ca), magnesium (Mg) and also serum albumin (Alb) were measured

Table 1: Mean±SD, minimum and maximum of age, duration and doses of hemodialysis and also laboratory results of patients

Total patients (N = 36)	Minimum	Maximum	Mean±SD	Median
Age years	16	80	46±16	43
DH* months	2	156	32±36	19
Dialysis (dose sessions)	36	1584	294±393	156
URR (%)	39	76	59±9	57.5
Creat (mg dL ⁻¹)	3	18	9±3	9.5
BUN (mg dL ⁻¹)	30	180	82±33	78
Ca (mg dL ⁻¹)	5	10	7.6±0.9	7.9
Alb (g dL ⁻¹)	2.4	4.8	3.8 ±0.5	3.95
PLT count [$\times 10^3 \mu\text{L}^{-1}$]	264	396	165±70	163
MPV (fl)	7	11	9±1	9.2
CRP (mg L ⁻¹)	3	40	8.7±6.6	8
Hgb (g dL ⁻¹)	5	13	9±2	9
HCT %	14	40	28±6	29.5
Leptin (ng mL ⁻¹)	0.1	73	9.4±14	5.75
Mg (mg dL ⁻¹)	1.6	3.5	2.5±0.4	2.4
iPTH p (g mL ⁻¹)	16	1980	434±455	309

*Duration of hemodialysis treatment

using standard kits. Intact serum PTH (iPTH) was measured by the radio immunoassay (RIA) method using DSL-8000kit of USA (normal range of values is 10-65 pg mL⁻¹). Serum Leptin (normal range of values for males is 3.84±1.79 and for females is 7.36±3.73 ng mL⁻¹) were measured by enzyme-linked immunosorbent assay (ELISA) method using DRGkit of Germany. Duration and doses of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 h. For the efficacy of hemodialysis the Urea Reduction Rate (URR) was calculated from pre and post blood Urea Nitrogen (BUN) data (Boag, 1994).

Statistical Analysis

Results are expressed as the mean±SD and median values. Statistical correlations were assessed using partial correlation test. All statistical analyses were performed using SPSS (version 12.00). Statistical significance was determined at a p<0.05.

Results and Discussion

The total patients were 36 (F = 15, M = 21). Table 1 shows patients' data. The mean patient's age was 46±16 years (Table 1). The mean length of the time patients had received hemodialysis was 32±36 months (median: 19 months). The MPV count was 165±70 [$\times 10^3 \mu\text{L}^{-1}$]. The mean of MPV as 9±1 fl. The mean serum CRP was 8.7±6.6 mg L⁻¹ (median: 8 mg L⁻¹). In this study a significant inverse correlation of PLT count with MPV ($r = -0.52$, $p = 0.003$) (adjusted for age) was seen. A significant inverse correlation of MPV with serum CRP ($r = -0.43$, $p = 0.0200$; Fig. 1) (adjusted for age, duration and doses of dialysis, serum leptin, iPTH, gender and serum Mg) was found. No significant association between PLT count with serum CRP was seen but this non significant association was positive ($r = 0.048$, $p = 0.803$). In Fig. 1 the result of correlations of this study were illustrated. In this study we found a significant inverse correlation of PLT count with MPV and a significant inverse correlation of MPV with serum CRP. No significant association between PLT count with serum CRP was seen but this non significant association was positive. The greatest role in the development of haemostasis disturbances in patients with chronic renal failure (CRF) is ascribed to the platelets. Although the platelet parameter, mean platelet volume have been routinely available

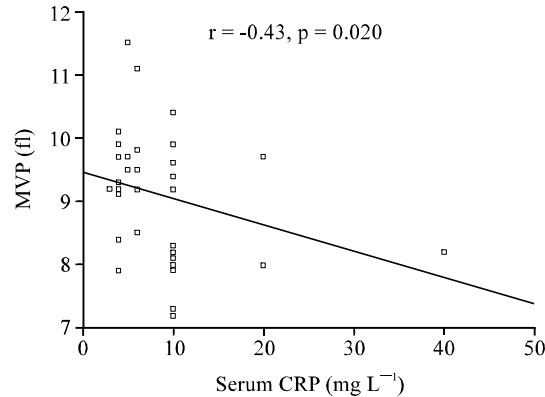


Fig. 1: Significant inverse correlation of MPV with serum CRP (partial correlation test after adjustment for age, duration and doses of dialysis, serum leptin, iPTH, gender and serum Mg)

to clinicians for some time, its role in the diagnosis and management of patients remains unclear. While factors affect PLT count and volume during hemodialysis is under investigation, it is believed that platelet activation and aggregation and coagulative activation are the earliest and most important phenomena that occur after contact between blood and artificial membranes (Coli *et al.*, 1995). Mean platelet volume is a physiological variable of hemostatic importance (Wolbink *et al.*, 1996) Large platelets are more reactive, produce more prothrombotic factors (Martin and Trowbridge, 1990; Jakubowski *et al.*, 1983; Martin *et al.*, 1983) and aggregate more easily. They also contain more dense granules and release more serotonin and β -thromboglobulin than do small platelets (Martin *et al.*, 1983; Haver and Gear, 1981; Thompson *et al.*, 1982). Platelets have no nuclei and their characteristics are determined by their progenitor cell, the bone marrow megakaryocyte. It is generally accepted that platelet volume and density are determined at thrombopoiesis and that, once in the circulation, platelets do not change in size (Thompson *et al.*, 1983a,b). The mechanisms controlling platelet production are obscure, although it has been suggested that both MPV and platelet counts are under independent hormonal control (Martin, 1989; Martin *et al.*, 1983; Sharpe *et al.*, 1994), however Larger platelets are more reactive (Sharpe *et al.*, 1994; Bancroft *et al.*, 2000). The inverse association of PLT counts and MPV which was shown in our study also was shown in the many studies (Sharpe *et al.*, 1994; Bancroft *et al.*, 2000; Lamparelli *et al.*, 1988). Lamparelli showed an inverse correlation between platelet volume and platelet number in 564 normal subjects and 297 pregnant women (Lamparelli *et al.*, 1988). Recently, markers of chronic inflammation have also been associated with adverse clinical outcome in chronic renal failure patients. CRP is a significant predictor of mortality as well as morbidity in both chronic hemo- and peritoneal dialysis patients (Ikizler *et al.*, 1999; Zimmermann *et al.*, 1999; Yeun *et al.*, 2000). Similarly, increased levels of proinflammatory cytokines are associated with increased risk of mortality in ESRD patients (Stenvinkel *et al.*, 2002; Bologa *et al.*, 1998). During hemodialysis session, several adverse reactions can occur on platelets, which are attributable to bioincompatibility of the dialysis membrane (Kuragano *et al.*, 2003). In this study no significant association between PLT count and CRP was seen, may be due to using biocompatible polysulfone membrane with has a lower complement cascade activation than cellulose cuperophane membrane. To our knowledge this is the first study, concerning the inverse association of MPV with serum CRP, which needs to more attention and investigation in hemodialysis patients.

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