

**RELATIONSHIP BETWEEN THE USE OF RECOMBINANT HUMAN
ERYTHROPOETIN AND MEAN PLATELET VOLUME IN DIALYSIS PATIENTS****Zeki Kemeç¹, Dr. Ali Gürel*², Mustafa Demir³ and Ayhan Doğukan³**¹Gümüşhane State Hospital Nephrology Clinic, Gümüşhane, Turkey.²Adıyaman University Medical Faculty Nephrology Clinic, Adıyaman, Turkey.³Fırat University Medical Faculty Nephrology Clinic, Elazığ, Turkey.***Corresponding Author: Dr. Ali Gürel**

Adıyaman University Medical Faculty Nephrology Clinic, Adıyaman, Turkey.

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ABSTRACT

Mean platelet volume (MPV) is predictive for many diseases and conditions. The effect of recombinant human erythropoietin (r-HuEPO) on MPV in hemodialysis (HD) or peritoneal dialysis (PD) patients is not well known. We aimed to determine MPV levels in four main groups and two subgroups. The main groups were respectively healthy control (HC), ischemic heart disease (IHD), PD, HD groups. The subgroups were PD and HD groups that received and did not receive r-HuEPO. There were no statistically significant changes in MPV levels in four groups ($p > 0.05$). On the other hand, MPV levels of r-HuEPO receiving patients were lower than those without ($p < 0.05$).

KEYWORDS: Mean platelet volume, recombinant human erythropoietin, peritoneal dialysis, hemodialysis.**INTRODUCTION**

MPV is a measure of platelet size. Although other markers of platelet activity require specific examination, MPV can be tested simple, inexpensive, easy to interpret, and routinely tested. MPV has been shown to be coded by chromosome 7q22.3.^[1] The activities and densities of circulating platelets are heterogeneous.^[2]

Platelets have important effects on atherosclerotic plaque formation. Therefore, they play a key role in atherothrombosis.^[3] A new meta-analysis revealed that MPV was a predictive and convenient biomarker in non-renal cardiovascular disease (CVD).^[4] High MPV levels have been found to be a predictive factor in ischemic events, myocardial infarction, fatal outcomes after myocardial infarction,^[5] cerebral infarction^[6] and preeclampsia.^[7] However, some other studies have shown no association between MPV and severity of conditions such as coronary artery disease, atherosclerotic vascular changes and platelet aggregation.^[8]

Chronic kidney disease (CKD) is associated with coagulation problems leads prolonged bleeding time and platelet aggregation disorders.^[9] A significant increase in MPV was observed during r-HuEPO treatment in two studies.^[10,11] Platelet aggregation increases and hemostasis improves in patients receiving r-HuEPO.^[11,12] One of the side effects of r-HuEPO is increased thrombosis risk.^[13] In experimental models, erythropoietin receptor is shown on megakaryocytes^[9,14]

and platelet size is determined before and during the megakaryocyte bud stage.^[15] In addition, r-HuEPO has been shown to have humoral growth factor effects and therefore supports the differentiation of megakaryocytes.^[16] In contrast, five studies^[10,11,17-19] showed a significant decrease in MPV during r-HuEPO treatment.

Although MPV was investigated in patients receiving r-HuEPO undergoing hemodialysis, r-HuEPO was rarely studied in patients with peritoneal dialysis and was not compared with hemodialysis patients. In addition, dialysis patients (HD, PD), IHD and healthy controls have not been previously compared. Platelet functions are key stone in the process of atherosclerosis. One of the platelet-activation criteria is the large platelet. Both IHD and dialysis patients are predisposed to atherosclerosis. The exact effect of r-HuEPO treatment on platelets is still a mystery. There are confusing data in some studies that r-HuEPO enlarges or shrinks platelets. These issues need to be clarified and we have done this study to clarify this. In this study, four main groups respectively HC, IHD, PD, HD among themselves, and sub-groups r-HuEPO receiving and non-receiving PD-HD participants compared in terms of MPV levels among themselves. Our work with these aspects will be the first.

MATERIAL AND METHODS

KDOQI defines CKD either a renal injury or a reduced glomerular filtration rate (GFR) of <60 mL/min/1.73 m² and takes three months or more. Typically, the course is

towards stage 4-5 (GFR <30 mL/ min/ 1.73 m²). In these stages, water and electrolyte balance disorders or endocrine/ metabolic disorders are clinically evident. Renal replacement modalities include treatments such as PD, HD, transplantation. Anemia occurs when the hemoglobin level falls below 10 g/ dL.^[20] All patients had normochromic normocytic anemia associated with kidney disease; and other causes of anemia were excluded by regular measurements of ferritin, vitamin B12 and folate.

Ethical approval was obtained from the local ethics committee.

The main group 1 included HC participants. A total of 100 participants (66 male, 34 female) were included in the age range of 18-81 (mean age 43.2 ± 16.3 years).

The main group 2 included IHD participants. A total of 110 participants (69 male, 41 female) were included in the age range of 28-91 (mean age 64.9 ± 12.4 years).

The main group 3 included a total of 24 PD patients (13 male, 11 female) with a mean age of 22-77 (mean age 48.3 ± 18.7 years). All participants received PD treatment for more than 6 months. Patients were randomly recruited regardless of whether they received or not received erythropoietin. Primary renal diseases were diabetic nephropathy (4), chronic glomerulonephritis (2), interstitial kidney disease (1), autosomal recessive polycystic kidney disease (1), hypertensive glomerulosclerosis (8), idiopathic (6), Alport syndrome (2). All patients received either continuous ambulatory peritoneal dialysis [CAPD] or instrumental automated peritoneal dialysis (APD).

Main group 4 included a total of 130 HD patients (76 male, 54 female), ages 19-89 (mean age 56 ± 17.6 years). All participants were receiving HD treatment for more than 6 months. Patients were randomly recruited regardless of whether they received or not received erythropoietin. Primary renal diseases were diabetic nephropathy (32), chronic glomerulonephritis (15), interstitial kidney disease (5), glomerular diseases (7), obstructive uropathy (7), recurrent kidney stone diseases (7), congenital defects of the bladder and kidney (3), polycystic kidney disease (3), hypertensive glomerulosclerosis (37), idiopathic (13), Alport syndrome (1). All patients were regularly receiving HD 3 times a week.

Subgroup 1. The PD patients who receive and do not receive r-HuEPO. Of 24 patients, 9 were receiving r-HuEPO. Their age was between 24 and 68 years (mean age 45.6 years). Of the 24 patients, 15 did not receive r-HuEPO. Their age was between 22-77 years (mean age 49.8 years). Patients who received r-HuEPO for more than 6 months were included.

Subgroup 2 consists of HD patients who receive and do not receive r-HuEPO treatment. Of the 130 patients, 86 were receiving r-HuEPO. Their age was between 19-89 years (mean age 58.3 years). Of the 130 patients, 44 did not receive r-HuEPO. Their age ranged from 21 to 88 years (mean age 54.1 years). Patients who received r-HuEPO for more than 6 months were included.

Little is known about the effects of various drugs on platelet size. Clopidogrel significantly inhibits adenosine diphosphate and induces increase in MPV in vitro,^[21] but also aspirin is associated with high MPV.^[22] For these reasons, we excluded patients who receive platelet inhibitor in the first week of the study. In addition, platelets are known to be affected by smoking,^[22] smokers were excluded.

There is a clear relationship between antihypertensive drugs and MPV. In studies, doxazosin treatment has been shown to decrease MPV while amlodipine have no effect.^[23] On the other hand, selective or non-selective B-blockers have no effect on MPV during the acute using period.^[24] While angiotensin II increases MPV, various types of ARBs and ACEIs have different effects. For example, losartan increases MPV, but candesartan and perindopril do not alter.^[25-27] There is a relationship between antihypertensive drugs and MPV. That's why we haven't taken any antihypertensive users in a week period to our study. Thrombotic and hematologic diseases, symptomatic IHD, cerebral artery disease, cerebrovascular disease, peripheral artery disease, aortic aneurysm, and heart failure were also excluded. Acute or chronic active inflammatory diseases and disorders were not included in the study.

The variables studied were hemoglobin (HGB), platelet count (PLT), platelet distribution width (PDW), hematocrit (HCT), and mean platelet volume (MPV).

The Statistical Package for the Social Sciences (IBM-SPSS 22, Chicago, IL, USA) was used for statistical analysis. Quantitative data are presented as mean ± standard deviation. Statistical differences between the main groups were determined by one-way analysis of variance (ANOVA) followed by the Tukey's Post Hoc test. The importance of nonparametric data between r-HuEPO and non-receiving subgroups was investigated by Mann Whitney U test. Chi-square test was used to compare categorical variables. Correlation analysis was performed using Pearson correlation analysis. p values less than 0.005 were accepted as significant.

RESULTS

A total of 364 patients consisting of 4 different main groups and two subgroups were included in the study. The age, gender and laboratory parameters of main groups are shown in Table 1 comparatively. MPV values of two subgroups and their comparisons are shown in Table 2. The age values of main group 2 were significantly different in comparison with the other 3

main groups ($p < 0.01$). Interestingly, the mean MPV values among all major groups were not statistically significantly different from each other ($p = 0.075$).

In subgroups 1 and 2; MPV values of r-HuEPO receiving patients were significantly lower than non-receiving patients ($p = 0.035$, $p = 0.012$, respectively). The HCT,

HGB values of the main groups 3 and 4 were significantly lower than main groups 1 and 2 ($P < 0.01$). The platelet and PDW values of the main group 4 were lower than the other main groups ($P < 0.001$). PDW values of main groups 3 and 4 were lower than the main groups 1 and 2 ($P < 0.001$). There was no positive correlation between PDW and HCT in groups 1 and 2.

Table 1: Age, gender and laboratory measurements in main groups (ANOVA and posthoc Tukey test).

	HC (main group 1) (n:100)	IHD (main group 2) (n:110)	PD (main group 3) (n:24)	HD (main group 4) (n=130)	P (Anova)
Age	43,2±16,3	64,9±12,4*	48,3±18,7 ⁺	56,9±17,6* ⁺	<0.001
Gender (M/F)	34/66	69/41	13/11	76/54	<0.001 ^X
Hb	14,1±1,5	13,8±1,7	10,9±1,9* ⁺	10,9±1,7* ⁺	<0.001
PLT	273,8±75,8	248,5±75,6	255,2±97,9	202,2±91,5* ^{+,‡}	<0.001
PDW	40,9±10,8	35,6±16,2**	29,4±14,5*	19,4±10* ^{+,‡‡}	<0.001
HCT	43,1±4,2	41,7±5,1	32,9±6,3* ⁺	33,2±5,1* ⁺	<0.001
MPV	8,6±1	8,8±0,9	8,3±0,8	8,5±1,1	0.075

It was compared with HC group; * $p < 0.001$, ** $p < 0.05$.

Compared with the IHD group; + $P < 0.001$

It was compared with the PD group; ‡ $p < 0.05$, † $P < 0.01$

XChi-Square Test shows.

* $p < 0.001$ The main group 1 has a statistically significant difference in comparison with other main groups with similar parameters.

** $p < 0.05$ Main group 2 shows a statistically significant difference in comparison with main group 1 with similar parameters.

+ $p < 0.001$ The main group 2 shows a statistically significant difference in comparison with other main groups with similar parameters.

‡ $p < 0.05$ The main group 3 shows a statistically significant difference in comparison with the main group 4 with similar parameters.

† $p < 0.01$ The main group 3 shows a statistically significant difference in comparison with the main group 4 with similar parameters.

Abbreviations: Hb hemoglobin; PLT platelet; PDW platelet distribution width; HCT hematocrit; MPV mean platelet volume; HD hemodialysis; PD peritoneal dialysis; IHD ischemic heart disease; HC healthy control.

Table 2: Comparison of MPV levels in the subgroups (Mann whitney U test).

	PD (Subgroup 1)			HD (Subgroup 2)		
	r-HuEPO+(n=9)	r-HuEPO-(n=15)	P	r-HuEPO+(n=86)	r-HuEPO-(n=44)	P
MPV	7,9±0,5	8,6±1	0.012	8,4±1	8,9±1,1	0.035
Age	45.6	49.8	<0,05	58,3	54.1	<0,05
Gender (M/F)	3/6	10/5	<0.001 ^X	48/38	28/16	<0,001 ^X

XChi-Square Test shows.

Abbreviations: MPV mean platelet volume; HD hemodialysis; PD peritoneal dialysis; r-HuEPO + recombinant human erythropoietin receiving; r-HuEPO- recombinant human erythropoietin non-receiving.

DISCUSSION

Tests determining PLT activity are often time consuming and expensive. MPV is a measure of platelet size that can be found in a complete blood count and is considered an important marker of platelet activity. High MPV may be a marker for risky cardiovascular events in subjects with CVD. In the largest researches to date, researchers have reported that MPV levels are an independent risk factor for death or recurrent ischemia after myocardial infarction.^[5,28] Very few studies on HD patients have shown that MPV is associated with cardiovascular

disease.^[29] MPV was not investigated in patients receiving HD, PD, HC, IHD comparatively. In previous studies,^[30] PD and HD groups were compared. But dialysis groups have not been compared in detail with HC and IHD in this way. In addition, the number of participants in our study is quite high. Our study is unique in the literature. In our study, there was no significant difference in MPV levels between the main groups of dialysis and control groups. In a study by Sakalli H. et al.,^[30] who contradicted our study, the HD group had a higher MPV level than the PD group and the

non-dialysis group before transplantation. They thought that HD treatment was associated with increased platelet turnover due to chronic platelet activation. In another study that contradicted our study, Asanuma *et al.*^[31] reported that MPV was slightly higher than in HC in HD patients. In a study conducted by Bilen Y. *et al.*,^[37] they compared MPV, CRP and sedimentation in patients with renal transplantation, HD, PD and CKD. They found no statistically significant difference. This heterogeneity between studies may depend on the genetic and ethnic background differences of the participants. New large genome studies have identified the loci of MPV.^[11] It is hoped that this study will reveal the relationship between the genetic determinants of MPV and the actual MPV values and the other human phenotype in detail. To address these important questions, there is a need for prospective studies of MPV and CVD among healthy and dialysis populations. In the literature, we found very few studies on MPV and PD.^[10] Our study is unique in the literature because it covers many different patient groups.

There are few data on dialysis patients receiving r-HuEPO. The level of MPV and related factors are not fully known in PD and HD patients receiving r-HuEPO. In PD and HD patients receiving r-HuEPO, larger and detailed randomized controlled trials are needed to clarify the effect of r-HuEPO on hematological parameters. MPV levels have been shown to increase or decrease in patients with CKD after administration of r-HuEPO^[38,39] or no significant change.^[10] In patients with CKD, a significant increase in MPV levels is suggested when r-HuEPO is given. The increase in MPV caused by r-HuEPO is not dose dependent.^[10] According to one view, young PLTs are larger than old ones, and r-HuEPO increases the number of younger and larger platelets with its thrombopoietin-like effect.^[5,15,40-41] The PLT production process is not fully known yet, but known to be produced by megakaryocyte fragmentation^[3] and MPV appears during megakaryocyte fragmentation.^[16] There is a relationship between platelet volume and erythropoietin administration. In our study, MPV levels of PD and HD groups not receiving r-HuEPO were higher than those receiving r-HuEPO. In other words, we observed that MPV levels decreased with r-HuEPO use. Sowade O *et al.*^[17] Conducted a double-blind, randomized, placebo-controlled study. They evaluated the effects of r-HuEPO on patients who had undergone cardiac surgery. They observed that preoperative hematocrit increase was accompanied by an decrease in MPV and increase in PDW epoetin users. The interesting thing in their study was that the low MPV was associated with weak aggregation capacity and high blood loss in the perioperative process in the erythropoietin arm. They concluded that MPV decline after r-huEPO treatment did not increase the risk of thromboembolism and could have positive effects on postoperative platelet functions. Our study results are similar to Sharpe PC.,^[10] Akizawa T.,^[11] Stenver D.^[18] In a study by Fabris F^[19] *et al.*, 9 patients with ESRD were studied in terms of platelet count and

functions during r-HuEPO use. All children observed improvement in hemoglobin and hematocrit levels with marked decrease in bleeding time. They also observed a marked increase in platelet count after both 6 and 12 weeks of treatments. At the same time, MPV decreased with r-HuEPO treatment and normal platelet mass was maintained. The hemostatic balance with r-HuEPO treatment improves not only the anemia but also the platelet count and functions. Since the study participants were children (Fabris *et al.* 13 + _ 3.7 years), it is unclear whether age contributed to r-HuEPO's effect on MPV.^[23]

In our study PDW, PLT, HCT levels were found to be lower in PD and HD treatment groups compared to other main groups. Macdougall IC. in his study stated that thrombocytopenia was frequently seen in patients with CKD but could not find a cause.^[42] Low HCT levels are associated with renal failure. The relationship between the number of PLT and MPV is not clear yet, but it has been shown that MPV is non-linearly and inversely associated with PLT count.^[38,43] The secondary increase in PDW is due to the inverse relationship between platelet volume and PDW.^[44] In our study, there was no relationship between HCT, PDW, PLT count and MPV.

pH,^[45] type of HD membrane,^[46] anticoagulation type, duration of blood in invitro EDTA tube,^[47] inflammation load,^[48-49] erythropoietin treatment, changes in serum sodium and potassium levels, ultrafiltration during HD may interact with MPV and change the predictive power of MPV as a marker in various disorders.^[50] MPV levels interacting with these variables are discussed as a risk factor for thrombosis or atherosclerosis in dialysis group patients.

As a result, MPV is an important marker and it is a cheap and easy to work method. We found that r-HuEPO receiving patients with PD and HD had lower MPV levels than those without r-HuEPO. It has been reported that there is a significant relationship between r-HuEPO use and MPV. Some studies^[11-13] showed the relationship between the use of r-HuEPO and thrombosis. In our study, we could not explain the relationship between thrombosis and r-HuEPO treatment. The relationship between thrombosis and the use of r-HuEPO is not fully understood yet. The larger platelets are more active and are prone to thrombosis, but after use of r-HuEPO platelets shrink and MPV falls. Small platelets cause less aggregation and bleeding time shortens.^[17,19] CKD and its numerous metabolic effects predispose to thrombosis.^[9] In this way, the risk of thrombosis can be minimized by using r-HuEPO in CKD patients and vascular access life may be prolonged in HD patients. In our study, there was no significant difference in MPV levels between the main groups and the IHD group. Correlation of coronary artery disease and MPV levels can also be discussed. To address these questions, large-scale prospective controlled studies are needed.

In our study, we can list a number of shortcomings. First, the blood samples were stored in EDTA tube, and this

may influence MPV.^[51] MPV measured from citrate samples are lower than that measured from EDTA tubes.^[43] An optimal measuring time of 120 minutes was recommended after venous puncture in both types.^[22] Second, study participants have heterogeneous clinical status and types of different instruments used to measure MPV. For example, high body mass index, hypertension, diabetes mellitus, hyperlipidemia in our participants affect MPV. Since hypertension and diabetes mellitus were the main causes of kidney disease, we could not exclude them when random groups were distributed. Third, the number of PD patients was inadequate and this may negatively affect gender and age statistical match.

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