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The Effect of Hypermagnesemia on Platelet Aggregation in Peritoneal Dialysis Patients: An in Vitro Study

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Abstract Magnesium has a reducing effect on platelet aggregation. Decreased glomerular filtration rate in chronic renal failure contributes to elevation of serum magnesium level. The aim of our study was to measure platelet aggregation in peritoneal dialysis (PD) patients who developed hypermagnesemia by the addition of magnesiun sulphate ($MgSO_4$) in vitro. Forty-three patients with PD were included in the study. Multiple electrode aggregometry method capable of measuring platelet aggregation in whole blood was used and adenosine diphosphate (ADP) was selected as the agonist. Only ADP was used in the first test cell (C1) of the device; ADP was added as a whole blood agonist, which was raised to magnesium level 3-3,5 mg/dl by the addition of in vitro $MgSO_4$ in the second test cell (C2). Three criteria were used to measure platelet aggregation: aggregation, area under the curve (AUC), and velocity. There was a statistically significant positive correlation between C1-C2 AUC and C1-C2 velocitiy. There was no statistically significant relationship between C1-C2 aggregation. PD patients were divided into two groups, male and female, and the results were examined. In male patient group; there was no statistically significant difference between C1-C2 AUC, C1-C2 aggregation and C1-C2 velocity. In the female patient group; there was no statistically significant difference between C1-C2 AUC. There was a statistically significant positive correlation between C1-C2 aggregation and C1-C2 velocities. Serum magnesium levels may be effective on platelet aggregation in female patients with PD but no similar effect was found in male patients.

Keywords: *hypermagnesemia, platelet aggregation, multiple electrode aggregometry, chronic renal failure, peritoneal dialysis*

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1. Introduction

Magnesium has platelet aggregation lowering effect [1]. This effect is due to stimulation of prostacyclin (PGI2) and nitric oxide (NO) production [2]. Experimental hypermagnesemia induced by magnesium sulphate ($MgSO_4$) inhibits platelet aggregation [3]. Depending on the dose, magnesium may inhibit platelet aggregation [4,5]. There are, however, studies in the literature in which the effect of magnesium therapy on platelet aggregation is absent [6,7].

Loss of renal function in moderate chronic renal disease (eGFR>30 ml/min) is compensated by an increase in fractional excretion of magnesium; hypermagnesemia develops in stage 5 chronic renal disease where the mechanism of compensation is insufficient [8]. However, in patients with chronic renal failure serum magnesium may be normal or low [9,10]. Cardiovascular diseases and its adverse events are the most important causes of morbidity and mortality in uremia [11]. Platelets have a substantial role in the pathogenesis of thrombotic disorders [12]. The aim of our study was to measure platelet aggregation in peritoneal dialysis (PD) patients who developed hypermagnesemia by the addition of $MgSO_4$ in vitro.

2. Materials and Methods

2.1. Patients

Forty-three patients who underwent continuous ambulatory

peritoneal dialysis (CAPD) and automatic peritoneal dialysis (APD) due to end-stage renal disease were included in the study. All patients were followed in the nephrology clinic of Kocaeli University Faculty of Medicine between October 2011 and April 2012.

Inclusion criteria: To apply PD for at least 3 months, to volunteer for research and to be between 18-80 years of age.

Exclusion criteria: Previous diagnosis with type 1 or type 2 diabetes mellitus; von Willebrand's disease, a platelet adhesion defect such as Bernard-Soulier syndrome or a platelet aggregation defect such as Glanzmann's thrombasthenia, afibrinogenemia; having an infectious disease within the last 10 days; using at least one of acetylsalicylic acid, clopidogrel, ticlopidine, beta lactam group antibiotics, nonsteroidal anti-inflammatory drugs and corticosteroids in last 2 weeks.

Approval of the clinical research ethics committee of Kocaeli University School of Medicine was obtained and written informed consents were taken from all subjects participated in the study. Following at least 8 hours of fasting, blood samples were taken for routine biochemical markers from all patients admitted to the nephrology clinic and the results were determined at the central laboratory. Adenosine diphosphate (ADP) solution used as a platelet agonist in our study was provided by the manufacturer of Multiplate company (Siemens PFA Collagen / ADP Test Cartridge, Marburg / Germany) and added to the test cells at a final concentration of 10 µmole/L. Blood samples from each patient were analyzed by enzymatic reaction method on Abbott Architectc 16000 for serum magnesium (Mg) determination.

2.2. Multiple Electrode Aggregometry

Platelet aggregation measurements were performed on whole blood of each patient with multiple electrode aggregometry using Multiplate instrument. After the analysis, three values were calculated:

a) Aggregation [Unit: Aggregation unit (AU)]
b) Area under the curve (AUC) [Unit: AU x minute]
c) Velocity [Unit:AU/minute].

2.3. Study Design

Two hirudinated tube blood samples taken from each patient were assessed in the Multiplate machine in the laboratory between 30th-120th minutes. One of the tubes was used for the 1.test cell and the other for the 2. test cell.

First test cell (C1): 300 µL of whole blood was taken into the test cell and 300 µL of isotonic NaCl was added. After incubation for 3 minutes, 31 µL (10 µmole/L) ADP was added and platelet aggregation was measured.

Second test cell (C2): Serum magnesium level was adjusted to between 3-3,5 mg/dl by adding the appropriate amount of MgSO4 solution in whole blood to each patient. Serum magnesium measured before addition to blood sample. Subsequently, the amount to be added from MgSO4 solution (15 mg/dl) was calculated to be 3-3,5 mg/dl of serum magnesium. After the procedure, serum magnesium level was between 3 and 3,5 mg/dl was confirmed by the second analysis. 300 µL of isotonic NaCl was added to 300 µL of whole blood, Mg concentration of which was increased to 3-3.5 mg/dL by addition of MgSO4. After incubation for 3 minutes, 31 µL (10 µmole/L) ADP was added and platelet aggregation was measured.

C2 platelet aggregation study of patients with C1 AUC < 530 AU x minute was not performed. This value was obtained from measurements of ADP induced platelet aggregation of healthy individuals at a concentration of 6.4 µL and remains below the 5th percentile [13].

2.4. Statistics

The data of the study were entered in the SPSS 20.0 statistical package program in electronic environment. Data control and analysis were done in this program. The mean ± standard deviation and the median (minimum-maximum) values were used to show continuous variables. The Spearman correlation analysis and the Mann Whitney U test were used for statistical evaluation. Statistical significance level was accepted as $p < 0.05$.

3. Results

Forty-three PD patients (21 men, 22 women) were included in the study. Forty-one patients underwent continuous ambulatory peritoneal dialysis and two patients underwent automatic peritoneal dialysis. In the evaluation according to etiology of chronic kidney disease; 23 patients had hypertension, 11 had chronic glomerulonephritis, 3 had autosomal dominant polycystic kidney disease, 1 had amyloidosis, 1 had obstructive nephropathy, and 4 had unknown causes.

C1 platelet aggregation values of all patients participating in the study were measured. C2 platelet aggregation values were not measured in 13 patients since they had a C1 AUC <530 AU x minute and in one patient since serum magnesium level was > 3.5 mg/dl. Demographic values and laboratory findings of PD patients are shown in Table 1, and C1-C2 platelet aggregation values are shown in Table 2. The C1-C2 platelet aggregation values of male patients receiving PD are shown in Table 3 and the same values of female patients are given in Table 4.

Table 1. Biochemical and demographic findings of PD patients

	Mean ± standard deviation	Median (minimum-maximum)
Age (year)	48,12±11,2	48 (20-78)
Dialysis duration (month)	40,74±22,75	39 (4-96)
Total Kt/V (week)	2,37±0,94	2,23 (1,0-4,97)
Serum BUN (mg/dl)	53,35±14,97	54 (29-101)
Serum albumin (g/dl)	3,55±0,4	3,58 (2,61-4,26)
PLT (mm^3)	254333±72779	245000 (92300-439000)
Serum PTH (pg/ml)	568,42±637,63	341,9 (12,6-3310,1)
Serum calcium (mg/dl)	9,09±0,84	9,0 (7,0-11,6)
Serum magnesium (mg/dl)	2,26±0,39	2,30 (1,5-3,2)

No statistically significance (p values of 0.136, 0.123, 0.178, respectively) were found when the mean serum

magnesium levels of patients and C1 platelet aggregation values (AUC, aggregation and velocities) were compared. PD patients were divided into two subgroups, male and female, in terms of gender, and statistical analyses were performed for the same variables. In the male patient subgroup, there was no statistically significant correlation difference between mean serum magnesium level and C1 platelet aggregation values (AUC, aggregation, velocity) (p values 0.942, 0.92, 0.783, respectively). In the female patient subgroup, there was a statistically significant positive correlation between mean serum magnesium level and C1 AUC and C1 aggregation (r = 0.47, p = 0.032; r = 0.473, p = 0.03, respectively). In females, there was no statistically significant correlation between mean serum magnesium level and C1 velocity (p = 0.121).

In PD patients, C1-C2 platelet aggregation measures were measured. There was a statistically significant positive correlation between C1-C2 AUC (r = 0.396, p = 0.027). There was no statistically significant correlation between the aggregation of C1-C2 (p = 0.188). There was a statistically significant positive correlation between C1-C2 velocities (r = 0.538, p = 0.002) (Table 2).

Table 2. Comparison of C1-C2 platelet aggregation values of PD patients (male and female)

Platelet aggregation values	C1	C2	p
Aggregation (AU)	130,69±49,68	112,39±25,97	0,188
AUC (AUxminute)	708±254,35	653,1±168,06	0,027
Velocity (AU/minute)	15,72±5,9	15,79±5,87	0,002

In the male patient subgroup there was no statistically significant difference between C1-C2 AUC, C1-C2 aggregation and C1-C2 velocity (p values were 0.579, 0.542, 0.276, respectively) (Table 3). In the female patient subgroup there was no statistically significant difference between C1-C2 AUC (p = 0.06). There was a statistically significant positive correlation between C1-C2 aggregation and velocities (r = 0.575, p = 0.025; r = 0.771, p = 0.001, respectively) (Table 4).

Table 3. Comparison of C1-C2 platelet aggregation values of male PD patients

Platelet aggregation values	C1	C2	p
Aggregation (AU)	128,68±38,24	104,29±23,08	0,542
AUC (AUxminute)	697,05±216,36	609,13±156,08	0,579
Velocity (AU/minute)	15,31±5,66	15,0±5,42	0,276

Table 4. Comparison of C1-C2 platelet aggregation values in female PD patients

Platelet aggregation values	C1	C2	p
Aggregation (AU)	132,62±59,46	121,03±26,83	0,025
AUC (AUxminute)	718,45±290,82	700,0± 172,79	0,06
Velocity (AU/minute)	16,1±6,23	16,64±6,4	0,001

4. Discussion

In both in vitro and ex vivo studies, magnesium was shown to reduce platelet aggregation [3,4,5]. However, there are studies in the literature in which the effect of magnesium therapy on platelet aggregation is absent [6,7]. In this study addition of MgSO4 caused statistically significant decrease in platelet aggregation in whole blood in which magnesium level was raised to 3- 3.5 mg/dL. PD patients were divided into two subgroups as male and female, and it was seen that in females in vitro magnesium addition was effective decrease on platelet aggregation causing a statistical significant difference but in male gender this did not reach statistical significance. AUC has the highest diagnostic value among three parameters (aggregation, AUC, velocity) measured after the multiplate assay [14]. Despite statistical significance of correlations between C1-C2 aggregation and C1-C2 velocities in female patients applying PD there was not a correlation between C1-C2 AUC meaning that addition of magnesium to whole blood in vitro does not have a strong effect on platelet aggregation. Unlike male patients, there were statistically significant positive correlations between mean serum magnesium level and C1 AUC and C1 aggregation in female patients applying PD but there was no statistically significant correlation between mean serum magnesium level and C1 velocity.

The pathogenesis of platelet dysfunction in the uremia is multifactorial. Platelet adhesion to the vascular endothelium is reduced. In patients with end-stage renal failure, dialysis treatment may partially correct platelet abnormalities because uremic toxins (methylguanidine, guanidinosuccinic acid, phenolic acid, etc.) can not be removed effectively. PGI_2 and NO inhibit platelet function. Uremic platelets produce more NO than platelets of healthy individuals. The predisposition to bleeding in uremic patients is associated with an increase in platelet nitric oxide synthase (NOS) activity [15].

Estriol leads to an increase in NOS activity in platelets [16]. The intraplatelet cyclic guanidine monophosphate (cGMP), a bioactive NO index, is increased by 17β-estradiol [17]. Increase in intraplatelet cGMP was detected in postmenopausal women getting hormone replacement therapy [18]. In a study of healthy and recently menopausal women, the proportion of PGI2 / thromboxane A2 was found to be higher in the group receiving hormone replacement therapy than in the placebo group by measuring stable intraplatelet metabolites [19]. In an experimental study in postmenopausal women, 17β-estradiol was found to increase NO release and cyclic adenosine monophosphate (cAMP) and cGMP levels and inhibit phospholipase C gamma2 (PLCγ2) [20]. PGI2 in platelets inhibits ATP secretion from dens granules by increasing cAMP (18). PLCγ2 causes intracellular calcium increase in platelets [21,22]. However; estrogen can increase platelet activation. Production of L-arginine, a NO precursor, is reduced by estrogen and the NO concentration decreases [23].

Some factors limit this study. In our study, patients' serum ionized magnesium, platelet magnesium and cGMP levels could not be evaluated. In addition, serum estrogen levels in female patients could not be assessed. There was also no control group formed from healthy people.

5. Conclusions

Establishment of experimental hypermagnesemia in female patients with PD led to a decrease in platelet

aggregation; a similar effect was not observed in male patients. Prospective studies involving more patients are needed to determine whether there is an association between serum magnesium level and platelet aggregation in this patient group.

Statement of Competing Interests

The authors have no competing interests.

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