Original Article

Does Hemodialysis Need to be Initiated to Improve Platelet Function in CKD G5 Patients? A Pilot Prospective, Observational Cohort Study

Abstract

Introduction: We previously showed that patients with chronic kidney disease (CKD) Stage G4-5 have normal bleeding times. This made us question whether hemodialysis (HD) initiation was really necessary solely to improve platelet function. **Methods:** In this prospective observational study, two 5 ml citrated blood samples and one 2 ml EDTA blood sample were collected from incident HD patients fulfilling inclusion criteria prior to HD initiation (baseline sample) and after three sessions of short duration, low flow, counter-current HD. In each instance, one sample was used to perform Collagen adenosine diphosphate closure time (CADPCT) using the Platelet function analyzer (PFA 200, normal range 68-142 seconds) and the second for light transmission aggregometry (LTA) with ADP as agonist (normal ≥50%). **Results:** This study included 20 patients between October 2017 and February 2019. Overall, and in the subgroup with normal baseline CADPCT or LTA, there was no statistically significant improvement after HD. However, of the 30% of patients who had an abnormal baseline CADPCT, 50% attained a normal value after three HD sessions, and the overall reduction in CADPCT in this group was statistically significant (P = 0.02). Of those with a baseline normal CADPCT, 21% developed abnormal prolongation post HD. **Conclusion:** HD for the sole purpose of improving platelet function is only of benefit in the subgroup of patients with an abnormal CADPCT at baseline, with close to 50% normalizing their platelet function after three sessions of low flow, short duration, counter-current HD.

Keywords: *Aggregometry, CKDG5, hemodialysis, PFA 200, platelet function*

Vinod B. Murakonda, Anjali Mohapatra, Tulasi Geevar[1], Ramya Vijayan[1], Shailesh Kakde, Shibu Jacob, Suceena Alexander, Vinoi G. David, Sukesh C. Nair[1], Santosh Varughese, Anna T. Valson

Departments of Nephrology and [1]Transfusion Medicine and Immunohaematology, Christian Medical College, Vellore, Tamil Nadu, India

Introduction

Haemorrhagic complications are commonly seen in patients with chronic kidney disease (CKD) Stage G5, mainly due to abnormal primary hemostasis, secondary to platelet dysfunction.[1] Several uremic toxins that are cleared by dialysis have been implicated in the impaired platelet function seen in uremia.[2,3] These findings suggest that dialysis should improve platelet function. However, all previous studies that have shown a beneficial effect of hemodialysis (HD) on platelet function have been done in maintenance HD patients.[4,5] This has been the basis for initiating patients with CKD Stage G5 on dialysis before an invasive procedure such as a kidney biopsy, even if they have no emergency indication for dialysis. On average, at least three sessions of HD are given to the patient, which entails a huge added financial burden on the patient and may be unnecessary if the effect of HD on platelet function is marginal.

A study done in our center[6] showed that patients with severe renal failure, who were not yet on dialysis, had normal bleeding times, von Willebrand Factor levels and von Willebrand Factor: Ristocetin cofactor activity. This made us question the need to initiate dialysis for dialysis naïve patients who had no emergency indication for dialysis and were only scheduled for an invasive procedure. For this, we would have to prove that the standard three HD sessions that are prescribed to these patients have a limited effect on platelet function.

Our study, therefore, aimed to study the effect of three HD sessions on platelet function in incident CKD Stage G5 patients with CKD EPI eGFR less than 10 ml/min/1.73 m^2 who had not yet been initiated on regular HD.

Materials and Methods

Patient cohort and study design

This prospective observational cohort study included CKD Stage G5 patients with

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Address for correspondence:
Dr. Anna T. Valson, Department of Nephrology, Christian Medical College, Vellore - 632 004, Tamil Nadu, India.
E-mail: ceruleus@gmail.com

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CKD EPI eGFR <10 ml/min/1.73 m^2 for at least 3 months, who were to initiate HD for uremic symptoms (intractable nausea, vomiting, pruritus, and anorexia) between October 2017 and February 2019 after obtaining written informed consent. The study was approved by the Institutional Review Board and Ethics Committee of our institution on 3rd October 2017.

The exclusion criteria are given below:

1. Patients already on HD or peritoneal dialysis
2. Patients with platelet count <1.0 lakh/mm^3
3. Patients on antiplatelet agents, anticoagulants or antibiotics
4. Patients with chronic liver disease
5. Patients with history of bleeding diathesis
6. Those with ongoing or recent (<2 weeks) infection
7. Patients who had received a blood transfusion within 2 weeks of enrolling into the study
8. Patients who were initiating dialysis for an emergency indication

Patients received three sessions of HD (1st session: 1.5 hours, 2nd session: 2.5 hours, 3rd session: 4 hours on days 1, 2 and 4, respectively) with blood flow (Qb) of 150 ml/min and counter-current dialysate flow, using a polysulfone dialyzer (FX8, Fresenius Medical Care AG & Co., Bad Homburg, Germany) as per standard protocol for incident HD patients at our center. Dialyzers were not reused. The first HD session was a no-heparin session, but in the second and third HD sessions patients received unfractionated heparin according to the following protocols: second HD session - 1000 U bolus followed by 700 U/hour, third HD session - 3000 U bolus followed by 1000 U/hour. Heparin infusion was discontinued 30 minutes and 1 hour before the end of dialysis for patients on a catheter and AV fistula, respectively. Platelet function was analyzed before and after three sessions of HD by platelet function analyzer 200 (PFA 200) and light transmission aggregometry (LTA) with adenosine diphosphate (ADP) as an agonist.

Blood sampling and measurements

Two 5 ml blood samples in sodium citrate vials and one 2 ml blood sample in EDTA vial were collected from all the study participants prior to HD initiation and after three sessions of HD. Blood was immediately transported to the laboratory and processed within 4 hours. EDTA vial was used for preparation of smear and to check blood counts. One citrated vial was used to perform PFA 200 and the second vial was used for LTA with ADP as agonist. The post HD sample was collected the morning after the third dialysis (approximately 12 hours after the end of the third HD session), since an immediate post HD sample may be abnormal due to dialyzer induced platelet dysfunction, which may normalize by the next morning. Also, most invasive procedures are carried out the morning after the last dialysis, and therefore, results would be relevant to current clinical practice.

Platelet function analyzer 200

Platelet function analyzer 200 or PFA 200 is a global *in vitro* assay to measure platelet-dependent hemostasis by simulating the process of platelet adhesion and aggregation after vascular injury. It does so by measuring CADP closure times (the time required for flowing whole blood to occlude an aperture in a membrane treated with ADP), which are surrogates for platelet-platelet and platelet-vessel wall interactions. Normal range of PFA 200 CADP closure time is 68-142 seconds.[7]

LTA with ADP as an agonist

LTA was performed using whole blood on an aggregometer. Platelet-rich plasma (PRP) was prepared by centrifugation of anticoagulated blood at 180 g for 10 minutes. Platelet aggregation was determined by measuring the change in the optical density (light transmittance) of stirred PRP after addition of the aggregating agent to the aggregometer cuvette. Aggregating agonist ADP at final concentrations of 2.5 and 50 μmol/mL was used. Results were expressed as the percent change in light transmittance after agonist addition. A normal platelet control was performed with each aggregation study. Abnormal aggregation in response to an agonist was considered present if there was less than 50% aggregation.[8]

Statistical analysis

Quantitative variables were expressed as mean ± standard deviation for normally distributed and median with range for skewed data. For continuous variables, means were compared using Student's *t*test for normally distributed and Mann-Whitney Utest for skewed data. Significance was determined by Chisquare test, with the Yates continuity correction factor being used where at least one cell of the 2 × 2 table had an expected count <5.

Results

A total of 26 patients were enrolled in the present study. Six patients were excluded from the study as their baseline platelet count on peripheral smear prior to processing of platelet function tests was less than 1 lakh, which affects interpretation of the PFA 200 assay. Mean age of the study population was 45.4 years (range of 25-70 years). The majority (65%) of study participants were male, 65% were diabetics, 75% were on iron and folic acid supplements, 30% on B12 supplements, 35% on statins, and 85% on erythropoietin stimulating agents (darbepoetin 40 mcg once a week or erythropoietin 4000 units s.c. thrice a week), the dose of which remained unchanged during the study.

Approximately 65% patients were initiated on HD via temporary jugular catheter, while 20% were initiated via permanent jugular catheter. Two patients were initiated

on HD via arteriovenous fistula. Diabetic nephropathy and chronic glomerulonephritis were the native kidney disease in 25% (5/20) cases each and tubulointerstitial disease (obstructive uropathy, ADPKD) in 2 (10%). In the majority (40%), the native kidney disease was unknown as kidneys were not biopsiable at presentation. Baseline biochemical parameters of the study population are given in Table 1.

Overall effect of HD on platelet count and platelet function tests

The mean baseline PFA 200 CADP closure time was 153.9 ± 72.2 seconds, which decreased to 127.3 ± 57.5 seconds after three sessions of HD though this change was statistically insignificant (P = 0.16). Further, the proportion of patients with an abnormal CADP closure time remained the same (30%) before and after three sessions of HD. Baseline mean LTA with ADP as agonist was 61.6 ± 14.5%, which improved to 67.2 ± 12.1% after three sessions of HD. This change, although suggesting a trend towards improvement, was also statistically insignificant (P = 0.07). The median platelet count increased from 1.43 to 1.73 lakh/mm^3 between the first and third HD sessions, a median increase of 11,000/mm^3 (25th, 75th quartile: −10,750, +18,000/mm^3), though this change was not significant (P = 0.287). In seven patients (35%), there was a fall in platelet count in the range of 5000-1,63,000/mm^3.

Effect of HD on patients with normal and abnormal baseline platelet function tests

There was no significant change in the CADP closure time with three sessions of HD in the subgroup with normal baseline CAPD closure time (114.4 ± 19.9 to 121.3 ± 57.7 seconds, P = 0.66). However, in those with an abnormal CADP closure time at baseline, it decreased from 245.8 ± 65.7 to 141.3 ± 60 seconds, a change that was statistically significant (P = 0.02). The median platelet count increased by 51,000/mm^3 in those with a baseline abnormal CADP closure time and 7000/mm^3 in those with a baseline normal CADP closure time after three sessions of HD, though this change was not significant (P = 0.628).

In terms of overall proportions, of the 6 patients (30% of the study population) who had an abnormal baseline CADP closure time, the closure time normalized with three sessions of HD in 3/6 (50%) patients. Interestingly, of the 14 patients who had a normal CADP closure time at baseline, 3/14 (21.4%) patients developed an abnormal CADP closure time after initiation of HD. The median increase in platelet count in those with a baseline normal CADP closure time that became abnormal after three sessions of HD and those with a baseline normal CADP closure time that remained normal after three sessions of HD was 10,000 and 4000/mm^3, respectively (P = 1.000).

Likewise, the subgroup with an abnormal LTA at baseline also showed a trend towards improvement post HD, however, this change did not achieve statistical significance [Table 2]. LTA was abnormally low at baseline in 20% (4/20) cases. Among them, LTA normalized with three sessions of HD in 2/4 (50%) of patients.

Predictors of an abnormal CADP closure time at baseline

We wanted to identify any factors that may help predict the subgroup of patients who were likely to have an abnormal CADP closure time at baseline as this group would be most likely to benefit from initiation of HD. While those with an abnormal CADP closure time had a trend towards higher BMI and albumin and a higher aPTT, these factors were not statistically significant [Table 3].

Predictors of failure to improve CADP closure time with HD

Table 4 compares various baseline and follow-up parameters between patients who showed an improvement in CADP closure time with HD and those who did not. While diabetics, those who did not receive iron and folic acid supplementation, and those with higher baseline blood urea showed a trend towards failure to normalize CADP closure time, none of these factors attained statistical significance.

Discussion

The impairment in hemostasis in uremic patients is multifactorial and includes defects in platelet function

Table 1: Baseline patient characteristics

Patient characteristic	Median (range) or mean±SD
CKD EPI eGFR (ml/min/1.73 m^2)	4.2±1.3
Duration of CKD (months)	17 (3-70)*
Hemoglobin (g/dl)	8.7±1.8
Platelet count (lakh/cumm)	1.8±0.8
Blood urea (mg/dl)	188.8±41
Serum creatinine (mg/dl)	12.6±4.1
Serum Albumin (g/dl)	3.8±0.7
Prothrombin time (sec)	9.7±0.9
aPTT (sec)	31.1±3.6
Ferritin (ng/ml)	216.8 (50-1975)*

aPTT - Activated partial thromboplastin time, CKD EPI - Chronic kidney disease epidemiology collaboration, *Median (range)

Table 2: Differential effect of HD in patients with normal and abnormal baseline LTA

	Pre HD mean±SD median (range)	Post HD mean±SD median (range)	*P*
Baseline Normal LTA (>50%), *n*-16 (80%)	67.2±7.4 68.6 (54.5-77.9)	69.9±10.8 68.2 (52.4-95)	0.36
Baseline abnormal LTA (<50%), *n*-4 (20%)	39±14.6 45.1 (17.4-48.9)	56.5±12.2 54.7 (46.1-70.7)	0.09

LTA: Light transmission aggregometry

Table 3: Comparison of baseline characters between patients with normal and abnormal baseline CADP

Patient characteristic	Baseline Normal CADP *n*-14 (70%) Mean±SD or Median (range)*	Baseline abnormal CADP *n*-6 (30%) Mean±SD or Median (range)*	*P*
Age (years)	45.6±15.9	44.8±15.7	0.92
Male (%) (*n*-13)	8/14 (57.1%)	5/6 (83.3%)	0.35
DM (%) (*n*-7)	5/14 (35.7%)	2/6 (33.3%)	0.99
BMI (kg/m^2)	21.4±2.8	23.7±1.98	0.09
CKD EPI eGFR (ml/min/1.73 m^2)	4.2±1.5	4.4±0.8	0.73
Duration of CKD (months)	25.1±20.5	24±13.7	0.9
Hemoglobin (g/dl)	8.4±1.8	9.4±1.9	0.27
Platelet count (lakh/cumm)	1.7±0.6	2±1.3	0.46
Blood urea (mg/dl)	198±41.9	167.3±32.2	0.13
Serum creatinine (mg/dl)	12.8±4.6	12±2 3	0.79
Serum Albumin (g/dl)	3.6±0.7	4.2±0.5	0.09
Prothrombin time (sec)	9.7±1.0	9.7±0.7	0.97
aPTT (sec)	30.2±2.7	33.2±4.6	0.08
Ferritin (ng/ml)	216.8 (50.1-197)*	217 (102-809)*	0.55
ESA use (%)	35.7%	33.3%	1.00
Blood group O (%)	35.7%	16.7%	0.61
Iron supplement use (%)	71.4%	83.3%	1.00
Folic acid use (%)	71.4%	83.3%	1.00
B12 supplement use (%)	21.4%	50%	0.30
Statin use (%)	28.6%	50%	0.61

DM: Diabetes mellitus; BMI: Body mass index; aPTT: Activated partial thromboplastin time; ESA: Erythropoiesis stimulating agent

Table 4: Comparison of baseline and follow up characteristics between patients who did and did not show an improvement in CADP closure time with 3 sessions of low-flow, low-flux counter-current HD

Patient characteristic	Improvement in CADP closure time *n*-13 (65%) Mean±SD or Median (range)*	No improvement or worsening of CADP closure time *n*-7 (30%) Mean±SD or Median (range)*	*P*
Age (years)	45.6±15.7	44.7±15.8	0.89
Male (%)	61.5%	71.4%	1.00
DM (%)	23.1%	57.1%	0.17
BMI (kg/m^2)	22.5±2.9	21.2±2.0	0.32
Baseline CKD EPI eGFR (ml/min/1.73m^2)	4.2±1.3	4.1±1.4	0.82
Baseline prothrombin time (sec)	9.5±0.7	10.0±1.2	0.27
Baseline aPTT (sec)	31.0±4.1	31.2±2.4	0.92
Blood urea (mg/dl)	181.8±45.0	201.7±29.8	0.31
Serum creatinine (mg/dl)	12.4±4.5	13.0±3.5	0.77
Duration of CKD (months)	27.2±19.2	20.1±17.0	0.42
Iron supplement use (%)	84.6%	57.1%	0.29
Folic acid use (%)	84.6%	57.1%	0.29
B12 supplement use (%)	23.1%	42.9%	0.61
Statin use (%)	30.8%	42.9%	0.65
ESA use (%)	84.6%	71.4%	0.58
Median haemoglobin change (g/dl, Q1, Q3)*	0.4 (-0.4, 1.1)	0.7 (-0.2, 1.3)	0.58
Median hematocrit change (%, Q1, Q3)*	1.6 (-0.5, 3.6)	1.8 (0, 3.8)	0.87
Median platelet count change (/cumm, Q1, Q3)*	12,000 (-14,000, 51,000)	10,000 (-10,000, 18,000)	0.87

*Before and after 3 sessions of HD

such as abnormalities in platelet number, dense granule content, concentration of intracellular ADP, serotonin and cyclic AMP, release of platelet α granules, calcium ion mobilization, arachidonic acid metabolism, cyclooxygenase activity, GPIIbIIIa binding, platelet aggregation and adhesion.[9] Uremic toxins such as phenol, phenolic acid, and guanidinosuccinic acid (GSA) play a major role in the pathogenesis of uremic platelet dysfunction[10] and accumulate in patients with renal failure due to reduced clearance.[11]

In the present study, the effect of three sessions of HD on platelet function was investigated in CKD G5 patients with

CKDEPI eGFR less than 10 ml/min/1.73 m^2 who were naïve to HD. We found that PFA 200 CADP closure time was abnormally elevated at baseline only in 30% and LTA was abnormally low in 20% of patients despite their presenting with severe renal failure (mean eGFR <5 ml/min/1.73 m^2). Thus, the prevalence of clinically significant uremic platelet dysfunction in *incident* HD patients is low. This is different from the situation in *prevalent* HD patients - Mekawy *et al*.[4] showed that PFA100 was abnormally high in the predialysis sample in 90% of cases.

The second finding overall was that the initiation of HD did not have a statistically significant effect on either CADP closure time or LTA in terms of change from baseline value or proportion of patients with abnormal values before and after HD.

However, a closer look at the data showed that the subgroup of patients with an abnormal baseline PFA200 may indeed benefit from the initiation of HD. At least part of this improvement can be attributed to an improvement in platelet count, as was seen in this study (though the sample size was too small to demonstrate statistical significance). There is evidence that HD may be associated with an increase in the immature platelet fraction and serum thrombopoetin resulting in an absolute platelet count increase from baseline.[12] It is hypothesized that platelet consumption on exposure to the dialysis membrane, stimulates release of immature platelets from the bone marrow.[13] However, even in the group with abnormal baseline CADP closure time, only 50% normalize their platelet function after three sessions of short duration, low flow, counter-current HD. This is supported by literature in prevalent HD patients, in whom CADP closure time normalized after HD only in 22% of patients.[4] There are many reasons why not all patients with an abnormal closure time may show a clinically significant improvement with dialysis. Studies have shown that serum levels of dialyzable uremic toxins that have been implicated in platelet dysfunction do not correlate with bleeding time or platelet adhesion, and therefore, even though they may be removed in HD, their removal alone may not improve the platelet function.[14] Second, HD improves platelet aggregation and secretion of aggregatory substances such as thromboxane but may not correct existing storage pool defects.[15] Third, it is likely that a larger number of dialysis sessions may be required to observe a meaningful effect on platelet function.

A more important finding in our study is that 21% of patients with baseline normal CADP closure time developed an abnormal prolongation of CADP post HD initiation. In these patients, there was no decrease in platelet count to explain this phenomenon; hence, it is unlikely that heparin-induced thrombocytopenia was the cause. As the post HD sample was obtained at least 12 hours after the last HD session, there is unlikely to have been a carry-over effect of heparin. Even in the absence of heparin, HD itself can contribute to bleeding, as the dialyzer surface and blood tubing can cause transient platelet activation leading to degranulation and loss of glycoprotein receptors (GpIb and IIb-IIIa) immediately post dialysis, which may take up to 24 hours to normalize.[16-18] Thus HD initiation for the sole purpose of improving platelet function should not be taken lightly, and should only be carried out in those most likely to benefit from it.

These findings prove that the initiation of dialysis has a variable effect on platelet function. This is borne out by available literature, with some authors showing a detrimental effect[16-18] and others a beneficial effect.[4,5,14,15] It is likely that many factors, such as incident or prevalent HD status, number of dialysis sessions provided, degree and duration of renal failure (which determines the accumulation of platelet function inhibitory middle molecules), low- versus high-flux dialysis, type of dialyzer membrane and sterilization method used, type of platelet function defect (secretory, aggregatory, and storage pool), intensity of complement and platelet activation on exposure to the dialyzer membrane and the ability of the bone marrow to respond adequately to platelet consumption, as well as timing of the samples used for testing platelet function, may determine the net effect of dialysis on platelet function.

We could not identify any factors that would help identify patients likely to have an abnormal CADP closure time at baseline. CADP closure times are prolonged by platelet count <1 lakh/mm^3 and hematocrit less than 25%, but platelet count and hemoglobin were not different between those with and without an abnormal CADP closure time. The trend towards abnormal CADP in those with a higher aPTT raises an intriguing hypothesis. The combination of normal platelet count with high normal aPTT and abnormal CADP raises the possibility of an acquired vWF defect. In the context of uraemia, this suggests vWF Type 2A, in which there is a paucity of high molecular weight multimers of vWF. It is likely that the initiation of HD, much like the administration of desmopressin, may benefit such patients.[19,20]

We could also not identify any factors that could identify patients who failed to show improvement in CADP closure time with initiation of dialysis, though this was more likely to be seen in diabetics, those with higher baseline blood urea and those who had not received iron and folic acid supplementation. There is evidence to show that in the setting of iron deficiency, folate and B12 deficiency may result in thrombocytopenia instead of the frequently seen thrombocytosis.[21] However, change in hemoglobin, hematocrit and platelet count after HD initiation were not significant predictive factors of improvement of CADP closure time.

Limitations

In view of the small sample size, we cannot generalize the results to a larger population, however, this study provides

some important insights that can be studied in greater detail with a larger sample size. Our strict inclusion criteria, exclusion of patients who had received recent blood transfusions or had emergency indications for HD severely limited the number of patients who could be recruited for our study. Our study population did not undergo any invasive procedure, and so the correlation between the bleeding risk, as determined by the tests performed, and clinical bleeding episodes, could not be determined. Another limitation is that serum vitamin B12, which is a thrombopoietic factor, was not measured in the study participants. Our protocol for dialysis initiation, specifically the duration of HD, may vary from those at other centers; however, this protocol is in place to minimize the risk of dialysis disequilibrium, which is a very real risk in severely uremic patients. It can be argued that the quantum of HD provided may not have been adequate to normalize platelet function, which affects the findings of the study, however, the aim of the study was only to assess whether a limited number of HD sessions solely to improve platelet function prior to an invasive procedure, would have a beneficial effect on platelet function. The dialysis protocol was therefore reflective of real-life practice. Because CADP closure time showed a tendency towards failure to correct in those with higher baseline blood urea, it is very likely that such patients may require more sessions to demonstrate an improvement in CADP closure time.

Conclusion

Only 30% of CKD Stage G5 patients with CKDEPI eGFR less than 10 ml/min/1.73 m^2 who are naïve to HD have abnormal platelet function at baseline and approximately half these patients will normalize their platelet function after three sessions of short, low blood flow, counter-current HD. Around 21% of patients with normal CADP closure time may experience a worsening after HD initiation. HD for the sole purpose of normalizing platelet function prior to an invasive procedure should only be initiated in the subgroup of patients with an abnormal baseline CADP closure time.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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