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Impact of Hemodialysis on Bleeding Tendency in End Stage Renal Disease Patients

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ABSTRACT

Background: Heparin is a potent anticoagulant with diverse pleiotropic effects through cytokine release. In hemodialysis, anticoagulation using systemic heparinization is crucial to prevent blood clots within the extracorporeal circuit. However, the risk of bleeding complications and substantial hemostatic defects are increased in maintenance HD patients.

Aim of work: the aim of the presented study was to investigate the effect of systemic heparinization during hemodialysis on the coagulation profile of maintenance HD patients and whether heparin free dialysis would be beneficial for specific patients.

Patients and Methods: Sixty ESRD patients on regular HD three times per week and 25 healthy controls were recruited to this study. Complete blood count, prothrombin time, activated partial prothrombin time, fibrinogen and D-dimer levels pre and post hemodialysis were evaluated.

Results: The presented study demonstrated prolongation of PTand APTT associated with increased levels of fibrinogen and D- dimer post HD (p-value: <0.0001).

Conclusion: The prolonged PT and APTT in addition to the rise in fibrinogen and D- dimer levels post HD indicates that the prophylactic anticoagulantion using unfractionated heparin could increase the risk of bleeding in HD patients.

Keywords: *Bleeding tendency - D-dimer - fibrinogen - hemodialysis-ESRD- heparin free dialysis*

INTRODUCTION

Hemodialysis (HD) is a renal replacement therapy (RRT) for long term control in End-Stage Renal Disease (ESRD) patients. HD is a blood filtering process through a semi-permeable membrane to get rid of toxic materials from the plasma as well as correcting the electrolyte abnormalities in the body (Suwitra et al., 2006).

Chronic uremia by itself induces functional platelet defect that can only be partially reversed with repeated HD (Mohamed et al., 2008). Further more, systemic heparinization during HD, can cause substantial hemostatic defects,

which together with the high blood flow in the arteriovenous fistula (AVF), may cause prolonged bleeding from the puncture site (Hernaningsih et al., 2019).

Bleeding can significantly contribute to mortality and morbidity and blood transfusions can lead to alloimmunization and thereby limit options for transplantation (Kim, 2003).

In HD, anticoagulation is necessary to prevent blood clots within the extracorporeal circuit. Several anticoagulation techniques, apart from heparin tailored according to the patients' conditions have been tested(Dara , 2009). However, based on its simple administration, high-molecular-weight Unfractionated Heparin (UFH) is still considered the standard anticoagulant used in HD process (Lohr and Schwab, 2008).

Hemodialysis will repair abnormal hemostasis in uremia, but heparinization during HD procedures increases the risk of bleeding (Lohr and Schwab, 2008). The bleeding risk can be minimized in extracorporeal circulation during HD through the administration of minimum heparin dose or heparin-free dialysis as well as the use of low-molecular-weight heparin (LMWH) anticoagulation (Prasanto, 2007).

Coagulation parameters such as prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen are screening tests for extrinsic, intrinsic and common pathway clotting factors (Hernaningsih et al., 2019). Intravascular coagulation and thrombotic disease are associated with increased fibrinolysis, and elevated blood concentration of D-dimer the degradation product of cross-linked fibrin. D-dimer levels were found to correlate with the level of impairment of renal function.

SUBJECT AND METHODS

This cross-sectional study included 60 patients with ESRD on regular HD from the hemodialysis unit, Theodor Bilharz Research Institute, Giza, Egypt. All patients received 4-hour HD session 3times a week Anticoagulation during dialysis was established with a stable, clinically efficient dose of UFH, administered as a single bolus, before starting HD (doses ranged between 20 and 60 mg). In addition to, 25 healthy volunteers who matched the ages, genders, and demographics of the patients were included as a control group.

The presented study was approved by the institution review board, TBRI (IBR noxxxx) and all subjects gave written informed consent to participate. Inclusion criteria: ESRD on regular HD for at least 6 months. Exclusion criteria: congestive heart failure, chronic inflammatory diseases, autoimmune disease, malignancy, sepsis, patients with liver disease, hemostatic disorder or on anticoagulants, patients have signs of acute illness or DIC and smokers. All participants were subjected to the following: (1) Full History taking (age, alcohol consumption, smoking, sexual, medical and drug history, hemodialysis duration). (2) Clinical examination including general examination with emphasis on signs of liver disease, signs of autoimmune diseases and signs of heart failure. (3) Routine and specific laboratory tests (4) Abdominal ultrasonography using Hitashi Hi vision avius with Using Hitachi EUP-L65 Small Parts to exclude liver cirrhosis.

Blood sampling

Venous blood samples were collected from healthy volunteers via aseptic clean venipuncture using vacutainer tube system. On the other hand, blood samples were obtained from patients' vascular access immediately before start of hemodialysis session and immediately post hemodialysis. Six ml of blood were withdrawn each time; one EDTA vacutainer (2 ml) for CBC and one citrate vacutainer (2 ml) for coagulation tests, one serum separator vacutainer (2ml) for blood chemistry. The serum and citrated Platelet Poor Plasma were prepared by centrifugation at 3000 rpm for 10min in centrifuge, (5804 Eppendorf, Germany).

Laboratory Tests

The following parameters were assessed: (A) Complete blood count (hemoglobin, WBCs and platelet counts) was performed on 3-part differential automated counter Medonic (Boule Diagnostics, Sweden). (B) Liver functions (aspartate transaminase, alanine transaminase,

serum albumin and bilirubin) and kidney functions (s. creatinine and urea) pre-hemodialysis were performed on serum samples using chemistry autoanalyzer (AU 480, Beckman Coulter, USA). (C) Screening hemostatic tests: Prothrombin Time (PT) and International Normalized Ratio (INR) performed using Thromborel® S Reagent (Cat. No. OUHP29), activated Partial Thromboplastin Time (APTT) performed using Pathromtin® SL Reagent (Cat. No. OQGS29), Quantitative determination of fibrinogen using modification of the Clauss method was performed using Multifibren® U Reagent (Cat. No OWZG19) Fibrinogen calibrator kit (Cat. No. OQVK11) was used for calculating the reference curve. Internal quality control was performed at start of the test run by measuring control plasma Normal (Cat. No. ORKE41) & Pathological (Cat. No. OUPZ17) (Siemens, Germany). These tests were carried out on semi-automated coagulation analyzer BFT II (Siemens, Germany). (D) Assay of Ddimer was carried out using ZYMUTEST DDimer ELISA kit (Cat No. RK023A) (Hyphen BioMed, France). The manufacturer's instructions were srtictly followed. ELISA plate reader SunriseTM (Tecan, Switzerland) was used to measure the absorbance at 450nm. MagellanTM data analysis software generated the concentration of Ddimer in every tested sample.

STATISTICAL ANALYSIS

Data were analyzed using SPSS software (USA). The data expressed as mean ± SD or number (%). The statistical comparison was done using independent student t test or paired student t test for parametric data. Categorical data was analyzed using Chi square test. P-values of less than 0.05 were regarded as statistically.

RESULTS

The study was conducted on 60 HD patients and 25 healthy volunteers as a control group.

TABLE 1: Demographic and clinical data of the HD patients and Healthy subjects

	HD patients (N=60)	**Healthy subjects (N= 25)**	**P value**
Age (Years)	55.2 ± 14.3	54.1 ± 13.7	0.76
Sex Male (1) Female (0)	 38 (63%) 22 (37%)	 13(52%) 12 (48%)	X2= 0.94 P= 0.33
Weight	75.9 ± 13.6	75 ± 12.3	0.77

Statistically significant at $p<0.05$ was considered significant.

TABLE 2: liver, kidney functions and abdominal ultrasound of the HD patients and Healthy subjects

	HD patients (N=60)	**Healthy subjects (N= 25)**	**P value**
Creatinine	7.8 ± 2.1	0.76 ± 0.14	<0.001*
Urea	143.9 ± 34.9	32.1 ± 3.3	<0.001*
Albumin	4.08 ± 0.33	4.04 ± 0.24	0.63
Total bilirubin	0.57 ± 0.21	0.52 ± 0.19	0.36
Direct bilirubin	0.5 ± 0.74	0.36 ± 0.15	0.38
AST	22.7 ± 11.2	21.3 ± 8.4	0.58
ALT	17.2 ± 6.04	15.9 ± 5.2	0.36
CRP	13.3 ± 2.9	2.8 ± 1.3	<0.001*
Abdominal Ultrasound Normal Fatty liver	 48 (80%) 12 (20%)	 19 (76%) 6 (24%)	0.68

J Popul Ther Clin Pharmacol Vol 30(7):e300–e306; 10 April 2023.

Statistically significant at p<0.05 was considered significant

CRP, C-Reactive protein; ALT, alanine transaminase; AST, aspartate aminotransferase.

The mean of serum creatinine, urea and CRP were much higher in the hemodialysis group compared to the healthy controls with a high statistical significance (P value <0.001) (Table 2)

TABLE 3: CBC, coagulation parameters, D-dimer and Fibrinogen of the HD patients and Healthy subjects

	Pre HD patients (N=60)	**Healthy subjects (N= 25)**	**P value**
RBCS	3.6 ± 0.59	4.5 ± 0.68	<0.001*
Hb	9.7 ± 1.3	11.4 ±0.88	<0.001*
HCT	28.7 ± 4.3	34.4 ± 4.6	<0.001*
MCV	81.07 ± 6.2	84 ± 2.2	0.01*
MCH	27.3 ± 2.4	28.7 ± 1.1	0.005*
MCHC	33.7 ± 1.08	34.7 ± 1.5	0.001*
RDW %	13.4 ± 1.1	14.2 ± 1.2	0.006*
TLC	6.8 ± 2.4	8.2 ± 1.6	0.01*
PLT	223.1 ± 85.1	270.5 ± 78.1	0.01*
PT	14.83 ± 4.48	13.3 ± 0.04	0.13
PC %	941.4 ± 132.71	100 ± 0	0.002*
INR	1.08± 0.4	1 ± 0	0.12
APTT	41.9 ± 11.1	24.2 ± 5.04	<0.001*
INR	± 0.36	1 ± 0	0.12
D- DIMER	3.4 ± 2.8	0.26 ± 0.1	<0.001*
FIBRINOGEN	2.8 ± 0.81	2.9 ± 0.53	0.66

Statistically significant at p<0.05 was considered significant.

Hb, hemoglobin; Hct, hematocrit, MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; TLC, total leucocytic count, PLT, platelets; PDW, platelet distribution width; RBC, red blood cells; INR, international normalization ratio; PT. prothrombin time; PC, prothrombin concentration; APTT, activated prothrombine time

TABLE 4: CBC, coagulation parameters, D-dimer and Fibrinogen of the HD patients before and after hemodialysis

	HD patients (N=60)		P Value
	Pre HD	Post HD	
RBCS	3.6 ± 0.59	3.9 ± 0.68	<0.001*
Hb	9.7 ± 1.3	10.6 ± 1.4	<0.001*
HCT	28.7 ± 4.3	30.9 ± 4.8	<0.001*
MCV	81.07 ± 6.2	77.4 ± 15.06	<0.001*
MCH	27.3 ± 2.4	27.6 ± 2.4	<0.001*
MCHC	33.7 ± 1.08	39.5 ± 39.6	0.87
RDW %	13.4 ± 1.1	13.5 ± 1.1	<0.001*
TLC	6.8 ± 2.4	6.9 ± 2.5	<0.001*
Platelets	223.1 ± 85.1	240.9 ± 89.1	<0.001*
PT	14.8 ± 4.8	14.3 ± 4.4	<0.001*
PT	14.3 ± 4.4	14.8 ± 4.8	<0.001*
PC %	91.4 ± 13.1	94.4 ± 12.7	<0.001*
PC%	94.4 ± 12.7	91.4 ± 13.1	<0.001*

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INR	1.08± 0.4	1.2± 0.36	<0.001*
APTT	41.9 ± 11.1	48.3 ±12.3	<0.001*
INR	± 0.36	1.08 ± 0.4	<0.001*
D- DIMER	3.4 ± 2.8	4.1 ± 3.6	<0.001*
FIBRINOGEN	2.8 ± 0.81	5.3 ± 1.1	<0.001*

Statistically significant at p<0.05 was considered significant

Hb, hemoglobin; Hct, hematocrit, MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; TLC, total leucocytic count; PLT, platelets; PDW, platelet distribution width; RBC, red blood cells; INR, international normalization ratio; PT. prothrombin time; PC, prothrombin concentration; APTT, activated prothrombin time.

Peripheral blood Erythrocytes data from HD patients and healthy controls

The differences between the mean of RBCs, Hb, Hct, MCV, MCH, MCHC, RDW % indices that occurred in renal failure patients before HD compared to the control group (Table 3).

There was a high statistically significant (p <0.001) decrease in RBCs count, Hb, Hct, MCH, MCHC and RDW % among patients before HD compared to after HD (Table 4).

Peripheral blood leukocyte and platelets data from HD patients and healthy controls

There was decrease in the TLC and platelets when compared to the controls with high statistical significance (P= 0.01) (Table 3). As regard the differences before and after HD, TLC and Platelets were statistically significant (p <0.001) of lesser values among patients before HD compared to the same patients after HD (Table 4).

Peripheral blood coagulation profile, D- dimer and fibrinogen from HD patients and healthy controls

A statistically significant (p =0.002) decrease in PC % was observed in HD patients as compared to the control group. In contrast a statistically significant (<0.001 and <0.001) increase in APTT and D- dimer was observed in HD patients as compared to the control group. As regards PT, INR and fibrinogen, there were no statistically significant differences between the two groups (Table 3).

There was a statistically significant (p <0.001) decrease in D- dimer and fibrinogen among patients before HD compared to after HD. On other hand, mean PT, PC, INR and APTT were prolonged in the patients after HD in comparison with before HD with high statistical significance (p <0.001) (Table 4)

DISCUSSION

The results of the present study showed that ESRD patients on maintenance HD displayed various degrees of changes in hematological parameters compared to the healthy controls.

Moreover, the present study indicated that the mean of each RBCs count, Hb, Hct, MCH, MCHC and RDW % levels show a statistically significant increase in ESRD patients' post-HD compared to pre-HD levels. This increase could be explained by the fact that before HD, patients are usually hypervolemic and the values of each RBCs count, Hb, Hct levels are also lower.

The findings of the presented study also indicated that there were statistically significant differences between the mean number of leukocytes in pre-HD when compared to the control group. Similar results were reported by Pereira et al., (2010) who showed that HD patients displayed a significantly lower leucocyte count. After the HD session, a statistically significant increase in leucocytes was found in agreement with results by Alghythan and Alsaeed, (2012). The increase of leukocytes after-HD could be explained by the fact that HD session, generates an abundance of growth factors, cytokines and other mediators of microinflammation. Also, heparin has many pleiotropic effects, some of which are mediated by cytokine release.

The present study revealed that there was a statistically significant decrease in the mean

platelet counts, though still within the normal range, in the patients on HD when compared to the results of the control group. Also, the mean platelet counts showed a significant decrease in patients after-HD when compared to pre-HD. This finding agreed with Daugirdas and Bernardo (2012), who reported a significant decrease in circulating platelets after-HD compared to the before-HD.

In the present study, the mean value of PC and APTT were significantly increased after-HD when compared to the before-HD levels. This finding could be explained by using systemic anticoagulation (heparin) during conventional HD for extracorporeal procedures which binds to the enzyme inhibitor antithrombin III. These finding were consistent with the work of Khalid and Zafar, 2015 who found a statistically significant increase of APTT after-HD. the current study showed that there were statistically significant differences between the mean of APTT in HD patients and those of the control group. This finding agreed with the results obtained by Ulusoy et al., (2004).

The presented study also clearly showed that there was no statistically significant difference in the mean of fibrinogen levels in HD patients when compared to healthy controls. However, it has been shown that the fibrinogen level increases significantly in patients after-HD when compared to before-HD levels and those results were supported by reports from Ciaccio et al., (2008). Increased levels of fibrinogen in HD patients could be explained in the context of the proinflammatory state induced by uremic milieu (Gackler et al.,2019).

A statistically significant increase in D- dimer and CRP was observed in HD patients compared to the control group. Similarly, fibrinogen and D-dimer levels increased significantly in patients after-HD when compared to before HD levels which was consistent with the work of Fritiwi et al., (2019). Increased D-dimer level in HD patients highlights the stimulation of the coagulation cascade and fibrinolytic system in the context of the proinflammatory state induced by the dialysis procedure.

CONCLUSIONS

The prophylactic anticoagulantion dose of UFH should be used in HD to maintain the balance between thrombosis and bleeding risk. Never the less, all HD patients should be screened appropriately before and after HD to avoid hypercoagulability or bleeding complications. Heparin free dialysis should be considered in HD patients with high risk of bleeding.

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