Prothrombotic Changes in Patients with End-Stage Renal Disease and its Relation to Thrombotic Cardiovascular Complication

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Abstract

Background: There is a great risk of cardiovascular disease (CVD) and vascular thrombosis in patients with End-Stage Renal Disease (ESRD). These patients exhibit numerous abnormalities in coagulation, fibrinolytic, inhibitory protein abnormalities in multiple levels.

Aim: To assess hypercoagulable changes by measuring the levels of Antithrombin, plasma fibrinogen and FXII activity in patients with ESRD, and to found correlation between and hematological parameters including: Hb level, WBC count, reticulocyte percentage and platelet count.

Method: A case control study conducted at Al-Hayat center, Al Karama teaching hospital during the period from the 1 of February to the 30 of October 2014 and **50** patients aged < 60 years of both genders with (ESRD) in addition to **20** apparently healthy controls were included in this study.

Results: Patients and controls were matched for age and gender. The mean hemoglobin level, total WBC count, absolute neutrophil count, reticulocyte percentage and platelets count were significantly lower in ESRD patients than controls, P<0.05. The mean values of the coagulation parameters, ProthrombinTtime (PT), Activated Partial Thromboplastin Time (APTT), Plasma Fibrinogen and Factor XII activity were significantly higher in patients than controls. Anti-thrombin activity was significantly lower in patients group than controls, (P<0.001). Cardiovascular complications and vascular thrombosis included Deep Venous Thrombosis(DVT), Cerebro-Vascular-Accident (CVA), Myocardial Infarction (MI), angina or heart failure reported in 62% of the patients. Patients with cardiovascular complication and vascular thrombosis had significantly higher PT, APTT, and factor XII activity, lower anti-thrombin activity as compared to those without cardiovascular complication and vascular thrombosis.

Conclusion: (ESRD) patients had coagulation abnormalities render them more liable to have cardiovascular complications and vascular thrombosis.

Keywords*: End stage renal disease, Antithrombin, Factor XII activity, plasma Fibrinogen, coagulation parameters.*

Introduction

The chance of occurance of cardiovascular disease and vascular thrombosis is far greater in patients with ESRD than in general population.[(1)] CVD was significantly more prevelant in uremic patients treated by hemodialysis (HD) or peritoneal dialysis (PD) compared with other populations of a similar age as proved by many studies.[(1,2)] ESRD poses a great cardiovascular fatality rate as it has been established. Cardiovascular consequences provide a mortality

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rate of about 10- to 100- fold higher among patients on dialysis than persons matched for age and sex in the general population.[3] Vascular sequele caused by atherosclerosis are the main cause of death in patients undergoing HD.[4] A modifications in the coagulation pathway and in fibrinolysis are the major cause of hyprcoagulability and these modifications may result from endothelial cell activation and damage.[5] Most of the traditional risk factors detected in the general population increase the risk of CVD in chronic kidney diseases (CKD) patients. In fact, numerous Framingham risk factors are more prevalent among CKD patients as compared to those with normal kidney function. Additionally, non-traditional risk factors which are related to CKD patients also contribute to the CVD burden.[6] CVDs are the leading cause of death among patients on HaemoDialysis HD.[7] Elevated levels of fibrinogen and D-dimer were associated with the presence of atherosclerotic disease independent of renal function and other risk factors. Changes in hemostatic parameters occur early in the course of renal failure in patients with arteriolar nephrosclerosis, suggesting a prothrombotic state that may contribute to the risk for atherosclerotic disease at all levels of renal function.[8] Persons with elevated plasma fibrinogen concentration in those with risk factors may further increase the risk of the development of atherothrombosis and subsequent CVD through the blood coagulation system.[9, 10]

Patients and Method

Study design setting and time:

This case-control study was conducted at Al-Hayat center in Al-Karama teaching hospital during the period from 1 February to 30 October 2014

Patients: After approval of study protocol from the scientific council of the Iraqi Board of Pathology and patients consents, a total of 50 adult patients aged < 60 years of both genders (35 males and 15 females) with ESRD on repeated sessions of HD, 2-3 times per week, were included in this case-control study.

Exclusion criteria:

- Patients aged above 60 years to avoid the risk of thrombosis.
- Patients with congenital or acquired thrombotic disorders, e.g. malignancy, DIC, liver disease or deranged liver enzymes, systemic lupus erythematosus or other immunologic diseases and pregnancy.
- Recent major surgery within the last 3 months.
- Active inflammation.
- Patients taking any type of anticoagulant therapy during the preceding one week.
- Women using oral contraceptives pills.
- Inability to give informed consent.
- Those who did not want to participate.

Control group: A 20 apparently-healthy participants aged (22-50) years, 14 males and 6 females were enrolled as controls and pre-constructed data collection sheet was filled for each participant (patients and controls) and included: personal information, and clinical and laboratory data.

Methods: This study test the hematological parameters including: Hb,WBC count, absolute neutrophil count and platelet count by using an automated electronic counter (Hematology auto-analyzer - BECKMAN COULTER, ACT. 5 diff. USA)and reticulocyte percentage by standerd method using new methylene blue solution.While Coagulation Tests which were done in current study include:

- **Prothrombin time (PT)**
- **Activated partial thromboplastin time (APTT).**
- **Fibrinogen assay (clauss technique) by using** commercially available kit FIBRI- PREST® 2 (DIAGNOSTICA STAGO, France).[11]
- **Coagulation factor XII activity:** was done using coagulation factor XII (STA Deficient XII) kit where the assay consists of measurements of the clotting time in the presence of cephalin and activator of a system in which all the factors are present and in excess except factor XII which is derived from the sample being tested.[12]
- **Antithrombin activity (AT):** LIATEST® AT Kit was used using antigenic assay of AT

concentration by the immunoturbidimetric method which based on the change in turbidity of a micro-particle suspension that is measured by photometry and the suspension is mixed with the test plasma whose AT antigen level is to be assayed. An antigen-antibody reaction takes place, leading to an agglutination of the latex micro-particles which induces an increase in turbidity of the reaction medium. This increase in turbidity is reflected by an increase in absorbance, the latter being measured photo-metrically.[13]

Statistical Analysis

Data of patients and participant were transformed and entered to the computer by using data base software with statistical utilities; The IBM statistical package for social sciences (SPSS), Chicago, USA, software version 20 was used for entering and analyzing the data. Level of significance (*P* value) was set at < 0.05 as cutoff point to be considered as significant difference. Results and findings presented in tables and figures with explanatory paragraphs.

Results

The hematological parameters of patients were significantly lower than that of the controls with a statistically significant differences with *P*- value <0.001 for Hb, total WBC count and reticulocyte percentage, and *P*= 0.005 regarding platelet count while there was no statistically significant difference found in absolute neutrophil count between both groups (Table 1).

The mean PT, APTT, fibrinogen and factor XII were significantly higher in patients than in control group with *P*-values of 0.008, 0.026, <0.001 and 0.001, respectively. While antithrombin was significantly lower in partient's group with *P*-value of <0.001 (Table 2**).**

Cardiovascular diseae (CVD) and vascular thrombosis was present in 62% of patients (31/50): deep venous thrombosis, cerebrovascular accident, angina, myocardial infarction, and heart failure were noticed in 20%, 16%, 16%, 8% and 8% respectively. Interestingly, 3 patients had 2 CVD at the same time.

The correlation between coagulation parameters and hemoglobin revealed a significant inverse relation between PT, APTT, fibrinogen and factor XII activity with Hb (*P*-values= 0.02, 0.001, 0.001 and 0.001 respectively), a significant direct relation between AT activity and Hb with *P*=0.001 (Table 3).

The comparison of the mean values of the coagulation parameters with CVD and vascular thrombosis had significantly prolonged mean PT, APTT, higher factor XII activity and lower anti-thrombin activity than the 19/50 patients with no CVD and vascular thrombosis as follows: the mean PT was 17.49 ± 6.06 versus 14.32±2.31 with *P*=0.013 (Figure 1); APTT 45.38 ± 23.90 versus 33.56 ± 9.51 with *P*=0.017 (Figure 2) and factor XII activity (%) 205.94 ± 110.99 versus 136.59 ± 65.82 with *P*=0.008 (Figure 3).

Table 1. The mean values of hematological parameters of studied groups (N=50)

	Patients (n=50)		Control (n=20)		P
	Mean	SD	Mean	SD	
Hb (g/dl)	9.67	1.46	14.05	1.02	< 0.001
Total WBC count (×109/L)	6.45	2.00	8.32	1.46	< 0.001
Absolute neutrophil count (×109/L)	4.17	1.69	4.70	1.02	0.11
Reticulocyte count (%)	1.35	0.37	1.88	0.21	<0.001
Platelet count (×109/L)	181.9	67.8	229.9	47.9	0.005

*p; pvalue is of significance if < 0.05, **SD: standard deviation.

***Stastical analysis was performed using t-test.**

Table 2. The Mean values of coagulation parameters of studied groups (N=50).

Coagulation parameters	Patients (n=50)		Control (n=20)		P
	Mean	SD	Mean	SD	
PT (seconds)	16.37	5.25	13.00	2.45	0.008
APTT (seconds)	41.36	20.84	30.58	5.16	0.026
Anti-thrombin activity (%)	48.03	23.12	78.55	14.60	<0.001
Plasma Fibrinogen g/l	4.28	1.70	2.75	0.73	<0.001
Factor XII activity	182.36	102.76	104.05	14.34	0.001

***Stastical analysis was performed using t-test.**

Table 3. The Pearson's correlation test between coagulation parameters and hemoglobin of the studied group (N=50)

Coagulation parameters		Hb (g/dl)
PT	r	-0.28
	P	0.02
APTT	r	-0.35
	P	0.001
Antithrombin activity	r	0.47
	P	0.001
Fibrinogen	r	-0.47
	P	0.001
Factor XII activity	r	-0.37
	P	0.001

* Correlation is significant at $P< 0.05$; minus sign indicated inverse correlation

***Stastical analysis was performed using Pearson's correlation test.**

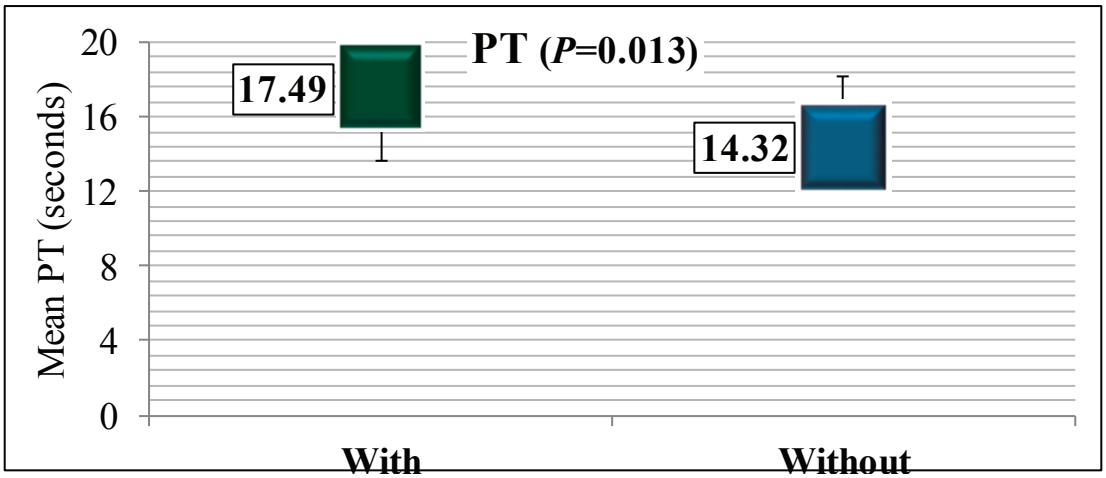

Figure 1. Box plot of the comparison of the mean values of PT of the patients with and without cardiovascular disorders and vascular thrombosis.

***Stastical analysis was performed using t-test.**

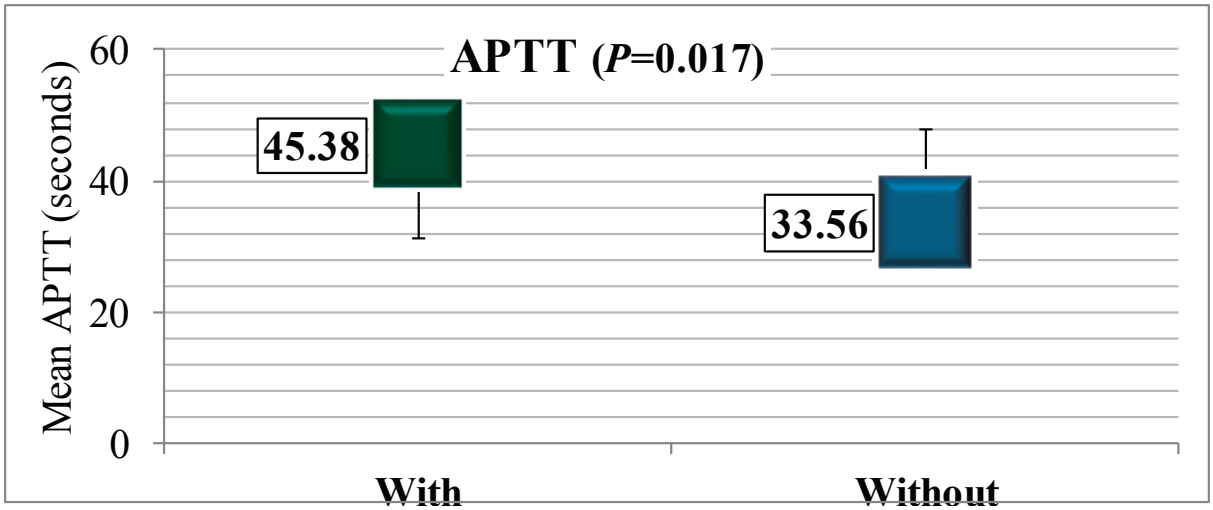

Figure 2. Box plot of the comparison of the mean values of APTT of the patients with and without cardiovascular disorders and vascular thrombosis.

***Stastical analysis was performed using t-test.**

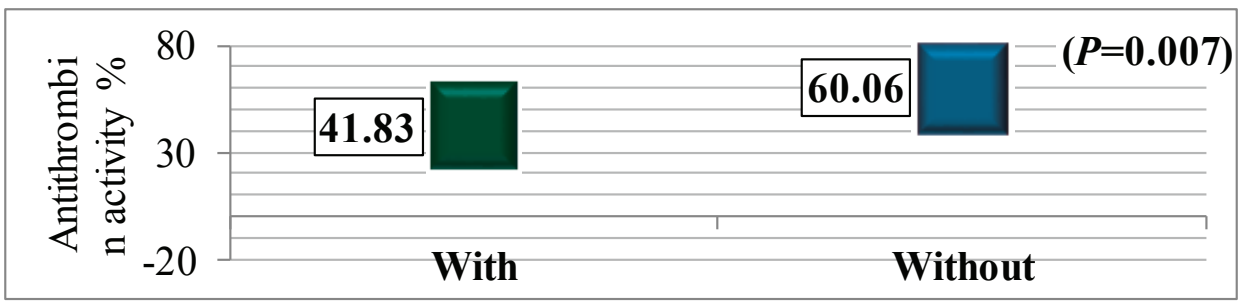

Figure 3. Box plot of the comparison of the mean values of anti-thrombin activity of patients with and without cardiovascular disorders and vascular thrombosis.

***Stastical analysis was performed using t-test.**

Discussion

In general ESRD patients not only experience an excess morbidity and mortality due to arteriosclerotic cardiovascular disease, but also thrombosis of the vascular access.[14] The ESRD patients exhibited numerous abnormalities of coagulation, fibrinolytic, and inhibitory proteins at multiple levels which involved in the pathogenesis of cardiovascular complications and vascular thrombosis in those patients. [15]

The current study found that the mean values of Hb level, total WBC count , reticulocyte percentage and platelets count of the 50 ESRD patients were significantly lower than their corresponding values of the controls (P<0.05). The mean value of the absolute neutrophil count was also lower in ESRD patients than controls in this study but the difference didn't reach the statistical significance. Anemia is a common complication of CKD which is associated with increased morbidity and mortality and this finding is supported by that of Al-Nozha et al.[16] The frequency of occurance of anemia correlates with the severity of Renal Failure, and is forming about 1% in stage 2 of CKD to almost 100% in ESRD or chronic HD patients. [17, 18]

Patients with ESRD in this study had a lower total leukocytes and differential counts when compared to that of control group and this finding was explained by Agarwal and Light who mentioned in their study that leukocyte counts change over time in people with CKD, and there was a decrease in lymphocytes and eosinophils. [19]

The current study found that PT and APTT were significantly elevated in patients as compared to controls (P<0.05). These findings were consistent with that reported by Alghythan and Al Saeed [20] who found that PT and APTT were significantly increased after-HD when compared to the before-HD levels and controls. The present study documented that mean plasma fibrinogen of ESRD patients was significantly higher than that of controls, (*P*<0.001), in line with the findings of a recent Egyptian study in 2013 which found a significant increase in plasma fibrinogen which might contribute to the hypercoagulability in ESRD patients.[21] The present study found that AT activity was significantly lower in ESRD patients than controls, (*P*<0.001). This finding agreed the findings of previous studies, Vaziri and his colleagues [23] which found that AT activity and concentration were significantly reduced in ESRD patients. Regarding factor XII activity, the current study reported that the mean factor XII activity of the ESRD patients was higher than that of controls (*P*=0.001). These findings were consistant with another study was conducted by Svensson et al involving ESRD patients on hemodialysis, it was reported that FXII activity in those patients was higher compared to normal subjects. [24]

Conclusions

End stage renal disease patients had significantly lower Hb levels (anemic), lower total WBC count, lower Reticulocyte count and lower platelets count as compared to healthy controls.Also we conclude that PT, APTT, plasma fibrinogen and factor XII activity levels were elevated in ESRD patients compared to healthy controls, while Anti-thrombin activity was reduced. Another conclusion we found in this study is that Higher levels of PT, APTT, Plasma Fibrinogen and Factor XII activity and the lower Anti-thrombin activity associated with higher prevalence of cardiovascular disorders and vascular thrombosis**.**

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pathology and all experiments were carried out in accordance with approved guidelines.

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