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Effect of Renal Failure and Hemodialysis on Some Procoagulant Aspects of Hemostasis

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ABSTRACT

Background: At present, the incidence of bleeding is CRF patients is apparently declining, whereas thrombotic complications have become the predominant causes of mortality. The objective of this study was to explore the effect of renal insufficiency and maintenance hemodialysis on some hemostatic parameters with possible role in increased risk of thrombosis.

Methods: This case control study was conducted on 30 patients with chronic renal failure under maintenance hemodialysis and 30 control subjects. An extended hemostatic assessment was performed including study of factor VIIa-rTF coagulant activity by clotting assay, factor VII Ag using ELISA technique, and assessment of monocyte procoagulant activity by measuring its tissue factor expression by flow cytometry before and immediately after dialysis.

Results: Thrombotic events were reported in 40% of patients. CRF patients showed increased monocyte tissue factor expression and increased activation of tissue factor pathway and factor VII Ag. Moreover the maintenance HD process significantly affected the levels of activated factor VII, monocyte tissue factor expression, and factor VII antigen with possible role in thrombotic events.

Conclusion: CRF patients under maintenance HD are at increased risk of thrombosis especially those with recurrent vascular access obstruction. An extended coagulation profile study including monocyte tissue factor expression, assay of (VIIa-rTF), factor VII antigen and protein C should be included in workup of these patients with possible role in considering prophylactic anticoagulant therapy.

Key Words: *Renal failure - Hemodialysis - Coagulation.*

INTRODUCTION

Although renal failure has classically been associated with a bleeding tendency, thrombotic events are common among patients with end stage renal disease (ESRD) under maintenance haemodialysis. Dialysis patients experience an exceedingly high incidence of thrombotic complications including cardiovascular disease, DVT, thrombotic cerebral accidents and vascular access-related complications **[1]**.

Studying of Hypercoagulability in ESRD patients and renal transplant recipients to identify patients at risk of thrombosis and evaluating strategies for prevention by Irish, (2004), recommended further studies to determine whether routine clinical screening for thrombophilic factors is justified **[2]**.

The objective of this study was to explore the effect of renal insufficiency and maintenance hemodialysis on some hemostatic parameters with possible role in increased risk of thrombosis.

SUBJECTS AND METHODS

This case control study was conducted on 30 patients with chronic renal failure under maintenance hemodialysis and 30 control subjects. The group under study was selected from patients on maintenance hemodialysis in Nephrology and Urology Unit (King Fahd Unit), Kasr Eleini Hospitals. Selection of the cases was randomly made with no segregation due to sex or age. All study participants were subjected to the following investigations:

1- Clinical Assessment included recording of age, sex, age of onset, clinical history with special emphasis on personal and family history of thrombosis, shunt operation and shunt failure, cardiac or cerebral accidents; intake of erythropoitien and anticoagulant drugs.

2- Routine laboratory tests included blood sugar, serum lipid profile, hemoglobin level, platelet count, kidney function and liver function tests.

3- Hemostatic screening: Hemostatic screening started with prothrombin time (PT), activated partial thromboplastin time (aPTT). Further coagulation assessment included assay of fibrinogen level, protein C (Quantitative determination of protein C by the synthetic chromogenic substrate method) **[3]**. In addition, protein S was measured (Quantitative determination of protein S by microlatex-mediated Immunoassay), using commercial kit from Diagnostica Stago (Cat. No. 00570), France **[4]**. Anticardiolipin IgG and IgM measurement by ELISA technique using commercial kit from Immco-Diagnostics, Inc. USA (Cat. No. 1118G for IgG and 1118M for IgM) **[5]**.

4- Specific coagulation parameter: Clotting assay of activated factor VII (Factor VIIarTF), using commercial kit from Diagnostica Stago (Cat. No. 00281), France **[6,7]**. Factor VII antigen (VII:Ag) Assay of factor VII antigen by enzyme immunoassay using commercial kit from Diagnostica Stago (Cat. No. 00241), France **[8]**. Monocyte tissue factor expression using Becton Dekenson "FACS Calibur" Flow cytometric analyse. Monocyte tissue factor expression assay included direct labelling of cells with FITC conjugated mouse antihuman CD14 antibodies and PE conjugated goat antihuman CD142 antibodies. Flow cytometric acquisition followed starting with isotypic control samples, followed by test samples. Two dot blots were created for each sample one representing FSC againest SSC and the other represents FL1 againest FL2. Analysis followed where Monocyte population was gated on forward scatter and side scatter blot. nonspecific fluorescence was excluded. Percent monocytes expressing TF were obtained as fluorescence in the upper right side of the dot blot FL1 againest FL2 **[9,10]**.

Statistical analysis:

The data were coded and entered using the statistical package SPSS version 11.01. The data were summarized using the mean and standard deviation (S.D.) for quantitative data and the frequency distribution for qualitative data.

The student's *t*-test was used to assess statistical differences between two groups of quantitative data, paired *t*-test was used to compare data before and after dialysis. ANOVA test was used to assess differences between multiple groups. As for the qualitative data, statistical associations were assessed using Chi-Square test.

RESULTS

Patient's characteristics are shown in Table (1). Frequency of shunt manipulation is illustrated in Table (2). The frequency distribution of other thrombotic events history (Deep venous thrombosis (DVT), ischemic heart disease is also summarized in Table (3). Comparison of routine coagulation parameters in CRF patients versus controls is summarized in (Table 4). Table (5) and Fig. (1-3) summarize the difference between procoagulant parameters in CRF patients compared to control subjects while Table (6), Figs. (4-6) show the effect of hemodialysis on the same parameters by comparing procoagulant parameters before versus immediately after hemodialysis session.

Comparison of procoagulant parameters in patients with history of thrombosis versus patients with no history of thrombosis is shown in (Table 7), calculated procoagulant level differences (before-After dialysis) were also compared in groups bases on history of thrombosis in Table 8 (Fig. 7).

Table (1): CRF patient characteristics and summary of history records.

Mean Age (years)	39.4±12.93
Mean age of onset (years)	7.6±4.69
Mean duration of dialysis (years)	6.4±3.9
Male / Female ratio	13/17
Family history of thrombosis	1/30 (3.1%)
Smoking	3/30 (10%)
Conraceptive pills	0/30 (0%)
Hypercholesterlemia	3/30 (10%)
DM	6/30 (20%)
Hypertension	8/30 (26.6%)
EPO Therapy	9/30 (30%)

Table (2): The Frequencies of shunt manipulation of the CRF patients under maintenance hemodialysis.

Number of shunt manipulation	Frequency	Percent
1	18	60.0
2	7	23.3
3	4	13.3
5	1	3.3

Table (3): Frequency distribution of thrombotic events among the CRF patients under maintenance hemodialysis.

Thrombotic events	Number	Percent
Deep venous thrombosis	3	10
Ischemic heart disease	2	6.6
Failure of shunt operation	12	40

Table (4): Comparison of coagulation parameters of the CRF patient group versus the control group under study.

Type of analysis	Patients Mean ± SD	Control Mean ± SD	*p* value
Prothrombin conc. (%)	92.8±9.38	97.9±3.67	0.173
Fibrinogen (mg/dl)	317.7±52.48	299.4±26.35	0.350
Protein C (%)	66.3±20.54	102.0±21.33	0.001/HS
Protein S (%)	112.1±19.51	100.8±13.91	0.304
Platelet count (x 1000/mm^3)	187.1±55.19	210.4±38.95	0.273

p value >0.05 is non significant (NS).
p value <0.05 is significant (S).
p value <0.01 is highly significant (HS).

Table (5): Effect of renal impairment on activated factor VII (VIIa-rTF complex), Monocyte TF Expression and Factor VII Antigen.

Sample status	VIIa-rTF level in mU/ml Mean ± S.D.	*p* value
Patients before dialysis Control	200.9±49.39 75.3±5.44	< 0.001/HS
	Monocyte TF Expression % Mean ± S.D.	
Patients before dialysis Control	5.2±4.14 1.5±1.08	< 0.001/HS
	Factor VII:Ag % Mean ± S.D.	
Patients before dialysis Control	108.7±10.96 101.3±13.80	NS

Table (6): Effect of hemodialysis on activated factor VII (VIIa-rTF complex), Monocyte TF Expression and Factor VII Antigen.

Sample status	VIIa-rTF level in mU/ml Mean ± S.D.	*p* value
Patients before dialysis Patients after dialysis	200.9±49.39 273.1±52.55	<0.001/HS
	Monocyte TF Expression % Mean ± S.D.	
Patients before dialysis Patients after dialysis	5.2±4.14 13.6±11.09	<0.001/HS
	Factor VII:Ag % Mean ± S.D.	
Patients before dialysis Patients after dialysis	108.7±10.96 116.2±11.18	<0.01/HS

Table (7): Specific procoagulant parameters measured after dialysis in patients with history of thrombotic events versus patients with no thrombotic events.

Procoagulant parameter	Patients with thrombotic events Mean ± SD	Patients with no thrombotic events Mean ± SD	*p* value
VIIa-rTF level (mU/ml)	289.4±51.05	260.7±51.66	NS
Monocyte TF Expression (percent)	14.4±12.38	13.0±10.34	NS
VII:Ag (%)	118.5±9.28	114.5±12.42	NS

Table (8): Difference between specific procoagulant parameters before and after dialysis (Δ change) in patients with thrombotic events versus patients with no thrombotic events.

Procoagulant parameter	Patients with thrombotic events Mean ± SD	Patients with no thrombotic events Mean ± SD	*p* value
VIIa-rTF level (mU/ml)	93.0±56.95	56.4±41.55	<0.05/S
Δ Monocyte TF Expression (percent)	9.5±11.43	8.3±9.19	NS
Δ VII:Ag (%)	9.2±10.06	6.3±12.26	NS

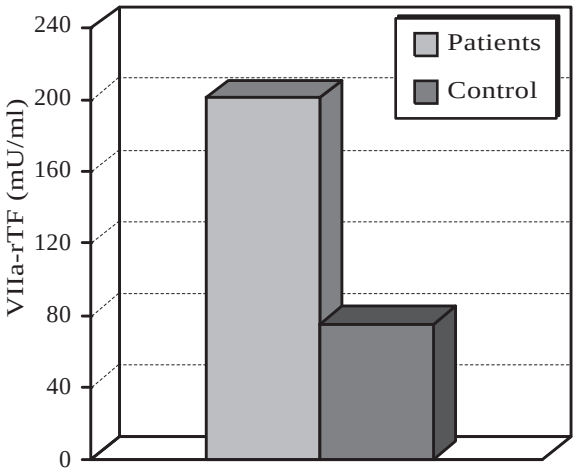

Fig. (1): Activated factor VII mean value before dialysis among CRF patients under HD versus control group.

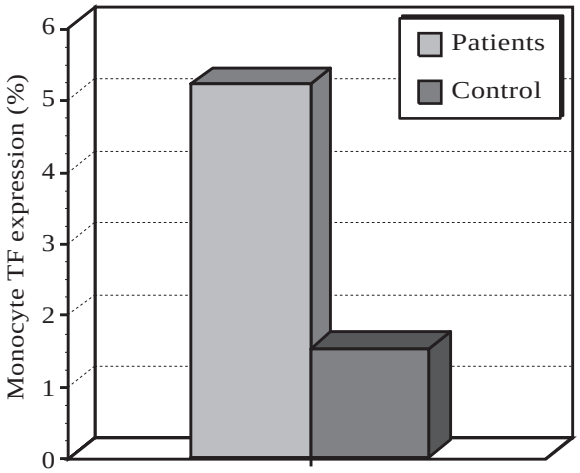

Fig. (2): Monocyte tissue factor expression mean value before dialysis among CRF patients under HD versus control group.

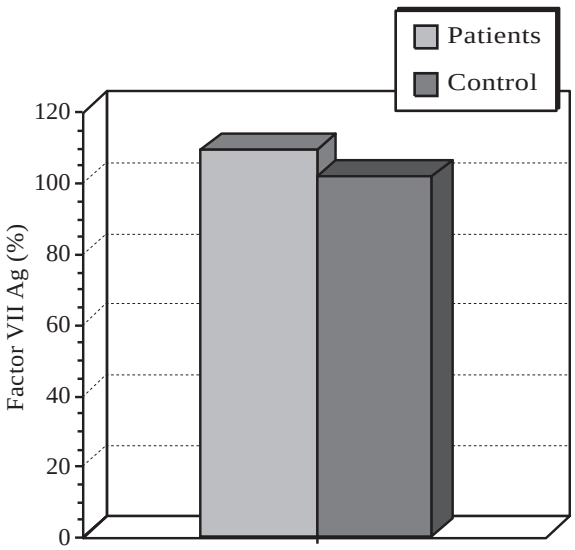

Fig. (3): Factor VII antigen mean value before dialysis among CRF patients under HD versus control group.

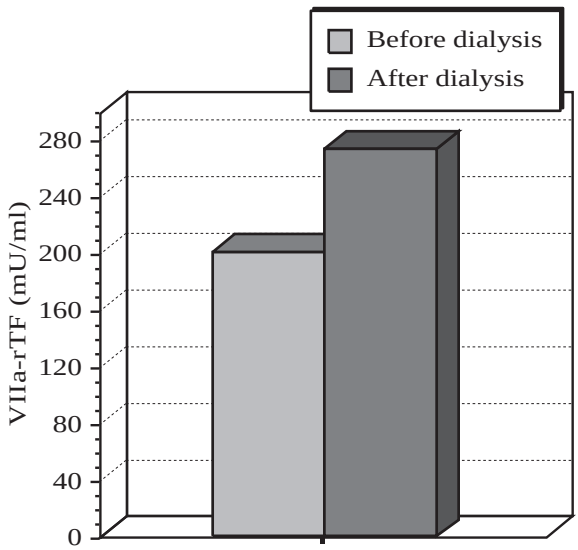

Fig. (4): Activated factor VII mean value before and after dialysis among CRF patients.

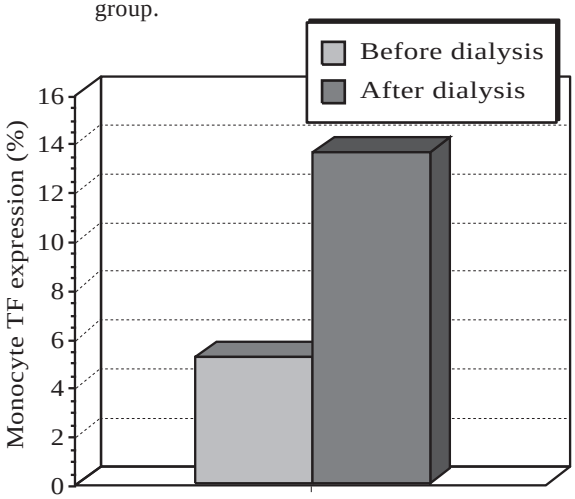

Fig. (5): Monocyte tissue factor expression mean value before and after dialysis among CRF patients.

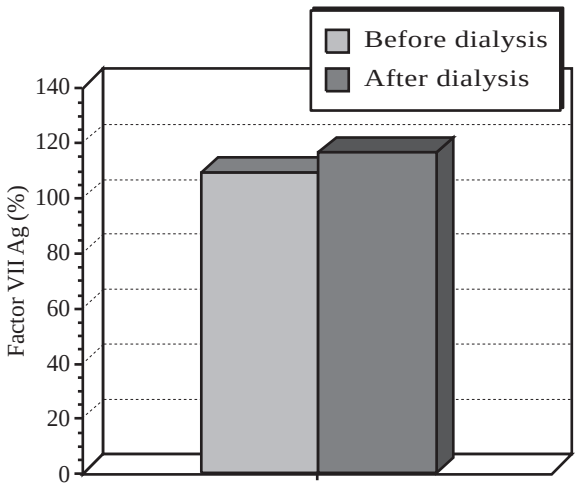

Fig. (6): Factor VII antigen mean value before and after dialysis among CRF patients.

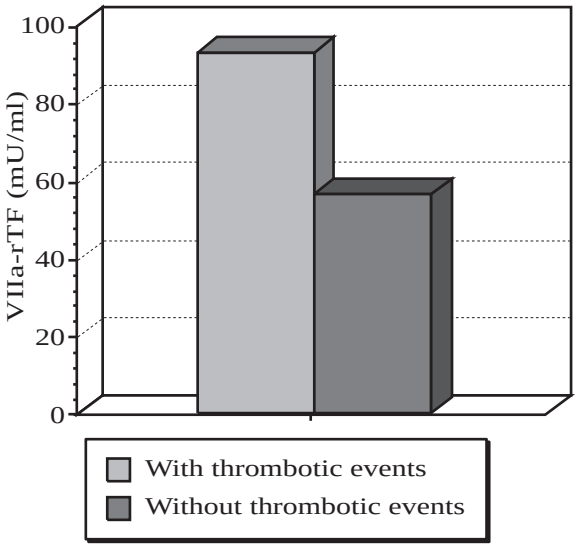

Fig. (7): Difference between activated factor VII mean value before and after dialysis in patients with thrombotic events versus patients with no thrombotic events.

DISCUSSION

At present, the incidence of bleeding is CRF patients is apparently declining, whereas thrombotic complications have become the predominant causes of mortality. The most important suggested determinants of the pathogenesis of the prothrombotic state in uremia are increased levels of clotting factors and decreased levels of clotting inhibitors, diminished fibrinolytic activity, hyperfibrinogenemia, and platelet hyper-aggregability **[11]**. Endothelial activation and endothelial injury in uremia can also induce TF exposure on its surface by a wide range of events, from inflammatory cytokines to mechanical arterial injury.

The objective of this study was to explore the effect of renal insufficiency and maintenance hemodialysis on hemostatic parameters with possible role in increased risk of thrombosis.

In the current study twelve CRF patients needed more than one shunt manipulation (40%), 3 (10%) of them had past history of DVT and 2 (6.6%) had past history of ischemic heart disease; where 18 CRF patients needed only one shunt operation and no past history of thrombosis (Tables 2,3). This finding suggest CRF and maintenance hemodialysis as a potential risk factor for thrombosis (Odds 19.33 CI 2.24-432.34 $p<0.01$).

Comparison of the coagulation parameters in ESRD patients versus controls showed no statistically significant difference in prothrombin concentration, fibrinogen level, Protein S or platelet count (Table 4). However, Protein C activity was significantly lower in ESRD patients compared to controls. Fifty eight percent of the patient group showed deficiency in protein C activity. These results agreed with the finding of Nampoory et al. (2003) who reported the presence of deficiency in protein C in end stage renal disease ESRD patients. When parameters were compared between patients with and without vascular access thrombosis (VAT) episodes. PC levels were significantly lower in those who experienced VAT. It was also reported that these deficiencies were completely corrected after renal transplantation **[12]**.

Values of VIIa-rTF in CRF patients under maintenance hemodialysis was significantly elevated compared to control group value. The same was observed comparing monocyte tissue factor expression percentage in patients versus controls where the mean value of the patient group showed significantly higher value compared to control value (Table 5, Figs. 1,2). Yu et al. (2003) confirmed the obtained results in this study when reported that elevated baseline levels of tissue factor in hemodialysis patients, compared to normal reference ranges **[13]**. In addition, Mercier et al. (2001) reported similar results of the current study showing increased values of FVIIa, VIIa/FVIIAg ratio, soluble tissue factor, and tissue factor monocyte procoagulant activity, along all the healthy control group, nondialyzed CRF group, and CRF on hemodialysis group in ascending manner (1). Level of factor VII antigen in plasma showed no significant difference in the patient group compared to control group (Table 5 and Fig. 3). This result agreed with the finding of Mercier et al. (2001) who did not detect any significant differences between factor VII antigen in CRF patients before dialysis and control group (1). The difference elicited comparing studied hemostatic parameters in ESRD patients versus controls suggest CRF as a hypercoagulable state.

To evaluate direct effect of hemodialysis on the same hemostatic parameters, comparative study of these parameters before and immediately after dialysis was performed. Activated

factor VII was significantly increased after dialysis compared to before dialysis values. (Table 6 and Fig. 4). The level of factor VII antigen in plasma was significantly elevated in patients immediately after dialysis compared to levels before dialysis. Procoagulant activity of monocytes, measured as increased expression of tissue factor (CD 142) on the surface of monocytes, showed a highly significant increase in TF expression immediately after dialysis compared to before dialysis values (Table 6 and Figs. 5,6). Theses results are in accordance with the data reported by Camici et al. (1997) who evaluated the behavior of factor VIIa before and after dialytic treatment in patients on maintenance hemodialysis and observed significant increase of factor VIIa after hemodialysis compared with before dialysis **[14]**.

Also Mercier et al. (2001) confirmed the result obtained from this study when they investigated FVIIa, soluble tissue factor, and monocyte procoagulant activity (represented by tissue factor expression of monocytes) before and immediately after dialysis and found that dialysis induced a significant increase of FVIIa, soluble tissue factor, and monocyte procoagulant activity. Fang et al. (2004) also showed enhanced levels of coagulation factor VII in chronic renal failure which might be aggravated by hemodialysis **[15]**.

The specific coagulation parameters performed in this study reflected abnormal coagulation parameters of CRF group on maintenance hemodialysis. Such abnormalities were more evident immediately after hemodialysis.

To evaluate potential role of studied parameters in thrombophilic events, patients were divided into 2 groups, group 1 with history of thrombosis and group 2 with no history of thrombosis. Procoagulant parameters measured before and after dialysis of these two groups of patients were statistically analyzed and no significant differences were observed (Table 7). The difference of the procoagulant parameters between before and after dialysis was then calculated and the new calculated data were statistically analyzed to detect the differences between the two above-mentioned groups. Factor VIIa change was the only parameter that showed significant difference between the two groups with or without history of thrombosis. VIIa-rTF change (Δ VIIa-rTF) was significantly higher in patients with history of thrombosis compared to patients with no history of thrombosis (Table 8, Fig. 7), a finding that can point to possible role of factor VII in dialysis induced enhanced coagulation.

LeSar et al. (1999) reported that warfarin therapy should be instituted when hypercoagulable states are found in CRF patients under maintenance hemodialysis, unless otherwise contraindicated, and prothrombin INR maintained at 2.7-3.0 to decrease morbidity and frequency of graft thrombosis **[16]**.

The study concluded that, chronic renal failure patients are more liable to thrombotic complications reflected by decreased protein C activity and increased procoagulant activity of monocytes and increased activation of tissue factor pathway and factor VII activation. Moreover the maintenance hemodialysis process increases the liability to thrombosis. Hypercoagulability has been a major etiologic factor in vascular access thrombosis which is a frequent cause of morbidity in patients on hemodialysis. Evaluation of CRF patients under maintenance hemodialysis should include an extended thrombophilia profile specially in patients with recurrent vascular access thrombosis (VAT). Thrombotic risk factors whether inherited or acquired should be investigated in all CRF patients as a step to identify high-risk patients and to consider anticoagulant therapy.

REFERENCES

1- Mercier E, Branger B, Vecina F, Al-Sabadani B, Berlan J, Dauzat M, Fourcade J, Gris J-C. Correspondence. Jean-Christophe Gris. Tissue factor coagulation pathway and blood cells activation state in renal insufficiency. The Hematology Journal. 2001, Vol. 2, No. 1 pp.18-25.

2- Irish A. Hypercoagulability in renal transplant recipients. Identifying patients at risk of renal allograft thrombosis and evaluating strategies for prevention. Am J Cardiovasc Drugs. 2004, 4 (3): 139-49.

3- Guglielmone HA, Vides MA. A novel functional assay of protein C in human plasma and its comparison with amidolytic and anticoagulant assays. Thromb Haemost. 1992, Jan 23; 67 (1): 46-9.

4- Gris JC, Toulon P, Brun S, Maugand C, Sarlat C, Schved JF, Berlan J. The relationship between plasma microparticles, protein S and anticardiolipin antibodies in patients with human immunodeficiency virus infection. Thromb Haemost. 1996, 76 (1): 38-45.

5- Harris EN, Pierangeli SS, Gharavi AE. Diagnosis of the antiphospholipid syndrome: A proposal for use of laboratory tests. 1988, Lupus 7 Suppl 2: S144-8.

6- Morrissey JH, Macik BG, Neuenschwander PF, Comp PC. Quantitation of activated factor VII levels in plasma using a tissue factor mutant selectively deficient in promoting factor VII activation. Blood. 1993, 81: 734-44.

7- Bourgeat P, Jasmin P, Migaud-Fressart M, Martinoli JL. Direct measurement of activated F.VII in plasma. development of an original assay. Thromb Haemostasis. 1995, 73 (6): 1204.

8- Broze GJ, Majerus PW. Purification and properties of human coagulation factor VII. J Biol Chem. 1980, 255: 1242-1247.

9- Leatham EW, Bath PM, Tooze JA, Camm AJ. Increased monocyte tissue factor expression in coronary disease. Br Heart J. 1995 Jan, 73 (1): 10-3.

10- Vickers J, Russwurm S, Dohrn B, Portele T, Spangenberg P, Reinhart K, Losche W. Monocyte tissue factor (CD 142) and Mac-1 (CD 11b) are increased in septic patients. Thromb Haemost. 1998 Jun, 79 (6): 1219-20.

11- Malyszko JS, Malyszko J, Pawlak K, Pawlak D, Buczko W, Mysliwiec M. Importance of serotonergic mechanisms in the thrombotic complications in hemodialyzed patients treated with erythropoietin. Nephron. 2000, 84: 305-311.

12- Nampoory MR, Das KC, Johny KV, Al-Hilali N, Abraham M, Easow S, Saed T, Al-Muzeirei IA, Sugathan TN, Al Mousawi M. Hypercoagulability, a serious problem in patients with ESRD on maintenance hemodialysis, and its correction after kidney transplantation. Am J Kidney Dis. 2003, 42 (4): 797-805.

13- Yu A, Egberg N, Jacobson SH. Haemostatic complications in hemodialysis patients. Effect of type of vascular access and dialysis filter. Scand J Clin Lab Invest. 2003, 63 (2): 127-33.

14- Camici M, Evangelisti L, Balestri P, Cioni L, Rindi P, Sagripanti A, Meriggioli M, Giordani R. Coagulation activation in extracorporeal hemodialysis. Int J Artif Organs. 1997 Mar, 20 (3): 163-5.

15- Fang J, Xia LH, Wei WN, Song SJ. Coagulation Factor VII levels in uremic patients and theirs influence factors. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2004 Dec, 12 (6): 730-2.

16- LeSar CJ, Merrick HW, Smith MR. Thrombotic complications resulting from hypercoagulable states in chronic hemodialysis vascular access. J Am Coll Surg. 1999, 189 (1): 73-81.