Hyperthrombotic State in Patients on Different Hemodialysis Membranes

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Abstract

Background. The patients on hemodialysis (HD) suffer from permanent hyperthrombotic condition. The aim of the study was to estimate hyperthrombotic state in HD patients regarding different HD membranes.

Methods. The biological activity of von Willebrand factor (vWf) was determined in 104 patients (64 males, 40 females, at age of 42±12 years) using the platelet agglutination in presence of ristocetin (Dade Behring, Germany) on different HD membranes: Cuprophan (CU) (n=30), Polymethylmethacrylate (PMMA) (n=30), Hemophan (HE) (n=24) and Polysulfone (PS) (n=20). Fibrinolysis activators (routine method with standardized fibrin plates) and prothrombine time (PT) (Dade Behring, Germany) were examined in 43 patients (28 males and 15 females at age of 40± 9 years) who were on: HE (n=24) and PS (n=19). In 40 patients (25 males and 15 females at age of 41±11 years) who were on HE (n=17), PS (n=11) and CU (n=12), nitric oxide (NO) level was examined (OXIS, USA). Patients' groups were compared to 30 healthy control subjects who were age and sex matched.

Results. The biological activity of vWf showed increased values even before the HD session in all examined groups with further increase after HD. The most significant increase of vWf activity during HD was observed in patients on PMMA membrane, 128±32% vs. 234±28%, ($p<0.001$) before and after HD, respectively. There was no significant increase of vWf activity in patients on PS membrane, 133±31% before, vs. 140±30% after HD. PT was shortened after HD in patients on the two examined membranes, for HE membrane from 10±1.9 to 9±0.6 seconds and for PS from 10±0.7 to 8.9± 0.6 seconds ($p<0.05$). Compared to controls, PT was shortened in HD patients before the HD. There was no significant difference in fibrinolytic response between patient' groups and controls, being at similar levels before and after HD. No statistical significance for NO was found before and after HD session in none of the patients groups on different HD membranes, but the patients had much higher value than the controls ($p<0.001$).

Conclusions. In conclusion, there was hemostasis activation in HD patients and the best membrane profile from all examined membranes was shown to be the PS type since there were only minimal changes in the examined parameters after HD session.

Key words: Von Willebrand factor, Hemostasis, Nitric oxide, Hemodialysis membrane

Introduction

The hyperthrombotic condition is common in patients undergoing hemodialysis (HD) with complications that contribute to morbidity and mortality. This condition may be caused by HD membrane bioincompatibility, its flow design and endothelial dysfunction (1). Furthermore, hyperthrombotic condition either originates on a ground of hypertensive, diabetic and inflammatory vasculopathies or occurs secondarily from coagulopathies due to proteinuria in the glomerular disease. A role of uremia promoting the hypercoagulable state by itself has been proposed (2, 3). The important role was assigned to the protein layer formation on HD membrane, followed by the platelet activation (4). Von Willebrand factor (vWf) as an important coagulation factor concerning factor VIII, enables adhesion and aggregation of platelets due to impaired blood vessel wall and due to an artificial surface contact, respectively. Beside the artificial surface action related to HD membrane, the turbulent blood flow in these patients has also an influence to the coagulation process (5). Simultaneosly, the fibrinolytic process (6) as well as complement and kalikrein-kinin systems, may be activated during HD session. Regarding the hemostasis imbalance, involvement of nitric oxide (NO) is obvious due to its action as a proinflamatory agent and its role in pathogenesis of many disorders as glomerulonephritis, arthritis and vasculities (7,8).

The aim of the study was to evaluate causes (regarding hemostasis parameters and endothelial derived substance) for prothrombotic state in HD patients dependent on different HD membranes that may contribute to the most appropriate HD membrane selected.

Patients and methods

A cohort of 104 HD patients (64 males and 40 females, at age of 42±12 years) were examined for the biological activity of vWf regarding different HD membranes: Cuprophan (CU) (n=30), Polymethylmethacrylate (PMMA) (n=30), Hemophan (HE) (n=24) and Polysulfone (PS) (n=20). The BC von Willebrand Reagent for determination of the ristocetin cofactor activity of vWf in human plasma using the platelet agglutination method was used (Dade Behring, Germany). It measures vWf (ristocetin cofactor) from the sample causes agglutination of stabilized platelets (provided by the von Willebrand Reagent) in the presence of ristocetin. The resulting agglutination decreases the turbidity of the reaction suspension. A coagulation instrument measures the change in absorbance and automatically determines the ristocetin cofactor activity in the sample as a percentage (%)

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of the normal value. Prothrombin time (PT) and fibrinolysis activators were examined in 43 HD patients (28 males and 15 females at age of 40±9 years) on: HE (n=24) and PS (n=19). A combination assay kit by Dade Behring, Germany was used for determination of PT and measured on the Behring Coagulation Timer.

Results for PT time are presented in seconds. Fibrinolysis activators were examined using the routine *in house* method with standardized fibrin plates modified by the Unit of Hemostasis, from the Institute of Blood Transfusion, Skopje, Macedonia. Results for the fibrinolysis activators are expressed as percentage (%). In 40 patients (25 males and 15 females at age of 41±11 years) nitric oxide (NO) level was examined on: HE (n=17), PS (n=11) and CU (n=12). For NO determination, microplate enzymatic method based on assay kit from OXIS, USA was used. A number of 30 healthy volunteers as age and sex matched controls were examined and compared to the HD patients.

The patients in our study were recruited from the Hemodialysis Unit at the Department of Nephrology, University Clinical Center in Skopje, Macedonia. The dialysis duration in all HD patients ranged between 5 to 10 years (8±3 years). They were undergoing a standard bicarbonate HD for 4 hours, 3 times per week. The patients were stratified according to the type of HD membrane they were exposed for the last 2 months. The patients with hypertension, diabetes mellitus and/or proinflamatory vasculopathies were appropriately matched within all examined groups. The blood sample was drawn from HD system tubes, before and after HD sessions.

Statistical analysis

The Student's t-test was used for statistical analysis, and p value less than 0.05 was considered significant. The results were expressed as mean value±SD.

Results

The biological activity of vWf in the dialysis patients showed increased values even before dialysis compared to the control group, 101±35 % (p<0.05). Moreover, the biological activity of vWf showed an additional increase after HD in all examined patients using different type of membranes. The most significant increase of vWf activity after HD (234±28 %) was observed in patients on PMMA type of membrane (p<0.001) but no statistical increase of vWf activity was noticed in patients on PS membrane (Table 1, Figure I).

Table 1. Biological activity of von willebrand factor related to different hd membranes before and after hd

HD membrane type	n	Biological activity of vWf (%)		p
		Before HD	After HD	
CU	30	132 ± 47	157 ± 42	<0.05
PMMA	30	128 ± 32	234 ± 28	<0.001
HE	24	128 ± 24	150 ± 25	<0.01
PS	20	133 ± 31	140 ± 30	ns
Controls	30	101± 35		*

ns, not significant, *****, p<0.05 compared to all patients' groups

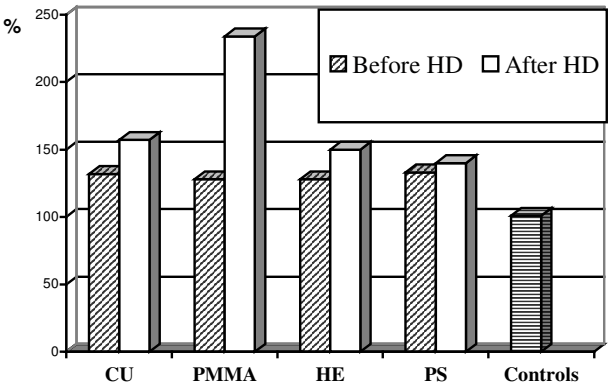

Fig. 1 - Biological activity of vWf related to different HD membranes before and after the HD session

All patients showed shortened PT even before HD session compared to the controls (11±0.5 seconds) (p<0.05). In addition, there was also a significantly shortened PT after HD session in patients related to the both examined membranes, HE and PS membrane (p<0.05) (Table 2, Figure II).

Table 2. Prothrombin time (in seconds) related to different hd membranes before and after hd

HD membranes		Protrombin time (sec.)	p
HE	Before	10 ± 1.9	<0.05
(n=24)	After	9 ± 0.6	
PS	Before	10 ± 0.7	<0.05
(n=19)	After	**8.9 ± 0.6**	
Controls (n=30)		**11** ± 0.5*	

*****, p<0.05 compared to both patients' groups

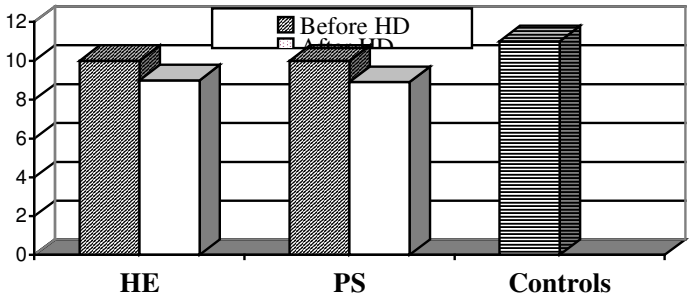

Fig. 2 - Prothrombin time (sec.) related to different HD membranes before and after the HD session

Neither significant difference in fibrinolytic response between patients and controls nor a significant difference in fibrinolytic response before and after HD regarding HE and PS membrane was found (Table 3, Figure III).

Table 3. Fibrinolysis activators (%) related to different hd membranes before and after hd

HD membranes		Fibrinolysis (%)	p
HE (n=31)	Before	109 ± 48	ns
	After	115 ± 43	
PS (n=24)	Before	90 ± 26	ns
	After	85 ± 25	
Controls (n=30)		90 ± 20	*

ns, not significant, *, not significant compared to any HD patients' group

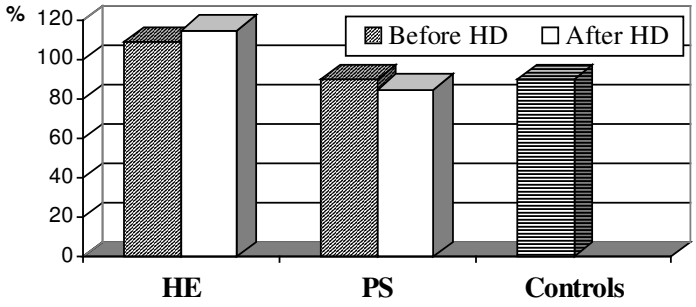

Fig. 3 – Fibrinolytic activators (%) related to different HD membranes before and after the HD session

No statistical significance for NO was found before and after HD session in patients on different HD membranes, although the patient's value was more than twice higher than in the controls ($p<0.001$) (Table 4, Figure IV).

Table 4. Nitric oxide level related to different hd membranes before and after hd

HD membranes type		n	NO (µmol/l)	p
PS	Before	11	271 ± 59	ns
	After		282 ± 48	
HE	Before	17	238 ± 27	ns
	After		215 ± 59	
CU	Before	12	272 ± 32	ns
	After		271 ± 49	
Controls		30	57 ± 38	*

ns, not significant , *, $p<0.001$, compared to all patients' groups

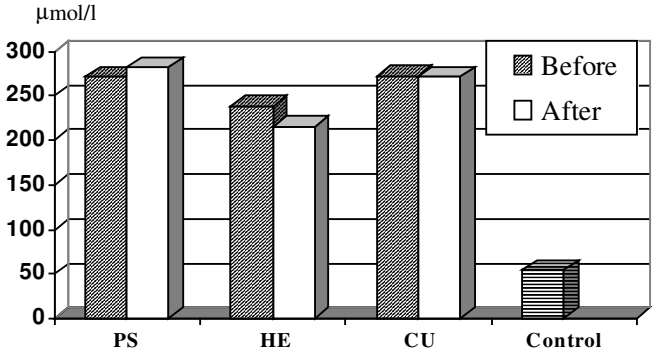

Fig. 4 – Nitric oxide level related to different HD membranes before and after the HD session

Discussion

At present, there is a great improvement in the biocompatibility condition of HD membranes due to their physicochemical quality, confirmed by some recent reports showing lower level of complement and leukocyte activation (9). However, some synthetic HD membranes (PMMA) still show rapid cell activation for platelets and leucocytes and increased production of reactive oxygen species (ROS) (10, 11). A higher level of vWf biological activity in dialysis patients was noticed even before HD session compared to controls, which might be explained by the hyperthrombotic state in this group of patients. Due to its elevated level after HD session, we found similar behavior of all examined HD membranes. However, PS membrane showed somewhat better biocompatibility due to the similar levels of vWf biological activity before and after HD session. Because of the prolonged contact to the artificial surface and subsequent platelets and leukocytes activation dialysis patients are situated to be in a sustained procoagulant state (12, 13). Our results are in line with the increased vWf activation regarding CU membrane reported by Schmidtt et al. (14) and also with the reported promotion of an increased hypercoagulability in the treatment with cellulose and synthetic HD membranes by Ishii et al. (15). According to the results of Nakamura Y et al. (3), an activated fibrinolytic response was found in these patients. In contrast, our results did not show an activation of fibrinolysis. In addition and apart from the other HD membranes, for PS membrane we did not find significant changes for both parameters, neither for fibrinolytic activation, nor for vWf activity. Prothrombin time in our patients was found to be shortened even before HD session which might be related to the hyperthrombotic state. Furthermore, the PT after HD session was even shorter probably caused by a cascade of changes in the normal coagulation processes associated with the close contact of the blood and the artificial surface of the membrane. PT was significantly shortened in both examined HD membranes, HE and PS, respectively. Due to the shortened PT in these patients, it is obvious that activation of extrinsic system occurred, provoked by cell activation (macrophages, polymorphonuclear cells) and appearance of released procoagulant agents as: factor VII and tissue factor as reported by Hoffbrand et al. (16). As regard to our results before and after HD session, there was no a membrane impact on NO molecule, although an increased NO level in HD patients compared to the controls was found. There are some reports that demonstrate an increased levels of NO in such patients. According to Yokokawa et al. (17), high nitrite and nitrate metabolites of NO were found after heparin administration *in vivo*. The basal NO production can be up regulated by physical forces such as shear stress as well as by several receptors-operating transmitters and hormones (18). Treatment with erytropoietin and increased blood viscosity due to an increased hematocrit might increased the shear stress resulting in an increased NO formation in renal patients (19). As for the increased NO level in HD patients, there might be some other explanations such as: cytokine appearance (TNF, IFN, IL-1, IL-6, etc.) from the activated polymorphonuclear leucocytes, macrophages and platelets; microbial products (LPS, peptidoglycan, etc.); and immune complexes. The NO molecule might exert a beneficial, but at the same time and harmful effect. Regarding its physiological

values, it is a vasodilatative and antiaggregation agent but extremely high NO values in HD patients may damage the endothelial surface via production of peroxynitrite, compound formed by NO and superoxide radical (20).

In conclusion, all examined HD membranes showed hemostatic activation in HD patients due to the increased vWf biological activity, shortened PT from one side and not changed fibrinolytic activity on the other side. Therefore the fibrinolitic activity might have not been sufficient to overcome the changes of increased vWf biological activity and shortened PT time which in turn could lead to the hypertrombotic state in these patients. Endothelial surface may be damaged by the harmful effect of the excessive NO levels via peroxynitrite formation, followed by an endothelial dysfunction, which also may contribute to the hyperthrombotic state. The best membrane profile from all examined membranes was shown to be the PS type since there were only minimal changes in the examined parameters after HD session.

References

1. Sperschneider H, Deppisch R, Beck W, Wolf H, Stein G. Impact of membrane choice and blood flow pattern on coagulation and heparin requirement – potential consequences on lipid concentrations. *Nephrol Dial Transplant* 1997; 12: 2638-46.
2. Ambuhl PM, Wutrich R, Korte W, Schmid L, Krapf R. Plasma hypercoagulability in haemodialysis patients: impact of dialysis and anticoagulation. *Nephrol Dial Transplant* 1997; 12: 2355-64.
3. Nakamura Y, Tomura S, Tachibana K, Chida Y, Marumov F. Enhanced fibrinolytic activity during a course of hemodialysis. *Clinical Nephrol* 1992; 38(2): 90-6.
4. Notohamiprodjo M, Andrassy K, Bommer J, Ridz E. Dialysis membranes and coagulation system. *Blood Purif* 1985; 4: 130-41.
5. Mohammad SF. Extracorporeal thrombogenesis: mechanisms and prevention. In: Replacement of renal Function by Dialysis; Boston, Kluwer Academic Publishers, 1989: 229.
6. Speiser W, Voita J, Korninger C, Kirchheimer JC, Zazgornik J, Binder BR. Enhanced fybrinolysis caused by tissue plasminogen activator release in hemodialysis. *Kidney Int* 1987; 32: 280-3.
7. Weinberg JB, Granger DL, Pisetsky DS, at al. The role of nitric oxide in pathogenesis of spontaneous murine autoimmune disease: increased nitric oxide production and nitric oxide synthase expression in MRL-lpr mice and reduction of spontaneous glomerulonephritis by orally administered NG-monomethyl-L-arginine. *J Exp Med* 1994; 179: 651-60.
8. Oates JC, Ruiz P, Alexander A, Pippen AMM, Gilkeson GS. Effect of late modulation of nitric oxide production on murine lupus. *Clin Immunol Immunopathol* 1997; 83: 86-92.
9. Hoenich NA, Woffindin C, Ronco C. Haemodialysers and associated devices. In: Replacement of renal function by dialysis. Kluwer Acadamic Publishers, Dordrecht, 1996: 189-230.
10. Stuard C, Bonomini M, Settefrati N, Albetrazzi A. Platelet neutrophil interactions during hemodialysis: A proposed biocompatibility approach. *Int J Artif Organs* 1998; 21 (2): 75-82.
11. Bosch T, Schimdt B, Samtleben W, Gurland HJ. Biocompatibility and clinical performance of a new modified cellulose membrane. *Clin Nephrol* 1986; 26 (1): S22-9.
12. Sagripanti A, Cupisti A, Baicchi U, Ferdehini M, Morelli E, Barsoti G. Plasma parameters of the prothrombotic state in chronic uremia. *Nephron* 1993; 63: 273-8.
13. Gordge MP, Leaker BR, Rylance PB, Neild GH. Hemostatic activation and proteinuria as factors in the progression of chronic renal failure. *Nephrol Dial Transplant* 1991; 6: 21-6.
14. Schmidtt GW, Moake J, Rudy CK, Vicks SL, Hamburger RJ. Alteration in hemostatic parameters during hemodialysis with dialysers of different composition and flow design. *Am J of Med* 1987; 83: 411-18.
15. Ishii Y, Yano S, Kanai H, Maezawa A, Tsuchida A, Wakamatsu R, Naruse T. Evaluation of blood coagulation-fibrinolysis system in patients receiving chronic hemodialysis. *Nephron* 1997; 77 (4): 486-7
16. Hoffbrand AV, Pettit JE. Platelets, blood coagulation and haemostasis. In: Essential Haematology. Blackwell Scientific Publications 1993; 299-317.
17. Yokokawa K, Tahara H, Kohno M, Mandal AK, Yanagisawa M, Takeda T. Heparin regulates endothelin trough endothelium-derived nitric oxide in human endothelial cells. *J Clin Invest* 1993; 92: 2080-5.
18. Luscher TF, Nell G, The endothelium as a regulator of vascular tone and growth. In: The endothelium in cardiovascular disease. Luscher TF Springer Verlag Berlin Heidelberg New York 1995; 1-24.
19. Linde T, Sandhagen B, Danielson BG, Wikstrom I. Impaired erythrocyte fluidity during treatment of renal anemia with erytropoietin. *J Inter Med* 1992; 231: 601-6.
20. Beckman JS, Beckman TW, Chen J at al. Apparent hydroxyl radical production by peroxynitrite: implication for endothelial injury from nitric oxide and superoxide anion. *Proc Natl Acad Sci USA* 1990; 87:1620-24.