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Original Article

Plasma Fibronectin A Potential Risk Factor For Extracorporeal Or Vascular Access Thrombosis in Children on Regular Hemodialysis Treatment

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ABSTRACT
Background: Fibronectin (FN) is a glycoprotein present in body fluids and tissues and is known to have a binding property involved in the haemostatic mechanism, among other functions. FN is known to be low in chronic renal failure (CRF) patients undergoing regular hemodialysis treatment (RDT).
Objectives: To asses the role of FN in the hypercoagulability of some children with CRF on RDT, particularly clotting in the hollow fibre or occlusion of their arterio-venous fistula (AVF).
Methods: FN was studied in 12 CRF children on RDT having hypercoagulability and requiring higher heparin doses on dialysis, 12 similar children with no coagulation problems and receiving usual heparin doses, and in 10 normal controls.
Results: In all the patients the examined coagulation tests were within the normal ranges and showed no significant differences among the patient groups. Anti-thrombin III was significantly higher in the hypercoagulability group than the healthy controls and insignificantly higher than the normal RDT group. Plasma FN in the normal heparin group (233,75 ± 84.5 mg/L) was significantly lower than in the control cases (328.5 ± 36.5 mg/L) ($p < 0.005$). The level in the hypercoagulability group (549.17 ± 220.68 mg/L) was significantly higher than the level in the healthy controls $p < 0.005$, and consequently more significantly higher than the level in the normal heparin group ($p < 0.001$). FN was above the normal range in 7/12 cases with hyperaiagulability suggesting an important role in inducing coagulation and/or antagonising the anticoagulant.
Conclusions: The coagulation profile study for RDT patients should include plasma FN as one of its parameters, which could predict higher heparin requirements on dialysis.

INTRODUCTION

Occlusion of the vascular access is a frequent complication encountered in patients on RDT. It may be related to hypotension, infection, mechanical obstruction or as a consequence of a hypercoagulable state[1,2].

Presence of an abnormality in the coagulation pathway including deficiency of protein C, protein S or resistance to the action of activated protein C probably due to mutation of factor V gene "factor V Leiden" resulting in shortening of activated partial thromboplastin time (APTT) are additional risk factors for vascular thrombosis particularly in younger patients lacking atherosclerotic disease[3].

Prevention of extracorporeal thrombosis during hemodialysis is routinely achieved by heparinisation. The dose of heparin required varies among patients for reasons that are not completely understood, each requiring a different heparin profile. Occasional blood

clotting in the dialyser still remains a common complication[4]

Heparin exerts its anti-coagulant effect mainly through activation of antithrombin III (AT **III).** It is also known to reduce platelet aggregability and to induce thrombocytopenia. Yet it was reported that heparin in doses as used during dialysis may enhance platelet aggregability by induction of adenosine diphosphate and adrenaline, stimulating the adherence of platelets to the dialyser membrane). Furthermore platelet factor IV released from activated platelets would neutralize the anticoagulant effect of heparin [6].

FN is one of the factors involved in the hemostatic mechanisms. Plasma FN concentration was found to be lower in patients with chronic renal failure (CRF) than in normal individuals[7]. Its role in the coagulation disorders in children with CRF was not thoroughly studied.

AIM OF WORK

The aim of this work was to measure the predialysis coagulation profile and plasma FN level in CRF children under RDT who have frequent hypercoagulation problems mainly dialyser clotting and/or occlusion of their arterio-venous fistulae (AVF) and to compare these results with a similar group of patients without such complications.

PATIENTS AND METHODS

The study included three groups of children:

Group I: comprised 12 CRF children on RDT who underwent either frequent extracorporeal clotting during dialysis and were consequently prescribed higher heparin doses during the dialysis sessions (9 cases), or had occlusion of their A-V fistulae (3 cases).

Group II: comprised 12 children on RDT receiving regular heparin doses on dialysis in the form of 20 units/Kg body weight as an initial bolus followed by 5 unitsiKg body weight every 30 minutes.

Group III: included 10 normal age- and sex-matched healthy children as controls.

The samples were taken from the patients before the start of the dialysis session and were subjected to routine laboratory investigations including complete blood count (CBC), serum creatinine, blood urea nitrogen, serum albumin, and globulin.

They were also tested for the coagulation profile including:

- Clotting time (CT) using the Lee and White method, activated partial thromboplastin time (APTT), bleeding time (BT) using Duke method and dire& platelet count.
- Plasma fibrinogen level.
- Plasma ATIII was quantitatively measured by the simple radial imcnuno-diffusion technique using agarose norpartigen plates with monospecific antiserum supplied by Behring company[8].
- Estimation of protein C and protein S was done to the three cases who had occlusion of their A-V fistulae. Protein C was measured by the intensity of colour produced after adding a chromogenic substrate to plasma to which a specific protein C activator had been added, and

expressed as a percentage of normal (normal range is between 70 - 130%) [9]. Protein S was tested by colorimetric measurment of the aggregates formed by latex coated antibodies to protein S added to the plasma, and expressed as a percentage of normal (normal range is between 70 - 140%)° °)

- Activated protein C (APC) resistance test was considered for cases with short clotting time or April: to determine the need to test factor V Leiden if no prolongation of the clotting time occurs. It was done for 6 cases of group I (cases 2, 4, 5, 7, 8 and 12) and 3 cases of group II (cases 2, 3, 8) using the "Diagnostica Stago" coagulometer calculating the ratio between APTT with and without APC, .and denoted APC - SR (sensitivity ratio) (normal > ow)
- Plasma FN was quantitatively determined using the commercially prepared immunodiffusion plates provided by Behrinl company[2).

The control samples were tested for CBC, ATIII, and FN.

The results were analyzed for statistical comparison using the Student "t" test of significance')

RESULTS

Results are shown in tables 1- 5. The normal values for each studied parameter are shown under the corresponding heading of each column in the tables.

Table 1: Clinical data of RDT patients with hypercoagulable events (gp I)

No	Sex and age (years)	Weight (Kg)	Original . disease	Duration on RDT. (months)	Serum creatinine (mg%)	S. albumin 32-45 g/L	S.globulin 23-35 g/L	Heparin unifs/Kg/ session
1	**F** 13	33	ESRD	18	5.4	32	38	80
2	**M** 11	27	ESRD	17	4.9	30	33	75
3	M 17	42	Obstr. Ur.	14	5.1	29	28	75
4	**M** 13	40	VUR	**10**	6.3	28	36.4	80
5	**F** 14	36	Lithiasis	8	4.8	27.5	58.5	70
6	M 8	20	Obstr. Ur.	4	7.3	35	35	80 ,
7	M 6	29	**Int. Neph**	6	6.2	31	43	75
8	M 17	32	Obstr. Ur.	12	5.9	35	40	75
9	F 12	30	ESRD	18	6.4	32.5	29.5	80
10	**M** 15	37	FSGS	14	7.1	31.5	35.5	70
11	**M** 11	28	ESRD	5	6.5	32	36	70
12	**M** 6	17	ESRD	4	. 8.2	36	40	80
M **S.D.**	11.92 ± 3.75	30.92 ±7.48		10.8 ±5.4	6.18 ± 1.03	31.63 ±2.75	37.7 ±7.81	75.83 ±4.17

ESRD = ei d stage renal disease, Obstr. Ur. =. obstructive uropathy, VUR = vesicoureteric reflux,
Int. Neph. = interstitial nephritis, FSGS = focal segmental glomerulosclerosis

Table 2: Clinical data of RDT patients without hypercoagulable events (gp II)

No	Sex and age (years)	Weight (Kg)	Original disease	Duration on RDT (months)	Serum creatinine (mg%)	S.albumin 32-45 g/L	S.globulin 23-35 g/L	Heparin units/Kg/ session
1	M / 12	37	FSGS	16	9.1	32.6	32	55
2	F / 14	35	ESRD	16	6.5	30	35	55
3	M / 11	30	ESRD	9	8.1	28	29	55
4	F/ 10	19	ESRD	9	6.2	33	32.4	55
5	F/ 12	32	FSGS	6	5.9	30.5	48.5	55
6	M/ 6	16	RTA	12	7.4	33	34	55
7	F/ 13	29	ESRD	16	5.9	29.5	43	55
8	F/ 17	49	SLE	8	6.2	31	40	55
9	M/ 11	21	Obst. Ur.	10	5.7	36	35	55
10	M/ 14	37	ESRD	6	6.5	32	35	55
11	M/ 7	18	ESRD	5	7.2	29.9	37	55
12	M/ 12	28	ESRD	5	5.9	32	38	55
M S.D.	11.58 + 2.99	29.25 + 9.67		9.8 ± 4.26	6.7 ± 1.04	31.46 ± 2.11	36.58 ± 5.28	55 ± 0.00

SLE = systemic lupus erythematosus

Table 3: Coagulation profile of RDT patients with hypercoagulable events (gp I)

No	Platelet count	P.T. Pt	C.T. 5-10 min	APTT 32-42 sec	Fibri-nogen 2-4 g/L	AT III 210-310 mg/L	Prot. C	Prot. S	APC-SR	FN 200-400 mg/L
1	190000	4	7	35	2.8	542.5	108	116	-	460
2	195000	3.50"	6.30"	25	3.0	793.5	-	-	1.7	240
3	190000	3.30"	6	33	2.6	376	-	-	-	780
4	230000	4.15"	9	27	2.1	406.5	-	-	1.9	.860
5	200000	4.30"	8	29	1.9	379.2	98	106	1.35	690
6	230000	4	6	42	2.9	435		-	-	800
7	200000	3.15"	7	29	3.1	658.3	-	-	1.65	380
8	230000	3	3	39	3.3	388	-	-	2.3	360
9	220000	4.40"	8	34	3.5	560	103	111	-	600
10	240000	4	7	34	2.6	342	-	-	-	750
11	200000	4	9.30"	40	3.2	273	-	-	-	350
12	180000	3	4.15"	27	2.5	420.5	-	-	1.45	320
M S.D.	208750 ± 20014.2	3.45" ± 0.30"	6.45" ± 1.50"	32.8 ± 5.56	4.22 ± 4.67	464.54 ± 147.65			1.73 ± 0.34	549.17 ± 220.68

able 4: Coagulation profile of ROT patients without hypercoagulable events (gp II)

No	Platelet count	B.T. 4-7 min	C.T. 5-10 min	APTT 32-42 sec	Fibrinogen 2-4 g/L	AT III 210-310 mg/L	APCSR	FN 200-400 mg/L
	210000	3.30"	8.30"	38	2.7	460.3		230
2	290000	3.50"	4.30"	- 33	;.2	285	1.85	180
3	200000	4.10"	3.30"	29	{.I	490	1.38	260
4	190000	3.15"	5.30"	39	1 6	512		90
5	170000	3.30"	5	38		295	- '	325
6	190000	4.30"	8	37		360	-	300
7	190000	5	6	40	3 6	501	-	140
8	190000	4	5	27	2.5	290	1.6	310
9	300000	5	7.30"	40	2.9	310	-	120
10	200000	2.30"	9	35	3.1	420	-	250
11	140000	3	8.15"	40	4.2	340	-	350
12	230000	4.30"	6	36	2.6	275	-	250
M S.D.	208333.3 ±45891.8	3.50" ±0.45"	6.20" ± 1.40"	36.0 ±4.33	3.13 ±0.48	380.28 ±90.89	1.61 ±0.24	233.75 ±84.5

Table 5: Statistical comparison of the studied groups

		Gp I RDT with hypercoagulability N = 12	Gp II RDT without hypercoagulability N = 12	Healthy controls N = 10
Heparin	**M** S.D.	75.83 ± 4.17	55.0 ± 0.00	
	t P	17.358 < 0.001		
AT III	M **S.D.**	464.54 ± 147.65	380.28 ± 90.89	- 341 ± 32.7
	t P	1.674 N.S.		
	1# cotitrols P	2.816 < 0.02	1.391 N.S.	
FN	M S.D.	549.17 ± 220.68	233.75 ± 84.5	328.5 ± 36.5
	t P	4.624 < 0.001		
	t # **controls** P	3.408 < 0.005	3.528 < 0.005	

DISCUSSION

FN is a high molecular weight glycoprotein existing in two major forms which are in dynamic equilibriuni The first is the soluble form present in body fluids and the other is the insoluble form present in the tissues. It is synthesized by the hepatocytes, platelets, endothelial cells, fibroblasts and different cells of the reticuloendothelial system" [4].

FN being a binding molecule explains most of its functions including its role in hemostasis. Several factors may be involved in the thrombogenic activity of FN, including its known action of inducing platelet aggregability. It was also believed to enhance red cell adhesiveness[15]. It was reported that FN interacts with some glycosaminoglycans including heparin[16] and would consequently oppose its activity.

Our group of children with renal failure on RDT requiring higher heparin doses during their dialysis sessions had an elevated plasma FN level (549A 7 ±.220,68 mg/L) significantly higher than the control value (328.5 ± 36.5 mg/L, $p < 0.005$), and both were significantly higher than the value of FN in the usual heparin RDT group (233.75 ± 84.5 mg/L, $p < 0.001$ and < 0.005 respectively). This expected low level of FN in the Usual heparin group of CRF patients on RDT has been described by several investigators [7,17,18]. The high FN in the RDT patients with hypercoagulability; being elevated in 7/12 of the studied group in the face of normal other studied parameters; suggests a pathogenic role. This does not exclude the possible roles of hemoconcentration during dialysis, trauma or infection to the A-V fistula as a cause of occlusion of the fistulae of the three cases with this event. The blood pressures of our patients with hypercoagulability were under control comparable to those of the usual heparin group.

The cause of this paradoxical elevation of FN in some of the renal failure_ patients on RDT is not clear. It might indicate continuation of the inflammatory process responsible for their renal failure, as FN levels were found to be elevated in nephrotic patients with normal renal functions, and is believed to be related to the compensatory increase in hepatic protein synthesis [19].

On the other hand the high FN level might reflect an ongoing hpercoagulable tendency as it is known to be released from stimulated platelets and would be a reflection of this hypercoagulable state rather than being a cause of it. But as the samples were drawn before the dialysis sessions and the main problem was extracorporeal thrombosis of the dialyser, we favor its pathogenic role. Several authors have demonstrated the contribution of elevated serum FN level to the occlusion of vascular access [20].

The plasma AT III of the hypercoagulability group was significantly higher than the control values excluding its possible role in inducing heparin resistance. This elevation of the AT III levels supports the hypothesis of excess hepatic protein synthesis in some of the patients on RDT. Yet its mean value in the group was insignificantly higher than the usual heparin group(21).

The fibrinogen levels among the studied groups were normal and is not consequently a possible cause of the hypercoagulable tendency encountered. High levels have been reported in cases with nephrotic syndrome [22].

We did not encounter deficiency of either protein C or protein S in the 3 cases with fistula occlusion, and they might be more related to other forms of hypercoagulability as deep venous thrombosis' [23]

Furthermore, we did not encounter cases with APC resistance among the cases who had either a shortened clotting time or activated partial thromboplastin time. It is reported to be found among 20 -- 28% of cases with thrombotic events in populations characterized by a high prevalence of the factor V mutation(' 25)

In conclusion, the predialysis plasma FN levels were generally found to be loWer than normal in CRF patients undergoing RDT. Yet it might be elevated in some of these patients having hypercoagulable problems particularly extracorporeal thrombosis. This elevated FN might have a pathogenic role or is simply a reflection of the hypercoagulability encountered. We recommend including plasma FN as one of the routine coagulation profile parameters done for RDT patients as it might have an important predictive value in preventing hypercoagulable events in this critical category of patients. It would also be beneficial to test for parameters of platelet activation during the dialysis session among these patients as a possible cause of extracorporeal thrombo'sis of the dialyzer.

REFERENCES

1. **Kanterman, RY.; Vesely, T.M. and Pilgram, T.K. (1995):** Dialysis access grafts: anatomic location of venous stencsis and results of angioplasty. Radiology, 195, 135-9.
2. **Johannes, H.M.; Linden, J.V. and Blankcstijn, P.J. (2000):** Coag&tion and hemodialysis access. Neph. **Dial. Transpl.,** 15, 1755-60.
3. **Eskandari, M.K.; Bontempo, F.A.** and **Hassett, A.C. (1993): .Arterial thromboembolic events in patients with** the factor V Leiden mutation. Am. J. Surg., 176 (2), 122-5.
4. **Lindsay, RM.; Prentice, R.M.; Davidson, J.F.; Burton, J.A. and McNicol, G.P. (1972):** Haemostatic changes during dialysis associated with thrombus formation on dialysis membranes. Br. Med. J., 454-458.
5. **High, K.A. (1988): AT III, protein** C and protein S. Naturally occurring anticoagulant proteins. Arch. Path. Lab. Med., 112 (1): 28-36.
6. **Rucinski, B.; Niewiarovvski, J.; James, P.; Walz, D.A. and Budzynski, A.Z. (1979): Antiheparin proteins secreted** by human platelets. Blood, 53, 47-62.
7. **Hou, F.; Zhang, X.; Wang, A. and Wu, J. (1990): Fibronectin in patients with chronic renal** failure undergoing dialysis. Nephron, 55 **(1), 45-**48.
8. Fagerhol, M.K. **and Abilgaard, V.. (1970):** Immunological studies on human AT III. Scand. J. Heinatol., 7, 10-17.
9. **Gugliemoae, H.A. and Vides, M.A. (1992): A novel functional** assay of protein C in human piasina. Thromb. Homeostasis, 67 (1), 46-49.
10. **Bertina, RM.; KoOeman, B.P.C. and Koster, T. (1994):** Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature, 369, 64-7.
11. **Horst Kamp, B.S.; Kiess, H. and Kramer, I (1999):** Activated protein C resistance shows an association with pregnancy induced hypertension. Hum Reprod, 14 (12): 3112-5.
12. **Mancini, G.; Cahonara, A.O. and Hereman, J. F. (1965): Immunological quantitation of antigens** by single radial im.munodiffusion. Immunochemistry, 2, 235-249
13. **Armitage, P. (1974): Statistical methods** in medical research. Blackwell scientific publications. Oxford, London, Edinburgh, Melbourne, 120-156.
14. **Mosher, D.F. (1984): Physiology of Fibronectin.**

Ann. Rev. Med. 35, 561-75.
15. Mosher, D.F.; Schad, P.E. and Xleinman, H.K. (1979): Cross linking of fibronectin to collagen by blood coagulation factor XIII a. J. Clin. Invest., 64, 181-187.
16. Yamada, KM.; Kenedy, D.W.; Kimata, K and Pratt, RM. (1980): Characterization of fibronectin interactions with glycosaminoglycans and identification of active proteolytic fragments. J. Biol. Chem., 255, 6055-63,
17. Schena, F.P. and Pertosa, G. (1988): Fibronectin and the kidney. Nephron, 48, 177-182.
18. Malyszko, J.; Malyszko, J.S.; Pawlak, D.; Buczko, W. and Mysliwiec, M. (1996): Hemostasis, platelet function and serotonin in acute and chronic renal failure. Thromb. Res. Sep 1, 83 (5), 351-61.
19. Cosio, F.G. and Bakaletz, A.P. (1984): Abnormal plasma fibronectin levels in patients with proteinuria. J. Lab. Clin. Med., 104 (6), 867-72.
20. Goldwasser, P.; Avram, M.M.; Collier, J.T.; Michel, M.A.; Gusik, S.A. and Mittman, N. (1994): Correlates of vascular access occlusion in hemodialysis. Am. J. Kidney Dis., 24 (5), 785-94.
21. Jorgensen, **M.; Ericksen, H.O. and** Tranebjoerg, L (1985): Plasma antithrombin III concentration in patients on regular hemodialysis treatment. Nephron, 40, 22-24.
22. Vaziri, N.D.; Gonzales, E. and Borton, C.H. (1991): Factor XIII and its substrates fibronectin, fibrinogen and a2 anti-plasmin in plasma and urine of patients with nephrosis. J. Lab. Clin. Med., 117, 152-156.
23. Segal, G.B. and Francis, C.W. (2001): Anticoagulant proteins in childhood venous and arterial thrombosis. Blood Cells Molecules and Diseases, 26 (5), 540-560.
24. Bertina, R.M. (1986): Hereditary protein S deficiency. Homeostasis, 15, 241-245.
25. Svensson, **P.J.; Zoller, B. and Muttiasson, I.** (1997): The factor V R506Q mutation causing APC resistance is highly prevalent among unselected outpatients with clinically suspected deep venous thrombosis. J. Intern, Med. 241, 379-85.