Plasminogen activator inhibitor-1 activity and 4G/5G polymorphism in hemodialysis

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Abstract: ***Introduction:*** **Chronic insufficiency alters homeostasis, in part due to endothelial inflammation. Plasminogen activator inhibitor-1 (PAI-1) is increased in renal disease, contributing to vascular damage. We assessed PAI-1 activity and PAI-1 4G/5G polymorphism in hemodialysis (HD) subjects and any association between thrombotic vascular access (VA) events and PAI-1 polymorphism.**
Methods: **Prospective, observational study in 36 HD patients: mean age: 66.6 ± 12.5 yr, males n=26 (72%), time on HD: 28.71 ± 22.45 months. Vascular accesses: 10 polytetrafluoroethylene grafts (PTFEG), 22 arteriovenous fistulae (AVF), four dual lumen catheters (CAT). Control group (CG): 40 subjects; mean age: 60.0 ± 15 yrs, males n=30 (75%). Group A (GA): thrombotic events (n=12), and group B (GB): No events (n=24). Groups were no different according to age (69.2 ± 9.12 vs. 65.3 ± 14.5 yrs), gender (males: 7; 58.3% vs. 18; 81.8%), time on HD (26.1 ± 14.7 vs. 30.1 ± 38.7 months), causes of renal failure. Time to follow-up for access thrombosis: 12 months.**
Results: **PAI-1 levels in HD: 7.21 ± 2.13 vs. CG: 0.42 ± 0.27 U/ml (p<0.0001). PAI-1 4G/5G polymorphic variant distribution in HD: 5G/5G: 6 (17%), 4G/5G: 23 (64%); 4G/4G: 7 (19%) and in CG: 5G/5G: 14 (35%); 4G/5G: 18 (45%); 4G/4G: 8 (20%). C-reactive protein (CRP) in HD: 24.5 ± 15.2 mg/L vs. in CG 2.3 ± 0.2 mg/L (p<0.0001). PAI-1 4G/5G variants: GA: 5G/5G: 3; 4G/5G: 8; 4G/4G: 1; GB: 5G/5G: 3; 4G/5G: 15; 4G/4G: 6. Thrombosis occurred in 8/10 patients (80%) with PTFEG, 3/22 (9%) in AVF, and 1/4 (25%) in CAT. Among the eight PTFEG patients with thrombosis, seven were PAI 4G/5G.**
Conclusions: **PAI-1 levels were elevated in HD patients, independent of their polymorphic variants, 4G/5G being the most prevalent variant. Our data suggest that in patients with PTFEG the 4G/5G variant might be associated with an increased thrombosis risk. (J Vasc Access 2008; 9: 142-7)**

Key words: *Plasminogen activator inhibitor-1, Hemodialysis, Endothelium, C-reactive protein, Thrombosis, 4G/5G polymorphism*

INTRODUCTION

Patients on maintenance hemodialysis (HD) frequently develop thrombotic events along the entire vascular tree, particularly at the site of the vascular access (VA), coronary, cerebral and renal arteries. Available data have disclosed both a state of hypofibrinolysis characterized in part by elevated plasminogen activator inhibitor-1 (PAI-1) levels and a condition of endothelial dysfunction and chronic inflammation, characterized by increased plasma concentrations of PAI-1 and C-reactive protein (CRP) or reduced endothelium-dependent vasodilatation mediated by nitric oxide (1-3). PAI-1 is synthesized mainly in endothelial cells; it inhibits plasminogen activator activity and is considered the most important physiological regulator of the fibrinolytic system. Several studies have shown that PAI-1 blood levels depend on many different pathophysiological factors, contribute to cardiovascular morbidity and to the de-

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velopment of perivascular fibrosis. In normal subjects, higher PAI-1 levels are associated with a polymorphic variance in the number of guanine bases (4G rather than 5G) at position -675 upstream of the transcription start site. Some studies have shown that the homozygosity for the 4G allele could be an independent risk factor for the development of atherosclerosis, thrombosis and cardiovascular disease, highly prevalent in HD patients (4), while other studies could not confirm this finding (5).
This study aimed to investigate PAI-1 activity levels and PAI-1 4G/5G polymorphism in 36 chronic HD patients and compare them with the occurrence of thrombotic VA events. We also investigated the correlation of PAI-1 levels with PAI-1 4G/5G polymorphism and CRP levels.

METHODS

Study design

Prospective, observational study in which in 36 chronic HD patients PAI-1 blood levels, the 4G/5G polymorphic variant and CRP concentrations were determined. Subsequently, patients were divided into two groups according to the occurrence of VA thrombotic events after 1 year of follow-up.

Patient characteristics

Thirty-six chronic HD patients were included. Exclusion criteria were: malignancy, end-stage chronic heart disease, active liver or thyroid disease, uncontrolled diabetes mellitus, severe malnourishment, patients with hematocrits (Hct) <32%, evidence of infection or of genetic or acquired thrombophilic defects.
Patients were then divided into two groups according to the development of HD access thrombosis.
Group A (GA) included patients with VA thrombotic events (n=12, 33%), while group B (GB) consisted of patients free of this complication (n=24, 67%). Groups were no different according to age, gender, time on HD and causes of renal failure (Tab. I). Moreover, antithrombin III blood concentration and proteins C and S activity were within normal limits in all patients, and no factor V Leiden or protein 20210 mutations were present. Homocysteine blood measurements were not different between both groups.

Hemodialysis aspects

HD was performed in a high-flux manner with a bicarbonate bath, mean Qd: 500 mL/min and mean Qb: 350 ± 50 mL/min; biocompatible membranes were used: polyamide dialyzers (Polyflux 6L or 10L®, Gambro Sweden). Each HD session averaged 3.5 ± 0.5 hr thrice weekly. In each HD session 3000 U of heparin were used. All patients were on clopidogrel 75 mg/day. Control subjects were free of medication.

Vascular access characteristics

VAs: ten polytetrafluoroethylene grafts (PTFEG), 22 arteriovenous fistulae (AVF), four dual lumen catheters (CAT) (Tab. I).

Biochemical measurements

PAI-1 activity and genotype determinations
Blood was collected before dialysis in fasting conditions with sodium citrate 3.2% for PAI-1 activity and with EDTA for PAI-1 polymorphism. Citrate samples were centrifugated at 3000 g for 15 min and plasma was stored at -80 C until testing. PAI-1 activity (normal: 0.3-2.5 U/mL) was determined by chromogenic methods according to the manufacturer's instructions (Berichrom PAI®, Dade Behring, Newark, USA). PAI-1 4G/5G polymorphism was identified by polymerase chain reaction (PCR) amplification and digestion with Bs11 restriction enzyme according to Margaglione et al (6).
CRP (normal: 0-3 mg/L) was determined by an immunoturbidimetric assay (Vitros® 5.1 FS Chemistry system, Orthoclinical Diagnostics, New Jersey, USA).

TABLE I - PATIENT CHARACTERISTICS

Group (%)	Male (%)	Age (years)	Time on HD (months)	AVF	PTFEG	CAT	Hct (%)	GN	DM	ANG	PKD	AN
A=12 (33)	7 (58.3)	69.2 ± 9.12	26.1 ± 14.7	3	8	1	33.2 ± 0.8	5	1	6	0	0
B=24 (67)	18 (75)	65.3 ± 14.5	30.1 ± 38.7	19	2	3	32.9 ± 0.7	6	4	7	6	1

Group A: patients with VA thrombotic events; Group B: no thrombosis. Abbreviations: HD, hemodialysis; Hct, hematocrit; AVF, arteriovenous fistulae; PTFEG, polytetrafluoroethylene grafts; CAT, catheters; GN, glomerulonephritis; DM, diabetes mellitus; ANG, angiosclerosis; PKD, polycystic kidney disease; AN, analgesic nephropathy

Statistical analyses

Results are expressed as the mean ± standard deviation of the mean (SD), unless specified otherwise. The Mann-Whitney U test was used for differences between groups of quantitative variables. The Chi square or Fisher test was used for qualitative variable comparisons.

Ethical aspects

The Institutional Review Board of the Hospital Británico approved the study.

RESULTS

Overall results

In the 36 patients included, these were the results obtained: mean age 66.6 ± 12.53 yrs, males n=26 (72%), time on HD: 28.71 ± 22.45 months. PAI-1 activity in HD patients was significantly higher than in the control group: 7.21 ± 2.13 vs. 0.42 ± 0.27 U/mL, p<0.0001. CRP in HD: 24.5 ± 15.2 mg/L vs. in the control group 2.3 ± 0.2 mg/L, p<0.0001 (Tab. II) PAI-1 4G/5G polymorphic variant distribution in HD patients: 5G/5G (normal): 6 (17%), 4G/5G (heterozygous): 23 (64%); 4G/4G (homozygous): 7 (19%); and in the control group: 5G/5G: 14 (35%); 4G/5G: 18 (45%); 4G/4G: 8 (20%) (Tab. III).

TABLE II - PAI-1 ACTIVITY AND CRP BLOOD LEVELS IN HD PATIENTS

Group	PAI-1 (U/mL)	CRP (mg/mL)	p
Patients (n=36)	7.21 ± 2.13	2.45 ± 1.52	<0.0001
Control (n=40)	0.42 ± 0.27	0.23 ± 0.02	<0.0001

Abbreviations: PAI-1: plasminogen activator inhibitor-1; CRP: C-reactive protein

Intergroup results

PAI-1 levels: GA vs. GB: 6.81 ± 2.3 vs. 7.42 ± 1.53 U/mL (p=ns); CRP in GA vs. GB: 14.4 ± 11.8 vs. 11.8 ± 8.4 mg/L (p=ns). PAI-1 4G/5G polymorphic variant distribution: GA: 5G/5G: 3; 4G/5G: 8; 4G/4G: 1; GB: 5G/5G: 3; 4G/5G: 15; 4G/4G: 6. VAs: GA: PTFEG: 8; AVF: 3, CAT: 1; GB: PTFEG: 2, AVF: 19, CAT: 3. There were no differences in PAI-1 polymorphic variants between patients with and without thrombosis but 7/8 patients with thrombosis and PTFEG were PAI 4G/5G.

DISCUSSION

This study aimed to assess PAI-1 activity in steady chronic HD patients, a condition of continuous systemic inflammation, and its relationship with PAI-1 4G/5G polymorphism, a potential association between PAI-1 polymorphisms and VA thrombosis and the inflammatory state of HD patients with CRP levels. PAI-1, a member of the SERPIN (SERine Protease INhibitor) family, is a 50-kDa glycoprotein with a half-life of 8-10 min and unstable in structure due to the lack of cysteine in its molecule. PAI-1 is present in trace amounts in normal conditions and is involved in thrombotic processes as a physiologic inhibitor of the tissue-type and urokinase-type plasminogen activators that exists in conformationally active and latent forms (7, 8). With the exception of platelets, in which it can be largely stored, PAI-1 is rapidly secreted after synthesis, binding rapidly to t-PA and forming a stable complex in which the PAI-1 to t-PA ratio is 1:1. Within the circulation, active PAI-1 is unstable unless bound to vitronectin (9). However, PAI-1 is known to mediate other actions beyond fibrinolysis. PAI-1 levels are increased in several chronic inflammatory states acting as a powerful fibrosis-promoting molecule and can contribute to the pathogenesis of vascular disease (10-15). PAI-1 synthesis occurs mainly in endothelial cells and platelets, although other sites include macrophages, vascular smooth muscle cells, liver, spleen and adipose tissue, and although it does not take place in the

TABLE III - POLYMORPHISM AND PAI-1 ACTIVITY LEVELS IN HD PATIENTS

Genetic variants	Argentinian prevalence	HD patients (n=36)		
		Frequency	PAI-1 activity (UI/ml)	Thrombotic events
5G/5G	36%	6 (17%)	5.69 ± 2.01	3
4G/5G	43%	23 (64%)	6.87 ± 1.97	8
4G/4G	21%	7 (19%)	7.19 ± 2.17	1

kidneys, synthesis by both resident and intrarrenal inflammatory cells occurs in several acute and chronic renal states, such as diabetic nephropathy, focal necrotizing glomerulonephritis, focal segmental glomerulosclerosis, nephroangiosclerosis, membranous nephropathy and chronic allograft nephropathy (9, 16). PAI-1 has impressive fibrosis-promoting effects in the kidney, with high PAI-1 levels predicting a bad long-term outcome (14). Exactly how PAI-1 promotes renal fibrosis is not completely understood. In addition to inhibiting serine protease activity within vascular and extracellular compartments, PAI-1 directly modulates cellular behavior, leading to a vicious cycle of inflammatory cell recruitment, fibroblast activation, and scar tissue accumulation (14). In lesions that are characterized by the presence of fibrin, such as occurs in certain glomerulonephritis, the inhibition of fibrinolysis is closely associated with chronic damage. Switching off PAI-1 synthesis has been shown experimentally to prevent chronic kidney disease (CKD) progression. By contrast, in the renal interstitium, where PAI-1 can accumulate as a result of the presence of vitronectin, its primary fibrogenic effects relate more closely with its ability to facilitate cell migration of monocytes and myofibroblasts, albeit other potential mechanisms remain to be elucidated. Among the many renoprotective properties of the angiotensin II inhibitors is their ability to suppress PAI-1 (14).

Regarding PAI-1 activity levels, our results show that they were uniformly elevated in our patients. Albeit it has been described that higher PAI-1 levels are associated with the 4G/5G homozygosity variant in the general population, we have found that in uremic patients PAI-1 measurements are uniformly increased, independent of this genetic variance, suggesting that the inflammatory state present in these patients, also shown by elevated CRP levels, could be the cause of such a disturbance. On the one hand, renal insufficiency causes an increase in PAI-1 levels (7, 14). On the other hand, PAI-1 has emerged as a critical mediator of tissue damage and renal interstitial fibrosis, which could worsen residual renal function in HD patients. It appears that PAI-1 expression may be induced by renin, angiotensin II, aldosterone, shear stress, TGF-β and CRP (2, 7, 15, 16). In addition, uremic toxins induce free radical production by renal tubular cells and activate NF-B, which, in turn up-regulate PAI-1 expression (17). Therefore, elevated PAI-1 activity levels in HD patients could contribute to endothelial damage. This damage could be clinically expressed in the form either of atherosclerosis or of thromboses, two of the most frequent and feared complications in the HD population.

Several factors, including genetic and epigenetic ones, influence plasma PAI-1 levels in humans. In the general population, the 4G variant is associated with higher plasma PAI-1 levels (4). However, in our study this phenomenon could not be observed. Our results agree with those found by Ando R et al, who found that plasma PAI-1 levels were no different among the three PAI-1 genotypes in HD patients (18). The question is why in HD patients PAI-1 levels are not influenced by the genotype encountered, which occurs in the general population, or whether this polymorphism can influence or determine the type of response of the PAI-1 gene to certain cytokines. This could be explained in part due to uremia itself as the main cause of such a disturbance. In this regard, PAI-1 synthesis is rapidly induced by a variety of factors, the vast majority of which are increased in renal disease and particularly in HD (6, 16-27). Finally, HD patients present an inflammatory milieu as shown by high CRP levels, which is independent of the genotype, and could explain the elevation of PAI-1 activity (16).

Another vascular damage marker is CRP, which as expected was increased in our study, suggesting that the inflammatory state that governs in renal failure could be in part the origin of the thrombotic events that affects HD patients. Evolving data suggest that CRP affects fibrinolysis by decreasing tissue plasminogen activator, which converts plasminogen to plasmin resulting in fibrin degradation (2, 14, 28, 29). Moreover, recent data show that CRP promotes tissue factor expression and activity in mononuclear cells and induces thrombomodulin release (30). Finally, CRP induces the expression and activity of PAI-1 and decreases prostacyclin production, suggesting a prothrombotic role for CRP (2, 29) Taken together CRP and PAI-1 could potentiate their actions and contribute to the endothelial damage found in HD patients.

As previously addressed, in our patients other main causes of acquired or genetic thrombotic diathesis were discarded. When thrombotic and non-thrombotic groups were analyzed in relation to PAI-1 polymorphism and VA type, PTFEG accesses were more prevalent in the thrombotic group and 7/8 patients who presented with thrombotic events and had PTFEG accesses showed PAI 4G/5G polymorphism (Tab. III). PTFE molecular characteristics, mechanical and biochemical interactions between blood constituents and graft surface could certainly play an important role in the thrombotic predisposition encountered in this setting.

When the overall results are analyzed, all patients displayed an inflammatory state with elevated PAI-1 and CRP blood levels (Tab. II), but not in all these patients could a history of thrombosis be found.

This could be attributed to a short follow-up period, to silent episodes of thrombosis that did not involve the VAs or that were clinically covert, or to the administration of heparin or clopidogrel, which could have certainly diminished or prevented thrombotic episodes.

Based on our findings, PAI-1 plasma levels are increased in HD patients independent of the type of PAI-1 4G/5G polymorphic variant and correlate with high CRP levels. Both molecules could contribute to the vascular and fibrotic damage found in HD patients. PAI-1 4G/5G polymorphism was the most prevalent variant encountered and is more frequent than in the normal Argentinian population (Tab. III) (31). PTFEG patients with the 4G/5G polymorphism presented an increased risk of thrombosis. Therefore, were this finding to be confirmed in future studies, PTFEG should be avoided, if possible, in patients with the 4G/5G polymorphic variant.

However, much remains to be learnt about the role of PAI-1 in CKD and HD. Will PAI-1 genotype prove to be a useful and practical risk marker predictor of CKD or of bad outcome in HD patients? If angiotensin II, aldosterone and TGF-β activities are therapeutically blocked, can PAI-1 still be synthesized and promote thrombosis or fibrosis? Is PAI-1's role as an inhibitor of fibrinolysis relevant to CKD? Much needs to be learnt about the specific therapeutic inhibition of PAI-1 and the clinical relevance of such an impact on fibrosis and thrombosis.

Conflict of interest statement: None of the authors who participated in this study have any conflicts of interest with any company that may have a financial interest in the information contained in the manuscript.

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