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Alternatively Spliced Human Tissue Factor and Thrombotic Tendencies in Hemodialysis Patients

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Abstract

Background and Aims: Chronic hemodialysis (HD) patients are associated with an increased thrombotic tendency, a frequent and costly cause of morbidity in this patient population. HD patients often have multiple defects in their hematologic/coagulation factors; however, few convincing associations have been made between these abnormalities and clinical thrombotic events. Alternatively spliced human tissue factor (asHTF), a recently discovered soluble form of tissue factor (TF), circulates in blood and exhibits procoagulant activity. Therefore, the present investigation was designed to initially determine if asHTF levels are correlated to thrombotic tendencies in HD patients.

Methods: Pre-dialysis blood samples were drawn from a cohort of 84 hemodialysis patients immediately prior to dialysis. Plasma asHTF levels were quantified and then compared to a variety of patient parameters collected for each patient.

Results: Mean plasma asHTF levels for HD patients varied significantly compared to 30 healthy normal subjects. We found a positive correlation between asHTF concentration and access thrombosis in hemodialysis patients (r=0.31, p=0.0046). When patients without any episodes of thrombosis were excluded, correlation increased (r=0.59, p=0.0001). The patient with the highest number of incidences of thrombosis (n=25) also had the highest plasma asHTF concentration (1066.61 pg/ml), over seven standard deviations above the mean.

Conclusions: These initial results suggest that plasma asHTF antigen levels may be associated with access thrombosis in HD patients. Further studies are needed to confirm our findings and to determine whether elevated asHTF levels indicate a causal or responsive role in HD associated access thrombosis.

Keywords: Hemodialysis, Thrombosis, Tissue Factor

Introduction

A bleeding tendency has been associated with renal failure, with impaired platelet function as the predominant abnormality underlying uremic bleeding. Despite this bleeding tendency, thrombotic complications are common among patients, and end stage renal disease (ESRD) has been labeled a hypercoagulable state (1). There are a wide spectrum of thrombotic events in ESRD, including deep vein thrombosis and pulmonary embolism, renal allograft-associated thrombosis, heparin-induced thrombocytopenia-associated thrombosis, coronary artery disease, and vascular access-associated thrombosis (2).

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Hemodialysis (HD) patients have a cardiovascular disease mortality rate 10-20 times greater than that of the general population (3). Venous access thrombosis is the most frequent thrombotic event among chronic HD patients (4) and is a major source of morbidity for patients (2). Thrombotic occlusion leads to permanent failure in 10% of arteriovenous fistulas (AVF) and 20% of grafts each year (5).

Chronic HD patients often have multiple defects in their hematologic/coagulation factors (2). These observations have prompted numerous studies on abnormalities of clotting factors in ESRD (6-10). Abnormalities in the protein-C, protein-S, and anti-thrombin III systems have been identified in HD patients (11). However, few convincing associations have been made between these abnormalities and clinical thrombotic events in ESRD patients (2).

Tissue factor (TF), a transmembrane glycoprotein normally anchored to vascular cells, is the primary cellular initiator of the extrinsic pathway of coagulation (12). Recently, the presence of soluble tissue factor (as opposed to membrane or vessel wall bound TF) in human circulation and its participation in the formation of ex-vivo thrombi has been demonstrated (13). Studies characterizing the circulating population of tissue factor have determined one form to be a plasma soluble isoform of human TF that is generated by alternative splicing of the of the primary RNA transcript of vessel wall TF (14), thus it is designated as alternatively spliced human tissue factor (asHTF). In asHTF, exon 5 of full length TF is absent and exon 4 is spliced directly to exon 6. This fusion creates a frameshift that results in a C terminus region unique to asHTF. Thus asHTF lacks transmembrane and cytoplasmic domains. However, the 165–166 lysine doublet involved in FVIIa binding in full length TF is maintained in asHTF. Preliminary investigations demonstrate that asHTF exhibits in vivo biological activity, is incorporated within developing thrombi by association with platelets, and may promote the rapid growth of thrombotic masses through activation of other coagulation proteins (14). In addition, asHTF released from endothelial cells have been shown to be a marker for and contributor to imbalanced hemostasis (15).

In humans, increased levels of blood-borne TF have been reported in diverse pro-thrombotic syndromes including unstable angina, myocardial infarction, trauma, sepsis, disseminated intra-vascular coagulation, antiphospholipid antibody syndrome, sickle cell disease, and cancer (16-19). In addition, plasma TF levels have been shown to be elevated in ESRD patients (20-23). Thus asHTF represents a potential marker to predict thrombotic tendencies in HD patients.

In the present investigation, we quantified plasma asHTF levels in chronic HD patients and retrospectively correlated this data with various patient parameters. Our aim was to elucidate whether asHTF, which has proven to be a highly prothrombotic protein and which has never been investigated in HD patients, plays a role in HD and ESRD.

Materials and Methods

The Institutional Review Board at the University of Chicago Hospital, Chicago, Illinois, approved the study protocol. Eighty-four patients regularly attending the outpatient hemodialysis unit at the University of Chicago were evaluated in 2007. After obtaining informed written consent from each patient, blood was collected from the patient's venous access site immediately prior to dialysis. Samples were also drawn from 30 subjects without known health problems including renal failure or hypertension. Blood was collected into 3.2% buffered sodium citrate and spun at 2000xg for 15 minutes to obtain platelet poor plasma. Samples were then stored at -80 degrees C prior to testing. The levels of plasma asHTF antigen were measured by a sandwich-style enzyme-linked immunoassay (ELISA) developed by Bogdanov et al (14). Briefly, each plasma sample was added to microplate wells pre-coated with a capture antibody

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(a polyclonal antibody targeting the extracellular domain of TF, common to all forms of TF). Once captured, asHTF was detected using a polyclonal antibody that specifically recognizes asHTF. A horseradish peroxidase (HRP) conjugated goat-anti-rabbit antibody completed the antibody-enzyme detection complex. The addition of O-Phenylenediamine substrate buffer and its reaction with HRP yields a yellow colored solution. Sensitivity was increased by the addition of stop solution (2.5 M H_2SO_4). Microplates were immediately read in a kinetic ELISA plate reader at a wavelength of 490 nm. Purified recombinant asHTF served as a standard.

asHTF levels and patient data were collected independently of each other. After asHTF levels in each sample were attained, they were retrospectively correlated with age, sex, weight, duration of dialysis, incidence of diabetes, incidence of hypertension, incidence of coronary artery disease (CAD), previous transplant, total number of episodes of access thrombosis since the start of dialysis, heparin administration (USP units/ml/week), coumadin administration, recent platelet count (within 30 days of assay), reuse number, and access type. A thrombotic event was defined as a thrombosed vascular access which required either a declot procedure or catheter change in Interventional Radiology or Surgery.

Statistical analysis was performed using Systat 11 (SYSTAT Software Inc, Richmond, CA, U.S.A). Pearson Product Moment Correlation was used for correlation analysis. The significance between normal and ESRD subjects was estimated by Student's t-test. A multiple linear model was used with post hoc pairwise comparison. A 2-tailed p-value ≤ 0.05 was considered significant.

Results

Plasma asHTF levels were determined for 84 hemodialysis patients (37 males, 47 females, mean age 57.2 years). Table 1 gives the profile of our study population. Mean plasma asHTF for all patients was 82±130 pg/ml. Samples were drawn from 30 subjects without known health problems including renal failure or hypertension. The average age was 33 years with 19 females and 11 males. In 30 healthy normal subjects, mean plasma asHTF was 85±21 pg/ml (unpublished data, 2006). Student t-test confirmed that the difference between mean asHTF levels in normal versus hemodialysis patients is significant (p=0.024).

Using a general linear model with HTF as a dependent variable, with co-variables of type of age, sex, weight, type of access (fistula, graft or catheter), number of episodes of access thrombosis, age, duration of dialysis, previous transplant, reuse of dialyzer, use of heparin or Coumadin, history of hypertension or diabetes, and platelet count drawn within one month of asHTF assay , access thrombosis (multiple R=0.544, squared multiple R=0.296, F=34.447, P=0.000) and sex (multiple R=0.589, squared multiple R=0.346, F=6.263, P=0.014) when entered were significant.

There was a significant positive correlation between asHTF concentration and access thrombosis in hemodialysis patients (r=0.31, p=0.0046). When patients without a thrombotic event were excluded, correlation increased (r=0.59, p=0.0001). Individual results comparing asHTF levels to incidences of thrombosis are presented in Figure 1. In our patient population, other parameters showed significant correlations with each other. asHTF was found to be higher in male versus female subjects (p=0.0270). Access thrombosis was positively correlated with duration of dialysis (r=0.29, p=0.0071) and negatively correlated with hypertension (r=-0.275, p=0.0113). Access thrombosis was more prevalent in graft access than fistula access (F=3.87, p=0.0249). Diabetes was positively correlated to CAD (r=0.292, p=0.0071). Significant correlation results are summarized in Table 2.

Discussion

Hemodialysis access thrombosis is associated with

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Table 1. HD Population Profile

	HD Patient Data
Male	37
Female	47
Age (years)	
Mean ± SD	57.7 ± 17.2
Range	22 - 88
Weight (kg)	
Mean ± SD	73.8 ± 21.9
Range	40.5 - 150
Duration of Dialysis (months)	
Mean ± SD	67.4 ± 65.9
Range	7 - 283
Diabetes	32
Hypertension	68
CAD	37
Previous Transplant	17
Coumadin	12
Heparin (units/ml/week)	
Mean ± SD	3027 ± 2169
Range	0 - 10000
Access Thrombosis (episodes)	
Mean ± SD	2.2 ± 4.2
Range	0 - 25
Platelet Count	
Mean ± SD	210 ± 64
Range	61 to 398
Reuse number	
Mean ± SD	22 ± 14
Range	0 - 51
Access Type	
Fistula	46
Graft	30
Catheter	8

asHTF levels. In vivo, levels of blood-borne TF have been reported to increase in diverse pro-thrombotic syndromes. The mechanism by which asHTF gains activity in prothrombotic conditions and its contribution to thrombosis in vivo is not known. We aimed to detect and measure the levels of asHTF in a patient population prone to thrombosis. As the mechanism of access thrombosis is poorly understood, the findings presented in the study can lead to a greater understanding of the role of asHTF in it.

ESRD is associated with a hypercoagulable state that contributes to a wide spectrum of thrombotic events and increased morbidity and mortality in HD patients. A variety of hemostatic abnormalities have been confirmed in ESRD patients; however, the in vivo consequences of these abnormalities have been difficult to establish. asHTF, a soluble TF isoform, has recently been discovered in human blood. Its presence in occlusive thrombi points to asHTF as a new procoagulant component in the pool of coagulation factors. The close association between asHTF and thrombogenesis makes it reasonable to investigate whether asHTF plays a role in thrombosis in HD patients.

Our findings provide initial evidence that plasma asHTF levels are independently linked to the incidence of hemodialysis access thrombosis. asHTF was significantly positively correlated to access thrombosis in our study population. Interestingly, the patient with the highest number of incidences of thrombosis (n=25) also had the highest plasma asHTF concentration (1067 pg/ml), over seven standard deviations above the mean. Furthermore, this patient was on dialysis for 126 months, which is within one standard deviation of the mean duration of dialysis for our population. When this patient was excluded, asHTF levels remained significantly positively correlated to access thrombosis ($r=0.35$, $p=0.026$) in subjects with ≥1 thrombotic incidences. However, correlation did not remain significant ($p>0.05$) when this patient was excluded and all subjects (including

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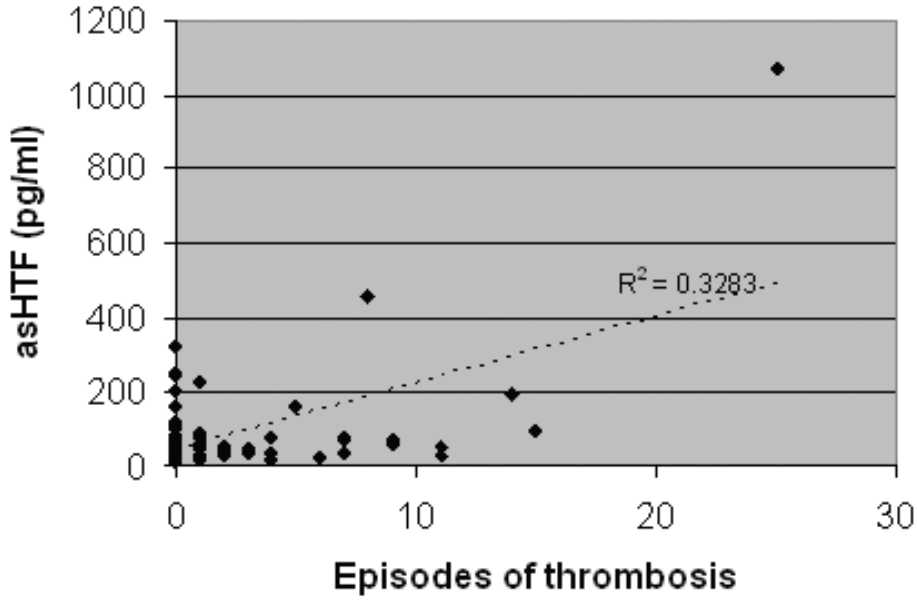

Figure 1. Individual Results: Episodes of Thrombosis vs. asHTF Levels

those without thrombotic incidences) were assessed. It is clear that this outlying value influenced our correlation data considerably. Therefore, further studies need to be done to gain better insight into the extent of the relationship between asHTF and thrombotic incidences. Furthermore, as our samples were randomly drawn, we are not able to assess causation. We are not able to conclude whether elevated asHTF levels indicate a causal or responsive role in HD access thrombosis. The present study was designed to initially assess asHTF as a novel biomarker. Future studies will be aimed at identifying the independent predictive effect of asHTF.

Our study population experienced a high event rate (42 patients had at least one event) of access thrombosis. Furthermore, our population had a reduced risk of thrombotic events in those with fistula versus graft access, regardless of asHTF level. These findings are in agreement with earlier retrospective studies on thrombotic incidences in HD (24-26). Access thrombosis was negatively correlated with hypertension, however 81% of our population

Table 2. Significant Correlations in HD Patient Data

	Pearson Product Correlation	
	Correlation Coefficient (r)	p-value
asHTF and Access Thrombosis (All Subjects)	0.31	0.0046
asHTF and Access Thrombosis (Subjects with ≥1 Thrombotic Incidences)	0.59	0.0001
asHTF and Sex (Males > Females)	0.24	0.0270
Access Thrombosis and Duration of Dialysis	0.29	0.0071
Access Thrombosis and Hypertension	-0.28	0.0113
Access Thrombosis and Access Type (Graft > Fistula)	(F-value) 3.87	0.0249
Diabetes and CAD	0.29	0.0071

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(n=68) was hypertensive, which is in accordance with previous reports (2). Finally, diabetes was positively correlated with CAD in our patient population; diabetes has been reported as a risk factor for CAD (27). However, despite these agreements with previous HD patient populations, further studies are needed to confirm our results, given the number of patients investigated (n=84).

In conclusion, thrombosis is an important cause of morbidity and mortality in HD patients. asHTF is present in significant concentrations in human plasma and our initial results indicate that this protein can be a candidate marker for or contributor to thrombosis in HD patients. Plasma asHTF may potentially be used a unique diagnostic target to identify HD patients at risk for access thrombosis. In addition, inhibition of asHTF but not full-length TF (which aids in normal coagulation) may represent a novel means to combat thrombotic events in HD patients without triggering bleeding problems. Additional studies need to be done to define further the relationship of asHTF to thrombosis in long-term HD patients.

Conflict of Interest

None declared.

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