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Plasma Total Homocysteine and Hemodialysis Access Thrombosis: A Prospective Study

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Abstract. Mild hyperhomocysteinemia, a putative risk factor for atherothrombotic cardiovascular disease morbidity and mortality, may contribute to the excess incidence of atherothrombotic outcomes in the dialysis-dependent end-stage renal disease population. Hemodialysis access (fistula or graft) thrombosis is an unfortunately common and costly morbidity in this patient population. In this study, using a prospective design, the potential relationship between baseline nonfasting, predialysis plasma total homocysteine (tHcy) levels and vascular access-related morbidity was examined in a cohort of 84 hemodialysis patients with a fistula or prosthetic graft as their primary hemodialysis access. Vascular access thrombotic episodes were recorded over a subsequent 18-mo follow-up period. Forty-seven patients (56% of the total) had at least one access thrombosis during the 18-mo follow-up period (median follow-up, 13 mo; rate, 0.6 events per patient-year of follow-up). Proportional hazards modeling revealed that each 1 μM/L increase in the tHcy level was associated with a 4.0% increase in the risk of access thrombosis (95% confidence interval, 1.0 to 6.0%, $P = 0.008$). This association persisted after adjustment for type of access (fistula *versus* graft), age, gender, time on dialysis, diabetes, smoking, hypertension, nutritional status, urea reduction ratio, dyslipidemia, and the presence of previous vascular disease. Elevated tHcy levels appear to confer a graded, independent increased risk for hemodialysis access thrombosis. A randomized, controlled trial examining the effect of tHcy-lowering intervention on hemodialysis access thrombosis appears to be justified.

Hemodialysis vascular access dysfunction due to thrombosis is the most common cause of hospitalization among maintenance hemodialysis patients (1). The annual cost of this serious morbidity is approximately $1 billion in the United States (2). Although their pathogenesis is unclear, thromboses of arteriovenous fistulae or synthetic hemodialysis access grafts are thought to result from stenotic lesions in the venous outflow system, or inadequate arterial inflow (2). Other than lower thrombotic event rates in matured arteriovenous fistulae relative to synthetic grafts (2,3), and the possible adverse influence of diabetes on synthetic graft survival (4), risk factors for hemodialysis access thrombosis remain ill-defined (2–4).

There is an excess prevalence of mild-to-moderate hyperhomocysteinemia, a putative thrombotic risk factor (5–10), in the dialysis-dependent end-stage renal disease population (11). Recently, conflicting retrospective analyses (12,13) have been reported regarding the potential association between total homocysteine (tHcy) levels and hemodialysis access thromboses. However, intractable survivorship effects resulting from the excess yearly mortality in hemodialysis patients (14) render hazardous *any* inference about tHcy access thrombosis associations derived from such retrospective (12,13) studies. The potential relationship between tHcy levels and hemodialysis access thrombosis requires more rigorous validation via prospective observational studies, and ultimately, randomized, controlled clinical trials of the effect of tHcy-lowering on access thrombosis event rates. Accordingly, using a prospective design, we examined the potential relationship between nonfasting, prehemodialysis session plasma tHcy levels and vascular access-related morbidity in a study group of 84 hemodialysis patients with an arteriovenous fistula or synthetic graft as their primary hemodialysis access. Vascular access thrombotic episodes were recorded over a subsequent 18-mo follow-up period.

Materials and Methods

The Institutional Review Board at Rhode Island Hospital, Providence, Rhode Island, approved the study protocol, and each participant provided written informed consent. All 99 patients undergoing chronic outpatient hemodialysis treatment at the Rhode Island Renal Institute, a nonprofit dialysis facility affiliated with Rhode Island Hospital, who had a functional arteriovenous fistula or polytetrafluoroethylene (synthetic) arteriovenous access graft, were offered enrollment in the study. Twelve patients did not participate due to either hospitalization for acute illness at the time of enrollment, or lack of interest. Of the remaining 87 patients, three transferred to other

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hemodialysis centers and were then lost to follow-up, leaving a total of 84 in the final analyses. All baseline data collection occurred during October 1996, with follow-up through April 1998. As per the standard of hemodialysis patient care in Rhode Island, 100% of patients were prescribed a multivitamin preparation that typically contained 1.0 mg of folic acid, 10 mg of vitamin B6, and 12 μg of vitamin B12.

Nonfasting prehemodialysis blood samples were collected for determination of plasma tHcy, folate, vitamin B12, and pyridoxal 5′-phosphate levels, as well as serum levels of creatinine, glucose, albumin, and total cholesterol, and blood urea nitrogen levels. tHcy was determined by HPLC with fluorescence detection, and pyridoxal 5′-phosphate by radioenzymatic assay, as described earlier (13). Folate and vitamin B12 were assessed by radioassay (BioRad, Hercules, CA). Creatinine, glucose, albumin, total cholesterol, and blood urea nitrogen were measured by standard automated clinical chemistry laboratory methods. Calculation of the urea reduction ratio has been described elsewhere (15).

Immediately after the baseline data and blood specimen collection, a record was initiated for all arteriovenous fistulae or synthetic graft thromboses, all morbid events requiring hospitalization, and all deaths. This record was reviewed monthly for the subsequent 18 mo. Physicians and allied health personnel carefully monitored pre-pump arterial and static venous pressures, and examined accesses, at each dialysis session. The urea reduction ratio was measured and recorded monthly. Recirculation studies, Doppler flow studies of the access, and fistulograms were ordered and performed at the discretion of the attending nephrologist. A fistula or synthetic graft thrombosis was defined as a sudden cessation of function of the arteriovenous access rendering hemodialysis impossible and requiring thrombectomy, thrombolysis, or the acute placement of another hemodialysis access. Detailed operational definitions for other clinical arteriosclerotic outcomes, diabetes or glucose intolerance, hypertension, smoking, and hypercholesterolemia are provided elsewhere (15). Outcome determinations and subsequent data entry were performed completely blinded to all laboratory and other relevant clinical data.

Statistical Analyses

Skewed continuous data were appropriately transformed, and comparisons of baseline continuous or categorical data between those who subsequently developed access thromboses *versus* those who did not were performed by *t* tests and χ^2 tests. Spearman's ρ was used to assess unadjusted rank order correlations between untransformed tHcy levels, and potential predictor variables. Subjects contributed person-time (months) until the development of access thrombosis, death, or the end of the follow-up period. Unadjusted and adjusted relative risk estimates (with 95% confidence intervals) for access thrombosis were generated by proportional hazards modeling with tHcy levels (untransformed or transformed) as the main independent variable. All analyses were performed using SAS software (version 6.12, Cary, NC).

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Results

Baseline characteristics of the 47 patients who experienced one or more vascular access thromboses during follow-up *versus* the 37 who did not are depicted in Table 1. Patients who developed access thromboses were less likely to have an arteriovenous fistula *versus* a synthetic graft (34% *versus* 68%, $P = 0.002$), more likely to have diabetes (60% *versus* 41%, $P = 0.083$), and tended to have been undergoing dialysis for a

Table 1. Baseline characteristics[a]

Parameter	Developed Access Thrombosis during Follow-Up		*P* Value[b]
	Yes	No	
n	47	37	
Fistula access (%)	34	68	0.002
Previous access thrombosis (%)	43	35	>0.2
Previous cardiovascular disease (%)	64	54	>0.2
Diabetes mellitus/glucose intolerance (%)	60	41	0.083
Current smoking (%)	38	37	>0.2
Hypertension (%)	91	81	>0.2
Hypercholesterolemia (%)	17	16	>0.2
Gender (% women)	55	49	>0.2
Age (yr)	69 [13]	66 [17]	>0.2
Time on dialysis (mo)	39 [48]	59 [50]	0.079
Urea reduction ratio (%)	69.7% [5.2%]	71.1% [4.5%]	>0.2
Total homocysteine (μM/L)	26.1 [11.6]	22.9 [2.8]	0.016
Folate (nmol/L)	39.3 [39.3]	84.8 [133.3]	0.051
Vitamin B12 (pmol/L)	583 [277]	899 [567]	0.003
Pyridoxal 5′-phosphate (nmol/L)	53.7 [68.4]	63.1 [67.2]	>0.2
Creatinine (mg/dl)	7.8 [2.5]	8.2 [2.4]	>0.2
Albumin (mg/dl)	3.8 [0.3]	3.7 [0.4]	>0.2

[a] SD is given in brackets.
[b] Based on χ^2 or unpaired *t* test.

shorter period of time (39 ± 48 *versus* 59 ± 50 mo, $P = 0.079$). Furthermore, higher mean baseline tHcy levels (26.1 ± 11.6 *versus* 20.9 ± 7.8 μM/L, $P = 0.016$) and lower mean vitamin B12 (583 ± 277 *versus* 899 ± 567 pmol/L, $P = 0.003$) and folate levels (39.3 ± 39.3 *versus* 84.8 ± 133.3, $P = 0.051$) were also observed in those patients who subsequently thrombosed their fistulae or grafts. Unadjusted correlation analyses revealed that tHcy levels were associated with serum albumin (+0.241, $P = 0.027$) as well as plasma B12 (−0.319, $P = 0.003$), and (marginally) plasma folate (−0.179, $P = 0.104$), but not ($P > 0.2$) with plasma pyridoxal 5′-phosphate, age, months on dialysis, creatinine, or the urea reduction ratio (data not shown). In additional unadjusted analyses, tHcy levels were found to be higher among those with a history of previous access thrombosis (27.1 ± 12.0 *versus* 21.7 ± 8.7 μM/L, $P = 0.030$), and (marginally) among current smokers (27.0 ± 13.2 *versus* 22.2 ± 9.5, $P = 0.126$), but did not differ ($P > 0.2$) by gender, type of access, or history of cardiovascular disease, diabetes, dyslipidemia, or hypertension (data not shown). During a total of 18 mo of follow-up (median follow-up, 13 mo), 47 patients experienced at least one access thrombosis (rate, 0.6 per person-year of follow-up). Unadjusted and adjusted relative risk estimates (hazards ratios) for dialysis access thrombosis during follow-up, derived from proportional hazards modeling, are displayed in Table 2. Results were identical using transformed or untransformed tHcy data, so only the untransformed data analyses are reported herein. In an unadjusted analysis, each 1 μM/L increase in plasma tHcy was associated with a 4% increase (95% confidence interval, 1 to 6%) in the risk for access thrombosis. This association persisted after individual adjustment for access type, diabetes, time on dialysis, history of previous access thrombosis or cardiovascular disease, smoking, hypertension, dyslipidemia, gender, age, urea reduction ratio, creatinine, or albumin. Predictably, adjustment for homocysteine levels attenuated the unadjusted association between folic acid or vitamin B12 levels, and access thrombosis. Modeling of interaction terms revealed no evidence for effect modification by gender, access type, or previous history of access thrombosis or clinical cardiovascular disease (all $P > 0.2$) (data not shown). Finally, there was no evidence that the assumption of constant proportional hazards was violated ($P > 0.2$) (data not shown).

Discussion

Our findings represent the initial prospective evidence that tHcy levels are independently linked to the development of hemodialysis access thrombosis. In three earlier prospective studies involving end-stage renal disease patients (16–18), tHcy levels were independently associated with the occurrence of clinical arteriosclerosis, including coronary heart, cerebrovascular, and peripheral vascular disease. The most recent of these reports (18) pooled arteriovenous fistulae thromboses ($n = 22$ events), but not synthetic graft access thromboses, with the typical arteriosclerotic events. However, no separate data regarding this thrombotic outcome were provided.

Although our study group experienced a characteristically (1–4) high event rate (*i.e.*, 47 patients with at least one access thrombosis during follow-up), the external validity of these results requires confirmation given the modest overall number of individuals investigated ($n = 84$). Despite this potential limitation, our finding of reduced risk for thrombotic events in those with arteriovenous fistulae *versus* synthetic grafts, regardless of homocysteine level, is in accord with the most consistent observation from earlier prospective studies of hemodialysis access thrombosis (2,3). Moreover, the apparent independent association between tHcy levels and hemodialysis access thrombosis is compatible with data linking hyperhomocysteinemia to thrombotic outcomes in other patient popula-

Table 2. Relative risk estimates for hemodialysis access thrombosis ($n = 47$ events) during follow-up (median of 13 mo), derived from proportional hazards modeling

Parameter	Relative Risk Estimate (95% Confidence Interval) [*P* Value]
Total homocysteine (per μM/L increase), unadjusted	1.04 (1.01 to 1.06) [0.008]
Total homocysteine (per μM/L increase), adjusted for	
fistula *versus* graft access	1.03 (1.00 to 1.05) [0.049]
previous access thrombosis	1.03 (1.00 to 1.05) [0.012]
previous cardiovascular disease	1.03 (1.00 to 1.05) [0.004]
time on dialysis	1.03 (1.00 to 1.05) [0.016]
diabetes mellitus	1.04 (1.01 to 1.06) [0.008]
gender	1.04 (1.01 to 1.06) [0.008]
age	1.04 (1.01 to 1.06) [0.008]
smoking	1.04 (1.01 to 1.06) [0.009]
hypertension	1.03 (1.00 to 1.05) [0.014]
dyslipidemia	1.04 (1.01 to 1.06) [0.009]
urea reduction ratio	1.04 (1.01 to 1.06) [0.004]
creatinine	1.04 (1.01 to 1.06) [0.007]
albumin	1.03 (1.00 to 1.05) [0.020]

tions (5–10). For example, the clinical course of children and young adults with homozygous cystathionine synthase deficiency is characterized by both marked hyperhomocysteinemia (fasting or random tHcy levels of 100 to 400 μM/L) and a dramatic increase in the risk, primarily, for thromboembolic sequelae, such as deep venous thrombosis, and pulmonary and cerebrovascular emboli (5). Post mortem examination of such patients has routinely revealed thrombi and emboli in nearly all major arteries and veins, as well as many smaller vessels (19). More recently, much milder hyperhomocysteinemia (tHcy levels of 14 to 50 μM/L) has been associated with thrombotic outcomes among adult populations in several retrospective studies (6–8), and two prospective studies (9,10). Curiously, the relationship between tHcy and idiopathic venous thromboembolism ($n = 73$ events) noted by Ridker and colleagues (10) apparently occurred at a "threshold" tHcy level (above approximately 17.3 μM/L) uncommon in their population (above the 95th percentile), but quite common in maintenance dialysis patients (11), including those we studied (17.3 μM/L = the 23rd percentile).

As recently reviewed by Selhub and D'Angelo (20), there are at present no data from appropriately designed mechanistic studies that explain how homocysteine may contribute to thrombogenesis. Hopefully, follow-up mechanistic investigations of a physiologic minipig model in which mild, dietary-induced hyperhomocysteinemia resulted in frank (21) thrombotic sequelae will prove more illuminating.

The current report might have important clinical implications given the serious costly morbidity associated with hemodialysis access thrombosis (1–4). Earlier strategies designed solely to lower grossly elevated tHcy levels in homozygotes for cystathionine synthase deficiency appear to have reduced thromboembolic event rates among these patients (5). Accordingly, even suboptimal lowering (22) of tHcy levels in hemodialysis patients (using high dose B vitamin regimens featuring folic acid) may reduce access thrombosis event rates, if, as the present data suggest, the risk for access thrombosis associated with tHcy levels is continuous.

In summary, elevated plasma tHcy levels appear to confer a graded, independent risk for vascular access thrombosis in maintenance hemodialysis patients. Controlled clinical trials evaluating the tenable hypothesis that lowering tHcy levels may reduce the rate of hemodialysis access thromboses appear warranted.

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