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Plasma Total Homocysteine Levels among Patients Undergoing Nocturnal *versus* Standard Hemodialysis

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Abstract. Mild hyperhomocysteinemia, a putative risk factor for arteriosclerotic outcomes, is seen in >85% of hemodialysis patients. Therapeutic strategies, including pharmacologic-dose B vitamin supplementation and "high-flux" or "super-flux" hemodialysis, have consistently failed to normalize total homocysteine (tHcy) levels in these patients. Predialysis plasma tHcy levels in 23 patients who were undergoing nocturnal hemodialysis (NHD) six or seven nights/wk were compared with those in 31 patients from the same Canadian dialysis unit who were undergoing chronic standard hemodialysis (SHD) (all <65 yr of age, undergoing thrice-weekly treatments). The SHD patients were similar to typical North American chronic hemodialysis patients with respect to B vitamin status and albumin, creatinine, and tHcy levels. Geometric mean tHcy levels for the NHD patients were significantly lower (12.7 *versus* 20.0 μM, $P < 0.0001$), as was the prevalence of mild-to-moderate hyperhomocysteinemia (>12 μM; NHD, 57%; SHD, 94%; $P = 0.002$). Analysis of covariance adjusted for plasma folate, vitamin B_{12}, and pyridoxal 5'-phosphate levels, age, and gender confirmed that NHD was independently associated with 6.0 μM lower geometric mean tHcy levels ($P = 0.001$). It is concluded that tHcy levels are significantly lower among NHD patients, compared with SHD patients. Clinical trials will be necessary to confirm that NHD is effective in reducing tHcy levels among patients with dialysis-dependent end-stage renal disease.

Homocysteine (Hcy) is an amino acid that, at elevated levels, may be an independent risk factor for arteriosclerotic disease and atherothrombosis (1). Mild-to-moderate elevations in plasma total Hcy (tHcy) levels (*i.e.*, hyperhomocysteinemia) are observed in the great majority (>85%) of patients with end-stage renal disease who are undergoing maintenance dialysis (2). Attempts to normalize tHcy levels in this population by using pharmacologic-dose folic acid-, pyridoxal 5'-phosphate (PLP) (vitamin B_6)-, or vitamin B_{12}-based regimens have been unsuccessful (3). Manipulation of the hemodialysis modality itself has also failed to significantly reduce tHcy levels (4). However, a preliminary report noted that fasting tHcy levels among patients undergoing daily hemodialysis were significantly lower than levels among patients undergoing standard hemodialysis (SHD) (5).

Nocturnal hemodialysis (NHD) is a relatively novel form of dialysis in which the patient undergoes prolonged overnight treatments six or seven nights each week, usually at home. Advantages over SHD include greater solute clearance, normalization of metabolic derangements, and improved quality of life (6).

We performed a cross-sectional study of a cohort of Canadian NHD subjects to ascertain tHcy levels among patients undergoing this intensive mode of dialysis. We then compared the values with those obtained for a SHD cohort from the same hemodialysis unit.

Materials and Methods

Study Population

Approval for the study was obtained from the Humber River Regional Hospital Ethical Review Board, and all participants provided written informed consent. All NHD patients affiliated with the Humber River Regional Hospital hemodialysis unit who were free of acute illness and who lived within a 1-h radius from the hospital were considered potential study subjects and were recruited into the study in October 2000.

The SHD subjects were randomly recruited from the same hemodialysis unit. Inclusion criteria were an age of >18 and <65 yr and no acute illness. All except two of the NHD subjects had previously undergone SHD. The NHD and SHD cohorts had been dialysis-dependent for 100 ± 83 mo (mean ± SD) and 29 ± 17 mo, respectively. Seven and one of the SHD subjects and five and zero of the NHD subjects had diabetes mellitus and coronary artery disease, respectively.

All NHD patients underwent home dialysis. The majority of these subjects underwent dialysis six nights/wk, with two undergoing nightly dialysis. Information on NHD dialysis techniques and prescriptions, access, and dialyzer reuse was previously provided (7). All of the SHD subjects underwent dialysis thrice-weekly, with high-flux

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membranes (Polyflux 21S or 17S: Gambro, Lakewood, CO). The urea Kt/V per treatment for the SHD group was 1.52 ± 0.23 (mean ± SD), whereas that for the NHD group was 1.48 ± 0.21. The latter value did not include urea production during dialysis or take into account the increased frequency of dialysis sessions. All NHD subjects received two Diavite tablets daily (folic acid, 1 mg; vitamin B_6, 6 mg; vitamin B_{12}, 6 μg: R & D Labs, Marina Del Rey, CA), whereas SHD subjects received one tablet daily.

Laboratory Data

Nonfasting prehemodialysis blood samples were obtained from all study subjects. Samples were immediately placed on ice, and the plasma was separated within 1 to 2 h. Plasma was stored at −70°C for several days and then transported to the Human Nutrition Research Center Vitamin Metabolism and Aging laboratory on dry ice. Plasma tHcy levels were determined by using HPLC with fluorescence detection (8), plasma PLP levels were measured enzymatically by using a radioenzymatic (tyrosine decarboxylase) assay (9), plasma folate levels were measured by using a microbial (*Lactobacillus casei*) assay, and plasma vitamin B_{12} levels were measured by using a RIA (Quantaphase II; BioRad, Hercules, CA). Plasma creatinine and albumin levels were measured by using standard automated clinical chemistry techniques.

Statistical Analyses

The positively skewed tHcy levels were transformed by using natural logarithms and are reported as geometric means. Folate and creatinine levels were transformed in the same manner, to conform with the assumption of linearity. Continuous variables were compared by using *t* tests, and categorical variables were compared by using χ^2 tests. Unadjusted analyses were assessed by using a Pearson correlation matrix. Forced multivariable modeling using analysis of covariance was then performed to determine the independent associations between potential covariables [NHD *versus* SHD, gender, age, PLP levels, vitamin B_{12} levels, and folate level] and prehemodialysis tHcy levels. Two-tailed *P* values are reported. All statistical analyses were performed by using SYSTAT software (version 7.0.1; SPSS, Chicago, IL).

Results

To confirm that the Toronto SHD reference population reflected a reasonably typical North American hemodialysis population, it was compared with a previously reported United States SHD cohort (10) (data not shown). Although our study SHD cohort was significantly younger than the comparison SHD cohort (45 *versus* 62 yr, $P < 0.001$), there was no difference between the groups with respect to gender, folate, PLP, or vitamin B_{12} status, or albumin, creatinine, or tHcy levels.

A comparison of the NHD and SHD cohorts (Table 1) demonstrated that the former group exhibited significantly higher albumin levels ($P < 0.001$) and lower creatinine ($P < 0.001$) and tHcy ($P < 0.001$) levels than did the SHD subjects. The difference in PLP levels approached statistical significance, although the 95% confidence intervals for both groups were well within the normal range.

Simple unadjusted analyses using tHcy levels as the outcome variable revealed significant linear correlations with PLP levels ($r = -0.41$), folate levels ($r = -0.31$), ln(creatinine levels) ($r = 0.53$), albumin levels ($r = -0.33$), and modality ($r = 0.52$), as indicated in Table 2. Geometric mean tHcy levels were 6.0 μM lower for the NHD subjects after analysis of covariance modeling, which controlled for the major determinants of tHcy levels, including gender, age, PLP levels, vitamin B_{12} levels, and folate levels ($P < 0.001$) (Table 2). Approximately 75% of the NHD patients exhibited homocysteine levels only slightly higher (≤14.6 μM) than the upper limit of the normal range (*i.e.*, ≤12 μM), and 50% exhibited tHcy levels within the normal range.

Discussion

This is the first study to measure tHcy levels in the NHD population. Available data demonstrate that the vast majority of SHD patients exhibit mild-to-moderate hyperhomocysteinemia (2), with mean predialysis tHcy levels being approxi-

Table 1. Biochemical and demographic profiles of SHD subjects and NHD subjects[a]

	SHD Cohort	NHD Cohort	*P* Value (Confidence Interval)[b]
n	31	23	
Age (yr)[c]	45 (25 to 64)	43 (20 to 65)	0.59
Gender (% women)	42	30	0.39
PLP (nM)[d]	133 (95 to 171)	193 (142 to 145)	0.052
Vitamin B_{12} (pg/ml)[d]	732 (586 to 880)	858 (715 to 1001)	0.23
Folate (ng/ml)[d]	42 (27 to 56)	51 (32 to 69)	0.45
Creatinine (mg/dl)[d]	9.5 (8.1 to 10.9)	5.1 (4.6 to 5.7)	<0.001 (2.7 to 6.0)
Albumin (g/dl)[d]	3.9 (3.8 to 4.1)	4.5 (4.3 to 4.6)	<0.001 (0.34 to 0.74)
tHcy (μM)[e]	20.0 (17.5 to 22.8)	12.7 (10.7 to 15.1)	<0.001 (1.27 to 1.94)[f]

[a] SHD, standard hemodialysis; NHD, nocturnal hemodialysis; PLP, pyridoxal 5′-phosphate; tHcy, total homocysteine.
[b] Based on two-sample *t* test.
[c] Mean (full range).
[d] Mean (95% confidence interval).
[e] Geometric mean (95% confidence interval).
[f] Confidence interval for ratio.

Table 2. Unadjusted and multivariable-adjusted analyses with tHcy levels as the outcome variable

Variable	Unadjusted Analysis, *P* Value (*r*)	Multivariable-Adjusted Analysis,[a] *P* Value	Multivariable-Adjusted Geometric Mean Difference in tHcy Levels, SHD *versus* NHD (μM)
Gender	0.35	0.71	
Age	0.23	0.22	
PLP	0.002 (−0.41)	0.32	
Vitamin B_{12}	0.26	0.90	
Folate[b]	0.02 (−0.31)	0.31	
Creatinine[b]	<0.0001 (0.53)		
Albumin	0.02 (−0.33)		
Modality[c]	<0.0001 (0.52)	0.001	6.0

[a] Forced analysis of covariance model adjusted for modality, age, gender, vitamin B_{12} levels, PLP levels, and folate levels ($R = 0.38$).
[b] Log transformed.
[c] NHD *versus* SHD.

mately ≥20 μM among patients receiving supplementation with B vitamin regimens featuring supraphysiologic doses of folic acid (4). Our SHD cohort exhibited similar tHcy levels and was not different from a North American SHD reference cohort with respect to other biochemical or demographic indices. In contrast, mean levels in our NHD population were 12.7 μM. Furthermore, predialysis tHcy values (which were essentially the "peak" tHcy levels for these patients) were near the normal range (≤14.6 μM) for 75% of NHD subjects and within the normal range (≤12 μM) for approximately 50%. This dramatic increase in the percentage of vitamin-replete hemodialysis patients with normal or near-normal predialysis tHcy levels has not been observed with any other type of tHcy-reducing therapy, including pharmacologic-dose vitamin supplementation or manipulation of the dialysis procedure (4,11).

NHD is a relatively novel form of hemodialysis that is increasingly being recognized as being superior to SHD in numerous respects (6,12,13). Dialytic removal of both small- and middle-molecule substances is markedly enhanced compared with SHD, often by as much as fourfold or more (12,13). Hypertension is ameliorated to the point that antihypertensive agents often are no longer required, and serum phosphate levels decrease so dramatically that supplementation actually becomes necessary. Patients report significant improvements in subjective feelings of well-being, end all dietary restrictions, seem to become anabolic (14), and exhibit normalized albumin levels (12). Consistent with the latter findings are the normal-range albumin levels of our NHD cohort. In our clinical experience, such levels are infrequently observed in SHD patients.

Plasma tHcy levels increase in proportion to decreases in GFR (3), but the cause of hyperhomocysteinemia in the setting of renal disease has not yet been fully elucidated. There are data to support the premise that healthy kidneys play a major role in the clearance and metabolism of Hcy and that deranged kidney function reduces plasma tHcy clearance, leading to tHcy accumulation and increased plasma levels (3). Conflicting data suggest that the kidney may not significantly metabolize Hcy and that the hyperhomocysteinemia of renal disease could be a consequence of retained inhibitory substances that interfere with normal extrarenal Hcy metabolism (15).

The reason for lower tHcy levels in the NHD cohort is not yet understood. One biologically plausible reason could be that Hcy is directly cleared in greater amounts by the dramatically enhanced solute clearance observed with NHD. Fully 30% of tHcy (*i.e.*, the non-protein-bound portion) can be easily and rapidly removed by dialysis. A fraction of protein-bound Hcy (the remaining 70%) would also likely be removed from the circulation by adsorption onto the dialysis membrane. This may be even more likely to occur with prolonged exposure of the membrane to circulating plasma, such as in NHD. Arnadottir *et al.* (16) measured tHcy levels in small dialysate samples from five SHD patients and concluded that SHD was unable to remove sufficient Hcy (*i.e.*, 1.2 mmol) to maintain tHcy levels within the normal range. However, the effects of prolonged intensive dialysis, such as NHD, on direct Hcy clearance and protein binding may be markedly different from those of SHD (7). Shorter periods between dialysis sessions may also prevent predialysis tHcy levels from reaching higher peaks.

Arnadottir *et al.* (16) also measured tHcy levels in six subjects at several time points after a SHD session and observed that levels began to increase only after 8 h, in contrast to plasma creatinine levels, which began to increase immediately. On the basis of those findings, the investigators surmised that the reduction in tHcy levels during dialysis results from the removal of inhibitory uremic toxins, rather than direct Hcy clearance. However, conflicting data demonstrate that tHcy levels begin to increase only 2 h after hemodialysis (17). It should also be recognized that these putative inhibitory toxins have not been identified and that there is good evidence that even mild reductions in GFR (*e.g.*, >60 ml/min) result in higher tHcy levels (18) in the absence of significant accumulation of uremic toxins. Of note, the multivariable analysis revealed no significant effect of the additional daily vitamin supplement given to NHD subjects on the difference in tHcy levels.

One potential limitation of this study is that data on tHcy levels were not available before subjects began NHD therapy, which prevented us from excluding the possibility that the patients had preexisting lower tHcy levels. However, because hyperhomocysteinemia is observed for the majority of dialysis patients, regardless of their biochemical profiles or comorbidities, we think that this is unlikely to be the case. Mean serum albumin and creatinine measurements from the NHD cohort before NHD initiation support our premise; the values were not statistically different from those in the study SHD cohort (NHD *versus* SHD albumin, 3.8 *versus* 3.9 g/dl; creatinine, 9.9 *versus* 9.5 mg/dl). It seems that the dramatic biochemical changes that occur after NHD initiation are most likely the result of the dialysis modality itself, rather than patient selection. In light of this and previous data (19), the 50% lower mean plasma creatinine levels for the NHD cohort, compared with the SHD cohort, should be interpreted as resulting from increased dialytic clearance of creatinine and not from reduced lean body mass.

We did not include albumin and creatinine levels in the final multivariable-adjusted model, because we did not want to overcorrect for the type of dialysis modality (*i.e.*, NHD *versus* SHD). tHcy levels have previously been associated with PLP and folate levels among dialysis patients (for the former, possibly because of its high level of albumin binding).

In conclusion, this study suggests that, among patients <65 yr of age, NHD is independently associated with 6 μM lower tHcy levels compared with SHD patients, and that the proportion of NHD patients with hyperhomocysteinemia is dramatically reduced, compared with the SHD cohort. Clinical trials will be necessary to confirm that NHD is effective in reducing tHcy levels among patients with dialysis-dependent end-stage renal disease.

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