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Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients

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Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients.

Background. We prospectively tested the prediction power of homocysteinemia for all-cause and cardiovascular outcomes in a cohort of 175 hemodialysis patients followed for 29 ± 12 months.

Methods. Survival analysis was performed by the Cox's proportional hazard model and data were expressed as hazard ratio and 95% confidence interval (CI).

Results. During the follow-up period 51 patients died, 31 of them (61%) of cardiovascular causes and 16 patients developed non-fatal atherothrombotic complications. Plasma total homocysteine was an independent predictor of cardiovascular mortality ($P = 0.01$). Combined analysis of fatal and non-fatal atherothrombotic events showed that homocysteine was a strong and independent predictor of these outcomes because the risk of these events was 8.2 times higher (95% CI 1.9 to 32.2) in patients in the third homocysteine tertile than in those in the first tertile ($P = 0.005$).

Conclusions. There is a clear association between hyperhomocysteinemia and incident cardiovascular mortality and atherothrombotic events in hemodialysis patients. Intervention studies are needed to determine whether the accumulation of this substance has a causal role in the pathogenesis of cardiovascular damage in patients undergoing hemodialysis.

Cardiovascular disease accounts for approximately 50% of mortality among patients on chronic dialysis and a similarly high mortality rate has been observed in the United States and in Europe [1]. The appreciation of the relative role of traditional (that is, Framingham risk factor) and nontraditional cardiovascular risk factors in determining the high mortality of patients with end-stage renal disease (ESRD) is complex because of the high prevalence of multiple risk factors [2]. Hyperhomocysteinemia was suspected as a cardiovascular risk factor in chronic renal failure in the eighties [3], and the problem was examined in several cross-sectional and mechanistic studies [4–18]. In this regard, the observations that plasma homocysteine is independently related to aortic stiffness [19] and left ventricular hypertrophy [20] are of particular interest. However, apart from the seminal studies by Bostom et al [21] and Moustapha et al [22] in a total of 240 patients, there is a lack of prospective investigations linking hyperhomocysteinemia to cardiovascular mortality in hemodialysis patients. The issue is relevant mainly because a recent study suggested that reduced plasma homocysteine concentration, rather than hyperhomocysteinemia, predicts survival in these patients [23].

Our current study prospectively tested the association between plasma total homocysteine and all-cause and cardiovascular outcomes in a large cohort of hemodialysis patients. Since cardiovascular risk is inherently multifactorial, the role of hyperhomocysteinemia was analyzed in multivariable models encompassing traditional and nontraditional risk factors and factors peculiar to ESRD.

METHODS

Protocol

The protocol conformed to the ethical guidelines of our Institutions and informed consent was obtained from each participant. All studies were performed during a non-dialysis day, between 8 a.m. and 10 a.m.

Study cohort

One hundred seventy-five patients with ESRD (98 males and 77 females, all Caucasians) who had been on

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regular dialysis treatment (RDT) for at least six months and without clinical evidence of overt cardiovascular congestion [24] were eligible for the study. These patients represented about the 70% of the entire hemodialysis population of four dialysis units. The remaining patients were excluded from the study because they were taking folic acid, vitamin B_6 and B_{12} supplements (11%), or because of frank cardiovascular congestion or overt infections (9%), or because they were hospitalized for inter-current illnesses (10%). The prevalence of diabetes mellitus in this cohort was 16% (that is, 28 out of 175 patients).

All patients were virtually anuric (24-hour urine volume <200 mL/day) and were being treated thrice weekly with standard bicarbonate hemodialysis (Na 138 mmol/L, HCO_3 35 mmol/L, K 1.5 mmol/L, Ca 1.25 mmol/L, Mg 0.75 mmol/L) or with high-flux hemodialysis either with cuprophan or semi-synthetic membranes (dialysis filter surface area 1.1 to 1.7 m^2). Dry weight was targeted in each case to achieve a normotensive edema-free state.

Sixty-one patients were on anti-hypertensive treatment [46 on mono-therapy with angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 (AT-1) receptor antagonists, calcium channel blockers, alpha- and beta-blockers, and 15 were on double therapy with various combinations of these drugs]. Sixty-five patients were habitual smokers (20 ± 15 cigarettes/day).

Follow-up study

After the initial assessment patients were followed up for 29 ± 12 months. During the follow-up period fatal and non-fatal cardiovascular events [electrocardiogram (ECG)-documented anginal episodes, myocardial infarction, heart failure, arrhythmia, transient ischemic attach, stroke, and major arterial and venous thrombotic episodes (except arteriovenous fistula thrombosis)] were accurately recorded. Myocardial infarction was confirmed by serial ECG evidence of a Q-wave or non-Q–wave myocardial infarction and appropriate creatine phosphokinase MB fraction elevations. Thrombotic stroke was confirmed by computerized tomographic imaging evidence of cerebral infarction and/or neurological examination findings consistent with new-onset focal neurological deficits lasting >24 hours. Each death was reviewed and assigned an underlying cause by a panel of five physicians who were blinded to the potential independent predictor variables. As a part of the review process, all available medical information about each death was collected. This information always included the study and hospitalization records. In the case of an out-of-hospital death, family members were interviewed by telephone to better ascertain the circumstances surrounding death.

Laboratory measurements

A fasting blood sample for serum lipids, albumin, calcium and phosphate, C-reactive protein (CRP), plasma fibrinogen, total homocysteine, B_6, B_{12}, folate and hemoglobin was obtained from all patients during a mid week non-dialysis day. Plasma total homocysteine was determined by a high-pressure liquid chromatography (HPLC) method based on a derivative of SBD-F (ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate) fluorescence [25]. CRP was measured by using a commercially available kit (immunoturbidimetric method, lower limit of detection ≤3.5 mg/L; Behring, Scoppito, L'Aquila, Italy). Vitamin B_6 was measured by a radioenzymatic assay for direct measurement of plasma pyridoxal 5′-phosphate [26] and plasma folate and vitamin B_{12} by commercially available competitive immunoassays (direct chemiluminescence; Chiron Diagnostic, San Francisco, CA, USA).

Blood pressure measurements

Blood pressure (BP) was measured before dialysis. The average value of all recordings (12 measurements, that is, 3 measurements/week) taken during the month preceding the study was considered as a representative of the BP of each patient [27].

Statistical analysis

Data are reported as mean ± SD or as median and inter-quartile range. Comparisons between groups were made by the *t* test or by the Mann-Whitney test, as appropriate. Independent correlates of plasma homocysteine were identified by univariate and multivariate linear regression analysis. Variables that had an independent relationship with plasma homocysteine were ordered according to their standardized effect, defined as regression coefficient/standard error of the regression (β).

The independent prediction power of plasma total homocysteine for all-cause and cardiovascular outcomes was tested in Cox's proportional hazards models adjusted for a series of traditional risk factors [age, sex, diabetes, smoking, body mass index (BMI), systolic pressure and cholesterol], for emerging risk factors (CRP and fibrinogen) and factors peculiar to ESRD [duration of regular dialysis treatment (RDT), hemoglobin, albumin and calcium*phosphate product]. Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated with the use of the estimated regression coefficients and their standard errors in the Cox's regression analysis. All calculations were made using a standard statistical package (SPSS for Windows Version 9.0.1, 11 Mar 1999; SPSS, Chicago, IL, USA).

RESULTS

The demographic, anthropometric, clinical and biochemical parameters of patients are given in Table 1. In the entire group, plasma total homocysteine (median 28.9 μmol/L, interquartile range 20.5 to 46.7 μmol/L) was above the upper limit of the normal range (cutoff

Table 1. Demographic, somatometric, clinical and biochemical characteristics of the study population

Demographic and somatometric data	
Age *years*	59.5 ± 15.1
Duration of RDT *months*	37.0 (18.0–99)
Males/females	98/77
BMI kg/m^2	24.7 ± 4.4
Cardiovascular risk factors	
Systolic pressure *mm Hg*	141.1 ± 24.7
Diastolic pressure *mm Hg*	77.2 ± 13.1
Diabetics	16%
Smokers	37%
Hypercholesterolemia	52%
On treatment with erythropoietin	52%
Biochemical data	
Plasma homocysteine *μmol/L*	28.9 (20.5–46.7)
Plasma B_6 *nmol/L*	15.7 (7.3–23.3)
Plasma B_{12} *pmol/L*	434.5 (297.8–984.6)
Plasma folate *nmol/L*	15.4 (10.3–21.1)
Serum total cholesterol *mmol/L*	5.2 (4.3–6.0)
Serum triglycerides *mmol/L*	1.7 (1.3–2.3)
Serum calcium *mmol/L*	2.27 ± 0.25
Serum phosphate *mmol/L*	1.94 ± 0.39
Serum CRP *mg/L*	5.4 (3.4–16.0)
Plasma fibrinogen *mg/dL*	342.0 (243.0–465.0)
Serum albumin *g/dL*	4.2 ± 0.4
Hemoglobin *g/dL*	10.8 ± 1.8
Kt/V	1.20 ± 0.26

Data are mean ± SD, median (inter-quartile range), or percent frequency as appropriate. Abbreviations are: RDT, regular dialysis treatment; CRP, C-reactive protein; Kt/V, fractional urea clearance.

Table 2. Fatal cardiovascular events and causes of death in the study cohort

Variable	*N*
Fatal cardiovascular events	
Myocardial infarction	9
Thrombotic stroke	3
Hemorragic stroke	3
Heart failure	6
Sudden death	3
Mesenteric infarction	3
Arrhythmia	2
Pulmonary embolism	2
Other causes of death	
Sepsis/infection	5
Cachexia	4
Neoplasia	3
Hyperkalemia	3
Gastrointestinal hemorrage	2
Respiratory failure	1
Diabetes, hyperosmolar coma	1
Treatment withdrawal	1
Total	51

>15 μmol/L) in the majority of hemodialysis patients (155 of 175, 89%).

Univariate and multivariate correlates of plasma total homocysteine

The univariate analysis showed that plasma total homocysteine was inversely related to plasma folate ($r = -0.30$, $P = 0.0001$) and plasma B_{12} ($r = -0.21$, $P = 0.02$), and directly related to serum albumin ($r = 0.17$, $P = 0.03$) and male sex (plasma total homocysteine in males was 32.2 μmol/L, interquartile range 22.1 to 53.8 μmol/L, and in females was 26.2 μmol/L, 19.2 to 41.9 μmol/L; $P = 0.03$). In multivariate analysis plasma folate ($\beta = -0.28$, $P = 0.001$), serum albumin ($\beta = 0.28$, $P = 0.009$) and male sex ($\beta = 0.18$, $P = 0.05$) were the only significant correlates of plasma homocysteine (multiple $R = 0.48$, $P = 0.0001$) while plasma B_6, plasma B_{12}, serum cholesterol, serum CRP, Kt/V, age, duration of RDT, smoking, diabetes, BMI, systolic pressure, and previous cardiovascular events were unrelated to plasma homocysteine.

Plasma total homocysteine, all-cause mortality and cardiovascular outcomes

During the follow-up period 51 patients died, 31 of them (61%) of cardiovascular causes (Table 2), and 16 patients developed non-fatal atherothrombotic complications (myocardial infarction in 2, angina in 3, retinal, femoral or iliac artery thrombosis in 5, thrombotic stroke in 5 and transient ischemic attack in 1). In a COX regression analysis all-cause mortality was explained only by age (HR, 1.06, 95% CI 1.02 to 1.08, $P = 0.0009$) and previous cardiovascular events (HR, 2.53, 95% CI 1.20 to 5.36, $P = 0.01$). In this model plasma total homocysteine failed to independently predict survival ($P = 0.15$). On the other hand, plasma homocysteine turned out to be an independent predictor of cardiovascular mortality, and the prediction power of plasma homocysteine for cardiovascular outcomes was also true when the analysis was confined to atherothrombotic events (Table 3) such as myocardial infarction, thrombotic stroke, mesenteric infarction and pulmonary embolism (these events were given in detail in Table 2). In this analysis a 10 μmol/L increase in plasma homocysteine was associated with a 35% increase in the incident risk of fatal atherothrombotic events. Combined analysis of fatal and non-fatal atherothrombotic events confirmed that homocysteine was an independent predictor of these outcomes (Table 4). When patients were divided into three tertiles according to plasma homocysteine concentration, the hazard ratio for fatal and non-fatal atherothrombotic events was progressively higher from the first tertile onwards, and the hazard ratio of patients with plasma homocysteine in the third tertile was 8.2 times (95% CI 1.9 to 32.2) higher than in those in the first tertile ($P = 0.005$; Fig. 1).

DISCUSSION

In a large cohort of patients with ESRD total plasma homocysteine predicted fatal cardiovascular events and established that the link between this risk factor and

Table 3. COX proportional hazard model for cardiovascular mortality and for cardiovascular mortality due to atherothrombotic events

	Units of increase	Hazard ratio	95% CI	*P*
Cardiovascular events				
Significant predictors				
Age	1 year	1.06	1.01–1.10	0.01
Homocysteine	10 μmol/L	1.20	1.04–1.38	0.01
Fibrinogen	10 mg/dL	1.03	1.00–1.05	0.03
Previous CV events	Yes/no	2.96	1.12–7.81	0.03
Male sex		2.79	1.07–7.32	0.04
Non-significant predictors				
Cholesterol	1 mmol/L	1.25	0.95–1.66	0.11
Albumin	1 g/dL	0.42	0.14–1.29	0.13
Systolic pressure	1 mm Hg	1.01	0.99–1.03	0.30
Diabetes		1.78	0.59–5.36	0.31
BMI	1 kg/m^2	1.05	0.95–1.15	0.38
Duration of RDT	1 month	1.00	0.99–1.01	0.60
Smoking	1 packet of cigarettes/month	1.00	0.99–1.02	0.69
Calcium × phosphate	1 $mmol^2/L^2$	0.91	1.00–1.01	0.71
Hemoglobin	1 g/dL	0.96	0.75–1.23	0.76
CRP	1 mg/L	1.00	0.98–1.02	0.80
Atherothrombotic events				
Significant predictors				
Age	1 year	1.16	1.05–1.29	0.003
Male sex		15.0	2.19–103.4	0.006
Homocysteine	10 μmol/L	1.35	1.05–1.73	0.02
Duration of RDT	1 month	1.01	1.00–1.03	0.03
Hemoglobin	1 g/dL	0.61	0.37–1.00	0.05
Non-significant predictors				
Smoking	1 packet of cigarettes/month	0.96	0.91–1.01	0.14
Albumin	1 g/dL	4.96	0.54–45.9	0.16
Cholesterol	1 mmol/L	1.41	0.85–2.33	0.18
BMI	1 kg/m^2	1.08	0.94–1.25	0.27
Previous CV events		3.33	0.38–29.38	0.28
Systolic pressure	1 mm Hg	1.02	0.98–1.05	0.31
Diabetes		2.31	0.39–13.76	0.36
Fibrinogen	10 mg/dL	1.01	0.96–1.06	0.65
CRP	1 mg/L	1.00	0.98–1.02	0.72
Calcium × phosphate	1 $mmol^2/L^2$	1.03	0.49–2.17	0.94

Data are expressed as hazard ratios, 95% CI (confidence interval) and *P* values. In the analysis of fatal atherothrombotic episodes deaths due to myocardial infarction, thrombotic stroke, mesenteric infarction and pulmonary embolism were included (see also Table 2).

Table 4. COX proportional hazard model for fatal and non-fatal atherothrombotic events

	Units of increase	Hazard ratio	95% CI	*P*
Significant predictors				
Age	1 year	1.06	1.02–1.11	0.004
Homocysteine	10 μmol/L	1.18	1.05–1.33	0.005
Non-significant predictors				
Systolic pressure	1 mm Hg	1.02	0.99–1.03	0.07
Duration of RDT	1 month	1.01	0.99–1.01	0.07
Diabetes		1.92	0.68–5.43	0.22
BMI	1 kg/m^2	1.05	0.97–1.13	0.23
Fibrinogen	10 mg/dL	1.01	0.99–1.04	0.32
Male sex		1.58	0.59–4.21	0.36
Cholesterol	1 mmol/L	0.89	0.68–1.18	0.42
Albumin	1 g/dL	1.59	0.42–5.95	0.49
Smoking	1 packet of cigarettes/month	1.01	0.98–1.03	0.64
Previous CV events	Yes/no	1.24	0.40–3.82	0.71
Calcium × phosphate	1 $mmol^2/L^2$	1.09	0.69–1.73	0.71
Hemoglobin	1 g/dL	0.97	0.75–1.25	0.81
CRP	1 mg/L	1.00	0.98–1.02	0.99

Data are expressed as hazard ratios, 95% CI (confidence interval) and *P* values.

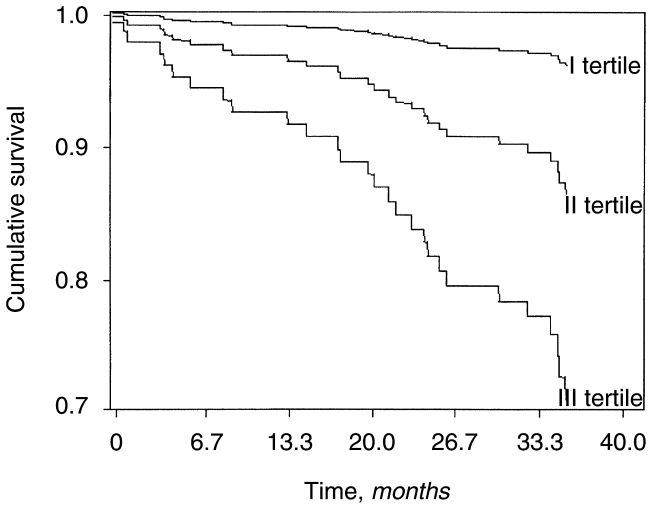

Fig. 1. Cox proportional hazard survival curves for fatal and non fatal atherothrombotic events. Patients were divided into three tertiles according to their plasma homocysteine concentration: I tertile <22.9 μmol/L; II tertile ≥ 22 μmol/L; III tertile ≥ 37.8 μmol/L. Data were adjusted for the other independent predictors of fatal and non-fatal atherothrombotic cardiovascular events (refer to Table 4).

cardiovascular outcomes was largely independent of traditional [21, 22] and non-traditional risk factors such as serum CRP [28–30] and of risk factors peculiar to chronic renal failure like anemia [31], hypoalbuminemia, hyperphosphatemia and high calcium-phosphate product [32].

Hyperhomocysteinemia is found very frequently in dialysis patients [4–8, 13, 15, 21–23]. In the present study, multivariate analysis based on the main clinical and biochemical indicators of the severity of uremia and on the vitamins that regulate the metabolism of this amino acid confirmed that serum folate is an independent correlate of circulating homocysteine, which is in keeping with previous observations [8, 1, 15]. Serum albumin is the main carrier of circulating homocysteine, and a univariate relationship between homocysteine and serum albumin was noted in a recent study in hemodialysis patients [23]. Our multivariate analysis confirms that serum albumin is an independent predictor of plasma total homocysteine. The direct link between homocysteine and albumin appears particularly important, because hypoalbuminemia per se is a predictor of adverse cardiovascular outcomes and may therefore confound the effect of homocysteine on these outcomes. Such a relationship highlights the complexity and inherent weakness of cross sectional studies aimed at establishing a link between cardiovascular risk and the plasma concentration of this substance.

The issue as to whether hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients was examined only in two studies. In the first study the cardiovascular risk was 3.6 times higher in patients with plasma homocysteine greater than 27 μmol/L than in those displaying levels below this threshold [21]. In the second study, homocysteine was by 59% higher in dialysis patients who had fatal and non-fatal cardiovascular events, and the high cardiovascular risk conferred by this hyperhomocysteinemia was largely independent of traditional risk factors and unrelated to all-cause mortality [22]. In another study in hemodialysis patients homocysteine was associated with all-cause mortality [33]. In the current study, which was based on the largest hemodialysis cohort studied to date the trend towards total homocysteine predicting total mortality failed to attain statistical significance. Notably, in our study the risk of fatal cardiovascular complications was highly significant and coincided numerically (20% risk increase for each 10 μmol/L increase in plasma total homocysteine; Table 3) with the estimate made in the study by Moustapha et al [22]. Although the biological mechanism(s) whereby homocysteine may cause damage to organ systems in humans has not been fully elucidated, it seems likely that this substance triggers cardiovascular events by causing oxidative injury to the endothelium and by altering the coagulation properties of the blood. In this regard, the strong link between homocysteine and atherothrombotic events found in our study seems relevant because it has a plausible biological basis. Homocysteine predicted cardiovascular events in a study in transplant patients [34] but was unrelated to all-cause mortality and graft survival in another recent study [35]. Our study is the first to test the prediction power of total homocysteine by adopting a statistical approach taking into account other emerging risk factors (namely CRP) and well-established risk factors peculiar to the uremic state, like anemia [31] and hyperphosphatemia [32]. The fact that the relationship between hyperhomocysteinemia and cardiovascular mortality and atherothrombotic events is independent of these factors makes the epidemiological association between this substance and cardiovascular complications in the dialysis population even more compelling. Our observation also may help us better understand two recent studies reporting a paradoxical link between hyperhomocysteinemia and survival in hemodialysis patients [18, 23]. The first was a small-scale study with a short follow-up period and did not contemplate cardiovascular events and death as primary end-points [18], while no statistical adjustment for potential confounders and for traditional and non-traditional risk factors was performed in the second study [23].

In conclusion, there is a clear association between hyperhomocysteinemia and cardiovascular outcomes in dialysis patients. Homocysteine is, at least partially, a modifiable risk factor [36]. Intervention studies are needed to determine whether the accumulation of this substance has a causal role in the pathogenesis of cardiovascular damage in hemodialysis patients.

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