Relationship Between Homocysteine and Mortality in Chronic Kidney Disease

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Background—The relationship between total homocysteine (tHcy) and outcomes has not been investigated in patients with chronic kidney disease stages 3 to 4.

Methods and Results—The Modification of Diet in Renal Disease Study was a randomized, controlled trial of 840 patients. Serum tHcy was measured in frozen samples collected at baseline (n = 804). Survival status and cause of death were obtained from the National Death Index. To evaluate its association with all-cause and cardiovascular disease (CVD) mortality, tHcy was evaluated both as tertiles (<14.7, 14.7 to 19.5, ≥19.6 μmol/L) and as a continuous variable (per 10/μmol/L). Participants had a mean age of 52 ± 12 years and glomerular filtration rate (GFR) of 33 ± 12 mL/min per 1.73 m²; 60% were male, and 85% were white. During a median follow-up of 10 years, 195 (24%) died from any cause, and 118 (15%) from CVD. The level of GFR was lower and proteinuria higher in the highest tHcy tertile. There was no association between the highest tertile of tHcy and all-cause (hazard ratio [HR]; 95% confidence interval [CI], 1.32, 0.94 to 1.85) or CVD (HR; 95% CI, 1.50, 0.96 to 2.34) mortality in univariate analyses; this association was further attenuated by adjustment for GFR (HR; 95% CI all-cause, 1.04, 0.72 to 1.51; CVD, 1.20, 0.73 to 1.95). There was no association between tHcy as a continuous variable and all-cause (0.98, 0.83 to 1.16) or CVD (1.04, 0.85 to 1.27) mortality.

Conclusions—Hyperhomocystinemia does not appear to be a risk factor for all-cause or CVD mortality in the Modification of Diet in Renal Disease Study. Prior studies demonstrating an association between tHcy and CVD risk may have inadequately adjusted for the confounding effects of kidney function. (Circulation. 2006;113:1572-1577.)

Key Words: kidney mortality epidemiology

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kidney function may explain the heterogeneity of study results.

Levels of tHcy are correlated with GFR, proteinuria, and nutritional status, as well as with traditional CVD risk factors.1,11 Given these correlations and the inconsistency in the literature, it remains unclear as to whether tHcy is an independent risk factor for CVD or rather a marker of severity of kidney disease in patients with CKD. The objectives of this study were to evaluate whether tHcy was an independent risk factor for all-cause and CVD mortality in a cohort of patients with CKD stages 3 to 4 who had precise measures of kidney function.

Methods

The Modification of Diet in Renal Disease (MDRD) Study, conducted from 1989 to 1993, was a randomized, controlled trial to

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study the effect of dietary protein restriction and blood pressure control on the progression of kidney disease. Details of the study have been published previously.12 In brief, 585 patients with a baseline GFR of 25 to 55 mL/min per 1.73 m² were randomized in study A, and 255 patients with a baseline GFR of 13 to 24 mL/min per 1.73 m² were randomized in study B. Patients in study A and study B were combined for the current analyses.

As described previously, levels of total Hcy were measured in frozen samples collected at baseline from 804 participants of the MDRD Study cohort.13 Samples were assayed for tHcy by using high-performance liquid chromatography with fluorometric detection. GFR was assessed by the kidney clearance of 125I-iothalamate.14 Survival status and cause of death were ascertained from the National Death Index. A death was ascribed to CVD if the primary cause of death was International Classification of Diseases, 9th Revision (ICD-9) code 390 to 459 (n=96) or if kidney disease was listed as the primary cause of death and CVD was the secondary cause (n=22). Survival time was defined as time from randomization to death or end of follow-up (December 31, 2000). Data collection procedures were approved by the Cleveland Clinic and Tufts–New England Medical Center Institutional Review Boards.

Statistical Analysis

Demographic, CVD risk factors, and kidney disease factors were compared across tertiles of tHcy through the use of the χ² test for categoric data, ANOVA for approximately normally distributed continuous variables, and the Kruskall-Wallis test for skewed continuous variables

Results

Baseline Characteristics

The study cohort had an average age of 52±12 years, GFR of 33±12 mL/min per 1.73m², and median (interquartile range) tHcy of 16.8 (8) μmol/L. The sample was predominantly white (91%); 60% were male, and 15% were current smokers. Higher tHcy was associated with male gender, history of coronary artery disease, lower body mass index, and lower prevalence of diabetes (Table 1). Glomerular filtration rate was lower and level of proteinuria higher in the higher tHcy groups; however, there was no difference in the cause of kidney disease.

Outcomes

Median follow-up for survival analyses was 10 years (range, 3 to 140 months). All-cause mortality rate was 24% (n=195), and CVD mortality rate was 15% (n=118). There were 60 (22%), 58 (22%), and 77 (29%) deaths from all causes and 33 (12%), 37 (14%) and 48 (18%) deaths caused by CVD in the respective tertiles. Incidence rates for all-cause and CVD mortality, per tertile, are presented in the Figure.

There was a nonsignificant positive association between the highest tertile of tHcy and both all-cause and CVD mortality in unadjusted analyses that was largely attenuated by adjustment for GFR (Table 2). Additional adjustment for random assignments, demographic, CVD, and other kidney disease factors had little incremental effect on attenuating the association between the high tHcy tertile and each mortality outcome.

After adjustment for GFR, the Cox regression coefficient for high tHcy and all-cause mortality decreased from 0.278 to 0.037, a proportional change of 87%. Similarly, for CVD mortality adjusting for GFR decreased the Cox regression coefficient from 0.406 to 0.179, a proportional change of 56%.

The Cox model was repeated evaluating tHcy as a continuous variable (Table 3). Again, there was no association between tHcy and either all-cause or CVD mortality in unadjusted or adjusted analyses. In models examining all-cause and CVD mortality, respectively, interaction terms between study (study A versus study B) and tHcy (P=0.70; P=0.69) and GFR and tHcy (P=0.73, P=0.93) were not significant.

Additional Analyses

There was no association between tHcy and all-cause (0.89, 0.69 to 1.15) or CVD (0.82, 0.57 to 1.18) mortality in the subgroup of participants without history of prevalent CVD.

Substituting adjusted protein intake at 1 year for random assignments to low protein diet in model 3 did not alter the results for all-cause (1.00, 0.85 to 1.18) or CVD mortality (1.05, 0.86 to 1.29). The addition of CRP and albumin also as covariates did not come of death before reaching kidney failure (need for renal replacement therapy with dialysis or transplantation) with censoring at kidney failure or end of follow-up and using a composite outcome of death or kidney failure.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.
appreciably alter the HR for tHcy in models examining all-cause
(1.00, 0.85 to 1.17) or CVD mortality (1.05, 0.87 to 1.27).

Sixty-five participants died before reaching kidney failure. There
was no association between tHcy and death before kidney failure in
a univariate Cox model (1.06, 0.81 to 1.39). We did not perform
multivariate analyses because of limited power, given the small
number of events.

Five hundred ninety-three participants died or reached kidney
failure by December 31, 2000. There was no association be-
tween tHcy and the composite outcome of kidney failure or
defined as tHcy levels >15 \mu mol/L, was an indepen-
dent predictor of CVD mortality in a large cohort of patients with
type 2 diabetes.6 In a population-based cohort of women, partici-
pants in the highest quintile for Hcy were at increased risk for fatal
and nonfatal acute myocardial infarctions.16 Homocysteine was an
independent risk factor for all-cause mortality in Jewish men and
women aged 50 years and older,7 in patients with angiographically
confirmed coronary artery disease,5 and in the Physicians Health
Study.17 However, other studies suggest that the relationship be-
tween Hcy and CVD exists only in specific subgroups such as those
with a previous history of CVD,9,18 in the elderly,9 in patients with
type 2 diabetes,19 and in patients with hypertension.20

None of the studies that demonstrated an association between
tHcy and outcomes adjusted for level of kidney function with the
gold standard of GFR as in this study. One study used estimated
creatinine clearance,6 some used serum creatinine,5,7,16 and others
did not adjust for any marker of kidney function.9,18,19 The preva-
lence of CKD may have been high in several of these studies
because they included the elderly, hypertensive patients, and pa-
tients with heart disease and type 2 diabetes, all populations at high
risk for kidney disease. Underlying kidney dysfunction may thus
have confounded the association of tHcy and cardiovascular out-

Discussion

In this cohort of patients with CKD stages 3 to 4, tHcy does not
appear to be an independent predictor of all-cause or CVD mortal-
ity. In unadjusted analysis, a nonsignificant positive association was
observed between elevated tHcy and the mortality outcomes that
was largely attenuated with adjustment for GFR. These findings
raise the question of whether prior studies that found tHcy to be a
CVD risk factor adequately adjusted for kidney function.

The results of prospective cohort studies investigating hyperho-
mocystinemia as an independent risk factor for the development of
CVD have been conflicting. A recent study found that hyperhomo-
cystinemia, defined as tHcy levels >15 \mu mol/L, was an indepen-
dent predictor of CVD mortality in a large cohort of patients with

table 1. baseline characteristics by tertiles of homocysteine

<table>
<thead>
<tr>
<th>Tertile 1 [5.9–14.7 \mu mol/L]</th>
<th>Tertile 2 [14.7–19.5 \mu mol/L]</th>
<th>Tertile 3 [19.6–31.0 \mu mol/L]</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine, \mu mol/L*</td>
<td>12.43 (2.81)</td>
<td>16.84 (2.31)</td>
<td>23.71 (6.59)</td>
</tr>
<tr>
<td>Demographic factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y†</td>
<td>52.59 ± 11.96</td>
<td>51.03 ± 12.91</td>
<td>51.69 ± 12.09</td>
</tr>
<tr>
<td>Male, %</td>
<td>47.8</td>
<td>66.0</td>
<td>67.2</td>
</tr>
<tr>
<td>White, %</td>
<td>84.3</td>
<td>85.8</td>
<td>85.1</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>8.6</td>
<td>9.3</td>
<td>10.4</td>
</tr>
<tr>
<td>CVD risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>6.0</td>
<td>6.3</td>
<td>3.4</td>
</tr>
<tr>
<td>History of coronary artery disease, %</td>
<td>7.8</td>
<td>7.5</td>
<td>13.4</td>
</tr>
<tr>
<td>Body mass index, kg/m²†</td>
<td>27.67 ± 4.60</td>
<td>27.13 ± 4.45</td>
<td>26.70 ± 4.23</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg†</td>
<td>132.19 ± 17.61</td>
<td>131.87 ± 17.52</td>
<td>131.78 ± 17.74</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL†</td>
<td>216.38 ± 45.27</td>
<td>222.22 ± 47.36</td>
<td>212.01 ± 43.69</td>
</tr>
<tr>
<td>Kidney disease factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR, mL/min per 1.73m²†</td>
<td>38.72 ± 11.22</td>
<td>32.69 ± 11.18</td>
<td>26.31 ± 10.39</td>
</tr>
<tr>
<td>Proteinuria, g/d*</td>
<td>0.20 (0.99)</td>
<td>0.40 (1.63)</td>
<td>0.44 (1.49)</td>
</tr>
<tr>
<td>Kidney disease category, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerular disease</td>
<td>19.5</td>
<td>26.5</td>
<td>24.6</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>31.5</td>
<td>33.6</td>
<td>29.1</td>
</tr>
<tr>
<td>Other</td>
<td>49.1</td>
<td>39.9</td>
<td>46.3</td>
</tr>
</tbody>
</table>

*Median (interquartile range).
†Mean ± SD.

Incidence of all-cause and cardiovascular mortality according to
tertiles of baseline homocysteine levels in the Modification of
Diet in Renal Disease Study cohort.
comes in these studies because serum creatinine is a less sensitive marker of kidney function.21

In data from the Hoorn Study, tHcy was associated with increased risk of coronary events in diabetic but not nondiabetic individuals.22 The association in diabetics persisted after adjustment for estimated GFR, calculated using the MDRD Study formula, but was attenuated by adjustment for history of cardiovascular disease. In a study of patients with type 2 diabetes, tHcy was a significant predictor of all-cause but not CVD mortality after adjustment for albumin excretion rate and estimated creatinine clearance as measures of kidney function.23

In contrast to the previously cited studies, other studies have failed to demonstrate an independent relationship between Hcy and CVD. An analysis from the Atherosclerosis Risk in Communities Study8 and a population-based study from Australia did not find an association between Hcy and CVD.24 Investigators from the Multiple Risk Factor Intervention Trial found no association between Hcy and fatal and nonfatal CVD events.25 Of these studies with a null result, two adjustment for serum creatinine,20,24 whereas two others did not include markers of kidney function in their multivariate analyses.26,27 Of note, these studies finding no association were conducted in relatively healthy cohorts that were likely to have low prevalence of CKD.26

Two meta-analyses have examined the associations between Hcy levels and ischemic heart disease. A publication from the Homocysteine Studies Collaboration using 30 prospective and retrospective studies found a 3-μmol/L lower Hcy level was associated with an 11% lower risk of ischemic heart disease and 19% lower risk of stroke.27 In another meta-analysis that included 20 prospective studies, a 5-μmol/L increase in Hcy was associated with a 32% increase in the risk of ischemic heart disease.28 Neither of these analyses adjusted for level of kidney function.

There are limited data evaluating Hcy as a risk factor for CVD in patients with CKD before reaching kidney failure. In a cohort of 147 patients with creatinine clearances between 20 and 55 mL/min per 1.73 m², high Hcy was an independent risk factor for incident CVD.29 However, tHcy was only available in a subset of 93 patients, and estimated creatinine clearance was the proxy for kidney function. In a cohort of 227 stable kidney transplant recipients (average serum creatinine, 1.9 ± 0.8 mg/dL), Ducloux et al30 found an independent association between Hcy and a composite outcome of fatal and nonfatal CVD events. However, serum creatinine was used as the marker of kidney function in this analysis, leaving the possibility that actual GFR may still confound the association. One prospective study of 733 kidney transplant recipients using a body surface area–adjusted measure of kidney function estimated using the Cockroft-Gault formula (mean = 55.8 mL/min per 1.73 m²) found an association between Hcy levels and all-cause mortality that was independent of kidney function.31 Differences in the results from this study compared with our study may be attributable to cohort differences including different kidney disease severity and burden of preexisting CVD. Alternatively, Hcy may have a differential impact as a risk factor for CVD in patients who have received transplantation.

Several studies have examined the association between elevated levels of tHcy and outcomes in dialysis patients, with contradictory findings. Although a few studies have found high tHcy to be a risk factor for outcomes,32–34 others have failed to demonstrate this association,35 and some have described an inverse relationship between tHcy levels and outcomes.36–38 However, it must be acknowledged that the dialysis population is very different from patients with stage 3 to 4 CKD, and, moreover, it is not possible to study the effect of kidney function per se in this population.

### TABLE 2. Relationship Between Tertiles of Homocysteine and All-Cause and Cardiovascular Mortality

<table>
<thead>
<tr>
<th></th>
<th>All-Cause Mortality</th>
<th>CVD Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, 95% CI*</td>
<td>P</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.03 (0.91–1.18)</td>
<td>0.63</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1 †</td>
<td>0.95 (0.81–1.11)</td>
<td>0.52</td>
</tr>
<tr>
<td>Model 2 ‡</td>
<td>1.02 (0.87–1.19)</td>
<td>0.81</td>
</tr>
<tr>
<td>Model 3 §</td>
<td>0.98 (0.83–1.16)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Hazard ratios and 95% CI are calculated per 10-μmol/L increase in serum homocysteine levels.
†Adjusted for GFR.
‡Adjusted for model 1 covariates plus age, gender, race, and random assignment to diet and blood pressure strata.
§Adjusted for model 2 covariates plus cause of kidney disease, proteinuria, history of coronary artery disease and diabetes, and body mass index.
We and other investigators have reported that GFR is a major determinant of Hcy levels in the general population as well as in patients with CKD.\textsuperscript{1,2,3,10,39,40} In the present study, adjustment for GFR attenuated most of the relationship between tHcy and mortality, suggesting that GFR was the primary confounder for the observed association of high tHcy levels and the mortality end points. Reduced GFR is a known important risk factor for CVD\textsuperscript{41}; thus, the varied measurement and adjustment for kidney function in prior literature may largely explain the heterogeneity of study results.

It is also possible that tHcy may be a risk factor for progression of kidney disease and thereby promote the development of CVD. In support of this hypothesis, in a population-based cohort, tHcy was found to be a predictor of the development of microalbuminuria in nondiabetic individuals, and this relationship was independent of GFR.\textsuperscript{41} Conversely, studies in patients with kidney disease, including data from the MDRD Study, have failed to demonstrate any association between tHcy and progression.\textsuperscript{13,42,43} In our study, we did not find a relationship between tHcy and a composite outcome of death or kidney failure.

Another possibility is that our null findings could be specific to the population we studied. The MDRD Study cohort consists of nondiabetic, predominantly white patients, an overrepresentation of the population we studied. The MDRD Study cohort consists of patients with polycystic kidney disease, with few older adults and a nondiabetic, predominantly white patients, an overrepresentation of the population we studied. The MDRD Study cohort consists of patients with polycystic kidney disease, with few older adults and a low burden of CVD. In addition, we were unable to assess nonfatal CVD events, which may have different associations with Hcy than mortality end points. As described in a previous publication,\textsuperscript{10} a 2-hour delay in sample processing may in theory result in a systematic measurement error resulting in elevated tHcy levels caused by release of Hcy from red blood cells. However, we have previously demonstrated that the range of tHcy levels, prevalence of hyperhomocysteinemia, and magnitude of associations with known variables such as folic acid and GFR in our sample are very similar to those previously reported.\textsuperscript{1} We also acknowledge that the relatively wide confidence intervals surrounding the hazard ratios preclude definitively ruling out an association between tHcy and outcomes. Finally, it must be recognized that the majority of the study participants reached kidney failure requiring dialysis during follow-up. Kidney failure is known to be associated with increased tHcy levels, and this may have influenced the associations studied. However, the lack of association between tHcy and mortality in participants who died before reaching kidney failure suggests that baseline tHcy is not a major determinant of outcomes in this cohort of patients with stage 3 to 4 CKD.

The major strength of this study is the inclusion of a large cohort of patients with precise measures of kidney function. In addition, this study sample has a wide range of tHcy levels, includes a large proportion individuals with abnormal tHcy values,\textsuperscript{1} and a high number of outcomes during follow-up. This is a relatively healthy CKD population; however, this makes it an ideal population to assess a potentially independent effect of tHcy on mortality in the absence of powerful confounders such as diabetes and preexisting CVD.

In summary, tHcy does not appear to be a risk factor for all-cause or CVD mortality in the MDRD Study cohort. Homocysteine may be a marker for severity of kidney disease rather than a mediator for the development of CVD, in patients with CKD stages 3 to 4. Prior studies demonstrating an association between tHcy and CVD risk may have been confounded by their less precise measurements of GFR.

Acknowledgments

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Disclosures

None.

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