Protein carbamylation is associated with heart failure and mortality in diabetic patients with end-stage renal disease

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Serum carbamylated albumin (C-Alb) levels are associated with excess mortality in patients with diabetic end-stage renal disease. To gain insight into the pathophysiology of carbamylation, we determined associations between C-Alb and causes of death in patients on chronic hemodialysis. The Die Deutsche Diabetes Dialyse Studie (4D study) was a randomized controlled trial testing the effects of atorvastatin on survival in diabetic patients on dialysis during a median follow-up of 4 years. We stratified 1161 patients by C-Alb to see whether differences in carbamylation altered the effects of atorvastatin on survival. Baseline C-Alb significantly correlated with serum cardiac stress markers troponin T and N-terminal pro-B-type-natriuretic peptide and was associated with a history of heart failure and arrhythmia. C-Alb was strongly associated with 1-year adjusted risk of cardiovascular mortality, sudden cardiac death, and the 4-year risk of death from congestive heart failure (hazard ratios of 3.06, 3.78, and 4.64, respectively) but not with myocardial infarction or stroke. Patients with low C-Alb, treated with atorvastatin, experienced a significant improvement in their 4-year survival (hazard ratio 0.692). High C-Alb levels are associated with ongoing cardiac damage, risk of congestive heart failure, and sudden cardiac death. Thus, carbamylation and uremic cardiomyopathy are associated in patients with diabetes mellitus and kidney disease. In addition, statins were specifically beneficial to hemodialysis patients with low C-Alb.

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One of the consequences of chronically elevated urea levels in patients with kidney disease is the accelerated chemical modification of proteins by urea (carbamylation).[1–3] The accumulation of these modifications on albumin (as well as other serum proteins) has been shown to be proportional to blood urea concentrations, in a manner analogous to the relationship between hemoglobin A_{1c} levels and time-averaged glucose concentrations.[4,5] In addition to chronic uremia, amino-acid deficiencies are common in patients with chronic kidney disease and end-stage renal disease (ESRD),[6] and there is evidence that these amino-acid deficiencies may further exacerbate protein carbamylation.[3,5] As a result, the proportion of albumin that is carbamylated (C-Alb) represents a biomarker that simultaneously integrates time-averaged urea concentrations and possibly protein energy wasting in patients on hemodialysis.[5]

Recent work has demonstrated a strong and independent association between serum carbamylated protein levels and risk of death in patients on hemodialysis treatment.[4,5] However, the mechanisms linking uremia, protein carbamylation, and mortality remain poorly understood. A number of studies have suggested a direct role for protein carbamylation in the cardiovascular (CV) pathology associated with chronic kidney disease. For example, when nephrectomized ApoE-null mice were fed pure urea in their chow, it significantly accelerated rates of aortic atherogenesis.[7] It has also been shown that protein carbamylation has pro-atherogenic effects on lipoproteins. Animal studies have shown that uremia increases carbamylation of plasma low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and this modification inhibits receptor-mediated uptake and instead promotes foam cell formation and accumulation in atherosclerotic tissues.[8–13] A clinical study of patients undergoing cardiac catheterization and age-matched controls further demonstrated that when cases and controls were stratified by their serum carbamylated protein levels, subjects

with higher carbamylated protein levels were affected by CV disease in greater proportions.[14] Together these results suggest that protein carbamylation may contribute to the CV disease burden associated with kidney failure.

We recently reported strong independent associations between C-Alb levels and 12-month all-cause mortality in both incident and prevalent hemodialysis patient cohorts, even after adjusting for other significant risk factors.[5] In order to test whether C-Alb values are also associated with longer-term outcomes, herein we describe analysis of 4-year risk of death from specific causes associated with C-Alb. Furthermore, in order to search for specific pathologic sequelae associated with carbamylation, we reanalyzed the study database for associations between C-Alb and coexisting comorbidities, as well as future risk of death from specific causes. In addition, in light of previous studies suggesting a connection between protein carbamylation and atherogenesis, we investigated the relationship between C-Alb, serum cholesterol levels, and survival benefits from atorvastatin therapy.

RESULTS

Associations between C-Alb levels, clinical laboratory tests, and coexisting comorbidities

Baseline differences in clinical, demographic, and biochemical characteristics were compared in 4D subjects according to their tertile of baseline C-Alb levels (tertile 1: C-Alb <0.72, tertile 2: C-alb between 0.72 and 0.90, tertile 3: C-alb >0.90). The mean length of follow-up was 3.96 years in the atorvastatin group and 3.91 years in the placebo group. As shown in Table 1, analysis of variation found associations between higher tertile C-Alb levels and higher predialysis and postdialysis blood urea and creatinine levels. High C-Alb was also associated with elevated phosphate and potassium levels and lower urea reduction ratio values. These findings are consistent with the hypothesis that chronic uremia and lower efficiency of urea reduction therapy may be contributing to increased protein carbamylation. High C-Alb was also associated with higher average N-terminal pro-B-type natriuretic peptide, troponin T, intact parathyroid hormone, alkaline phosphatase, and HDL cholesterol and with lower average body mass index, lower total cholesterol, lower hemoglobin, and low ferritin. Baseline C-Alb also showed modest correlations with troponin T ($r = +0.183$, $P<0.001$) and N-terminal pro-B-type natriuretic peptide ($r = +0.227$, $P<0.001$). The data set was further analyzed for associations between high C-Alb levels and coexisting comorbidities, revealing that high C-Alb was associated with current diagnosis of congestive heart failure (CHF), atrial fibrillation, and hypertension. In contrast, there were no significant associations between high C-Alb and history of coronary artery disease (CAD), peripheral vascular disease, or cerebrovascular disease.

Associations between C-Alb levels and specific 1-year adverse events

4D subjects' baseline C-Alb values were analyzed for associations with 1-year risk of specific adverse outcomes using multivariable-adjusted Cox proportional hazards models. As shown in Table 2, higher baseline C-Alb values were found to be strongly associated with univariate risk of death from all causes, CV and non-CV mortality. The effect was strongest for CV mortality, in particular for sudden cardiac death. The risks associated with C-Alb were adjusted for potentially confounding variables, including age, time on dialysis therapy (dialysis vintage), systolic and diastolic blood pressure, serum albumin, phosphate, parathyroid hormone, cholesterol, HDL cholesterol, hemoglobin, C-reactive protein, potassium, creatinine, N-terminal pro-B-type natriuretic peptide, troponin T, treatment with angiotensin-converting enzyme inhibitors/calcium antagonists/diuretics, a history of hypertension, and a history of CVD (including CAD, CHF, and peripheral vascular disease). After adjusting for all these variables, it was still found that the risk of sudden cardiac death, all-cause mortality, and CV mortality remained significant. Interestingly, there was no specific association between C-Alb values and risk of myocardial infarction (MI).

We performed a sensitivity analysis in the subset of patients with available urea reduction rate (URR) values ($n = 471$). We compared the hazard ratios (HRs) for 1-year mortality when (a) adjustments were made for all variables mentioned above and (b) additional adjustments were made for URR. Of note, in both analyses, the risks were similar with HRs of (a) 1.77 (0.92–3.40) and (b) 1.78 (0.92–3.42), reassuring that URR is unlikely to act as a confounding parameter in our study.

Associations between C-Alb levels and specific 4-year adverse events

4D subjects' baseline C-Alb values were analyzed for associations with 4-year risk of specific adverse outcomes, using multivariable-adjusted Cox proportional hazards models. As shown in Table 2, higher baseline C-Alb values were found to be strongly associated with univariate risk of CV and all-cause mortality. Notably, there was an especially strong association between initial C-Alb values and risk of death from CHF (HR 7.26 (2.18–24.16), $P = 0.001$) and a significant association with sudden cardiac death (HR 2.29 (CI 1.23–4.24)). HR estimates were again calculated using a Cox proportional hazards model adjusted for the potential confounders mentioned above; the risk of death from CHF, sudden cardiac death, CV, and all-cause mortality remained significant even after accounting for these other risk factors.

Finally, although our multivariable model was adjusted for the effects of many potential confounders, in order to further exclude the possibility that comorbid conditions may have confounding effects on the association between C-Alb values and risk of death, we reanalyzed our data after stratifying subjects according to the presence or absence of a variety of baseline risk factors (including history of hypertension, CAD, CHF, and arrhythmias; see Supplementary Table S1). Furthermore, because of the observed association between high C-Alb values and shorter times on dialysis, we also wanted to exclude the possibility that differences in dialysis