OXIDATIVE DNA DAMAGE AND MORTALITY IN HEMODIALYSIS AND PERITONEAL DIALYSIS PATIENTS

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◆ **Background and Aims:** Increased oxidative stress in dialysis patients is thought to contribute to increased mortality; however, confirmatory data are scarce. We analyzed the serum concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative stress, in relation to mortality in hemodialysis (HD) and peritoneal dialysis (PD) patients.

◆ **Methods:** Serum 8-OHdG, interleukin 6 (IL-6), other biochemical markers, Davies comorbidity score, and protein-energy wasting (PEW) were assessed in 303 prevalent patients treated with HD ($n = 220$; age: 63 ± 14 years) or PD ($n = 83$; age: 64 ± 14 years). Mortality was assessed after a median follow-up of 31 months.

◆ **Results:** The median (25th – 75th percentile) concentration of 8-OHdG was higher in HD than in PD patients: 1.3 ng/mL (0.9 – 1.8 ng/mL) versus 0.5 ng/mL (0.4 – 0.6 ng/mL), $p < 0.001$. The HD modality (standard $\beta = 0.57$, $p < 0.001$) and dialysis vintage (standard $\beta = 0.12$, $p = 0.02$) were independent predictors of serum 8-OHdG in a multivariable linear regression model including age, sex, body mass index, dialysis modality (HD or PD), preceding time on dialysis (dialysis vintage), PEW, comorbidity score, IL-6, and use of angiotensin converting-enzyme inhibitors or angiotensin II receptor blockers or statins. During follow-up, 107 patients died. In multivariable Cox regression models including all 303 patients and adjusted for age, sex, body mass index, dialysis modality, dialysis vintage, and comorbidity score, 8-OHdG was significantly associated with all-cause mortality (adjusted hazard ratio: 1.40; 95% confidence limits: 1.05, 1.87 for 1 standard deviation increase of 8-OHdG). In subgroup analyses according to dialysis modality, 8-OHdG was associated with mortality in HD patients but not in PD patients.

◆ **Conclusions:** Oxidative stress as assessed by 8-OHdG is an independent predictor of all-cause mortality in dialysis patients. This association was seen in HD patients, but no such association could be demonstrated for PD patients.

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End-stage renal disease (ESRD) patients undergoing dialysis have a substantially increased risk of cardiovascular morbidity and mortality (1,2). Oxidative stress and inflammation are thought to be involved in the pathogenesis of atherosclerosis and cardiovascular disease (CVD) (3–5). On the other hand, vascular changes might induce oxidative stress and inflammation, thereby increasing the likelihood of morbidity and mortality from aggravation of CVD (6,7).

Oxidative stress is defined as a state of imbalance between excessive generation of oxidant compounds such as reactive oxygen and nitrogen species, and insufficient antioxidant defense mechanisms (8). However, it is difficult to detect reactive oxygen and nitrogen species directly *in vivo* because of their extreme instability. Among the many modifications of lipids, carbohydrates, proteins, and DNA that are induced by reactive oxygen species, the circulating level of 8-hydroxy-2'-deoxyguanosine (8-OHdG), first described in 1984 (9), reflects the oxidative decay (damage) of DNA and is thought to be a robust and sensitive marker of oxidative stress (10).

Circulating and urinary concentrations of 8-OHdG are increased in patients with cancer, chronic hepatitis,

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diabetes, and heart disease (11) and in patients with ESRD (12,13). After the first report of increased serum 8-OHdG in dialysis patients, many studies confirmed the finding. However, only a few trials have investigated the association between oxidative stress and risk of cardiovascular events or death in these patients (14–17). Also, it is not known whether oxidative stress varies with dialysis modality. In a few studies, increased levels of oxidative stress have been reported to be associated with hemodialysis (HD) compared with peritoneal dialysis (PD) (18,19), but other studies showed the opposite results (20–22).

The objective of the present study was to assess the association between circulating levels of 8-OHdG and all-cause mortality in patients treated with HD or PD, and to investigate the clinical and biochemical characteristics associated with 8-OHdG in those patients.

METHODS

The present study undertook *post hoc* analyses in 303 dialysis patients (60% men; mean age: 63.1 ± 14.1 years) from two cohorts of prevalent HD and PD patients.

HD PATIENT COHORT

The HD cohort consisted of 228 patients from a cross-sectional study on the variability of inflammatory markers in patients from five dialysis units in Stockholm and one unit in Uppsala, Sweden (23). For the present study, 8 of those patients were excluded because 8-OHdG results were not available at baseline; thus, the analysis included 220 patients (55% men; mean age: 62.9 ± 14.2 years). The HD patients underwent HD three times weekly (4–5 hours per session) using bicarbonate dialysate; 58% had an arteriovenous fistula, and 22% and 20% had a graft or central dialysis catheter respectively. Most of the HD patients were functionally anuric, with a residual glomerular filtration rate (GFR) equal or close to zero, and the median Kt/V per session was 1.5 [interquartile range (IQR): 1.3–1.8].

PD PATIENT COHORT

The PD cohort consisted of patients from a cross-sectional study on the variability of inflammatory markers in prevalent PD patients at Karolinska University Hospital and Danderyds Hospital, Stockholm, Sweden. Of 97 eligible patients, 13 did not start the study for various reasons, including transfer to HD, transplantation, and mortality. For the present study, 8-OHdG could not be measured in 1 of the remaining 84 patients because

stored serum was lacking, and thus the analysis was performed in 83 patients followed for assessment of all-cause mortality (68% men; mean age: 63.7 ± 14.1 years). The PD treatment consisted of continuous ambulatory PD in 77% of the patients and automated PD in 23%. The median urine volume for the PD patients was 950 mL (IQR: 500–1300 mL), with a GFR (calculated as the average of renal creatinine and urea clearances from a 24-hour urine collection) of 3.2 ± 2.2 mL/min/1.73 m². The weekly total (renal plus peritoneal) Kt/V was 2.2 (IQR: 1.8–2.5). A standard peritoneal equilibration test, with assessment of dialysate-to-plasma ratio of creatinine was performed using the method of Twardowski *et al.* (24).

Body mass index was calculated as weight in kilograms divided by the square of height in meters. Comorbidity was calculated as a Davies score, which comprises assessments of chronic and active conditions in seven domains (25): ischemic heart disease (angina pectoris, previous myocardial infarction, percutaneous coronary intervention, and coronary artery bypass grafting); diabetes mellitus; left ventricular dysfunction (congestive heart failure); peripheral vascular (arterial insufficiency of the extremities, carotid or renal artery stenosis, or aortic aneurysm) and cerebrovascular disease (stroke, transient ischemic attack, subdural hematoma, and intracerebral or subarachnoid hemorrhage); non-cutaneous active malignancy; systemic collagen vascular disease; and a group of chronic conditions classified as “other significant pathology” (chronic infection, chronic obstructive pulmonary disease, heart valve dysfunction, liver cirrhosis, and other conditions with a poor survival prognosis compared with the general population). Comorbidity is scored as the number of domains affected (minimum: 0; maximum: 7). Depending on comorbidity score, patients were defined as low-risk (score: 0), medium-risk (score: 1–2), or high-risk (score: ≥ 3). Subjective global assessment was used to evaluate overall nutrition status and the presence of protein–energy wasting (PEW) in the patients. The score assigned to the patients reflects nutrition status as follows: 1, normal nutrition status; 2, mild wasting; 3, moderate wasting; and 4, severe wasting. A subjective global assessment score greater than 1 was defined as PEW (26).

Most patients received phosphate binders; erythropoiesis-stimulating agents; vitamin B, D, and C supplementation; and antihypertensive drugs. Some were treated with statins. With respect to antihypertensives, 33% of the HD patients used angiotensin converting-enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs), 26% took calcium channel blockers, 50% took

beta-blockers, and 32% used statins. Of the PD patients, 56% used an ACEI or ARB (or both), 32% took calcium channel blockers, 70% took beta-blockers, 83% used diuretics, and 46% used statins.

A population-based group of 55 subjects (69% men; mean age: 61.4 ± 11.6 years) served as controls for comparative analyses of biochemical parameters. This control group consisted of individuals from the Stockholm region who were randomly selected by Statistics Sweden (a government agency) and who accepted to participate, as volunteers, in the group.

The Ethics Committee of the Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden, approved the study protocol. Informed consent was obtained from all patients and control subjects.

LABORATORY MEASUREMENTS

Venous blood samples were taken at the start of the study. In the PD patients, morning fasting samples were obtained without interruption to their ongoing continuous treatment with PD; in the HD patients, samples were obtained before the start of a HD session. All blood samples were centrifuged immediately for 15 minutes at 2300g and 4°C, and were stored at -70°C if not analyzed immediately. Plasma IL-6 was measured by an Immulite Automatic Immunoassay Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) using an assay manufactured for the analyzer. High-sensitivity C-reactive protein (hs-CRP), triglycerides, high-density lipoprotein cholesterol, hemoglobin, urea, and creatinine were analyzed by routine methods. All laboratory measurements were centralized to the Department of Laboratory Medicine, Karolinska University Hospital Huddinge, and to the Renal Laboratory, Department of Renal Medicine, Novum, Karolinska Institutet.

MEASUREMENT OF 8-OHDG

Serum 8-OHDG was measured using a commercial competitive enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Shizuoka, Japan). Following the manufacturer's instructions, serum samples from patients and controls were purified by centrifugal ultrafiltration for 15 minutes at 14 000g and 25°C with Amicon Ultra 10K filters (Millipore Corporation, Bedford, MA, USA). Blank samples (without primary antibody) were used as a negative control, and standards, as a positive control. Briefly, 50 µL of standard or sample and the reagent (50 µL 8-OHDG monoclonal antibody) were added to a microtiter plate pre-coated

with "competitive" 8-OHDG; the plate was then incubated at 4°C overnight. After the wells had been washed 3 times, horseradish peroxidase-conjugated secondary antibody was added, and the plate was incubated for 60 minutes. The wells were again washed 3 times, the enzyme substrate solution was added, and the plate was incubated for 15 minutes. The reaction was stopped with 1 mol/L phosphoric acid. The concentration of unknown samples was obtained from a standard curve of 450 nm, in which absorbance was plotted against the 0.125 – 10 ng/mL standards.

FOLLOW-UP AND MORTALITY

Mortality was assessed after a median follow-up of 31 months (IQR: 21 – 38 months). None of the participants were lost to follow-up.

STATISTICAL ANALYSIS

All normally distributed variables are expressed as mean $\pm$ standard deviation; non-normally distributed variables are reported as medians with IQR or as percentage frequencies. Comparisons between groups were analyzed using the Wilcoxon test or the Fisher exact test. A Spearman rank correlation (ρ) was used to determine correlations for 8-OHDG with other variables in each dialysis group. Multivariable regression models were fitted to assess the relationships of independent variables with 8-OHDG.

We examined the impact of mortality by testing the statistical significance of two-way interaction terms of 8-OHDG by dialysis modality; the interaction terms did not reach statistical significance (interaction $p = 0.90$). Cox regression analysis for 8-OHDG and all-cause mortality was therefore performed for the entire patient cohort ($n = 303$). The association between 8-OHDG and total mortality was assessed both by continuous models of the prognostic value of a 1 standard deviation increase of 8-OHDG, and by multi-category models of tertiles of 8-OHDG, using the lowest tertile of 8-OHDG as reference.

The crude and multivariate Cox regression analyses are presented as hazard ratios (HRs) with 95% confidence intervals (CIs). In the multivariate Cox regression models comprising all 303 patients, dialysis modality (HD or PD) was included as a confounder. A p value less than 0.05 from two-sided tests was considered to be statistically significant. The statistical analyses were performed using the SAS (version 9.3; SAS Institute, Cary, NC, USA) and Stata 12.1 (Stata Corporation, College Station, TX, USA) statistical software.

RESULTS

BASELINE CHARACTERISTICS

Table 1 shows the baseline demographic, clinical, and biochemical characteristics of the study population, categorized by dialysis modality. Briefly, plasma 8-OHdG was markedly elevated in HD and PD patients compared with control subjects randomly selected from the general population. Plasma concentrations of 8-OHdG, IL-6, and hs-CRP were higher in the HD patients than in the PD patients, and time on dialysis (dialysis vintage) in the HD patients was longer. The comorbidity burden was similar in the PD and HD patients (Table 1). According to the grading based on the Davies comorbidity score,

52% of the HD patients were graded as low risk; 24%, as medium risk; and 24%, as high risk. Of the PD patients, 62% were graded as low risk; 24%, as medium risk; and 14%, as high risk. The only difference between the two modalities with respect to comorbidities was that CVD was more common in HD than in PD patients (63% vs 29%, $p < 0.001$). The use of ACEIs, ARBs, or statins was more common in PD patients (73% PD vs 52% HD, $p = 0.001$). There were no differences in age, sex, body mass index, hemoglobin, or prevalence of PEW between the PD and HD patients.

Most PD patients (79%) were high (25%) or high-average (54%) transporters; 21% were low (3%) or low-average (18%) transporters. Median plasma 8-OHdG was not significantly different between the high and

TABLE 1
Clinical and Biochemical Characteristics in the Study Subjects

Parameter	Value ^a by participant group		
	Control subjects	HD patients	PD patients
Participants (n)	55	220	83
Mean age (years)	61.4±11.6	62.9±14.2	63.7±14.1
Sex (% men)	69	55	68
Mean BMI (kg/m ²)	25.9±4.2	24.5±5.2	25.5±4.0
Dialysis vintage (months)	—	28.4 ^b (14.3–56.9)	11.5 (5.9–28.8)
Comorbidity (%)			
By Davies score			
Low risk (0)	—	52	62
Medium risk (1–2)	—	24	24
High risk (≥3)	—	24	14
CVD (%)	9 ^{c,d}	63 ^b	29
Diabetes (%)	2 ^{c,d}	26	24
PEW (SGA>1)	6 ^{c,d}	46	37
Biochemical analysis			
Creatinine (μmol/L)	80.6±15.7 ^{c,d}	769.7±211.4	724.1±174.1
Triglycerides (mmol/L)	1.4±0.8 ^{c,d}	1.9±1.1	2.0±0.9
HDL cholesterol (mmol/L)	1.6±0.5 ^{c,d}	1.4±0.5	1.3±0.5
Hemoglobin (g/L)	142.1±8.6 ^{c,d}	118.8±13.0	118.4±11.2
High-sensitivity CRP (mg/L)	1.1 ^{c,d} (0.6–2.5)	6.5 ^b (2.5–21)	4.4 (1.4–10.6)
Interleukin 6 (pg/mL)	1.3 ^{c,d} (1.0–2.6)	8.6 ^b (5.2–15)	6.5 (3.9–9.6)
8-OHdG (ng/mL)	0.2 ^{c,d} (0.1–0.3)	1.3 ^b (0.9–1.8)	0.5 (0.4–0.6)
ACEI or ARB (%)	—	33 ^b	56
Statins (%)	—	32 ^b	46
ACEI, ARB, or statin (%)	—	52 ^b	73

HD = hemodialysis; PD = peritoneal dialysis; BMI = body mass index; CVD = cardiovascular disease; PEW = protein-energy wasting; SGA = subjective global assessment of nutrition; HDL = high-density lipoprotein; CRP = C-reactive protein; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; ACEI = angiotensin converting-enzyme inhibitor; ARB = angiotensin II receptor blocker.

^a Presented as mean and standard deviation, median and interquartile range (25th – 75th percentile), numbers, or proportions.

^b $p < 0.05$ for HD patients compared with PD patients.

^c $p < 0.05$ for control subjects compared with HD patients.

^d $p < 0.05$ for control subjects compared with PD patients.

Figure 1 — Associations between 8-hydroxy-2'-deoxyguanosine (8-OHdG) and (A) high-sensitivity C-reactive protein (hs-CRP) and (B) interleukin 6 (IL-6) in 303 dialysis patients. Open triangles = peritoneal dialysis; open circles = hemodialysis.

high-average group and the low and low-average group: 0.51 ng/mL (IQR: 0.46 – 0.63 ng/mL) and 0.64 ng/mL (IQR: 0.49 – 0.67 ng/mL) respectively, $p = 0.71$.

DETERMINANTS OF 8-OHDG

We first performed Spearman rank tests of the relations between plasma 8-OHdG and all the variables listed in Table 1 separately in the HD and PD patients. In the PD patients, plasma 8-OHdG was positively associated with IL-6 ($p = 0.35$, $p < 0.001$), hs-CRP ($p = 0.32$, $p < 0.01$), and negatively with GFR ($p = -0.35$, $p < 0.001$). In the HD patients, no significant association between 8-OHdG and IL-6 or hs-CRP was evident; the only association with 8-OHdG was a correlation with dialysis vintage ($p = 0.21$, $p < 0.01$). In the combined cohort (PD and HD patients together), plasma 8-OHdG was positively associated with hs-CRP [$p = 0.17$, $p = 0.004$; Figure 1(A)] and IL-6 [$p = 0.22$, $p < 0.001$; Figure 1(B)], and negatively associated with use of ACEIs or ARBs ($p = -0.16$, $p = 0.005$), of statins ($p = -0.12$, $p = 0.04$), and of ACEIs or ARBs or statins ($p = -0.16$, $p = 0.01$). However, for the two dialysis modalities separately, the association of plasma 8-OHdG with ACEIs or ARBs or statins was not statistically significant. No significant relation between plasma 8-OHdG and hemoglobin was observed in the HD and PD patients.

A multivariable regression analysis was fitted to the study determinants of plasma 8-OHdG in all 303 dialysis patients (Table 2). In the model, dialysis modality (HD vs PD: standard $\beta = 0.57$, $p < 0.001$) and dialysis vintage (standard $\beta = 0.12$, $p = 0.02$) were considered independent contributors to the variance of plasma 8-OHdG.

TABLE 2
Multiple Regression Models Predicting for 1 Standard Deviation Increment of Serum 8-Hydroxy-2'-deoxyguanosine (8-OHdG), in Nanograms per Milliliter, in 303 Dialysis Patients

Independent variable (adjusted $R^2=0.42$)	Standard β	p Value
Dialysis modality (% HD)	0.57	<0.001
Age (years)	0.02	0.71
Sex (% men)	-0.08	0.11
BMI (kg/m ²)	-0.03	0.57
Dialysis vintage (months)	0.12	0.02
Davies comorbidity score	0.04	0.45
PEW (% SGA>1)	-0.01	0.77
Interleukin 6 (pg/mL)	0.06	0.23
ACEI, ARB, or statin (%)	-0.02	0.70

HD = hemodialysis; BMI = body mass index; PEW = protein-energy wasting; SGA = subjective global assessment of nutrition; ACEI = angiotensin converting-enzyme inhibitor; ARB = angiotensin II receptor blocker.

COX REGRESSION MODELS FOR PLASMA 8-OHDG AND ALL-CAUSE MORTALITY

During follow-up, 107 patients died (all-cause mortality). In multivariate Cox regression models adjusted for age, sex, body mass index, dialysis modality (HD vs PD), dialysis vintage, PEW, and Davies comorbidity score, plasma 8-OHdG was significantly associated with all-cause mortality [adjusted HR: 1.40 (95% CI: 1.05 to

1.87) for each 1 standard deviation increase of 8-OHdG]. We did not include markers of inflammation as confounders because inflammation is part of the causal pathway of oxidative stress. Results of the multi-category models (linear trend across tertiles of 8-OHdG) consistently confirmed those findings (Table 3). In Kaplan–Meier curves, higher plasma 8-OHdG (within tertiles 2 and 3) was associated with worse all-cause mortality (log-rank chi-square: 9.9; $p < 0.001$; Figure 2). A spline curve analysis based on HRs for all-cause mortality associated with plasma 8-OHdG in all 303 dialysis patients showed a significant effect of plasma 8-OHdG on mortality risk (Figure 3).

In the assessment of mortality according to dialysis modality strata, survival in the HD group was determined after a median follow-up of 31.2 months (IQR: 20.6 – 37.8 months). During that period, 83 deaths (38%) occurred among the HD patients. Plasma 8-OHdG arose as an independent predictor of mortality among patients receiving HD [adjusted HR: 1.37 (95% CI: 1.02 to 1.85) per $\log_2(8\text{-OHdG})$]. Survival in the PD group was determined after

a median follow-up of 31.9 months (IQR: 21.9 – 41.7 months), during which time, 24 patients (29%) died. According to the analyses, which were performed for the PD patients as they had been for the HD patients, plasma 8-OHdG was not significantly associated with all-cause mortality in the PD patients [adjusted HR: 1.13 (95% CI: 0.34 to 3.72) per $\log_2(8\text{-OHdG})$].

DISCUSSION

Increased oxidative stress in ESRD patients is thought to contribute to poor clinical outcomes, but only a few studies so far have actually confirmed that hypothesis. Here, we report that an increased circulating level of 8-OHdG was independently associated with all-cause mortality in 303 ESRD patients undergoing dialysis.

Since the mid-1980s, interest in oxidative stress in chronic kidney disease (CKD) patients has undergone sustained growth, and oxidative stress is now well established to be progressively enhanced with advancing stages of CKD, and signs of higher oxidative stress have

TABLE 3
Cox Regression Analysis for 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and All-Cause Mortality in 303 Dialysis Patients

Model		HR	All-cause mortality 95% CI	p Value
Continuous models				
Crude	1-SD increase of 8-OHdG	1.54	1.21 to 1.97	<0.001
Adjusted model 1 ^a	1-SD increase of 8-OHdG	1.59	1.16 to 2.18	0.01
Adjusted model 2 ^b	1-SD increase of 8-OHdG	1.40	1.05 to 1.87	0.02
Multi-category models				
Cut-off value of 8-OHdG				
Crude model				
	Tertile 1	≤0.7 ng/mL	Reference	
	Tertile 2	0.7–1.4 ng/mL	1.17 to 3.36	0.01
	Tertile 3	1.4–3.2 ng/mL	1.43 to 4.08	0.001
	p for trend	0.01		
Adjusted model 1 ^a				
	Tertile 1	≤0.7 ng/mL	Reference	
	Tertile 2	0.7–1.4 ng/mL	1.32 to 4.88	0.01
	Tertile 3	1.4–3.2 ng/mL	1.31 to 5.37	0.01
	p for trend	0.01		
Adjusted model 2 ^b				
	Tertile 1	≤0.7 ng/mL	Reference	
	Tertile 2	0.7–1.4 ng/mL	1.00 to 3.44	0.05
	Tertile 3	1.4–3.2 ng/mL	1.03 to 3.92	0.04
	p for trend	0.09		

HR = hazard ratio; CI = confidence interval; SD = standard deviation.

^a Adjustments included age, sex, body mass index, dialysis modality, and dialysis vintage.

^b Adjustments included age, sex, body mass index, dialysis modality, dialysis vintage, Davies comorbidity score, and protein-energy wasting.

Figure 2 — Kaplan–Meier curves for all-cause mortality in 303 dialysis patients from the low, middle, and high tertiles of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG).

been demonstrated in dialysis patients—especially those of advanced age, with a higher comorbidity burden, or with higher plasma urea concentrations compared with the general population (12,27). The current study shows that, although the level of 8-OHdG was higher in both prevalent HD and PD patients than in control subjects, dialysis modality (and dialysis vintage) contributed to explain the variance of oxidative stress as assessed by plasma 8-OHdG.

Two recent studies comparing oxidative stress in various dialysis modalities showed increased levels of advanced oxidation protein products in HD patients compared with PD patients (18,19), and three other studies found similar or lower levels of oxidative stress markers (advanced oxidation protein products, myeloperoxidase, and 8-OHdG) in HD patients than in PD patients (20–22). Although the HD procedure might induce repetitive bouts of oxidative stress, primarily through dialyzer membrane incompatibility and endotoxin contaminants (28,29), glucose degradation products in glucose-based PD fluids are also thought to increase oxidative stress (30). The marker of oxidative stress used in the present study, 8-OHdG, is freely filtered in the glomeruli, partly reabsorbed in the proximal tubules, and then excreted in urine. The fractional excretion of this nucleotide is increased in CKD patients because of the adaptive suppression of tubular reabsorption capacity (31). Recent studies indicate that preservation of residual renal function (RRF) has a beneficial effect in reducing the oxidative and carbonyl stress in PD patients (32,33). Given that most of our PD patients had some RRF, but that the HD patients were essentially all functionally anuric, it is reasonable to assume that urinary excretion

Figure 3 — Spline curve showing hazard ratios (bold line) and 95% confidence intervals (dotted lines) for all-cause mortality associated with serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) in 303 dialysis patients. The model was plotted as restricted cubic splines with 5 knots. The shaded bars show the counts of patients in the 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentration categories.

of 8-OHdG in the PD patients could have contributed to lower serum levels.

Increased oxidative stress has been linked to accelerated atherosclerosis and CVD in dialysis patients (34–36). One study found increased levels of asymmetrical dimethylarginine (37), a compound reflecting oxidative stress, in patients who experienced cardiovascular events or who died (14). In the current study, CVD was more common and 8-OHdG levels were higher in the HD patients than the PD patients; that differential might perhaps explain why the mortality-predictive effect of 8-OHdG was significant only in the HD patients.

So far, large trials—including the Heart Outcomes Prevention Evaluation trial (38) and the Heart Protection Study (39)—have not supported the notion that antioxidants provide CVD protection in the general population. Relatively few studies have documented mortality prediction by oxidative stress in dialysis patients (14–17) and antioxidant therapy in dialysis patients to reduce CVD has in general been unsuccessful (40); however, two studies reported a positive effect of interventions targeting oxidative stress (41,42).

In the present study, although circulating 8-OHdG was, in the crude analysis, inversely associated with ACEIs or ARBs or statins, suggesting a possible antioxidative benefit of those medications, the correlation lost its statistical significance after adjustment for confounders. Unlike the case of many other conditions influencing survival in dialysis patients, a major problem for research in this area is the lack of a clinically accepted oxidative stress biomarker with a validated role as a mortality predictor. That

lack might be discouraging relevant research and might explain the scarcity of studies exploring the association between oxidative stress and clinical outcomes. Although the results of the present study have to be confirmed in other larger cohorts of dialysis patients, our study supports the proposition that strategies targeting oxidative stress by dietary, pharmaceutical, or other means should not be ruled out as potentially meaningful interventions in ESRD patients.

Several important limitations of our study should be noted. First, the cross-sectional design precludes conclusions with respect to causality. Second, we are limited by the restricted number of variables that were analyzed; inclusion of unmeasured potential confounders could have changed the conclusions. Third, comparisons between the HD and PD cohorts in the present study are questionable because of differences in patient characteristics such as dialysis vintage and RRF (most of the HD patients were functionally anuric, and most of the PD patients had RRF, with a GFR of 3.2 ± 2.2 mL/min/1.73 m²) and because of the smaller number of events in the PD patients. However, it should be noted that dialysis vintage, which could serve as a surrogate marker of RRF, was included as confounder, and we performed the effect modification by testing the statistical significance of two-way interaction terms of 8-OHdG by dialysis modality. Those interaction terms did not reach statistical significance. Thus, Cox regression analysis of the association between plasma 8-OHdG and all-cause mortality was performed in the entire population of dialysis patients. Fourth, from a theoretical viewpoint, it is possible that oxidative stress could be related especially to cardiovascular mortality; however, given that our data on cardiovascular mortality were imprecise or incomplete (or both) in the study cohorts, the association between plasma 8-OHdG and cardiovascular mortality was not evaluated.

CONCLUSIONS

Degree of oxidative stress, as assessed by plasma 8-OHdG, was a strong independent predictor of all-cause mortality in 303 prevalent dialysis patients. Differences between patients treated with PD and HD also suggest that dialysis modality as such, or possibly factors related to dialysis modality (for example, differences in dialysis vintage or RRF), might modify oxidative stress and its consequences in patients with ESRD. Thus, in the present study, plasma 8-OHdG was lower in the PD patients than in the HD patients, and we could demonstrate a mortality-predictive effect of plasma 8-OHdG in the HD patients, but not in the PD patients. Further research to confirm

those results and, if possible, to delineate the causes and implications of oxidative stress in larger cohorts of dialysis patients is warranted. Such data could be of value for the design of future interventional studies aimed at reducing oxidative stress in these patients.

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DISCLOSURES

BL is affiliated with Baxter Healthcare. PS is a past member of the scientific advisory board of Gambro AB. None of the other authors declared any financial conflict of interest.

REFERENCES

1. Eknoyan G. Cardiovascular mortality and morbidity in dialysis patients. *Miner Electrolyte Metab* 1999; 25:100–4.
2. Collins AJ, Foley RN, Gilbertson DT, Chen SC. The state of chronic kidney disease, ESRD, and morbidity and mortality in the first year of dialysis. *Clin J Am Soc Nephrol* 2009; 4(Suppl 1):S5–11.
3. Vaziri ND. Oxidative stress in uremia: nature, mechanisms, and potential consequences. *Semin Nephrol* 2004; 24:469–73.
4. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease—what have we learned in 10 years? *Semin Dial* 2010; 23:498–509.
5. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62:1524–38.
6. Guerin A, Pannier B, London G. Atherosclerosis versus arterial stiffness in advanced renal failure. *Adv Cardiol* 2007; 44:187–98.
7. Brunet P, Gondouin B, Duval-Sabatier A, Dou L, Cerini C, Dignat-George F, et al. Does uremia cause vascular dysfunction? *Kidney Blood Press Res* 2011; 34:284–90.
8. Del Vecchio L, Locatelli F, Carini M. What we know about oxidative stress in patients with chronic kidney disease on dialysis—clinical effects, potential treatment, and prevention. *Semin Dial* 2011; 24:56–64.

9. Kasai H, Tanooka H, Nishimura S. Formation of 8-hydroxyguanine residues in DNA by X-irradiation. *Gann* 1984; 75:1037–9.
10. Beckman KB, Ames BN. Oxidative decay of DNA. *J Biol Chem* 1997; 272:19633–6.
11. Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. *Clin Chim Acta* 2004; 339:1–9.
12. Tarng DC, Huang TP, Wei YH, Liu TY, Chen HW, Wen Chen T, *et al.* 8-Hydroxy-2'-deoxyguanosine of leukocyte DNA as a marker of oxidative stress in chronic hemodialysis patients. *Am J Kidney Dis* 2000; 36:934–44.
13. Mastalerz-Migas A, Steciwko A, Pokorski M, Pirogowicz I, Drobnik J, Bunio A, *et al.* What influences the level of oxidative stress as measured by 8-hydroxy-2'-deoxyguanosine in patients on hemodialysis? *J Physiol Pharmacol* 2006; 57(Suppl 4):199–205.
14. Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, *et al.* Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; 358:2113–17.
15. Bayes B, Pastor MC, Bonal J, Foraster A, Romero R. Oxidative stress, inflammation and cardiovascular mortality in haemodialysis—role of seniority and intravenous ferrotherapy: analysis at 4 years of follow-up. *Nephrol Dial Transplant* 2006; 21:984–90.
16. Grahl DA, Axelsson J, Nordfors L, Heimbürger O, Bárány P, Qureshi AR, *et al.* Associations between the CYBA 242C/T and the MPO-463G/A polymorphisms, oxidative stress and cardiovascular disease in chronic kidney disease patients. *Blood Purif* 2007; 25:210–18.
17. Kalantar-Zadeh K, Brennan ML, Hazen SL. Serum myeloperoxidase and mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2006; 48:59–68.
18. Marques de Mattos A, Marino LV, Ovidio PP, Jordao AA, Almeida CC, Chiarello PG. Protein oxidative stress and dyslipidemia in dialysis patients. *Ther Apher Dial* 2012; 16:68–74.
19. Zhou Q, Wu S, Jiang J, Tian J, Chen J, Yu X, *et al.* Accumulation of circulating advanced oxidation protein products is an independent risk factor for ischemic heart disease in maintenance hemodialysis patients. *Nephrology (Carlton)* 2012; 17:642–9.
20. Castoldi G, Antolini L, Bombardi C, Perego L, Mariani P, Vigano MR, *et al.* Oxidative stress biomarkers and chromogranin a in uremic patients: effects of dialytic treatment. *Clin Biochem* 2010; 43:1387–92.
21. Al-Hweish A, Sultan SS, Mogazi K, Elsammak MY. Plasma myeloperoxidase, NT-proBNP, and troponin-I in patients on CAPD compared with those on regular hemodialysis. *Hemodial Int* 2010; 14:308–15.
22. Samouilidou EC, Karpouza AP, Kostopoulos V, Bakirtzi T, Pantelias K, Petras D, *et al.* Lipid abnormalities and oxidized LDL in chronic kidney disease patients on hemodialysis and peritoneal dialysis. *Ren Fail* 2012; 34:160–4.
23. Snædal S, Heimbürger O, Qureshi AR, Danielsson A, Wikstrom B, Fellstrom B, *et al.* Comorbidity and acute clinical events as determinants of C-reactive protein variation in hemodialysis patients: implications for patient survival. *Am J Kidney Dis* 2009; 53:1024–33.
24. Twardowski ZJ, Nolph KD, Khanna R. Limitations of the peritoneal equilibration test. *Nephrol Dial Transplant* 1995; 10:2160–1.
25. Davies SJ, Phillips L, Naish PF, Russell GI. Quantifying comorbidity in peritoneal dialysis patients and its relationship to other predictors of survival. *Nephrol Dial Transplant* 2002; 17:1085–92.
26. Qureshi AR, Alvestrand A, Danielsson A, Divino-Filho JC, Gutierrez A, Lindholm B, *et al.* Factors predicting malnutrition in hemodialysis patients: a cross-sectional study. *Kidney Int* 1998; 53:773–82.
27. Dounousi E, Papavasiliou E, Makedou A, Ioannou K, Katopodis KP, Tselepis A, *et al.* Oxidative stress is progressively enhanced with advancing stages of CKD. *Am J Kidney Dis* 2006; 48:752–60.
28. Antonelou MH, Kriebardis AG, Velentzas AD, Kokkalis AC, Georgakopoulou SC, Papassideri IS. Oxidative stress-associated shape transformation and membrane proteome remodeling in erythrocytes of end stage renal disease patients on hemodialysis. *J Proteomics* 2011; 74:2441–52.
29. Himmelfarb J, McMonagle E, McMenamin E. Plasma protein thiol oxidation and carbonyl formation in chronic renal failure. *Kidney Int* 2000; 58:2571–8.
30. Huh JY, Seo EY, Lee HB, Ha H. Glucose-based peritoneal dialysis solution suppresses adiponectin synthesis through oxidative stress in an experimental model of peritoneal dialysis. *Perit Dial Int* 2012; 32:20–8.
31. Tamura Y, Murayama T, Minami M, Yokode M, Arai H. Differential effect of statins on diabetic nephropathy in db/db mice. *Int J Mol Med* 2011; 28:683–7.
32. Morinaga H, Sugiyama H, Inoue T, Takiue K, Kikumoto Y, Kitagawa M, *et al.* Effluent free radicals are associated with residual renal function and predict technique failure in peritoneal dialysis patients. *Perit Dial Int* 2012; 32:453–61.
33. Furuya R, Kumagai H, Odamaki M, Takahashi M, Miyaki A, Hishida A. Impact of residual renal function on plasma levels of advanced oxidation protein products and pentosidine in peritoneal dialysis patients. *Nephron Clin Pract* 2009; 112:c255–61.
34. Jiang J, Chen P, Chen J, Yu X, Xie D, Mei C, *et al.* Accumulation of tissue advanced glycation end products correlated with glucose exposure dose and associated with cardiovascular morbidity in patients on peritoneal dialysis. *Atherosclerosis* 2012; 224:187–94.
35. Kaya Y, Ari E, Demir H, Soylemez N, Cebi A, Alp H, *et al.* Accelerated atherosclerosis in haemodialysis patients; correlation of endothelial function with oxidative DNA damage. *Nephrol Dial Transplant* 2012; 27:1164–9.

36. Ikizler TA, Morrow JD, Roberts LJ, Evanson JA, Becker B, Hakim RM, *et al.* Plasma F2-isoprostane levels are elevated in chronic hemodialysis patients. *Clin Nephrol* 2002; 58:190–7.
37. Sydow K, Munzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; 4:41–51.
38. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000; 342:154–60.
39. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; 360:23–33.
40. Coombes JS, Fassett RG. Antioxidant therapy in hemodialysis patients: a systematic review. *Kidney Int* 2012; 81:233–46.
41. Boaz M, Smetana S, Weinstein T, Matas Z, Gaftor U, Iaina A, *et al.* Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. *Lancet* 2000; 356:1213–18.
42. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation* 2003; 107:992–5.