



Original Article

Serum 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, is associated with mortality independent of inflammation in chronic kidney disease

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ABSTRACT

Background: Oxidative stress and low-grade systemic inflammation are common interrelated sequelae of chronic kidney disease (CKD) that associate with mortality. We investigated the association of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, with mortality in CKD individuals and analyzed whether inflammation modifies the association.

Methods: In 376 individuals with a wide range of estimated glomerular filtration rate (eGFR); > 60 ml/min ($n = 53$), 15–60 ml/min ($n = 60$) and < 15 ml/min ($n = 263$), cut-off values of serum 8-OHdG, high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and tumor necrosis factor (TNF) as predictors of mortality were determined by ROC curves. We analyzed associations of 8-OHdG with inflammation markers and the overlapping effect of hsCRP, IL-6 and TNF on the association between 8-OHdG and all-cause mortality by multivariate generalized linear models.

Results: In separate individual exposure analyses, higher 8-OHdG, hsCRP, and IL-6 (but not TNF) were each independently associated with increased risk of death in multivariate models adjusted for age, sex, diabetes mellitus, cardiovascular disease, protein-energy wasting, cohort calendar year, blood sample storage time and eGFR. For 8-OHdG, the multivariate relative risk ratio, RR_{8-OHdG} (95% confidence interval) 1.17 (1.08–1.26), remained essentially unchanged when adjusting also for inflammation in three separate models including: hsCRP, $RR_{8-OHdG} = 1.15$ (1.06–1.25); IL-6, $RR_{8-OHdG} = 1.15$ (1.07–1.25); and TNF, $RR_{8-OHdG} = 1.16$ (1.07–1.26).

Conclusions: Serum 8-OHdG, a biomarker of oxidative DNA damage, is associated with increased all-cause mortality risk in individuals with a wide range of eGFR and this association is independent of inflammation.

1. Introduction

The risk of early vascular ageing [1] and death due to cardiovascular disease (CVD) is high among patients with chronic kidney disease (CKD) [2–4]. The underlying pathophysiological mechanisms remain unclear and traditional risk factors, such as hypertension, diabetes mellitus and dyslipidemia only partially explain the massive CVD burden in CKD [5–7]. In addition, non-traditional risk factors, such as

oxidative stress [8,9] and inflammation [10,11], may contribute to early vascular ageing with endothelial dysfunction, vascular remodeling and vascular calcification in patients with CKD [12–17].

Oxidative stress is thought to be a major contributor to increased atherosclerosis and cardiovascular morbimortality in advanced CKD [18]. 8-hydroxy-2-deoxyguanosine (8-OHdG), one of the most abundant oxidative products of DNA, is a sensitive biomarker of oxidative stress that is capable of reflecting extremely low levels of oxidative DNA

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damage [19]. An elevated level of serum 8-OHdG serves as a surrogate marker of oxidant-induced DNA damage in dialysis patients [20,21]. However, while an association between serum 8-OHdG and mortality is observed among dialysis patients [22,23], the mortality predictive capacity of 8-OHdG in patients with earlier stages of CKD is less well studied.

Low grade chronic inflammation with elevated circulating levels of inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF) and C-reactive protein (CRP) is highly prevalent and associates with increased mortality in CKD [24–26]. Additionally, according to the inflammation-catalyst hypothesis [27], inflammation may exacerbate the effect of other concurrent risk factors in CKD [28,29]. As “partners in crime” oxidative stress and inflammation are believed to play pivotal roles in the progression of CKD [30] and in the pathogenesis of the progeric uremic phenotype [31]. However, it is unknown if in CKD, a catalytic effect of inflammation enhances the association of oxidative stress with mortality, or if oxidative stress predicts mortality independent of inflammation.

The aim of this study was to test the hypothesis that the association of elevated serum 8-OHdG with increased mortality is modified by concomitant inflammation as assessed by inflammatory mediators (hsCRP, IL-6 and TNF) in an observational cohort of patients with a wide range of glomerular filtration rate (GFR).

2. Materials and methods

2.1. Patients

The current analysis of the association of baseline serum 8-OHdG with 5-year all-cause mortality included 376 individuals with CKD stage 1–5 including those with CKD stage 1–2 ($n = 53$) with estimated GFR (eGFR) > 60 ml/min/1.73m² comprising CKD stage 1 ($n = 21$; eGFR > 90 ml/min/1.73 m²) and CKD stage 2 ($n = 32$; eGFR 60–90 ml/min/1.73 m²); CKD stage 3–4 ($n = 60$) with eGFR 15–60 ml/min/1.73m² comprising those with CKD stage 3 ($n = 22$) and CKD stage 4 ($n = 38$), and 263 CKD stage 5 patients with eGFR < 15 ml/min/m². Exclusion criteria were age < 18 years, acute renal failure, signs of overt clinical infection and unwillingness to participate. Informed consent was obtained from each individual. The Ethics Committee of the Karolinska Institute (EPN) at the Karolinska University Hospital Huddinge, Stockholm, Sweden, approved study protocols. The studies were conducted in adherence to the Declaration of Helsinki.

The GFR categories were defined according to current guidelines based on estimated GFR (eGFR) calculated according to the CKD-EPI (CKD Epidemiology Collaboration) equation [32]. Individuals were classified into GFR categories 1–5 according to the National Kidney Foundation's K/DOQI guidelines [33]. The clinical and laboratory characteristics of patients according to eGFR groups (CKD stage 1–2, 3–4 and 5 respectively) are shown in Suppl. Table 1. The subjects from different cohorts and their underlying causes of CKD are briefly described below:

CKD stage 1–2 ($n = 53$), comprising CKD stage 1 ($n = 21$) and CKD stage 2 individuals ($n = 32$), were recruited from a population-based sample of individuals from the Stockholm region, randomly selected by Statistics Sweden (a government agency), who served as control subjects for a study on malnutrition, inflammation and atherosclerosis in patients with CKD 3–4, the PRIMA study [34], and who were found to have signs of CKD.

CKD stage 3–4 ($n = 60$) comprising CKD stage 3 ($n = 22$) and CKD stage 4 ($n = 38$) individuals were recruited from PRIMA study ($n = 51$), and from an ongoing study of associations between malnutrition, inflammation and atherosclerosis in CKD patients initiating dialysis therapy, the MIA study ($n = 9$) [34]. The causes of CKD were chronic glomerulonephritis ($n = 12$), diabetic nephropathy ($n = 14$), reno-vascular disease ($n = 2$), and others ($n = 32$).

CKD stage 5 patients ($n = 263$) were recruited from the PRIMA study ($n = 16$) and from the MIA study ($n = 247$). The causes of renal failure included: chronic glomerulonephritis ($n = 64$), hypertension and reno-vascular disease ($n = 51$), diabetic nephropathy ($n = 82$) and others ($n = 66$).

At baseline, 70 out of the 263 CKD stage 5 patients had initiated dialysis within median 9 (2–28) days prior to recruitment while most ($n = 193$) were being treated conservatively before initiating dialysis after variable periods of time.

2.2. Collection of clinical and laboratory data

Clinical data collected at baseline visits included demographics, comorbid conditions, causes of kidney diseases, blood pressure, body mass index, and nutritional status evaluated by subjective global assessment (SGA) [35]. Presence of clinical CVD was defined as a clinical history or signs of ischemic cardiac disease, and/or presence of peripheral vascular disease, cerebrovascular disease/presence of heart failure, and arrhythmia. Blood pressure is presented as mean arterial blood pressure defined as [diastolic pressure + (systolic pressure – diastolic pressure) / 3].

2.3. 8-OHdG and other laboratory analyses

All blood samples were obtained in the morning after an overnight fast and kept frozen at -70°C if not analyzed immediately. Serum 8-OHdG was measured with a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Ageing, Shizuoka, Japan) as described previously [36]. Briefly, serum samples were purified by centrifugal ultrafiltration at $14,000 \times g$ for 15 min at 25°C using Amicon Ultra-10 k (Millipore Corp., Bedford, MA, USA) according to the manufacturer's instruction. Blank samples (without primary antibody) were used as negative controls, and standards as positive controls. 50 μl of standards or sample and the reagent 50 μl 8-OHdG monoclonal antibody were added to microtiter plates, which had been pre-coated with “competitive” 8-OHdG and incubated at 4°C overnight. After washing the wells three times, horseradish peroxidase conjugated secondary antibody was added, followed by incubation for 60 min. The wells were again washed three times, the enzyme substrate solution was added, and the wells were incubated for 15 min. The reaction was stopped by 1 M phosphoric acid. The concentration of unknown samples was obtained from a standard curve of 450 nm where absorbance was plotted against the 0.125–10 ng/ml standards. The coefficient of variation, CV, was 5.8%.

Serum samples of creatinine, albumin (bromocresol purple), calcium, phosphate, intact parathyroid hormone (iPTH), ferritin, cholesterol, triglyceride (TG), hemoglobin, high-sensitivity CRP (hsCRP; by nephelometry assay; CV, 5%) were measured by routine methods at the Department of Laboratory Medicine, Karolinska University Hospital at Huddinge. Commercial ELISA kits were used to determine serum vascular cell adhesion protein-1 (VCAM-1) (R&D Systems, Minneapolis, MN). Plasma concentrations of IL-6 (CV, 4%), TNF, (CV, 2–5%) and insulin-like growth factor-1 (IGF-1, CV, 4.3%) were measured on an Immulite TM Automatic Analyzer (Siemens Healthcare; Diagnostics Products Ltd.) according to the manufacturer's instructions. Glomerular filtration rate (GFR) was assessed in CKD stage 5 patients by the mean of renal urea and creatinine clearances from a 24-h urine collection, and, in CKD stage 1–4 patients, by iohexol clearance. To allow comparisons, eGFR was assessed in all patients by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [32].

2.4. Statistical analyses

All variables are expressed as median (10th and 90th percentile) or percentage, or relative risk ratio (95% CI, confidence intervals), as

appropriate. Comparisons between two groups were assessed with the non-parametric Wilcoxon test for continuous variables and Fischer's exact test for nominal variables; differences among three groups were analyzed by Kruskal Wallis ANOVA test. Statistical significance was set at the level of $p < .05$ (multiple comparisons were un-adjusted and p -values were descriptive). Univariate Spearman's rank correlation was used to determine association between 8-OHdG and other variables. Cut-off values for biomarkers as predictors of all-cause mortality were calculated by receiver operating characteristics (ROC) curves. Survival was analyzed by generalized linear regression models the GENMOD procedure in SAS. We analyzed 8-OHdG, hsCRP, IL-6 and TNF in separate generalized linear regression models using identical multi-variable modeling strategy adjusted for confounders. We performed multiple imputation for the missing values. All statistical analyses were performed using statistical software SAS version 9.4 (SAS Campus Drive, Cary, NC, USA).

3. Results

3.1. Clinical and biochemical characteristics

The baseline characteristics of the 376 CKD individuals are presented in Table 1. Their median age was 57 years, 37% were women, 29% had diabetes mellitus (DM), 30% clinical signs of CVD, and 23% PEW (SGA > 1). Median hsCRP was 3.6 (0.6, 28.3) mg/l, median IL-6 4.2 (1.4, 14.4) pg/ml, median TNF 9.0 (4.6, 15.8) pg/ml, and median 8-OHdG 0.57 (0.21, 1.03) ng/ml. The individuals were divided into two groups by the ROC-curve-defined cut-off value of 8-OHdG (0.64 ng/ml). As shown in Table 1, patients with high 8-OHdG had higher prevalence of CVD and PEW, higher levels of serum creatinine, triglycerides, IGF-1, iPTH, CaPO₄, ferritin, VCAM-1, hsCRP, IL-6 and TNF, but lower eGFR,

serum albumin and hemoglobin. Baseline characteristics of individuals divided according to their eGFR levels into three groups (CKD 1–2, 3–4 and 5 respectively) are shown in Suppl. Table 1. As expected, the levels of 8-OHdG and inflammatory markers rose with the deterioration of renal function (Suppl. Table 1).

3.2. Univariate correlations and determinants of 8-OHdG

As shown in Suppl. Table 2, serum 8-OHdG was negatively related with eGFR and positively associated with inflammation markers hsCRP, IL-6, TNF, endothelial function marker VCAM-1 and iron status indicated by ferritin. In addition, 8-OHdG was also associated with CVD, PEW, serum creatinine, triglycerides, IGF-1, iPTH and CaPO₄, while albumin and hemoglobin showed an inverse relation with 8-OHdG. There were no significant associations between 8-OHdG and age, sex, DM, mean arterial blood pressure or BMI.

Determinants of 8-OHdG were explored in three multivariate generalized linear regression models including either hsCRP, IL-6 or TNF (Table 2). CVD, PEW, VCAM-1, ferritin, and CaPO₄, were identified as determinants of 8-OHdG, whereas among the three investigated inflammatory biomarkers, only IL-6 (relative risk ratio, RR: 1.13 [95% confidence interval, 1.03–1.24]), but not hsCRP or TNF, was significantly associated with 8-OHdG.

3.3. Individual effects of 8-OHdG and inflammatory markers on mortality

During 5 years of follow-up, median 45(10–60) months, 95 out of 376 individuals died. As shown in Table 3, high levels of 8-OHdG, hsCRP and IL-6 were independently associated with increased mortality risk in crude as well as in multivariate models, adjusted for confounders (age, sex, DM, CVD, PEW, cohort calendar year, blood sample storage

Table 1

Baseline demographic, clinical and biochemical characteristics of all 376 individuals divided according to the 8-OHdG cut off value.

	All (n = 376)	8-OHdG < 0.64 ng/ml (n = 218)	8-OHdG > 0.64 ng/ml (n = 158)	p-Value
Demography and clinical characteristics				
Age, years	57 (37, 70)	55 (36, 72)	59 (39, 69)	0.48
Male, %	63	62	64	0.76
Diabetes mellitus, %	29	27	33	0.19
Cardiovascular disease, %	30	21	42	< 0.001
BMI (kg/m ²)	24.5 (19.9, 31.1)	24.7 (20.3, 31.2)	24.1 (19.3, 31.2)	0.13
PEW (SGA > 1) (%; n = 369/215/154)	23	14	35	< 0.001
eGFR (ml/min/1.73 ²) ^a	8 (4, 75)	13 (5, 89)	7 (4, 12)	< 0.001
MAP (mmHg; n = 358/205/153) ^b	109 (90, 129)	108 (90, 127)	110 (91, 131)	0.68
Biochemical parameters				
Hemoglobin, g/l	112 (89, 144)	118 (91, 148)	104 (87, 130)	< 0.001
Creatinine, μmol/l	558 (89, 980)	375 (76, 885)	676 (380, 1120)	< 0.001
S-albumin, g/l	35 (27, 41)	37 (29, 42)	33 (25, 40)	< 0.001
Triglycerides (mmol/l; n = 371/214/157)	1.8 (0.8, 3.4)	1.6 (0.7, 3.4)	1.9 (0.9, 3.5)	0.02
Cholesterol (mmol/l; n = 375/217/158)	5.2 (3.6, 7.1)	5.2 (3.9, 7.0)	5.3 (3.5, 7.2)	0.71
IGF-1 (μg/ml; n = 359/214/145)	150 (74, 277)	147 (73, 254)	156 (76, 346)	0.11
iPTH (ng/l; n = 366/212/154)	133 (33, 486)	100 (30, 441)	200 (41, 544)	< 0.001
Ca × PO ₄ (mmol ² /l ² ; n = 355/209/146)	4.02 (2.17, 6.23)	3.47 (2.07, 6.03)	4.47 (3.15, 6.67)	< 0.001
Ferritin (mg/l; n = 352/196/156)	216 (68, 615)	172 (58, 457)	288 (88, 793)	< 0.001
VCAM-1 (ng/ml; n = 360/202/158)	1125 (678, 1773)	939 (600, 1552)	1389 (891, 2070)	< 0.001
8-OHdG (ng/ml)	0.57(0.21, 1.03)	0.41(0.12, 0.60)	0.84(0.67, 1.23)	< 0.001
Inflammatory biomarkers				
hsCRP, mg/l	3.6 (0.6, 28.3)	2.5 (0.5, 13.0)	6.9 (1.0, 49)	< 0.001
IL-6 (pg/ml; n = 358/200/158)	4.2 (1.4, 14.4)	3.3 (1.1, 8.8)	7.0 (2.3, 16.8)	< 0.001
TNF (pg/ml; n = 355/199/156)	9.0 (4.6, 15.8)	8.0 (3.6, 14.7)	10.5 (6.4, 17.2)	< 0.001

Data presented as median (10th - 90th percentile), number or percentage.

For parameters with missing values, the number of available measurements is presented in brackets.

Abbreviations: 8-OHdG, 8-hydroxy-2'-deoxyguanosine; BMI, body mass index; PEW, protein-energy wasting; SGA, subjective global assessment; eGFR, estimated glomerular filtration rate; MAP, mean arterial blood pressure; S-albumin, serum albumin; IGF-1, insulin-growth like factor-1; iPTH, intact parathyroid hormone; Ca × PO₄, calcium phosphate product; VCAM-1, vascular cell adhesion protein 1; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF, tumor necrosis factor.

^a eGFR was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

^b MAP was calculated as diastolic pressure + 1/3 (systolic pressure - diastolic pressure).

Table 2Predictors of oxidative stress (8-OHdG < 0.64 ng/ml as reference group) by generalized linear regression analysis in 376 individuals.^a

Variables ^b	Relative risk ratio (95%CI)		
	Model 1 ^a	Model 2 ^a	Model 3 ^a
Age ≥ 58 vs < 58 years	1.01 (0.92–1.12)	1.03 (0.93–1.13)	1.02 (0.92–1.12)
Gender, male vs female	1.02 (0.93–1.12)	1.02 (0.93–1.12)	1.02 (0.93–1.12)
Diabetes mellitus, yes vs no	0.93 (0.84–1.03)	0.91 (0.82–1.00)	0.93 (0.84–1.02)
Cardiovascular disease, yes vs no	1.12 (1.01–1.25)	1.13 (1.01–1.25)	1.13 (1.02–1.26)
PEW (SGA > 1), yes vs no	1.14 (1.01–1.28)	1.13 (1.01–1.26)	1.16 (1.04–1.31)
Calendar 1994–2001 vs 2002–2005	0.96 (0.79–1.17)	0.92 (0.76–1.13)	0.96 (0.78–1.17)
Blood sample storage time	0.93 (0.77–1.12)	0.94 (0.78–1.13)	0.94 (0.78–1.13)
eGFR > 21.1 vs < 21.1 ml/min/1.73 ²	0.92 (0.83–1.02)	0.95 (0.86–1.06)	0.91 (0.82–1.02)
VCAM-1 > 1048 vs < 1048 ng/ml	1.24 (1.12–1.38)	1.24 (1.12–1.39)	1.25 (1.13–1.39)
Triglycerides > 3.4 vs < 3.4 mmol/l	0.97 (0.84–1.13)	0.98 (0.85–1.14)	0.99 (0.85–1.14)
Ferritin > 272 vs < 272 mg/l	1.13 (1.03–1.24)	1.11 (1.01–1.22)	1.13 (1.03–1.24)
CaxPO ₄ > 3.35 vs < 3.35 mmol ² /l ²	1.21 (1.07–1.35)	1.29 (1.14–1.44)	1.17 (1.04–1.32)
iPTH > 105 vs < 105 ng/l	1.05 (0.95–1.16)	1.05 (0.95–1.16)	1.07 (0.97–1.18)
Inflammation: hsCRP or IL-6 or TNF ^c	1.09 (0.99–1.21)	1.13 (1.03–1.24)	1.04 (0.95–1.15)

Abbreviations: 8-OHdG, 8-hydroxy-2'-deoxyguanosine; 95% CI, 95% confidence interval; PEW, protein energy wasting; SGA, subjective global assessment; eGFR, estimated glomerular filtration rate; VCAM-1, vascular cell adhesion protein 1; Ca × PO₄, calcium phosphate product; iPTH, intact parathyroid hormone; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF, tumor necrosis factor.

Bold indicates p value < 0.05.

^a The three models are adjusted by all variables listed in the table except for the three inflammation which were included one by one as follows: Model 1: adjusted for inflammation marker hsCRP; Model 2: adjusted for inflammation marker IL-6; Model 3: adjusted for inflammation marker TNF.

^b For continuous variables, cut off levels were defined by ROC analysis.

^c Cut off levels for biomarkers of inflammation included in Model 1: hsCRP (< 4.5 vs > 4.5 mg/l); in Model 2: IL-6 (> 5.1 vs < 5.1 pg/ml); in Model 3: TNF (> 9.4 vs < 9.4 pg/ml).

time, and eGFR) while TNF was associated with mortality only in crude analysis. In the separate adjusted models, patients with higher levels of 8-OHdG, hsCRP and IL-6 had 1.17, 1.10 and 1.09-fold higher risks of death respectively than those with lower levels of each exposure.

3.4. Dual effects of 8-OHdG and inflammatory biomarkers on mortality

Next, we tested whether elevated levels of 8-OHdG and inflammatory biomarkers would exert an overlapping effect on 5-year mortality. In three separate models (Table 3), we added hsCRP, IL-6 and TNF respectively to the multivariable-adjusted 8-OHdG model (adjustments for age, sex, DM, CVD, PEW, cohort calendar year, blood sample

Table 3

Dual effects of 8-OHdG and inflammatory markers on all-cause mortality.

	Relative risk ratio (95% CI)			
	Crude	p value	Adjusted model ^a	p value
8-OHdG and hsCRP				
Separate generalized linear regression models of each individual exposure				
8-OHdG > 0.64 vs < 0.64 ng/ml	3.78 (2.47–5.79)	< 0.001	1.17 (1.08–1.26)	< 0.001
hsCRP > 4.5 vs < 4.5 mg/l	3.21 (2.11–4.90)	< 0.001	1.10 (1.02–1.19)	0.01
Generalized linear regression models that include both 8-OHdG and hsCRP				
8-OHdG > 0.64 vs < 0.64 ng/ml	2.85 (1.82–4.47)	< 0.001	1.15 (1.06–1.25)	< 0.001
hsCRP > 4.5 vs < 4.5 mg/l	2.24 (1.43–3.50)	< 0.001	1.10 (0.99–1.16)	0.06
8-OHdG and IL-6				
Separate generalized linear regression models of each individual exposure				
8-OHdG > 0.64 vs < 0.64 ng/ml	3.78 (2.47–5.79)	< 0.001	1.17 (1.08–1.26)	< 0.001
IL-6 > 5.1 vs < 5.1 pg/ml	3.36 (2.21–5.12)	< 0.001	1.09 (1.01–1.18)	0.02
Generalized linear regression models that includes both 8-OHdG and IL-6				
8-OHdG > 0.64 vs < 0.64 ng/ml	2.54 (1.63–3.96)	< 0.001	1.15 (1.07–1.25)	< 0.001
IL-6 > 5.1 vs < 5.1 pg/ml	2.54 (1.64–3.93)	< 0.001	1.07 (0.99–1.15)	0.06
8-OHdG and TNF				
Separate generalized linear regression models of each individual exposure				
8-OHdG > 0.64 vs < 0.64 ng/ml	3.78 (2.47–5.79)	< 0.001	1.17 (1.08–1.26)	< 0.001
TNF > 9.4 vs < 9.4 pg/ml	1.86 (1.24–2.80)	0.003	1.06 (0.98–1.14)	0.21
Generalized linear regression models that includes both 8-OHdG and TNF				
8-OHdG > 0.64 vs < 0.64 ng/ml	3.20 (2.03–5.03)	< 0.001	1.16 (1.07–1.26)	< 0.001
TNF > 9.4 vs < 9.4 pg/ml	1.31 (0.85–2.00)	0.22	1.04 (0.96–1.12)	0.34

Abbreviations: 95% CI, 95% confidence interval; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; TNF, tumor necrosis factor.

^a Adjusted by age, gender, diabetes mellitus, cardiovascular disease, protein energy wasting, cohort calendar year, blood sample storage time, eGFR and inflammation (hsCRP, IL-6 or TNF).

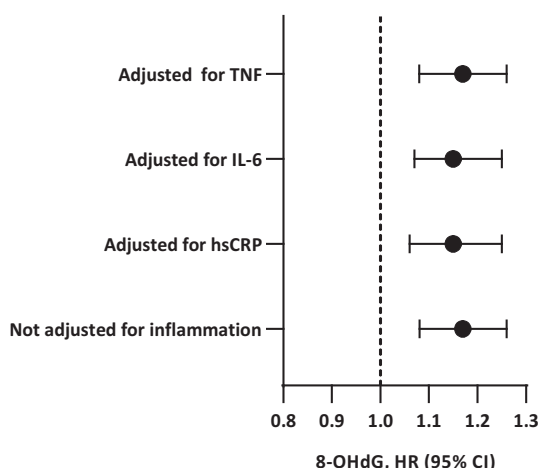


Fig. 1. An increased serum concentration of 8-OHdG (defined by ROC curve) was independently associated with increased all-cause mortality risk. The strength of this association was essentially similar in generalized linear regression models not adjusted for inflammation and in models adjusted for CRP, IL-6 and TNF. All models were adjusted for age, gender, diabetes mellitus, cardiovascular disease, protein energy wasting, cohort calendar year, blood sample storage time and eGFR.

storage time, and eGFR) to evaluate whether additional adjustments for any of the three inflammatory markers would attenuate the association between 8-OHdG and risk of death. In none of the three separate models, the association between 8-OHdG and mortality was weakened, and a higher level of 8-OHdG remained independently associated with all-cause mortality in all multivariate models. Thus, the association of 8-OHdG with mortality when the inflammatory mediators were not included in the adjusted model, RR_{8-OHdG} 1.17 (95% CI 1.08–1.26), remained essentially unchanged when adjusting also for hsCRP, RR_{8-OHdG} 1.15 (1.06–1.25); for IL-6, RR_{8-OHdG} 1.15 (1.07–1.25); and, for TNF, RR_{8-OHdG} 1.16 (1.07–1.26) respectively (Table 3 and Fig. 1).

4. Discussion

In this study, serum 8-OHdG levels increased with the deterioration of renal function and correlated with inflammatory markers hsCRP, IL-6 and TNF. We report that an elevated level of 8-OHdG was independently associated with increased risk of death during 5 years of follow-up, which was not affected by concomitant inflammation in models adjusted for hsCRP, IL-6 and TNF. Furthermore, none of the inflammatory markers were independently associated with increased mortality co-adjusted with 8-OHdG, regardless of a borderline statistical significance for hsCRP and IL-6 ($p = .06$ for both; Table 3). To the best of our knowledge, this is the first study reporting such an association in pre-dialysis CKD individuals representing a wide range of eGFR. The novel observation that inflammation did not attenuate the effects of 8-OHdG on mortality suggests that the severity of oxidative DNA damage is an independent risk factor for increased mortality in CKD.

In this study, the presence of CVD and PEW, higher levels of VCAM-1, Ca x PO_4 and ferritin turn out to be the independent determinants of increased levels of 8-OHdG, suggesting that oxidative stress is potentially involved with pathogenesis of cardiovascular risk, poor nutritional status, bone and mineral disorders, endothelial dysfunction and iron status in the context of CKD. The role of inflammation as a risk factor of increased mortality has been repeatedly demonstrated in CKD [24,25,29]. In accordance with a previous study [10], we showed that the inflammatory burden increased with the deterioration of renal function and as expected, all inflammatory markers correlated with serum 8-OHdG though only IL-6 emerged as an independent

determinant of 8-OHdG. Despite the complex molecular and cellular mechanisms of inflammation in CKD, redox-sensitive signaling pathways are known to have a major role in the processes of uremic inflammation [37]. Reactive oxygen species (ROS) produced intracellularly in response to CKD-associated factors act as second messengers, coupling molecules in signal transduction pathways to activate NF- κ B transcription factor and inducing cell adhesion molecules and inflammatory gene expression [38]. Considering the interactive signaling transductions between oxidative stress and inflammation, we hypothesized that they may have synergistic effects on mortality in CKD. Contrary to our hypothesis, we found an independent association between 8-OHdG and all-cause mortality risk, which was not attenuated by inflammation.

Based on these findings, we speculate that while 8-OHdG and inflammatory markers may directly increase one another, they may have distinct downstream effects accounting for the impact on mortality in the context of CKD. Moreover, the correlation between 8-OHdG and inflammatory markers lost significance in the subsequent adjusted multivariate regression analyses (except for IL-6), suggesting that the severity of oxidative DNA damage is not in parallel with circulating inflammatory markers. Our results revealed that 8-OHdG withstood the scrutiny of being concomitantly assessed together with inflammation and remained as a robust risk factor of all-cause mortality, which also implies that treatment strategies targeting oxidative stress should be tested in CKD. Whereas previous randomized controlled studies with anti-oxidants show either none (tocopherol) [39] or small (coenzyme Q10) [40] effects on surrogate markers of oxidative stress in CKD, bardoxolone, a potent anti-oxidant Nrf2 agonist, improved kidney function in a post-hoc analysis of diabetic CKD 4 patients [41]. The effects of Nrf2 agonist treatment on serum 8-OHdG levels need to be uncovered in CKD.

Several limitations should be considered in interpreting the results. Firstly, no conclusion can be made regarding causality among oxidative DNA damage and risk of death due to the observational study design. Secondly, we adjusted for numerous factors that may affect 8-OHdG, inflammation and mortality, but cannot rule out unmeasured or unknown residual confounding factors. Thirdly, single measurements of 8-OHdG and inflammation markers may not accurately reflect long-term exposure to the potential risk factors. Fourthly, long-term storage of blood samples may influence 8-OHdG levels; however, we applied adjustment for storage time as a potential confounder in all analyses. On the other hand, while oxidative stress and inflammation are intrinsically linked, separate models with three investigated inflammatory biomarkers verified an independent association between 8-OHdG and mortality.

In conclusion, serum 8-OHdG, a marker of oxidative DNA damage, was associated with all-cause mortality risk independent of inflammation and other confounders such as eGFR, in CKD patients with a wide range of renal function. Future studies should examine the impact of anti-oxidative 8-OHdG-lowering interventions on inflammatory markers, and the feasibility of studies designed to investigate the potential effect of anti-oxidative therapeutic strategies on clinical outcomes of CKD patients.

Disclosures

Bengt Lindholm is employed by Baxter Healthcare Corporation. None of the other authors declare any conflict of interest.

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Declaration of Competing Interest

Bengt Lindholm is employed by Baxter Healthcare Corporation. None of the other authors declare any conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejim.2019.07.035>.

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