



Urinary chloride concentration and progression of chronic kidney disease: results from the KoreaN cohort study for Outcomes in patients With Chronic Kidney Disease

Young Su Joo ^{1,2,*}, Jinseok Kim^{1*}, Cheol Ho Park¹, Hae-Ryong Yun¹, Jung Tak Park¹, Tae Ik Chang³, Tae-Hyun Yoo¹, Su-Ah Sung⁴, Joongyub Lee⁵, Kook-Hwan Oh⁶, Soo Wan Kim⁷, Shin-Wook Kang^{1,8}, Kyu Hun Choi¹, Curie Ahn⁶ and Seung Hyeok Han ¹

¹Department of Internal Medicine, College of Medicine, Institute of Kidney Disease Research, Yonsei University, Seoul, Republic of Korea, ²Division of Nephrology, Department of Internal Medicine, Myongji Hospital, Goyang, Republic of Korea, ³Department of Internal Medicine, National Health Insurance Service Medical Center, Ilsan Hospital, Goyang, Republic of Korea, ⁴Department of Internal Medicine, Eulji Medical Center, Eulji University, Seoul, Republic of Korea, ⁵Department of Prevention and Management, Inha University School of Medicine, Incheon, Republic of Korea, ⁶Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea, ⁷Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea and ⁸Severance Biomedical Science Institute, Brain Korea 21 PLUS, Yonsei University, Seoul, Republic of Korea

Correspondence to: Seung Hyeok Han; E-mail: hansh@yuhs.ac

*These authors contributed equally to this work.

ABSTRACT

Background. Urinary chloride is regulated by kidney transport channels, and high urinary chloride concentration in the distal tubules can trigger tubuloglomerular feedback. However, little attention has been paid to urinary chloride as a biomarker of clinical outcomes. Here, we studied the relationship between urinary chloride concentration and chronic kidney disease (CKD) progression.

Methods. We included 2086 participants with CKD from the KoreaN cohort study for Outcomes in patients With Chronic Kidney Disease. Patients were categorized into three groups, according to baseline urinary chloride concentration tertiles. The study endpoint was a composite of $\geq 50\%$ decrease in estimated glomerular filtration rate from baseline values, or end-stage kidney disease.

Results. During a median follow-up period of 3.4 years (7452 person-years), 565 participants reached the primary endpoint. There was a higher rate of CKD progression events in the lowest and middle tertiles than in the highest tertile. Compared with the lowest tertile, the highest tertile was associated with 33% [95% confidence interval (CI) 0.49–0.90] lower risk for the primary outcome in a cause-specific hazard model after adjustment for confounding variables. In addition, for every 25 mEq/L increase in urinary chloride concentration, there was 11% (95% CI 0.83–0.96) lower risk for CKD progression. This association was consistent in a time-varying model. Urinary chloride concentration correlated well with tubule function and

kidney injury markers, and its predictive performance for CKD progression was comparable to that of these markers.

Conclusions. In this hypothesis-generating study, low urinary chloride concentration was associated with a higher risk for CKD progression.

Keywords: CKD progression, urine chloride

INTRODUCTION

Chronic kidney disease (CKD) is a global health problem worldwide and has become a major medical, social and economic burden on patients and health care systems [1, 2]. Not only are CKD patients at risk for progression to end-stage kidney disease (ESKD), but they are also at an increased risk for cardiovascular events and death, even in early stages of CKD [3], which emphasizes the importance of identifying these high-risk patients.

The renal tubule contains numerous transport channels that reabsorb and excrete various ions. Chloride is the most abundant anion in the extracellular fluid compartment and is regulated by specific chloride channels and transporters [4, 5]. Clinically, low urinary chloride concentrations of <20 mEq/L may indicate volume-depleted metabolic alkalosis [6]. Urinary chloride concentration is generally used to calculate the urinary anion gap to distinguish among the different types of normal anion gap metabolic acidosis [7].

In the kidney, a well-coordinated tubuloglomerular feedback (TGF) mechanism exists between the glomerular and tubular compartments [8]. This is one of the mechanisms responsible for autoregulation of the glomerular filtration rate (GFR) and renal blood flow. TGF is mainly driven by an increased distal tubular chloride concentration that causes the basolateral release of adenosine from the macula densa cells [9, 10]. In response to this signaling, the glomerular afferent arteriole constricts, such that blood flow into the glomerulus decreases, thus resulting in reduced glomerular hypertension [11, 12]. This physiologic response can function as a defense mechanism against hyperfiltration- and proteinuria-induced injuries. In fact, renin-angiotensin system (RAS) blockers and sodium-glucose cotransporter-2 (SGLT-2) inhibitors exert their protective effects via modulating the afferent arteriolar tone [13, 14]. Many studies have consistently shown that use of these drugs results in decreased proteinuria and GFR preservation in diabetic and non-diabetic kidney disease [15, 16].

In this regard, abnormal urinary chloride concentrations could be implicated in various kidney diseases. In particular, tubular dysfunction is commonly seen in CKD, and thus, metabolic acidosis also commonly occurs together with low urinary chloride concentrations [17]. From the viewpoint of TGF response to increased flow of tubular fluid in the thick ascending limb, it can be inferred that high urinary chloride concentration may reflect the activation of TGF. However, to our knowledge, urinary chloride concentration has never been tested as a marker of tubular defects or TGF, and its clinical implication in CKD patients is unknown. Therefore, in this study, to test our hypothesis, we investigated the association between urinary chloride concentration and the progression of kidney disease.

MATERIALS AND METHODS

Study design and participants

The KoreaN cohort study for Outcomes in patients With Chronic Kidney Disease (KNOW-CKD) is a nationwide prospective cohort study in non-dialysis patients with CKD stages 1–5 in Korea (NCT01630486 at <http://www.clinicaltrials.gov>). The study rationale, design, methods and protocol summary have been previously detailed elsewhere [18]. Participants were recruited from nine tertiary care hospitals between February 2011 and February 2016. Of the 2238 participants initially recruited, a total of 152 were excluded due to missing data on urinary chloride concentration ($n = 93$) and loss to follow-up, i.e. patients participated in baseline examination only, and not in subsequent follow-up visits ($n = 59$), and these patients were excluded on the date of their last examination. Finally, a total of 2086 patients were enrolled in this study (Supplementary Figure S1).

Data collection and measurements

Detailed data collection methods are described in the Supplementary data. Demographic data, including age, sex, smoking history, comorbidities and drug history, were collected at enrollment. Laboratory variables were measured by each

participating center's laboratory using 8-h fasting blood samples. Serum creatinine was measured by an isotope dilution mass spectrometry-traceable method at the central laboratory. The CKD Epidemiology Collaboration equation was used to determine the estimated GFR (eGFR) [19]. Second voided urine samples were immediately sent to the central laboratory for analysis. Urinary chloride concentration was expressed as mEq/L, and urinary protein excretion was determined by calculating the urinary protein-to-creatinine ratio.

Exposure and outcome ascertainment

The exposure of interest was urinary chloride concentration, which was used as a categorical variable and divided into tertiles. We also used urinary chloride concentration as a continuous variable in baseline cause-specific models and time-varying models.

The primary outcome was a composite of $\geq 50\%$ decline in eGFR from the baseline value or the onset of ESKD during the follow-up period. ESKD was defined as the initiation of renal replacement therapy, including dialysis or renal transplantation. Patients were followed up until 31 December 2018. Patients with CKD stage 3 and above were under close observation and followed up at 1- to 3-monthly intervals by all participating centers. Regardless of the study protocol, patients were reported by each center on reaching the endpoints.

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Board of each participating hospital's clinical trial center. All participants provided written informed consent before participating in the study.

Statistical analysis

Detailed statistical analysis methods are described in the Supplementary data. Statistical analyses were performed using STATA version 15.1 (Stata Corporation, College Station, TX, USA) and R (R Foundation for Statistical Computing, www.r-project.org). To test the independent prognostic value of urinary chloride concentration on the primary outcome, multi-variable cause-specific hazard models were constructed and incremental adjustments were performed. Survival time was defined as the time interval between baseline measurements and the first onset of a renal endpoint. Patients who were lost to follow-up were excluded on the date of their last examination. Deaths that occurred before reaching the primary outcome were considered as competing risks and as censoring in cause-specific hazard models.

We also constructed a time-varying model, in which repeated measurements, including urinary chloride concentration, systolic blood pressure, low-density lipoprotein cholesterol and eGFR, taken during the follow-up period, were considered as time-varying exposures. Restricted cubic splines were used to reveal the association between urinary chloride concentration as a continuous variable and the hazard ratio (HR) for the composite kidney outcome. The rate of renal

function decline per year was determined by the slope of eGFR obtained from a generalized linear mixed model. Correlations between urinary chloride concentration and other kidney injury markers were examined using Pearson correlation.

Finally, we compared the predictive power of urinary chloride concentration with those of other prognostic parameters. We calculated the C-statistic, area under the receiver operating characteristics curve (AUC), categorical net reclassification improvement (cNRI) and integrated discrimination improvement (IDI) to compare the predictive power of the models. P-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Demographic, clinical and laboratory characteristics, according to urinary chloride tertiles, are presented in Table 1. Among the 2086 participants, 1274 (61.1%) were men. Their mean \pm SD age was 53.6 ± 12.2 years, and the median urinary chloride concentration was 95 (67–131) mEq/L. Patients with higher urinary chloride concentrations were younger, had higher eGFR and hemoglobin, albumin and urinary sodium levels, and had lower Charlson comorbidity index scores. In addition, these patients had lower proteinuria and more preserved kidney function than those with lower urinary chloride concentrations.

Urinary chloride concentration and CKD progression

During a follow-up period of 7452 person-years (median 3.4 years), 281 (129/1000 person-years), 216 (86/1000 person-years) and 68 (25/1000 person-years) patients from the lowest to the highest tertiles of urinary chloride concentrations, respectively, reached the primary endpoint (Table 2). Cumulative kidney events were significantly lower in the highest tertiles (Gray's test, $P < 0.001$) (Supplementary Figure S2).

The unadjusted HRs (95% confidence interval) for the cause-specific proportional hazard model were 0.65 (0.55–0.78) and 0.18 (0.14–0.24) for the middle and highest tertiles, respectively, as compared with the lowest tertile (Model 1, Table 3). The results were similar following sequential adjustments for confounding factors (Models 2–4). The fully adjusted model, including eGFR, proteinuria and use of diuretics, revealed that the highest tertile of urinary chloride concentrations had 33% lower risk for CKD progression than the lowest tertile (0.49–0.90) (Model 4). Similar findings were obtained when urinary chloride concentration was used as a continuous variable; for every 25 mEq/L increase in urinary chloride concentration, there was 11% lower risk for adverse kidney outcomes (0.83–0.96) (Table 4). Furthermore, the time-updated urinary chloride model yielded consistent findings (Table 4).

Spline regression analyses clearly showed a relatively linear inverse relationship between urinary chloride concentration and the risk for adverse kidney outcomes (Figure 1). We also compared the rates of kidney function decline among the tertile groups and found that the lowest tertile had a faster decline in eGFR than the highest tertile (Supplementary Table S1).

Subgroup analyses

To evaluate the modification effects of subgroups on the relationship between urinary chloride concentration and CKD progression, subgroup analyses were performed in subgroups stratified by age, sex, eGFR, proteinuria, body mass index, diabetes and primary renal disease (Figure 2). A significant interaction was observed between eGFR and urinary chloride concentration (P for interaction = 0.028), suggesting that the significance of urinary chloride concentration is affected by kidney function. In fact, lower risk for adverse kidney outcomes associated with higher urinary chloride concentrations was evident particularly for eGFR values of ≥ 45 mL/min/1.73 m². This association was also observed in generalized linear mixed models that were separately analyzed according to eGFR level (<45 or ≥ 45 mL/min/1.73 m²) (Supplementary Table S1).

Sensitivity analyses

To substantiate our findings, we performed sensitivity analyses on 1958 patients, excluding patients with eGFR of <15 mL/min/1.73 m² since these eGFR levels are almost equivalent to ESKD. We constructed multivariable cause-specific hazard and time-varying models with the same adjustment levels as described above and found that the highest tertile of urinary chloride concentrations was associated with a significantly lower risk for CKD progression (Supplementary Tables S2–S4). Given that urinary sodium concentration was higher in subjects with high urinary chloride concentration, we conducted further sensitivity analyses after adjustment for urinary sodium concentration in baseline and time-varying cause-specific models and obtained comparable results (Supplementary Table S5). Furthermore, an additional analysis showed a similar association with urinary chloride-to-creatinine ratio (Supplementary Table S6).

Association of urinary chloride concentration with other markers of kidney injury

To find a mechanistic link that would explain the significant association between urinary chloride concentration and CKD progression, we examined the correlation between urinary chloride concentration and other well-known markers of tubular dysfunction (Supplementary Figure S3). We found urinary chloride concentration positively correlated with eGFR ($\gamma = 0.4862$), serum bicarbonate concentration ($\gamma = 0.2607$) and urine osmolality ($\gamma = 0.7144$), while it inversely correlated with serum potassium concentration ($\gamma = -0.1610$), proteinuria ($\gamma = -0.2242$), urinary osmolar gap ($\gamma = -0.1604$) and urinary angiotensinogen level ($\gamma = -0.1109$). However, there was no correlation between urinary chloride concentration and urinary anion gap.

Predictive power of urinary chloride concentration

Finally, we compared the predictive power of urinary chloride concentration with that of other kidney injury markers. Individual additions of urinary chloride concentration and other kidney injury markers, except for urinary anion gap, to the basic model significantly increased all prediction indices. Among the markers, the eGFR-added model had the

Table 1. Baseline characteristics of cohort participants according to urinary chloride tertile groups

Variables	Overall <i>n</i> = 2086	Urinary chloride tertile (mEq/L)		
		Low (<77) <i>n</i> = 699	Middle (77–116) <i>n</i> = 703	High (>116) <i>n</i> = 684
Urinary chloride (mEq/L)				
Mean \pm SD	101.8 \pm 48.7	53.0 \pm 17.2	95.7 \pm 11.5	158.1 \pm 33.8
Median (interquartile range)	95 (67–131)	56 (41–67)	95 (86–105)	149 (132–178)
Range	6–298	6–76	77–116	117–298
Demographic data				
Age, years	53.6 \pm 12.2	53.8 \pm 12.1	54.5 \pm 12.2	52.4 \pm 12.3
Male, <i>n</i> (%)	1274 (61.1)	417 (59.7)	424 (60.3)	433 (63.3)
Smoker, <i>n</i> (%)	644 (30.9)	225 (32.2)	214 (30.4)	205 (30.0)
Married, <i>n</i> (%)	1752 (84.0)	562 (80.4)	607 (86.3)	583 (85.2)
Education, <i>n</i> (%)				
Low	502 (24.1)	179 (25.6)	181 (25.7)	142 (20.8)
Intermediate	728 (34.9)	239 (34.2)	245 (34.9)	244 (35.7)
High	856 (41.0)	281 (40.2)	277 (39.4)	298 (43.6)
Income, <i>n</i> (%)				
High	472 (23.3)	151 (22.0)	160 (23.6)	161 (24.3)
Intermediate	1083 (53.4)	363 (52.8)	350 (51.5)	370 (55.8)
Low	474 (23.4)	173 (25.2)	169 (24.9)	132 (19.9)
Body mass index (kg/m ²)	24.6 \pm 3.4	24.2 \pm 3.5	24.6 \pm 3.3	24.9 \pm 3.4
Systolic BP (mmHg)	128.6 \pm 16.4	128.9 \pm 17.3	128.8 \pm 16.1	128.1 \pm 15.6
Diastolic BP (mmHg)	76.9 \pm 11.1	76.5 \pm 11.2	77.1 \pm 11.2	77.2 \pm 10.9
Laboratory parameters				
Hemoglobin (g/dL)	12.8 \pm 2.0	12.2 \pm 2.0	12.7 \pm 1.9	13.7 \pm 1.9
Albumin (g/dL)	4.2 \pm 0.4	4.1 \pm 0.5	4.1 \pm 0.4	4.3 \pm 0.3
Phosphate (mg/dL)	3.7 \pm 0.7	3.9 \pm 0.8	3.7 \pm 0.6	3.5 \pm 0.6
Cholesterol (mg/dL)	174.2 \pm 38.9	172.0 \pm 41.6	174.2 \pm 39.7	176.6 \pm 34.9
Triglyceride (mg/dL)	133.0 (92.0–194.0)	133.0 (93.0–194.0)	133.0 (89.0–198.0)	131.0 (93.0–189.0)
LDL-C (mg/dL)	96.9 \pm 31.4	95.5 \pm 33.4	95.5 \pm 30.6	99.7 \pm 30.0
hs-CRP (mg/dL)	0.60 (0.21–1.70)	0.60 (0.20–1.80)	0.60 (0.30–1.60)	0.60 (0.21–1.50)
UPCR (g/g)	0.5 (0.1–1.5)	0.6 (0.2–2.0)	0.5 (0.2–1.7)	0.3 (0.1–0.9)
eGFR (mL/min/1.73 m ²)	53.2 \pm 30.8	40.4 \pm 27.9	47.3 \pm 26.8	72.4 \pm 28.1
Serum sodium (mEq/L)	140.8 \pm 2.4	140.4 \pm 2.7	141.0 \pm 2.3	141.1 \pm 2.2
Serum bicarbonate (mEq/L)	25.7 \pm 3.6	24.8 \pm 3.8	25.6 \pm 3.5	27.0 \pm 3.0
Serum potassium (mEq/L)	4.6 \pm 0.6	4.7 \pm 0.6	4.7 \pm 0.6	4.5 \pm 0.5
Urinary sodium (mEq/L)	84.5 \pm 38.2	48.5 \pm 17.5	81.9 \pm 15.9	124.0 \pm 31.4
Cause of CKD, <i>n</i> (%)				
Diabetic nephropathy	523 (25.1)	229 (32.8)	192 (27.3)	102 (14.9)
Hypertension	417 (20.0)	126 (18.0)	152 (21.6)	139 (20.3)
Glomerulonephritis	661 (31.7)	188 (26.9)	202 (28.7)	271 (39.6)
Polycystic kidney disease	341 (16.3)	119 (17.0)	107 (15.2)	115 (16.8)
Others	144 (6.9)	37 (5.3)	50 (7.1)	57 (8.3)
Comorbidities and drugs				
CCI score, points	2.3 \pm 1.6	2.7 \pm 1.5	2.5 \pm 1.5	1.6 \pm 1.6
Diabetes mellitus, <i>n</i> (%)	704 (33.7)	275 (39.3)	262 (37.3)	167 (24.4)
Cardiovascular disease, <i>n</i> (%)	1758 (84.3)	578 (82.7)	583 (82.9)	597 (87.3)
RAS inhibitors, <i>n</i> (%)	1786 (85.6)	590 (84.4)	611 (86.9)	585 (85.5)
Diuretics, <i>n</i> (%)	657 (31.5)	279 (39.9)	233 (33.1)	145 (21.2)
β -blocker, <i>n</i> (%)	530 (25.4)	208 (29.8)	202 (28.7)	120 (17.5)
Statin, <i>n</i> (%)	1079 (51.7)	371 (53.1)	374 (53.2)	334 (48.8)

Data are expressed as mean \pm SD, median (interquartile range) or proportion *n* (%).

BP, blood pressure; CCI, Charlson comorbidity index; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; UPCR, urinary protein-to-creatinine ratio.

best C-statistic, AUC, cNRI and IDI. These indices of urinary chloride concentration were comparable to those of proteinuria, urine osmolality, urinary anion gap and serum bicarbonate concentration (Supplementary Figure S4 and Table S7).

DISCUSSION

In this study, we tested our hypothesis that urinary chloride concentration can perform well as a useful marker of CKD

progression in a nationwide prospective cohort of Korean CKD patients. We found that urinary chloride concentration showed good correlations with other kidney injury markers and that higher urinary chloride concentrations were associated with a significantly lower risk for adverse kidney outcomes. This relationship was robust, as confirmed in multiple tests using cause-specific and time-varying models. Thus, our findings suggest the potential clinical usefulness of urinary chloride concentration as a predictor of adverse kidney outcomes.

Table 2. Outcome event rates according to urinary chloride tertile groups

Outcomes	Overall	Urinary chloride group (mEq/L)			P-value ^a
		Low (<77)	Middle (77–116)	High (>116)	
Number of subjects	2086	699	703	684	
Deaths, <i>n</i>					
Number of person-years	8547.4	2751.6	2937.8	2858.0	
Incidence of outcome, <i>n/n</i>	82/2086	42/699	26/703	14/684	
Incidence rate per 1000 person-years	9.6	15.3	8.9	4.9	<0.001
Composite renal outcome, <i>n</i>					
Number of person-years	7451.7	2180.2	2509.9	2761.7	
Incidence of outcome, <i>n/n</i>	565/2086	281/699	216/703	68/684	
Incidence rate per 1000 person-years	69.8	128.9	86.1	24.6	<0.001
eGFR halving, <i>n</i>					
Number of person-years	7451.7	2180.2	2509.9	2761.7	
Incidence of outcome, <i>n/n</i>	359/2086	135/699	167/703	57/684	
Incidence rate per 1000 person-years	48.2	61.9	66.5	20.6	<0.001
ESKD, <i>n</i>					
Number of person-years	7722.6	2259.4	2651.9	2811.3	
Incidence of outcome, <i>n/n</i>	445/2086	246/699	162/703	37/684	
Incidence rate per 1000 person-years	57.6	108.9	61.1	13.2	<0.001

^aP-value based on log-rank test.**Table 3. Cause-specific HRs for composite outcome according to urine chloride groups**

Urine chloride group	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Low	1.00	–	1.00	–	1.00	–	1.00	–
Middle	0.65 (0.55–0.78)	<0.001	0.63 (0.52–0.76)	<0.001	0.84 (0.69–1.01)	0.07	0.83 (0.69–1.01)	0.07
High	0.18 (0.14–0.24)	<0.001	0.23 (0.17–0.31)	<0.001	0.67 (0.50–0.91)	0.009	0.67 (0.49–0.90)	0.009

Model 1: unadjusted.

Model 2: adjusted for age, sex, CCI, primary renal disease, body mass index, systolic blood pressure, serum albumin, LDL-C, hs-CRP and smoking history.

Model 3: Model 2 + baseline eGFR and UPCR.

Model 4: Model 3 + economic status, RAS blockade use, diuretic use and statin use.

hs-CRP and UPCR were log-transformed due to skewed distributions.

CCI, Charlson comorbidity index; CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; UPCR, urinary protein-to-creatinine ratio.

Table 4. Cause-specific HRs for composite outcome according to urine chloride as a continuous variable

Models	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Cause-specific model								
Urinary chloride (per 25 mEq/L increase)	0.72 (0.68–0.76)	<0.001	0.73 (0.69–0.77)	<0.001	0.90 (0.83–0.96)	0.002	0.89 (0.83–0.96)	0.002
Time-updated cause-specific model ^a								
Urinary chloride (per 25 mEq/L increase)	0.66 (0.63–0.70)	<0.001	0.69 (0.64–0.73)	<0.001	0.88 (0.81–0.95)	0.001	0.89 (0.82–0.96)	0.003

Model 1: unadjusted.

Model 2: adjusted for age, sex, CCI, primary renal disease, body mass index, SBP, serum albumin, LDL-C, hs-CRP and smoking history.

Model 3: Model 2 + eGFR and UPCR.

Model 4: Model 3 + economic status, RAS blockade use, diuretic use and statin use.

^aUrinary chloride, SBP, LDL-C and eGFR were used as time-varying covariates.

hs-CRP and UPCR were log transformed due to skewed distributions.

CCI, Charlson comorbidity index; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; UPCR, urinary protein-to-creatinine ratio.

Several studies to date have identified numerous urinary biomarkers to predict adverse clinical outcomes, based on the assumption that these markers can reflect the local milieu in the kidney [20, 21]. Chloride is a major urinary anion that is regulated by transport channels along the nephron segments. Thus, it can be presumed that urinary chloride concentration could be affected as kidney function declines in CKD patients. However,

little attention has been paid to urinary chloride concentration as a marker of chronic tubular injury. This led us to test whether urinary chloride concentration can predict CKD progression in the long term. To prove our hypothesis, we sought to validate the prognostic value of urinary chloride concentration using various statistical tests and showed that it was an independent predictor of CKD progression, even after rigorous adjustment

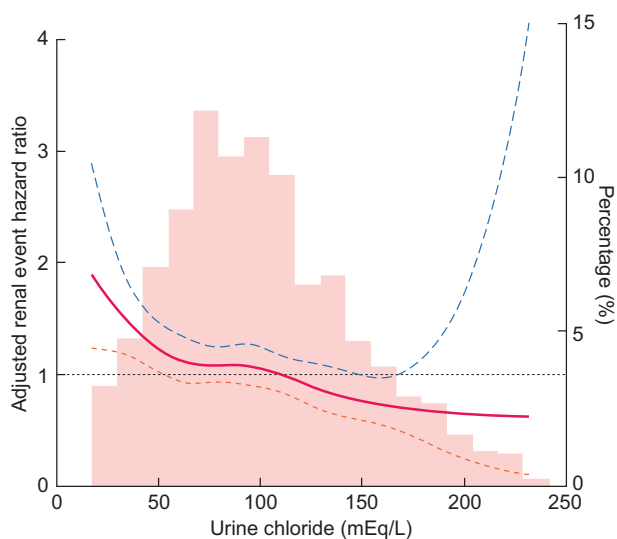


FIGURE 1: Restricted cubic spline curve. Adjusted for age, sex, primary renal disease, CCI, body mass index, SBP, serum albumin, LDL-C, hs-CRP, smoking history, UPCR, economic status, RAS blockade use, diuretic use and statin use. hs-CRP and UPCR were log-transformed due to skewed distributions. CCI, Charlson comorbidity index; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; UPCR, urinary protein-to-creatinine ratio.

for confounding factors. We also found that the predictive performance of urinary chloride concentration was similar to that of other well-known renal markers such as eGFR, proteinuria and bicarbonate concentration. Furthermore, we examined the relationship between urinary chloride concentration and other well-known kidney injury markers. Notably, urinary chloride concentration was well correlated with many parameters, including eGFR, proteinuria, serum potassium and bicarbonate concentrations, urinary osmolar gap and urinary angiotensinogen levels. Together, these findings suggest that urinary chloride concentration can be used as a possible surrogate marker of kidney injury in CKD.

There are several potential mechanisms to explain the association of higher urinary chloride concentrations with a lower risk for CKD progression. First, chloride is abundant in the urine and is used to determine the urinary anion gap. In general, a high urinary chloride concentration indicates a negative urinary anion gap, suggesting a normal distal tubular acidification process [7]. It is well known that tubulointerstitial fibrosis is a powerful marker of kidney function decline; impaired kidney tubules are involved in metabolic acidosis, which is a significant predictor of CKD progression [22–28]. In fact, urinary chloride concentration was positively correlated with serum bicarbonate concentration in our study. Therefore, elevated urinary chloride excretion can represent well-functioning kidney tubules. Second, increased delivery of chloride to distal tubules can trigger TGF. In CKD, activation of TGF can play a protective role by reducing proteinuria and glomerular hypertension [11, 29]. This notion has been recently validated by several randomized controlled trials on SGLT-2 inhibitors in diabetic patients [30, 31]. It should be noted that urinary sodium can

also mediate TGF response. However, in experimental studies, salt sensing in the macula densa is predominantly driven by chloride, rather than sodium, ion [9, 32]. To account for potential effects of sodium, we constructed an additional model adjusted for urinary sodium concentration and found a robust association between urinary chloride concentration and CKD progression. Third, in response to the increased flow of tubular fluid (i.e. high sodium chloride concentration) in the thick ascending limb, adenosine binds to the A1 receptor, which activates the inhibitory guanosine-5'-triphosphate (GTP) binding protein subunit and increases intracellular calcium release [33]. This cascade causes a decrease in cyclic adenosine monophosphate (cAMP), eventually resulting in the inhibition of renin release from the juxtaglomerular cells [34]. To support this notion, we showed that urinary chloride concentration was inversely correlated with urinary angiotensinogen level. Increased urinary excretion of angiotensinogen can mirror the local activation of RAS and is associated with an increased risk for CKD [35, 36]. Together, these findings could explain the significant association between higher urinary chloride concentrations and a lower risk for CKD progression in our study.

Because urinary chloride can represent a tubular marker, based on our findings, one interesting question is whether the association between urinary chloride concentration and kidney outcome is stronger in tubular disease than in proteinuric kidney disease. The KNOW-CKD comprised four representative cohorts, including patients with diabetic nephropathy, hypertensive kidney disease, glomerular disease and polycystic kidney disease. However, there was no significant interaction between urinary chloride concentration and these primary diseases in terms of CKD progression, suggesting that the association between urinary chloride concentration and kidney outcome universally exists across the different types of kidney disease. Notably, the mean eGFR was 43.6 mL/min/1.73 m² in two proteinuric kidney diseases, diabetic nephropathy and glomerular disease, suggesting that tubulointerstitial fibrosis was already present in this group. It is well known that tubulointerstitial fibrosis eventually occurs with progression of kidney disease of any type. On the other hand, the significant association between urinary chloride concentration and CKD progression in proteinuric kidney disease can also support the notion that urinary chloride plays a key role in TGF. Together, these findings could explain no significant effect of modification between urinary chloride and different types of kidney disease.

Although this study showed that urinary chloride concentration was an independent predictor of adverse kidney outcomes, it may not be a stronger marker than eGFR. In fact, when we adjusted for eGFR in the multivariable cause-specific model, the significance of urinary chloride concentration still remained, but was weaker. Notably, we found a significant interaction between urinary chloride concentration and eGFR, suggesting that urinary chloride excretion is largely dependent on kidney function. In addition, we showed that eGFR had a greater predictive performance than urinary chloride concentration. Therefore, the prognostic value of urinary chloride concentration should be interpreted in conjunction with kidney function.

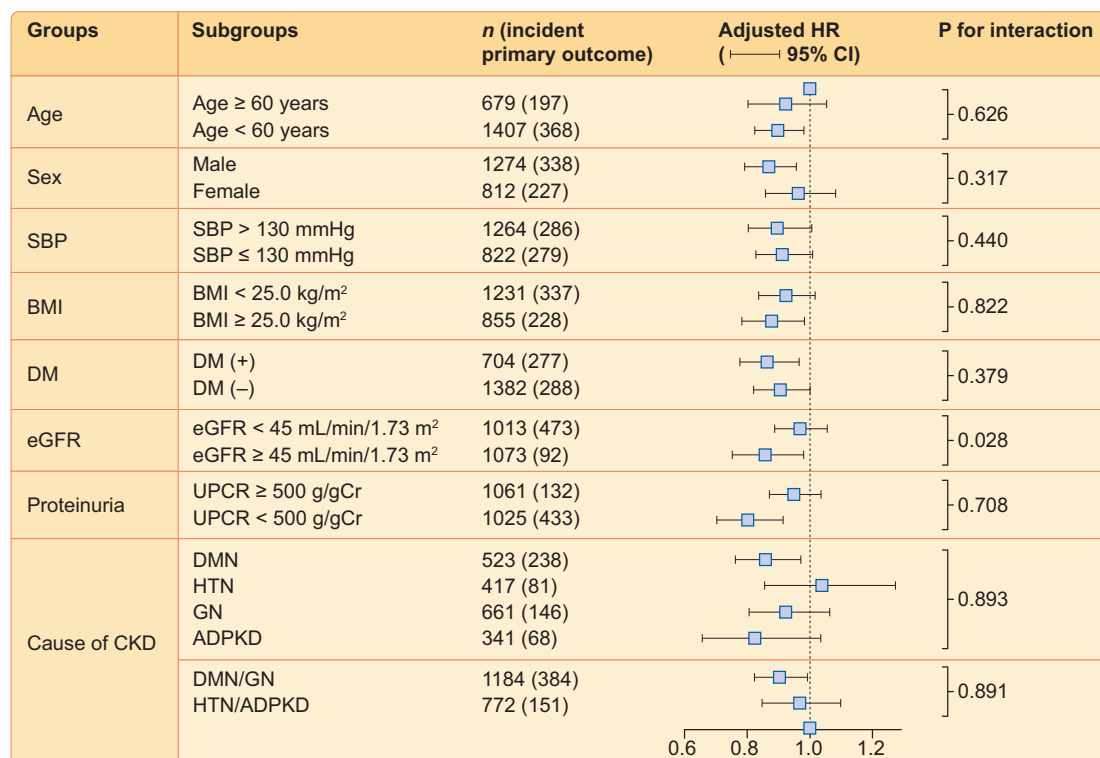


FIGURE 2: Forest plot. ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; DM, diabetes mellitus; DMN, diabetic nephropathy; GN, glomerular disease; HTN, hypertension; SBP, systolic blood pressure; UPCR, urinary protein-to-creatinine ratio.

This study has several limitations. First, due to the observational nature of the study, a confirmative causal link between urinary chloride concentration and adverse kidney outcomes cannot be demonstrated by our findings alone. For the same reason, the residual confounding effects cannot be entirely excluded. However, we performed multiple tests using various statistical models after rigorous adjustment and observed consistent results. Second, abnormal urinary chloride concentrations can be observed in conditions other than CKD. For example, low urinary chloride concentrations can be seen in patients with metabolic alkalosis accompanied by volume depletion, which can cause a pre-renal type of acute kidney injury [37]. In addition, urinary chloride excretion can be elevated in inherited tubular disorders such as Bartter syndrome or Gitelman syndrome [38]. However, we excluded these patients at enrollment and analyzed only stable CKD patients. Third, urinary chloride concentration did not correlate with urinary anion gap in our study. This was expected, because the standard urinary anion gap is determined by subtracting chloride from sodium plus potassium, making this value close to zero. Interestingly, two previous studies raised concern regarding this equation, as they found a lack of correlation between the urinary anion gap and urinary ammonia concentration [39, 40]. Furthermore, Raphael *et al.* showed that the urinary anion gap did not predict the development of ESKD or death, while the modified urinary anion gap including urinary phosphate and sulfate was well correlated with urinary ammonia and predicted adverse outcomes better than the conventional urinary anion gap [40]. In line with this finding, we showed that the predictive value of the urinary anion gap was not greater than that of the

basic model and was significantly inferior to that of eGFR and urinary chloride concentration, suggesting that the urinary anion gap is not a useful marker. Unfortunately, our study did not measure other urinary anions and thus could not test the validity of the modified urinary anion gap. It should be noted that there was a significant correlation between urinary chloride concentration and urinary osmolar gap. In a study by Kirschbaum *et al.*, the urinary osmolar gap was a more accurate surrogate for ammonia concentration than the urinary anion gap [39]. Nevertheless, measurement of urinary chloride concentration is simple and easily interpreted in clinical practice. Further studies are required to confirm our findings. Lastly, the inclusion of only Korean patients with CKD in our study limits the generalizability of our findings to other populations.

In conclusion, this study showed good correlation between urinary chloride concentration and other kidney injury markers and an association between higher chloride concentrations and a significantly lower risk for adverse kidney outcomes. Our hypothesis-generating study suggests that urinary chloride concentration could be used as a biomarker of CKD progression.

SUPPLEMENTARY DATA

Supplementary data are available at [ndt](https://academic.oup.com/ndt) online.

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AUTHORS' CONTRIBUTIONS

Y.S.J., J.K. and S.H.H. were responsible for the research idea and study design; Y.S.J., J.K., C.H.P., H.-R.Y., C.A. and S.H.H. carried out data acquisition; Y.S.J., H.-R.Y., C.H.P., J.K., J.T.P., T.I.C., T.-H.Y., S.H.H. and S.W.K. performed the statistical analysis; and supervision or mentorship was provided by J.T.P., T.I.C., T.-H.Y., S.-A.S., J.L., K.-H.O., S.-W.K., K.H.C., C.A. and S.W.K. All authors contributed to important intellectual contents during manuscript drafting or revision and accepted accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work were appropriately investigated and resolved.

CONFLICT OF INTEREST STATEMENT

All the authors declared no competing interests.

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