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Original Article

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Urinary cotinine as an objective measure of cigarette smoking in chronic kidney disease

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Abstract

Background. Smoking is a modifiable behaviour that may hasten the progression of chronic kidney disease (CKD). Cotinine, a nicotine metabolite, is measurable in body fluids, including urine, and can be utilized as an objective measure of smoking exposure. Its use has not been examined in the CKD population.

Methods. In this cross-sectional study, we evaluated use of 24-h urinary cotinine excretion (Ucot) as a quantitative index of smoking exposure in a CKD population. Methods of comparison included self-report and expired air carbon monoxide (eCO) as standard measures of smoking exposure. Assessments of kidney function included estimated glomerular filtration rate (eGFR) and 24-h urinary protein (Uprot) excretion.

Results. Sixty-one patients were enrolled, of whom 12 were excluded for incomplete urine collections. Of the remaining, 77% were active current smokers (mean cigarettes smoked: 12 ± 7 per day). The mean eGFR was 47 ± 25 ml/min/1.73 m^2 with no significant differences among non-smokers. The mean eCO and Ucot were significantly higher in smokers *vs* non-smokers (12.5 ± 6.9 ppm and 1.3 ± 1.1 ppm and 1685.87 ± 922.77 μg/d and 134.18 ± 445.03 μg/d, respectively, $P < 0.001$ for both). Ucot was weakly correlated with eGFR ($R = 0.40$, $P = 0.005$), but not with Uprot ($R = 0.09$, $P = 0.54$). In multivariate analyses, daily cigarette consumption and eCO were the only significant predictors of Ucot ($P < 0.05$ for both).

Conclusion. In this CKD cohort, Ucot is correlated with commonly used measures of smoking exposure and is minimally influenced by underlying renal function, demonstrating its potential utility in clinical trials examining change in smoking behaviour and effects on renal injury.

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Introduction

Given the poor outcome of persons with chronic kidney disease (CKD), it is important to identify strategies to alter the course of this disease. Cigarette smoking has been reported to exacerbate existing diabetic and non-diabetic kidney disease and may also be an etiologic factor triggering the onset of proteinuria and reduced renal function [1]. Despite the recognized relationship between cigarette smoking and kidney disease, it is unclear whether smoking cessation or reduction can attenuate the progression of renal injury in CKD.

The identification of objective measures of cigarette exposure among individuals with CKD is an essential prerequisite to conducting a clinical trial to assess the effect of cigarette reduction and/or cessation on renal injury and incidence of kidney failure in this disease population. Self-report of smoking habit is known to have limited utility in assessing smoking exposure accurately. Expired air carbon monoxide (eCO) measurement is an objective measure, but has a short half-life and only provides an indication of recent smoking exposure [2–3]. Another objective measure of smoking exposure is cotinine, a nicotine metabolite, which can be measured in several body fluids, including saliva, plasma, and urine, and its measurement imparts additional benefit to eCO as an objective measure of active smoking because it has a longer half-life, and represents a quantitative, daily measure of exposure to tobacco [4]. While urinary cotinine has been used as a quantitative measure of changes in smoking behaviour in the general population, its utility in the CKD population has not been studied and there is little known about the metabolism of nicotine in smokers with varying degrees of renal impairment [5].

The purpose of the current study was to evaluate the utility of 24-h urinary cotinine excretion (Ucot) as an additional objective measure of cigarette exposure in a CKD cohort.

Methods

Study design and population

This was a cross-sectional study conducted in a disease management program for CKD between October 2004 and January 2006. Eligible subjects were adults, aged ≥18 years with stages 1–4 CKD based on estimated glomerular filtration rate (eGFR) defined using NKF guidelines [6]. Smokers and non-smokers were enrolled at a ratio of 2:1. Self-reported non-smokers were included to (i) ensure the inclusion of potentially misclassified smokers; (ii) provide an adequate number of subjects without detectable markers of cigarette smoking and (iii) minimize the skew in the distribution of the key study measures. Exclusion criteria included current immunotherapy for renal disease or renal transplantation, need for renal replacement within the last month, and inability to provide consent.

Study protocol

After informed consent was obtained, participants were enrolled into the study. During the first visit, subjects were surveyed with a smoking questionnaire and evaluated for nicotine dependency utilizing the Fagerstrom Test for Nicotine Dependency (FTND); and subjects provided a breath test to measure eCO [7]. A 24-h urine collection was obtained to measure the excretion of cotinine, protein and creatinine. Participants were required to submit the urine collection within 1 month of study entry. To examine the intra-subject variability of Ucot, two urine samples were collected at different times in a subgroup of participants. The Institutional Review Board at the University of Maryland School of Medicine approved the study protocol.

Measurements

For this study, baseline assessments included patient demographics, weight, blood pressure and comorbidities. Smoking status and history were obtained using a smoking questionnaire that consisted of 14 questions for all study participants and six additional questions for smokers. Secondhand smoking exposure was not assessed in non-smokers. Study participants who reported 'yes' to current smoking were classified as smokers and participants who reported 'no' were classified as non-smokers. The Fagerstrom Test for Nicotine Dependence (FTND) was used to measure the level of nicotine dependency [8]. The FTND consists of six items. For each item, there is a score depending on the patient's response, and the final score (a number between 0–10) is used to categorize the level of dependency of the patient. A score of 0–2 indicates very low dependence, 3–4 low dependence, 5 medium dependence, 6–7 high dependence and 8–10 very high dependence to nicotine. The eCO, a smoking by-product, was measured via the EC50 Micro III Smokerlyzer (Bedfont Scientific) per the manufacturer's instructions. When possible, two measurements were taken and the average was used for data analysis. A 24-h urine sample was collected from all participants and after the total volume of urine was measured, an aliquot was retained and stored at −70°C until the time of analysis. The urine collections were considered adequate based on the following minimal rates of creatinine excretion: 20 mg/kg lean body weight for men ≤50 years old, 10 mg/kg lean body weight for men >50 years old, 15 mg/kg lean body weight for women ≤50 years old and 7.5 mg/kg lean body weight for women >50 years old [9]. Twelve participants were excluded from the final analysis for inadequate urine collections. High performance liquid chromatography (HPLC) was used to determine the cotinine concentration in the urine [10]. Cotinine concentrations were multiplied by the 24-h urine volume in order to determine the total amount excreted in the urine. The limit of quantification of cotinine with this method was 20 μg/l. Estimated glomerular filtration rate (eGFR) and excretion of urine protein (Uprot) were used as the indicators of renal function. The GFR was estimated based on a serum creatinine obtained within 4 weeks of the initial study visit and using the abbreviated Modified Diet in Renal Disease (MDRD) equation [11].

Statistical analysis

The primary reference of smoking exposure in this study was self-reported current cigarettes smoked per day (cigs/day). A secondary reference of cigarette exposure, eCO, was obtained. The principal measure being evaluated was Ucot. Two urine samples were collected at different times in a subgroup of participants to examine the intra-individual variability of Ucot.

Baseline characteristics were summarized using descriptive statistics. Means and standard deviations (SD) were obtained for continuous variables and compared using the Student's *t*-test. Categorical variables were reported as percentages and compared using the Chi-square test. Spearman's rho (non-parametric) correlation was used to determine the strength of the relationship between different measures of smoking exposure, including Ucot and eCO, and measures of renal injury, including eGFR and Uprot. Multiple linear regression analysis was performed to evaluate the association between the primary outcome of interest—Ucot, key independent variables—cigarettes smoked per day and eCO, and potential confounding variables—eGFR, Uprot and race. Race was included as a covariate because there is evidence that suggests different rates of nicotine metabolism in African Americans [12,13]. The level of statistical significance of all tests was a 2-tailed *P*-value <0.05. All procedures were performed with the Statistical Package of Social Science (SPSS, version 10.0; SPSS, Inc, Chicago, IL).

Results

Forty-nine CKD patients were included in the final analysis of this study, including 38 smokers and 11 non-smokers. The characteristics of the study participants are displayed in Table 1. When compared with non-smokers, there were no statistically significant differences in baseline characteristics, including age, gender, race, body weight and diabetic status.

The level of nicotine dependency among smokers, as determined by the Fagerstrom dependency score, was low. This low dependency on nicotine is consistent with the low amount of cigarettes reported among smokers in this study. As expected, smokers excreted more Ucot than non-smokers. There was no difference between smokers and non-smokers in the mean volume of urine collected, creatinine excreted (Ucr), Uprot or eGFR. Similarly, Uprot and eGFR did not differ significantly between those with and without diabetes mellitus (data not shown).

The smoking index, eCO was strongly associated with the self-reported number of cigarettes smoked per day and Ucot. Ucot had a similar degree of correlation with self-reported cigarettes smoked per day. This provides evidence that Ucot can also be utilized as an objective measure of cigarette smoking. The correlations between each measure of cigarette exposure and renal injury are demonstrated in Table 2. Of note, there was a weak, but significant correlation between current cigarettes smoked per day and renal function as measured by eGFR, and Ucot. However, when stratified by degree of cigarette consumption, there was no significant correlation between Ucot and eGFR except among those who consumed 6–12 cigarettes per day (Table 3). Ucot increased with increasing cigarette consumption, as expected. While declining renal function was associated with lower cigarette consumption, this finding is limited by the cross-sectional design of this study.

Table 1. Subject demographics, study characteristics, markers of renal injury and quantitative measures of nicotine exposure by smoking status

	Smokers $n = 38$ **(77%)**	**Non-smokers** $n = 11$ **(23%)**	***P*-value**[a]
Age, mean (years ± SD)	63 ± 11	63 ± 13	0.97
Gender			0.47
Male	27 (71)	4 (36)	
Female	11 (29)	7 (64)	
Race			0.57
African American	32 (84)	10 (91)	
Caucasian	6 (14)	1 (9)	
Diabetes			0.47
Yes	16 (42)	6 (55)	
No	22 (58)	5 (45)	
Cigs/d, (n ± SD)	12 ± 7	0	<0.001
FTND	4 ± 2	0	<0.001
Body weight (kg)	82 ± 16	93 ± 19	0.05
Urine volume, mean (ml ± SD)	1862 ± 841	1799 ± 599	0.82
eGFR (ml/min/1.73 m^2)	47 ± 25	36 ± 18	0.20
Uprot (g/d)	1.33 ± 1.57	0.77 ± 0.80	0.265
Ucr (g/d)	1.43 ± 0.51	1.20 ± 0.29	0.165
Ucot (μg/d)	1685.87 ± 922.77	134.18 ± 445.03	<0.001
eCO , mean (ppm)	12.5 ± 6.9	1.3 ± 1.1	<0.001

SD, standard deviation; Cigs/d, current cigarettes smoked per day, self-reported; FTND, Fagerstrom Test for Nicotine Dependence; kg, kilogram; eGFR, estimated glomerular filtration rate; Uprot, 24-h urinary protein excretion; Ucr, 24-hour urinary creatinine excretion; Ucot, 24-hour urinary cotinine excretion; eCO, expired air carbon monoxide, ppm, parts per million.
[a]Chi-square test.

A multivariate analysis was performed to evaluate the relationship between measures of smoking exposure, including self-reported cigarettes smoked per day and eCO, and Ucot, adjusting for eGFR, Uprot and race. Two linear regression models were constructed to evaluate separately the ability of each measure of smoking exposure to predict Ucot in cigarette smokers (Table 4). Model 1 contains the primary reference of smoking exposure, cigs/day, and covariates. Model 2 contains the secondary reference of smoking exposure, eCO and covariates. Covariates entered into the models included eGFR, Uprot and race. Stepwise linear regression revealed both cigs/day and eCO as the best predictive variables in each model. Model 2 explained more of the variance in Ucot than model 1; suggesting that when used alone, self-report is inferior to eCO as a predictor of Ucot. Markers of renal function (eGFR and Uprot) and race were not significant predictors of Ucot excretion.

The variability of Ucot excretion in a subgroup of participants ($n = 8$) was examined and is illustrated in Figure 1. The average time between each 24-h urine collection was 7.4 months. The mean difference in Ucot from sample 1 and sample 2 was -27.5 ± 122.1 mcg ($P = 0.94$) and the coefficient of variation $23 \pm 5\%$.

Table 2. Correlation between measures of cigarette smoking exposure and renal injury

	ρ	***P*-value**[b]
Cigs/d *vs* eCO	0.67	<0.001
Cigs/d *vs* Ucot[a]	0.74	<0.001
Cigs/d *vs* eGFR	0.25	0.08
eCO *vs* Ucot[a]	0.72	<0.001
Ucot[a] *vs* eGFR	0.40	0.005
Ucot[a] *vs* Uprot[a]	0.09	0.54

Cigs/d, current cigarettes smoked per day, self-reported; eCO, expired air carbon monoxide, parts per million (ppm); Ucot, 24-h urinary cotinine excretion, μg/day; eGFR, estimated glomerular filtration rate, ml/min/1.73 m^2.
[a]24-h sample, adjusted for creatinine excretion; Uprot, 24-h urinary protein excretion, g/day.
[b]Spearman's rho.

Table 3. Correlation between urine cotinine excretion and renal function among subjects, stratified by cigarette consumption

Cigs/d	n **(%)**	**Ucot (mean ± SD)**	**eGFR (mean ± SD)**	ρ	***P* value**[a]
0	14 (20)	134.18 ± 445.03	36 ± 18	−0.40	0.22
1–5	9 (13)	1125.14 ± 344.28	47 ± 25	0.57	0.10
6–12	16 (23)	1393.33 ± 615.76	35 ± 21	0.63	0.03
13–19	11 (16)	2101.73 ± 1360.84	54 ± 29	0.34	0.31
>19	11 (16)	2043.50 ± 528.04	54 ± 19	−0.26	0.53

Cigs/d, current cigarettes smoked per day, self-reported; Ucot, 24-h urinary cotinine excretion, μg/day; eGFR, estimated glomerular filtration rate, ml/min/1.73 m^2; SD, standard deviation.
[a]Spearman's rho.

Discussion

In this cross-sectional study, which examined measures of smoking in CKD, there was a strong relationship between Ucot and self-reported cigarettes smoked per day and eCO. Among subjects with similar cigarette use, there was no significant correlation between Ucot and eGFR. In the overall sample, Ucot was weakly correlated with eGFR; and eGFR was correlated with cigarettes smoked per day. However, when adjusting for eGFR, cigarettes smoked per day was the only significant predictor of Ucot. Furthermore, even when other variables that may influence the relationship between Ucot and eGFR were included in multivariate analysis, cigarettes smoked per day and eCO were the only significant predictors of Ucot. The results of this study suggest that Ucot can be used as an objective tool to measure smoking exposure in CKD patients.

Table 4. Multiple linear regression models including primary (Model 1) and secondary (Model 2) references of cigarette smoking exposure and covariates to evaluate predictors of urinary cotinine

	β	R^2	*P* value
Model 1		0.41	
Cigs/d	84.92		<0.001
eGFR	0.18		0.13
Uprot	0.20		0.09
Race	0.16		0.17
Model2		0.59	
eCO	105.41		<0.001
eGFR	0.13		0.19
Uprot	0.02		0.85
Race	0.11		0.25

Cig/d, current cigarettes smoked per day, self-reported; eGFR, estimated glomerular filtration rate, ml/min/1.73 m^2; eCO, expired air carbon monoxide; ppm, parts per million; Uprot, 24-h urinary protein excretion, g/day.

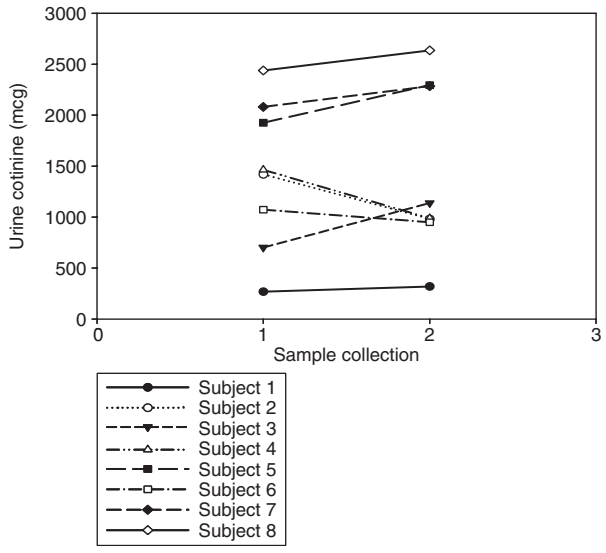

Fig. 1. Variability in 24-h urine cotinine among study participants (mean time between sample 1 and 2: 7.4 months)

It is known that nicotine is extensively metabolized in the liver to cotinine and other by-products by cytochrome P450 enzymes. For example, Molander *et al*. [14] evaluated the pharmacokinetics of single-dose nicotine in 24 subjects with varying degrees of renal impairment. Here, plasma nicotine levels were higher in patients with severe renal impairment, with minimal amounts of nicotine (<2%) excreted unchanged in urine and renal clearance of cotinine <16ml/min in all subject groups. This altered plasma pharmacokinetic profile of nicotine is likely explained by reduced metabolic capacity in the presence of renal failure [15,16]. In the present study, we report a weak association between eGFR and Ucot, which further supports the minimal role of renal function in Ucot excretion, and we extend these findings to include the association between 24-h urinary cotinine excretion and assessment of daily smoking habits during chronic exposure equivalent to steady state dosing of nicotine.

There is data from two clinical trials suggesting that cessation of smoking reduces progression of renal injury, albeit not back to the rate of a never smoker; however, these studies had homogenous study populations, limiting the generalizability of the study results [17–19]. Furthermore, these studies did not address quantification of smoking exposure. The current study is the only report of smokers and non-smokers with renal impairment to demonstrate that urinary cotinine excretion is a feasible, objective measure of smoking exposure in this disease population. The findings of this study should be interpreted recognizing the limitation of the cross-sectional design. However, we were able to demonstrate that markers of renal injury were not significant predictors of urinary cotinine excretion. We were also able to demonstrate limited variability of cotinine excretion in the urine samples of CKD patients who had not changed their smoking behaviour.

It is unknown whether reduction in smoking consumption could decelerate CKD progression. Our prior work indicates that smokers with CKD are unaware of the relationship between smoking and CKD; and when educated about the relationship, this population reported increased motivation to cease cigarette smoking [20]. This suggests that smoking reduction/cessation interventions among smokers with CKD should include education about smoking and CKD. Additionally, urinary cotinine measurements may be utilized as a key element to provide positive feedback in a targeted intervention to promote smoking behaviour modification. Future studies are needed to explore the relationship between smoking reduction/cessation and kidney disease progression. The findings in this study suggest that 24-hour urinary cotinine may be of additional benefit as a quantitative measure of smoking exposure in CKD with utility in both observational and interventional trials examining change in smoking behaviour and its effects on renal injury.

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Conflict of interest statement. None declared.

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