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Brief Report

Relationships Between Race, Gender, and Spot Urine Levels of Biomarkers of Tobacco Exposure Vary Based on How Creatinine Is Handled in Analyses

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Abstract

Introduction: We illustrate the differential impact of common analysis approaches to handling urinary creatinine, a measure for urine dilution, on relationships between race, gender, and biomarkers of exposure measured in spot urine.

Methods: In smokers, spot urine levels of total nicotine equivalents (TNE, sum of total nicotine, total cotinine, and total 3′-hydroxycotinine) and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) overall and per cigarette were examined. Relationships between race (African Americans [AA] $n = 373$, Whites $n = 758$) or gender (males $n = 629$, females $n = 502$) and TNE or NNAL were examined using the following approaches to handling creatinine: (1) unadjusted/unstandardized; (2) standardization; (3) adjustment as a covariate. Significance was considered at $p < .05$

Results: Creatinine was higher in AA versus Whites (1.19 vs. 0.96 mg/mL; $p < .0001$) and in males versus females (1.21 vs. 0.84 mg/mL; $p < .0001$). Independent of how creatinine was handled, TNE was lower among AA than Whites (TNE ratios AA vs. Whites: 0.67–0.84; p's < .05). Unadjusted TNE per cigarette was higher among AA versus Whites (ratio 1.12; $p = .0411$); however, the relationship flipped with standardization (ratio 0.90; $p = .0360$) and adjustment (ratio 0.95; $p = .3165$). Regarding gender, unadjusted TNE was higher among males versus females (ratio 1.13; $p = .0063$), but the relationship flipped with standardization (ratio 0.79; $p < .0001$) or adjustment (ratio 0.89; $p = .0018$). Unadjusted TNE per cigarette did not differ across gender (ratio 0.98; $p = .6591$), but lower levels were found in males versus females with standardization (ratio 0.68; $p < .0001$) and adjustment (ratio 0.74; $p < .0001$). NNAL displayed similar patterns.

Conclusions: Relationships between race, gender, and spot urine levels of biomarkers of exposure can vary greatly based on how creatinine is handled in analyses.

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Implications: Lack of appropriate methods can lead to discrepancies across reports on variability of urinary biomarkers by race and gender. We recommend that for any analyses of biomarkers of exposure measure in spot urine samples across race, gender, or other population subgroups that differ in urinary creatinine levels, sensitivity analyses comparing the different methods for handling urinary creatinine should be conducted. If methods result in discrepant findings, this should be clearly noted and discussed.

Introduction

Biological markers (biomarkers) are important tools that provide an objective measure of human exposure to and adverse effects of tobacco products and are frequently used to compare risk for tobacco-related disease across population subgroups.[1] A commonly used matrix for measuring biomarkers is urine. Although 24-hour urine collection is the gold standard for assessment of biomarker measurements, compliance with and ease of collecting 24-hour urine samples can be challenging and so spot urine samples, either first morning void or random spot collections, are commonly used.[1–3]

A disadvantage of using spot urine for measurement of biomarkers is that hydration status and thus urine flow rate can affect the concentration of some biomarkers. Different approaches have been suggested to control for these differences in spot urine samples with the most commonly used method involving urinary creatinine. Creatinine is a breakdown product of creatine phosphate in muscle and dietary meat. Creatinine is filtered through and secreted by the kidneys at a fairly constant rate over time and therefore can serve as an estimate of urine dilution.[3] A common approach to account for variation in urine flow rate is to standardize urinary biomarker values by creatinine (expressed as units of urinary biomarker per unit of creatinine).[4–6] Another frequently used approach is to adjust the biomarker for urinary creatinine by including urinary creatinine as an independent covariate in the multiple regression model with the biomarker as the outcome.[3,7] This approach allows for other variables in the model to be independent of the effects of creatinine.[3]

However, urinary creatinine itself is influenced by factors other than hydration status, examples of such factors are age, race, and gender.[8–10] For example, two large epidemiologic surveys, the Multiethnic Cohort Study and the National Health and Nutrition Examination Survey, reported significantly higher concentrations of urinary creatinine in African Americans (AA) versus Whites and in males versus females due to higher lean body mass.[3,8] For this reason, some studies comparing urinary biomarkers across racial groups and/or gender also present urinary biomarkers values that are unadjusted and unstandardized for creatinine[8,9] because these differences are inherent to the population.

The purpose of this report is to illustrate the impact of different approaches to handling urinary creatinine in data analysis on the relationships between race, gender, and biomarkers of tobacco exposure measured in spot urine samples. We utilize two urinary biomarkers of tobacco exposure, specifically total nicotine equivalents (TNE; sum of urinary nicotine, cotinine, and several metabolites in the nicotine metabolic profile and considered the gold standard biomarker for daily nicotine dose) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides (total NNAL; a biomarker for the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone). We are interested in these two biomarkers due to their prominence in the field for measuring tobacco exposure and prior studies showing differences in their levels by race and gender.[1,8,9]

Methods

Secondary data analysis was conducted on baseline data from a randomized clinical trial of reduced nicotine content cigarettes among adult daily smokers conducted at 10 sites throughout the United States between July 2014 and September 2016. Participants were eligible if they were at least 18 years of age; breath alcohol level < 0.02%; smoked five or more cigarettes per day; an expired carbon monoxide level of >8 ppm or if ≤ 8 ppm a urinary cotinine level of >1000 ng/mL. Participants were ineligible if they were breastfeeding, pregnant, or planning to become pregnant; reported intentions to quit smoking in the next 30 days; used roll-your own cigarettes exclusively or tobacco products other than machine-manufactured cigarettes for >9 days of the past 30 days; had previously used reduced nicotine content study cigarettes; had unstable mental or physical health conditions; and positive for illicit drug use with the exception of cannabis.

At baseline, all participants provided a first morning void urine sample and information on their smoking history and demographics, including self-report race and gender. Additional information on the randomized clinical trial methods and procedures can be found elsewhere.[11] Urine levels of TNE (molar sum of total nicotine, total cotinine, and total 3′-hydroxycotinine, where “total” refers to the unconjugated and glucuronide conjugated forms), total NNAL, and creatinine were quantified at the Analytical Biochemistry Shared Resource of the Masonic Cancer Center, University of Minnesota by methods previously described.[12,13]

Statistical Analyses

With race (African American, White) or gender (male, female) as the independent variable, the dependent variables were TNE or total NNAL. We examined overall levels of TNE and total NNAL, as a measure of total exposure, and per cigarette smoked (biomarker divided by average cigarettes per day), as a measure of smoking intensity. Due to skewness in the distributions of TNE, total NNAL, and creatinine, values were transformed using the natural logarithm to achieve approximately normal distribution and summarized using geometric means and 95% confidence intervals (CIs).

We examined three common approaches to handling urinary creatinine in the data analysis stage:

Method I, creatinine unstandardized/unadjusted: linear regression model with race or gender as the independent variable and the urinary biomarker as the dependent variable. This approach does not account for creatinine.

Method II, Creatinine standardization: linear regression model with race or gender as the independent variable and the urinary biomarker divided by urinary creatinine (expressed

as unit of biomarker per unit of creatinine) as the dependent variable.

Method III, creatinine adjustment: linear regression model with race or gender as the independent variable, urinary creatinine included as a covariate, and the urinary biomarker as the dependent variable.

Finally, we conducted sensitivity analyses that involved adjusting the models (methods I, II, and III) with race as the independent variable for the covariates gender and age (since age can affect kidney function) and adjusting the models with gender as an independent variable for the covariates race and age. Results (i.e., magnitude of the effects and significance) were similar to the models without these additional covariates. We present the results without these covariates for simplicity.

Results

Distributions of log-transformed urinary creatinine by race (African Americans [AA] $n = 373$; Whites $n = 758$) and gender (males $n = 629$; females $n = 502$) are displayed in Figure 1. Geometric mean values of urinary creatinine in AA and Whites were 1.19 mg/mL (95% CI: 1.11, 1.27) and 0.96 mg/mL (95% CI: 0.91, 1.00), respectively, ($p <$ .0001). Geometric mean value of urinary creatinine in males and females were 1.21 mg/mL (95% CI: 1.15, 1.26) and 0.84 mg/mL (95% CI: 0.79, 0.90), respectively ($p < .0001$). Race and gender comparisons of TNE and total NNAL per cigarette smoked and overall by the three methods for handling creatinine are displayed in Table 1.

AA Versus Whites

Independent of how creatinine was handled in analyses, TNE overall was significantly lower among AA versus Whites with the largest difference occurring when TNE was standardized by creatinine (44.55 vs. 65.87 nmol/mL; $p < .0001$) followed by adjustment for creatinine (47.99 vs. 66.30 nmol/mL; $p < .0001$). Unadjusted TNE per cigarette smoked was significantly higher among AA versus Whites (3.99 versus 3.56 nmol/mL; $p = .0411$); however, the direction of the relationship flipped when TNE was standardized by creatinine (3.35 vs. 3.71 nmol/mg creatinine; $p = .0360$). Adjustment for creatinine as a covariate in linear regression modeling resulted in levels of TNE per cigarette smoked that did not significantly differ across race (3.58 vs. 3.76 nmol/mL; $p = .3165$. The relationship between total NNAL and race across the different methods for handling creatinine displayed a similar pattern to TNE.

Males Versus Females

TNE overall was significantly higher among males versus females when unadjusted for creatinine (63.00 vs. 55.59 nmol/mL; $p = .0063$). However, the direction of the relationship flipped when TNE was standardized by creatinine (52.22 vs. 65.88 nmol/mg creatinine; $p < .0001$) or creatinine was adjusted for as a covariate in regression modeling (56.58 vs. 63.61 nmol/mL; $p = .0018$). Unadjusted TNE per cigarette smoked did not significantly differ across gender (3.66 vs. 3.74 nmol/mL; $p = .6591$). Creatinine standardization (3.03 vs. 4.43 nmol/mg creatinine; $p < .0001$) and adjustment (3.22 vs. 4.38 nmol/mL; $p < .0001$) resulted in significantly lower levels of TNE per cigarette smoked in males versus females. The relationship between total NNAL and gender across the different methods for handling creatinine displayed a similar pattern to TNE.

Discussion

Because urinary creatinine is commonly used to correct for individual variation in urine flow rate in studies of tobacco-related metabolites measured in spot urine samples and urinary creatinine itself is influenced by race and gender, it is important to review the impact of how urinary creatinine is handled in data analysis. Thus, we compared three common approaches to handling urinary creatinine in data analyses of biomarkers of tobacco exposure measured in spot urine samples by race and gender. Our results demonstrate that the relationships between urinary biomarkers of exposure and race or gender can vary greatly based on how urinary creatinine is accounted for in the data analyses. For example, an interpretation on the relationship between race and TNE per cigarette smoked unadjusted for creatinine would be that African Americans have higher nicotine exposure per each cigarette smoked than Whites. However, the opposite interpretation would have been made if creatinine standardization was performed. Lack of appropriate methods can lead to discrepancies across reports on variability of urinary biomarkers by race and gender.

Our results caution the field on the interpretation of results using urinary biomarkers of tobacco exposure in spot urine samples by race and gender. Researchers should seriously consider the impact these findings will have and be mindful of potential

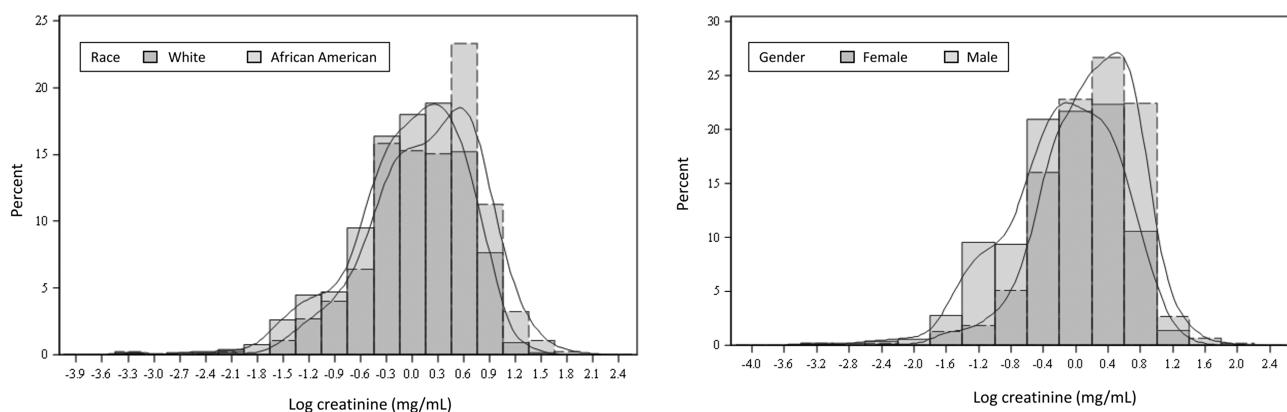

Figure 1. Distribution of log-transformed urinary creatinine (natural log of mg/mL) by race and gender in spot urine sample among cigarette smokers.

Table 1. Geometric Means and 95% Confidence Intervals of Urinary Biomarkers Across Different Approaches to Handling Urinary Creatinine by Race and Gender in First Morning Void Samples Among Cigarette Smokers

	African Americans (*n* = 373)	Whites (*n* = 758)	Ratio African Americans to Whites	*p*
TNE (nmol) overall				
Unadjusted	53.07 (48.65, 57.89)	63.09 (59.91, 66.44)	0.84	.0008*
Standardized	44.55 (41.12, 48.26)	65.87(63.19, 68.66)	0.67	<.0001*
Adjusted	47.99 (44.50, 51.74)	66.30 (63.73, 68.91)	0.72	<.0001*
TNE (nmol) per cigarette smoked				
Unadjusted	3.99 (3.63, 4.38)	3.56 (3.37, 3.76)	1.12	.0411*
Standardized	3.35 (3.06, 3.65)	3.71 (3.56, 3.88)	0.90	.0360*
Adjusted	3.58 (3.29, 3.89)	3.76 (3.60, 3.91)	0.95	.3165
Total NNAL (pmol) overall				
Unadjusted	1.20 (1.11, 1.31)	1.40 (1.31, 1.50)	0.86	.0066*
Standardized	1.01 (0.93, 1.10)	1.46 (1.37, 1.56)	0.69	<.0001*
Adjusted	1.12 (1.04, 1.20)	1.45 (1.37, 1.55)	0.77	<.0001*
Total NNAL (pmol) per cigarette smoked				
Unadjusted	0.090 (0.082, 0.099)	0.079 (0.074, 0.084)	1.14	.0170*
Standardized	0.076 (0.069, 0.083)	0.083 (0.078, 0.088)	0.92	.1627
Adjusted	0.083 (0.088, 0.091)	0.082 (0.078, 0.087)	1.01	.8363
	Males (*n* = 629)	**Females (*n* = 502)**	**Ratio males to females**	***p***
TNE (nmol) overall				
Unadjusted	63.00 (59.19, 67.06)	55.59 (52.10, 59.30)	1.13	.0063*
Standardized	52.22 (49.40, 55.21)	65.88 (62.38, 69.59)	0.79	<.0001*
Adjusted	56.58 (53.67, 60.40)	63.61 (60.40, 66.97)	0.89	.0018*
TNE (nmol) per cigarette smoked				
Unadjusted	3.66 (3.42, 3.91)	3.74 (3.48, 4.02)	0.98	.6591
Standardized	3.03 (2.86, 3.21)	4.43 (4.21, 4.67)	0.68	<.0001*
Adjusted	3.22 (3.05, 3.41)	4.38 (1.16, 4.62)	0.74	<.0001*
Total NNAL (pmol) overall				
Unadjusted	1.40 (1.30, 1.50)	1.26 (1.16, 1.36)	1.11	.0442*
Standardized	1.16 (1.08, 1.24)	1.49 (1.38, 1.61)	0.78	<.0001*
Adjusted	1.29 (1.21, 1.38)	1.39 (1.29, 1.49)	0.93	.1404
Total NNAL (pmol) per cigarette smoked				
Unadjusted	0.081 (0.076, 0.087)	0.085 (0.078, 0.092)	0.96	.4482
Standardized	0.067 (0.063, 0.072)	0.100 (0.093, 0.108)	0.67	<.0001*
Adjusted	0.073 (0.069, 0.078)	0.096 (0.089, 0.103)	0.76	<.0001*

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; TNE = total nicotine equivalents.
*Statistically significance difference at $p < .05$ between African Americans and Whites or males and females.

discrepancies. A commonly used dataset within our field that has relevance to our findings is the Population Assessment of Tobacco Use and Health (PATH) Study.[2] PATH includes a comprehensive panel of biomarkers of exposure measured in spot urine samples. PATH biomarker values are provided unadjusted for creatinine, but a measurement of urinary creatinine is available for researchers to conduct creatinine standardization or adjustment. While not reported in the present study, our discrepant results based on how urinary creatinine was handled was reproduced with the PATH data. Future research is needed to clarify which approach to handling urinary creatinine in data analyses may be most optimal. Such research could include collection of both spot urine samples and well-monitored 24-hour urine samples among participants balanced by race and gender and then identifying which method for handling urinary creatinine in the spot samples yields a similar interpretation as the 24-hour sample. Last, although the focus herein was on race and gender as independent variables, researchers should be mindful of other characteristics (i.e., age) which affect urinary creatinine and thus their relationship with spot urine levels of biomarkers of exposure may vary based on how creatinine is handled in analyses.

In conclusion, the relationships between urinary biomarkers of exposure and race or gender can vary greatly based on how urinary creatinine is accounted for in the data analyses. Our recommendation is that for any analyses of biomarkers of tobacco exposure across population subgroups that differ in creatinine levels, sensitivity analyses comparing the different methods for handling urinary creatinine should be conducted. If methods result in discrepant findings, this should be clearly noted and discussed. Recommending which approach to use in the presence of discrepant findings is beyond the scope of the present paper. However, we do recommend to try to discern which set of results are most in alignment with prior research studies (i.e., consistency) and whether they are biological feasible (i.e., plausibility) and compatible with the natural history or biology of the disease (i.e., coherence).

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

None declared.

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