Creatinine Normalization of Workplace Urine Drug Tests: Does It Make a Difference?

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Background: This study examines the effect of creatinine normalization on urine drug concentrations of 5 substances (amphetamines, cocaine, marijuana, opiates, and phencyclidine) and how this affects the proportion of reported positives.

Methods: The Wilcoxon matched-pairs signed-ranks test was used to compare the mean prenormalization urinary drug concentration with the mean postnormalization urinary drug concentration. Frequency analysis was performed on dichotomous drug test results and the information was used to complete McNemar testing for each drug to determine the difference of proportions for prenormalization positive drug tests to postnormalization positive drug test.

Results: Each drug tested (N = 4460) was found to have a statistically significant increase in mean urinary drug concentration after creatinine normalization with effect sizes ranging from small to medium with cocaine having the largest effect size ($r = 0.229$) and phencyclidine having the lowest effect size ($r = 0.121$). The differences in proportion of dichotomous results between study and control groups for drugs tested were compared with the McNemar test. Each drug had a statistically significant ($P = 0.0010$) increase of positive drug tests.

Conclusions: This result indicates that specimen dilution does affect the number of laboratory-positive results confirmed.

Key Words: creatinine normalization, drug testing, urine dilution

(*J Addict Med* 2013;7: 129–132)

U rine is the biological tool of choice for qualitative illicit drug detection (Wolff et al., 1999). Urine drug concentration depends on several biological variables such as the elapsed time since use, the quantity and regularity of use, fluid intake, body composition, hepatic function, and renal function (Standridge et al., 2010). Many illicit drug users will attempt to subvert the urine drug testing process by manipulative polydipsia, which is the in vivo dilution of the urine by ingestion of a large amount of water (George and Braithwaite, 1995).

The kidneys maintain plasma analyte homeostasis within a tight range of normality via filtration of the plasma, reabsorption of needed substances, and secretion of metabolic waste. The metabolic waste is then excreted in the urine. Creatinine is a metabolic waste product that is spontaneously formed from creatine and creatine phosphate in muscle. Creatinine production is dependent on muscle mass, age, and gender but remains constant from 1 day to the next (Loewenthal et al., 1995; Carrieri et al., 2001). Because of this property, urinary creatinine concentration serves as an indicator of urine dilution (Cook et al., 2000).

Creatinine normalization of urinary drug concentrations has been used by athletic organizations, and pain management programs to compensate for dehydration, excessive hydration, and variations of glomerular filtration rate. Similar procedures have not been adopted by workplace drug testing programs (Cone et al., 2009).

This study will attempt to answer several questions. Is there a difference between the mean urinary creatinine for a workplace drug testing population and the U.S. population that would be consistent with in vivo dilution? Is there a difference in the means of urine drug concentrations for workplace drug testing before and after creatinine normalization? Does creatinine normalization of urinary drug concentrations affect the final interpretation of workplace urine drug testing for marijuana, amphetamines, cocaine, opiates (morphine and codeine), and phencyclidine?

MATERIALS AND METHODS

This study examines the effect of creatinine normalization on urine drug concentrations of 5 substances (amphetamines, cocaine, marijuana, opiates, and phencyclidine) and how this affects the proportion of reported positives. The population consists of employees from various industries in Southern Indiana that have a routine urine drug testing policy and are contracted with our clinic for medical review officer services. No distinction is made regarding age or gender of the subjects. Institutional review board approval with an informed consent waiver was obtained from St. Mary's Medical Center, Evansville, Ind before data collection.

The data were obtained from an administrative database maintained by Clinical Reference Laboratory of Lenexa, Kan. The urine collections occurred between January 2, 2009, and December 30, 2010. Specimens were collected at several sites, each following rigorous collection procedures with a strict chain of custody algorithm originally established by the U.S. Department of Transportation. The samples were

all processed at the same Department of Health and Human Services certified laboratory (Clinical Reference Laboratory, Lenexa, Kan) following a standardized 2-step process. This procedure requires specimens to first be screened for the presence of the substances in question using the Siemens ADVIA 2400 immunoassay. Each positive screen is confirmed by gas chromatography–mass spectroscopy using an Agilent Instruments 5975 to eliminate the prospect of false positives.

The information was supplied as an Excel spreadsheet. Analyses were performed using "OpenStat (version June 2012)." The spreadsheet provided urine test results for amphetamines cocaine, marijuana, opiates, and phencyclidine. The spreadsheet also supplied the urinary creatinine concentration for each sample and a specimen identifier. No unique patient identifiers were supplied and no demographics were available. Six specimens that met established Department of Transportation substitution criteria (Creatinine <2 mg/dL and specific gravity ≤1.0010 or ≥1.0200) were eliminated from the study leaving the final tally of specimens, N = 4460 (Barbanel et al., 2002; Moeller et al., 2008).

The control group consisted of urinary drug concentrations before creatinine normalization. Creatinine normalization of each urine drug concentration was performed mathematically to create the study group. Normalization of drug excretion to urinary creatinine concentration was carried out as described by Cone and Associates in 2009:

$$\text{Concentration}_{\text{CR normalized}} = \text{Concentration}_{\text{specimen}} * (\text{CR}_{\text{reference}} / \text{CR}_{\text{specimen}})$$

(Cone et al., 2009).

The reference creatinine was established by referring to the Third National Health and Nutrition Examination Survey (NHANES III) database and using the mean urinary creatinine concentration for the U.S. population ($\text{CR}_{\text{reference}} = 130.4$ mg/dL) as the reference value (Barr et al., 2005). The drug concentrations for each group were assessed and deemed positive or negative in accordance with U.S. Department of Health and Human Services cutoff criteria (Table 1) (Federal Register, 2008).

Descriptive statistics were generated for each variable including means with the 95% confidence interval (CI), range, standard deviation, skewness, and kurtosis. A 2-tailed t test was used to compare the NHANES III mean urinary creatinine concentration with the study mean urinary creatinine concentration to evaluate the evidence of dilution in the study population. The data did not meet the distributional requirements for parametric analysis so the Wilcoxon matched-pairs signed-ranks

test was used to compare the mean prenormalization urinary drug concentration with the mean postnormalization urinary drug concentration (Altman and Bland, 2009; Okeh, 2009). The effect size was calculated as described by Cohen (1992). Frequency analysis was performed on dichotomous drug test results and the information was used to complete McNemar testing for each drug to determine the difference of proportions for prenormalization positive drug tests to postnormalization positive drug test (Gauvreau, 2006).

RESULTS

The mean urine creatinine is 119.045. Skewness is 1.127 and kurtosis is − 114.930 indicating non-normal distribution. Distribution parameters for all of the tested drugs did not meet criteria for normal distribution (Table 2) (Altman and Bland, 1995). Skewness ranged from 5.233 for opiates to 16.393 for cocaine in the prenormalization group and 9.679 for opiates to 25.555 for cocaine in the postnormalization group. Kurtosis ranged from − 16519.013 for cocaine to − 1553.731 for opiates in the prenormalization group and − 43512.657 for cocaine to − 6698.812 for opiates in the postnormalization group. Normalization for urine creatinine resulted in increased mean concentrations of each drug tested.

Established Department of Health and Human Services cutoff values were used to assign positive or negative values to each drug tested for each specimen collected within each group. The findings indicated an increase in positive results for each drug tested after normalization for urinary creatinine (Table 3).

NHANES III data were used to obtain a population estimate of mean urinary creatinine for the U.S. population. Race, ethnicity, and age were not factored into this value as demographics were not available for our study data. However, the sample size and the diversity of represented industries result in a representative sample of the working population of Southern Indiana that should be similar to the NHANES population. The reference material supplied the mean urinary creatinine (130.4 mg/dL), the 95% CI (128.2 mg/dL to 132.7 mg/dL), and the sample size (N = 22,245). This information was used to calculate the standard deviation (SD = 171.22) for use in comparison to the study mean urine creatinine.

The study mean urinary creatinine was compared with NHANES III mean urinary creatinine assuming unequal variances (Table 4). The calculation yielded a difference of 11.35 (95% CI, 8.13-14.57) with $P = 0.0000$. This is consistent with a statistically significant difference in urinary creatinine concentrations between the NHANES II population and the study population indicative of in vivo dilution.

The distribution of data required the use of Wilcoxon matched-pairs signed-ranks testing to compare the difference in mean urinary drug concentrations between the prenormalization and postnormalization groups. Each drug tested was found to have a statistically significant increase in mean urinary drug concentration after creatinine normalization with effect sizes ranging from small to medium with cocaine having the largest effect size (r = 0.229) and phencyclidine having the lowest effect size ($r = 0.121$) (Table 5).

The differences in proportion of dichotomous results between study and control groups for drugs tested were compared

TABLE 1. Cutoff Criteria for Federally Regulated Urine Drug Testing Programs*

Drug	Screening Cutoff	Confirmation Cutoff
Amphetamines	500 ng/mL	250 ng/mL
Cocaine metabolites	150 ng/mL	100 ng/mL
Marijuana metabolites	50 ng/mL	15 ng/mL
Opiate metabolites	2000 ng/mL	2000 ng/mL
Phencyclidine	25 ng/mL	25 ng/mL

*Adapted with permission from Federal Register, 2008.

TABLE 2. Descriptive Statistics With Distribution Parameters

Substances, N = 4460	Group	Mean (95% CI)	Range (Min-Max)	Standard Deviation	Skewness	Kurtosis
Urine creatinine		119.045 (116.74-121.35)	583.500 (2.40-585.90)	78.485	1.127	− 114.930
Amphetamines	Prenormalization	127.623 (119.33-135.91)	4382.000 (0.0-4382.00)	282.563	8.051	− 4607.316
	Postnormalization	208.763 (179.96-237.57)	37949.060 (0.0-37949.06)	981.474	22.134	− 36497.886
Cocaine	Prenormalization	7.031 (6.38-7.68)	478.000 (0.0-478.00)	22.059	16.393	− 16519.013
	Postnormalization	13.190 (11.05-15.33)	2725.130 (0.0-2725.13)	72.880	25.555	− 43512.657
Marijuana	Prenormalization	7.691 (7.30-8.08)	150.000 (0.0-150.00)	13.291	6.859	− 2913.807
	Postnormalization	13.261 (11.42-15.11)	2086.400 (0.0-2086.400)	62.860	21.232	− 31158.053
Opiates	Prenormalization	137.959 (122.88-153.04)	4439.000 (0.0-4439.00)	513.842	5.233	− 1553.731
	Postnormalization	226.397 (194.94-257.85)	21803.440 (0.0-21803.44)	1071.804	9.679	− 6698.812
Phencyclidine	Prenormalization	2.145 (2.08-2.20)	51.000 (0.0-51.00)	2.050	8.098	− 8570.209
	Postnormalization	2.854 (2.71-2.99)	184.730 (0.0-184.73)	4.713	19.054	− 32871.013

TABLE 3. Frequency of Positive Drug Tests Using Established Department of Health and Human Services Cutoff Values

Drug	Group (N for Each Group = 4460)	Positives	Percent Positive
Amphetamine	Prenormalization	338	0.0758 (7.58%)
	Postnormalization	533	0.1195 (11.95%)
Cocaine	Prenormalization	17	0.0038 (0.38%)
	Postnormalization	42	0.0094 (0.94%)
Marijuana	Prenormalization	242	0.0543 (5.43%)
	Postnormalization	496	0.1112 (11.12%)
Opiates	Prenormalization	124	0.0278 (2.78%)
	Postnormalization	146	0.0327 (3.27%)
Phencyclidine	Prenormalization	0.0	0.0
	Postnormalization	16	0.36 (36%)

TABLE 4. Comparison of Study Mean Urinary Creatinine to NHANES III Mean Urinary Creatinine Assuming Unequal Variances

Group	Mean Urinary Creatinine (mg/dL)	Standard Deviation
NHANES III (N = 22,245)	130.40	171.22
Study (N = 4460)	119.05	78.48

$t = 6.909$.
$P = 0.0000$.
df = 14401.38.
Difference = 11.35 (95% CI, 8.13-14.57).

TABLE 5. Wilcoxon Matched-Pairs Signed-Ranks Testing of Individual Drugs

Drug	Approximately Normal z	Probability (1-Tailed)	Effect Size*
Amphetamines	$z = 16.892$	$P = 0.0000$	$r = 0.179$
Cocaine	$z = 21.632$	$P = 0.0000$	$r = 0.229$
Marijuana	$z = 15.949$	$P = 0.0000$	$r = 0.169$
Opiates	$z = 18.172$	$P = 0.0000$	$r = 0.192$
Phencyclidine	$z = 11.429$	$P = 0.0000$	$r = 0.121$

*Small = 0.05; medium = 0.15; large = 0.25 (Cohen, 1992).

with the McNemar test. Each drug had a statistically significant ($P = 0.0010$) increase of positive drug tests when interpreted in accordance with Department of Health and Human Services criteria (Table 6).

DISCUSSION

Actions were taken to address potential systematic errors. Sample bias was diminished by including all individuals employed by the participating companies during the specified time period. Measurement bias was reduced by using a single laboratory that has achieved certification through the Department of Health and Human Services for participation in federally mandated drug testing programs (Moeller et al., 2008). Analysis bias was avoided by following strict inclusion criteria and no individual data point was excluded other than for those samples that met criteria for substitution.

Several measures were taken to limit random errors such as using only trained urine specimen collectors that follow a strict collection protocol. Specimen adulteration was supplanted by specimen validation. Department of Health and Human Services laboratory guidelines direct laboratories to test for adulterants and to validate that the specimen was not substituted with a commercial synthetic substitute (Moeller et al., 2008). Any specimen that was determined to be substituted or adulterated was removed from the study.

The Wilcoxon matched-pairs signed-ranks results are presented as a 1-tailed probability for comparison of mean urine drug concentrations. One-tail probabilities are prone to Type I (α) error (Rigby, 1998). The likelihood of this being the case is quite small as the probability reported for each comparison was at the software's limit of precision ($P = 0.0000$). The effect size was also reported as a method of clarifying any doubt regarding the result.

CONCLUSION

The results of this study support the conclusion that the mean urine creatinine concentration for the study population is statistically lower than that of the U.S. population. This finding is consistent with in vivo dilution of the study population's urine but cannot distinguish between unintentional dilution secondary to fluid ingestion to expedite the production of urine and volitional dilution as a means of subverting the drug testing process. This reinforces past studies sighting the importance of creatinine analysis of urine drug test specimens (Lafolle et al., 1991).

The difference in mean urine concentration and the proportion of positive drug tests were higher for the 5 drugs tested in the postcreatinine normalization group. This result indicates that specimen dilution does affect the number of laboratory-positive results confirmed. Corrections for urinary creatinine

TABLE 6. McNemar Test for the Difference in Proportion of Positive Drug Tests Between Study and Control Groups

Drug	Difference in Proportions (95% CI)	z for 95% Confidence Interval	Chi-Squared Statistic	*P*-Value 2-Tailed
Amphetamines	0.4023 (0.3899-0.4148)	z = 1.9600	$\chi^2 = 2766.1676$	$P = 0.0010$
Cocaine	0.4934 (0.4828-0.5040)	z = 1.9600	$\chi^2 = 4317.5736$	$P = 0.0010$
Marijuana	0.4172 (0.4049-0.4296)	z = 1.9600	$\chi^2 = 2937.7978$	$P = 0.0010$
Opiates	0.4697 (0.4587-0.4807)	z = 1.9600	$\chi^2 = 3916.0279$	$P = 0.0010$
Phencyclidine	0.4982 (0.4878-0.5087)	z = 1.9600	$\chi^2 = 4411.2288$	$P = 0.0010$

and urinary specific gravity have been shown to positively correlate (Cone et al., 1998). A similar technique utilizing urine specific gravity as a method of mathematically adjusting for dilution or concentration of specimens is worthy of investigation. This result is consistent with previous studies that determined that urinary dilution may lead to false negative urine drug tests for cocaine and marijuana (Cone et al., 1998; Fraser and Worth, 1999; Preston et al., 2002; Smith et al., 2009). This study expands that determination to conclude the in vivo dilution may also lead to false negative interpretations for other substances including amphetamines, opiates, and phencyclidine. Correcting for urine creatinine level may augment the effectiveness of deterrent programs by mitigating volitional in vivo dilution as a means of subverting workplace drug testing.

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