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Use of Urinary Thiocyanate as a Biomarker of Tobacco Smoke

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Abstract

Data from National Health and Nutrition Examination Survey for the years 2005-2010 were used to develop cut off levels for urinary thiocyanate (USCN) to distinguish smokers from nonsmokers aged ≥ 20 years. A cut off of (i) 1840 ng/ml for USCN was able to distinguish smokers from nonsmokers with a sensitivity of 80.7% and a specificity of 80.8%, (ii) 2630 ng/ml for USCN was able to distinguish smokers from nonsmokers with exposure to SHS at home with a sensitivity of 82.0% and a specificity of 82.1%, and (iii) 1550 ng/ml for USCN was able to distinguish smokers from nonsmokers without exposure to SHS at home with a sensitivity of 75.7% and a specificity of 75.6%. Adequacy of these cut offs was evaluated by applying them to NHANES data for 2011-2012. Sensitivities and specificities for 2005-2010 and 2011-2012 data were comparable. USCN levels for smokers were 4.6 times of what they were for nonsmokers (4102.6 *vs.* 890.6 ng/ml, p<0.01). Those aged 20-64 years had higher levels of USCN than those aged 65+ years (1390.0 *vs.* 855.9 ng/ml). Males had higher levels of USCN than females (p<0.01). Non-Hispanic whites had statistically significantly higher levels of USCN than non-Hispanic blacks (p<0.01).

Keywords: Specificity; Sensitivity; Thiocyanate; Smoking

Introduction

Thiocyanates are a group of compounds formed from a combination of sulfur, carbon, and nitrogen [1]. They are produced primarily from the reaction of free cyanide with sulfur [1]. This reaction may occur in the environment, for example, in industrial waste streams that contain cyanide and in human body after cyanide is swallowed or absorbed [1]. Thiocyanates are present in water because of discharges from coal processing, extraction of gold and silver, and mining industries [1]. They are found in soils from direct application of herbicides, insecticides, and rodenticides and disposal of byproducts from industrial processes [1]. Other sources of thiocyanates include damaged or decaying tissues of certain plants like mustard, kale, and cabbage [1].

Humans can be exposed to cyanides by breathing air, drinking water, touching soil or water that contains cyanide, or eating foods that contain cyanide. Plant materials like cassava roots, lime beans, and almonds naturally contain low to moderate levels of cyanide. Other sources of cyanide include breathing in smoke-filled air during fires, living near hazardous waste sites, and working in occupational settings where activities include electroplating, metallurgy, metal cleaning, tanning, photoengraving, and firefighting [1].

Cyanide is a potent toxic agent present in the cigarette smoke which is metabolized to USCN through sulfuration with thiosulfate by mitochondrial rhodanase [2]. It has been shown that urinary and/or serum thiocyanate levels among smokers are substantially higher than non-smokers [2-7].

These results suggest the possible utility of serum and urinary thiocyanate levels as potential biomarker of tobacco smoke. Zil-a-Rubab and Rahman [2] proposed serum thiocyanate levels of ≥ 60 µmol/L as a cut off to distinguish smokers from nonsmokers [8], Jarvis et al. [8], based on a study of 215 outpatients at St. Mary's Hospital, London, suggested a cut off of 78.0 µmol/L for plasma thiocyanate with a specificity of 78% and a specificity of 79% and a cut off of 118.0 µmol/L for urine thiocyanate with a specificity of 83% and a sensitivity of 63% to distinguish self-reported smokers from nonsmokers [8].

However, since the study published by [8], there has been a substantial change, not only in smoking prevalence but also a downward change in exposure to second hand smoke (SHS) that could affect cut off levels for distinguishing self-reported smokers from nonsmokers. For example, the proposed cut off as recommended by [2], was 60.0 µmol/L as compared to 78.0 µmol/L recommended [8]. However, cut off by [8] was for plasma and for serum by [2]. In addition, the cut offs proposed by Jarvis, were not necessarily based on a representative sample of smokers and nonsmokers.

Consequently, this study was undertaken to develop cut off levels for urinary thiocyanate (USCN) to distinguish smokers from nonsmokers aged ≥ 20 years by gender and race/ethnicity. In addition, geometric mean levels of USCN by age, gender, race/ethnicity, and smoking status will also be computed. Percentile distributions of USCN will be presented. Data from National Health and Nutrition Examination Survey (NHANES, www.cdc.gov/nchs/nhanes.htm) 2005-2012 were selected for analyses.

Materials and Methods

Data source and data description

Data from NHANES for the years 2005-2012 from demographic and USCN files, recent tobacco use questionnaire, family smoking questionnaire for those aged ≥ 20 years were downloaded and match merged. NHANES uses a complex, stratified, multistage, probability sampling designed to be representative of the civilian, non-institutionalized U.S population based on age, sex, and race/ethnicity.

Sampling weights are created in NHANES to account for the probabilities of selection and response as well as total U.S. population for selected combinations of gender, age, and race/ethnicity.

Total number of participants available with non-missing values of USCN as well recent tobacco use data for 2005-2010 was 10454 and 1474 for 2011-2012. Sample sizes by gender, race/ethnicity, and smoking status are given in (Table 1).

It should be noted that separate databases were created for 2005-2010 and 2011-2012. Data for 2005-2010 were used to develop thiocyanate cut offs to distinguish smokers from nonsmokers. These cut offs were then applied to the data for 2011-2012 to validate the cut offs developed for 2005-2010.

v	NHANES 2005-2010		NHANES 2011-2012	
	N	%	N	%
Total	10454	100	1474	100
Male	5249	50.2	767	52
Female	5205	49.8	707	48
Non-Hispanic White	5079	48.6	557	37.8
Non-Hispanic Black	2180	20.9	388	26.3
Mexican American	1942	18.6	130	8.8
Other Race/Ethnicities	1253	12	399	27.1
Smokers - Total	2682	25.7	340	23.1
Non-smokers - Total	7772	74.3	1134	76.9
Smokers with Exposure to SHS	1495	55.7	141	41.5
Smokers without Exposure to SHS	1171	43.7	198	58.2
Nonsmoker with Exposure to SHS	458	5.9	45	4
Nonsmoker without Exposure to SHS	7277	93.6	1088	95.9

Table 1: Un-weighted sample sizes by gender, race/ethnicity, smoking, and exposure to second hand smoke at home status (SHS) at home for those aged ≥ 20 years. Data from National Health and Nutrition Examination Survey (NHANES) 2005-2012.

Derived variables and definitions

All those who reported not smoking during the last five days were assumed to be nonsmokers. All those who reported using cigarettes, cigars, and pipes with or without using smokeless tobacco products and pharmaceutical products like nicotine gum etc. at least once during the last five days were assumed to be smokers.

Those who exclusively used smokeless tobacco products and/or pharmaceutical products like nicotine patch etc. were excluded from the analysis. All those who reported at least one person smoking inside the home were considered to be exposed to SHS at home and those who reported no one smoking inside the home were considered to be not exposed to SHS at home.

Statistical analysis

In order to develop optimal cut off points on a receiving operator characteristic curve, there are several criteria that can be used. These criteria, among others, include maximizing sensitivity levels, maximizing the area under curve, and minimizing the difference between sensitivity and specificity.

There is always a temptation to maximize sensitivity but since there is an inverse relationship between specificity and sensitivity, an acceptably high level of sensitivity may be associated with an undesirably low level of specificity. Consequently, a compromise must be made to have a pair of sensitivity and specificity levels that are acceptable. A SAS macro ROCPLOT (http://support.sas.com/kb/25/018.html) developed by SAS Institute that minimizes the difference between sensitivity and specificity is available. This macro uses Proc LOGISTIC which is already available as part of SAS's statistical package.

For the purpose of this study, a decision was made to select cut off points based on minimizing the difference between sensitivity and specificity because, in the opinion of this author, an effort should be made to maximize both specificity and sensitivity simultaneously and the criterion selected for this study seems to do that. All statistical analyses completed for this study used SAS University Edition software (www.sas.com).

SAS Proc SURVEYMEAN and SURVEYREG were used to compute model based creatinine corrected unadjusted geometric means (UGM) by age, gender, race/ethnicity, smoking status and t-test was used to do pairwise comparisons.

In order to compute creatinine corrected unadjusted levels of USCN, log10 transferred values of USCN were used as the dependent variable and urinary creatinine was used as the independent variable.

Separate simple regression models were used for age, gender, and race/ethnicity. Either age (20-64 years) or gender (male, female) or race/ethnicity (non-Hispanic white or NHW, non-Hispanic black or NHB, Mexican American or MA, other unclassified race/ethnicities or OTH was the other independent variable used in the models.

Results

Cut offs for urinary thiocyanate

USCN cut offs to distinguish (i) all smokers from nonsmokers, (ii) smokers from nonsmokers with exposure to SHS at home, and (iii) smokers from nonsmokers without exposure to SHS at home by gender and race/ethnicity are given in (Table 2).

Except for MA, sensitivity to distinguish all smokers from nonsmokers varied between 78.7% and 83.9%, and specificity varied from 78.8% to 83.9%. For MA, sensitivity and specificity were somewhat low at 69.9% and 70.1% respectively.

Sensitivity to distinguish smokers from nonsmokers with exposure to SHS at home varied between 77.4% and 83.5%, and specificity varied from 77.1% to 82.1%.

Sensitivity and specificity to distinguish smokers from nonsmokers without exposure to SHS at home was lower than 80%, sensitivity varying between 73.6% to 79.4% for all demographic groups except MA (sensitivity=67.9%), and specificity varying between 73.6% to 80.4% for all demographic groups except MA (specificity=68.1%).

Age		Smokers vs. Nonsmokers			Smokers vs. Nonsmokers with Exposure to SHS			Smokers vs. Nonsmokers with No Exposure to SHS		
		Cut off	Sensitivity	Specificity	Cut off	Sensitivity	Specificity	Cut off	Sensitivity	Specificity
≥ 20 Years	Total	1840	80.7	80.8	2630	82	82.1	1550	75.7	75.6
	Males	1960	78.7	78.8	3010	79.3	79	1660	73.6	73.6
	Females	1740	83.2	83.1	2300	83.5	83.9	1450	76.9	76.7
	Non-Hispanic White	2150	83.9	83.9	2910	77.4	77.1	1820	79.4	79.4
	Non-Hispanic Black	2370	81.5	81.6	2660	80.1	80.3	2230	80.3	80.4
	Mexican American	1080	69.9	70.1	1500	77.4	77.1	1030	67.9	68.1
	Other race/ethnicities	1390	80.6	80.5	3380	82.5	82.1	1270	77.4	77.5

Table 2: Urinary thiocyanate cut offs in ng/mL to distinguish smokers from non-smokers with and without exposure to second hand smoke (SHS) at home. Data from National Health and Nutrition Examination Survey 2005-2010.

Validity of cut offs for urinary thiocyanate

Cut offs used to distinguish all smokers from nonsmokers were able to generate sensitivity and specificity for 2011-2012 (Table 3) that were very comparable to those observed when developing these cut offs (Table 2). The only exception was for NHB for which newly computed sensitivity was 69.7% (Table 3) while the original sensitivity was 81.5% (Table 2). Use of the cut off of 1840 ng/mL developed for the total sample using 2005-2010 data was able to generate sensitivity and specificity of 80.7% and 81.4% respectively for the 2011-2012 data.

	Smokers *vs.* Nonsmokers		Smokers *vs.* Nonsmokers with Exposure to SHS		Smokers *vs.* Nonsmokers with No Exposure to SHS	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Total	80.7 (74-87.3)	81.4 (78.1-84.6)	81.5 (67.9-95)	72 (48.6-95.5)	77.2 (68.7-85.6)	75.3 (71.5-79.1)
Males	75.2 (64.7-85.8)	78 (72-84.1)	75.1 (57.5-92.8)	79.6 (57-102.1)	69.4 (55.4-83.4)	71.7 (65.4-77.9)
Females	88.1 (83.6-92.5)	84.3 (80.5-88)	86.8 (73.6-99.9)	90.8 (70.6-110.9)	86.7 (79.8-93.6)	78.8 (75.5-82.1)
Non-Hispanic White	81.3 (71.6-91)	83.4 (79.6-87.3)	74.8 (47.9-101.7)	56.3 (12.5-100)	78.3 (67.5-89)	78.8 (74.4-83.2)
Non-Hispanic Black	69.7 (59.5-79.8)	89.5 (87-92)	71.1 (60.8-81.3)	76.6 (53.1-100)	59.5 (39.5-79.5)	88 (85.4-90.7)
Mexican American	87 (72.2-101.8)	68.2 (58.7-77.7)	100 (100-100)	100 (100-100)	84 (77.4-90.6)	65.5 (52.9-78.1)
Other race/ethnicities	80.4 (72.5-88.3)	83.1 (78.3-87.8)	79.3 (51.5-107.1)	100 (100-100)	72.1 (59.3-85)	81.5 (76.9-86)

Table 3: Validity of proposed cut off for urinary thiocyanate based on NHANES 2005-2010 data to distinguish smokers from non-smokers with and without exposure to second hand smoke (SHS) at home for those aged ≥ 20 years verified by NHANES 2011-2012 data. Data from National Health and Nutrition Examination Survey 2011-2012.

Application of cut offs developed using 2005-2010 data to distinguish smokers and nonsmokers with exposure to SHS at home were able to generate sensitivity and specificity for the 2011-2012 data which were reasonably close to those for 2005-2010 with two exceptions. For total population, specificity using 2005-2010 data was 82.1%, for 2011-2012 data, it was 72%. For NHW, specificity using 2005-2010 data was 77.1%, for 2011-2012 data, it was 56.3%. Use of the cut off of 2300 ng/mL developed for females using 2005-2010 data was able to generate sensitivity and specificity of 83.5% and 83.9% respectively for the 2011-2012 data.

Application of cut offs developed using 2005-2010 data to distinguish smokers and nonsmokers with no exposure to SHS at home were able to generate sensitivity and specificity for the 2011-2012 data which were reasonably close to those for 2005-2010 with two exceptions. For NHB, sensitivity using 2005-2010 data was 80.3%, for 2011-2012 data, it was 59.5%. For MA, specificity using 2005-2010 data was 68.1%, for 2011-2012 data, it was 65.5%. Use of the cut off of 1450 ng/mL developed for females using 2005-2010 data was able to generate sensitivity and specificity of 86.7% and 78.8% respectively for the 2011-2012 data.

Model based creatinine unadjusted levels for USCN

Model based creatinine unadjusted UGMs for USCN are given in Table 4. Among smokers, the order of UGMs by type of smoker was:

cigarette only smokers>mixed use smokers>cigar only smokers>pipes only smokers>pharmaceutical product only users>tobacco chewers only>tobacco snuffers only (Table 4). Ratio of USCN for all smokers (excludes exclusive users of smokeless and pharmaceutical products) was 4.6 for all participants, 5.0 for those with exposure to SHS at home, and 3.5 for those without exposure to SHS at home. Cigarette only smokers, in general, had statistically higher USCN levels than pipes only, cigars only, and mixed use smokers (Table 4) irrespective of their exposure to SHS at home. It should be noted that those with missing smoking status had substantially higher USCN levels (1135.9 ng/mL) than pipes only smokers (1083.7 ng/mL), tobacco chewers only (532.8 ng/mL), tobacco snuffers only (436.6 ng/mL), and pharmaceutical product only users (963.2 ng/mL). Could it be that those who refuse to report their smoking status are primarily smokers? Those who had exposure to SHS at home had statistically significantly higher levels of USCN than those who were not exposed to SHS at home among nonsmokers (1160.9 *vs.* 874.1 ng/mL, p<0.01) as well as all smokers (5789.7 *vs.* 3048.9 ng/mL, p<0.01).

	Exposed to SHS at Home				
	Total	**Yes (SY)**	**No (SN)**	**Unknown**	**Statistically Significant Differences**
Nonsmokers (NSM)	890.6 (849.1-934)	1160.9 (1048-1285.9)	874.2 (830.2-920.4)	705.2 (485.4-1024.6)	SY>SN (p<0.01)
Cigarette only smokers (CIGS)	4319.2 (4048.5-4607.9)	5827.8 (5445.8-6236.5)	3228.8 (2968.9-3511.3)	2957 (1314.6-6651.1)	SY>SN (p<0.01)
Pipes only smokers (PIPES)	1083.7 (513.3-2288.2)	2314.9 (1338.4-4003.9)	710.3 (283.1-1781.9)	2030.3 (1489.6-2767.4)	SY>SN (p=0.01)
Cigars only smokers (CGRS)	2592.6 (2051.2-3277)	5942.9 (4367.7-8086.3)	2154.9 (1621.1-2864.4)	1166.4 (1039.7-1308.4)	SY>SN (p<0.01)
Tobacco chewers only (CHEW)	532.8 (391.6-724.9)	639.3 (373.3-1094.7)	491.6 (358.1-674.7)	1525 (1364.1-1704.8)	
Tobacco snuffers only (SNUFF)	436.6 (339.9-560.9)	596.9 (275.6-1292.8)	419.3 (326.5-538.4)		
Pharmaceutical product users only (GUM)	963.2 (458.4-2024)	758.4 (176.2-3263.5)	956.4 (447-2046.3)		
Mixed smokers (MIX)	3679.7 (2790.3-4852.7)	4686.2 (3901.1-5629.3)	3281.3 (2016.6-5339)	2112.4 (676.5-6596.6)	SY>SN (p=0.02)
Missing data (MISS)	1135.9 (1028.2-1254.8)	3646.3 (2702.2-4920.4)	910 (806.9-1026.3)	762.5 (471.7-1232.4)	SY>SN (p<0.01)
All smokers	4102.6 (3840.5-4382.6)	5789.7 (5433-6169.9)	3049 (2795.8-3325.2)	2465 (1343.2-4523.8)	SY>SN (p<0.01)
Statistically Significant Differences	NSM<CIGS (p<0.01), NSM<CGRS (p<0.01), NSM<CHEW (p=0.02), NSM<SNUFF (p<0.01), NSM<MIX (p<0.01), NSM<MISS (p<0.01), CIGS>PIPES (p<0.01), CIGS>CGRS (p<0.01), CIGS>CHEW (p<0.01), CIGS>SNUFF (p<0.01), CIGS>GUM (p<0.01), CIGS>MISS (p<0.01), PIPES<CGRS (p<0.01), PIPES<MIX (p<0.01), CGRS>CHEW (p<0.01), CGRS>SNUFF (p<0.01), CGRS<GUM (p=0.03), CGRS>MISS (p<0.01), CHEW<MIX (p<0.01), CHEW<MISS (p<0.01), SNUFF<MIX (p<0.01), SNUFF<MISS (p<0.01), MIX>MISS (p<0.01)	NSM<CIGS (p<0.01), NSM<PIPES (p<0.01), NSM<CGRS (p<0.01), NSM<MIX (p<0.01), NSM<MISS (p<0.01), CIGS>PIPES (p<0.01), CIGS>CHEW (p<0.01), CIGS>SNUFF (p<0.01), CIGS>GUM (p=0.04), CIGS>MISS (p<0.01), PIPES<CGRS (p<0.01), PIPES>CHEW (p=0.03), PIPES>SNUFF (p=0.03), PIPES<MIX (p<0.01), CGRS>CHEW (p<0.01), CGRS>SNUFF (p<0.01), CGRS<GUM (p=0.04), CGRS>MISS (p=0.02), CHEW<MIX (p<0.01), CHEW<MISS (p<0.01), SNUFF<MIX (p<0.01), SNUFF<MISS (p<0.01), MIX>MISS (p=0.04)	NSM<CIGS (p<0.01), NSM<CGRS (p<0.01), NSM>CHEW (p<0.01), NSM>SNUFF (p<0.01), NSM<MIX (p<0.01), CIGS>PIPES (p<0.01), CIGS>CHEW (p<0.01), CIGS>SNUFF (p<0.01), CIGS>GUM (p=0.03), CIGS>MISS (p<0.01), PIPES<CGRS (p<0.01), PIPES<MIX (p<0.01), CGRS>CHEW (p<0.01), CGRS>SNUFF (p<0.01), CGRS>MISS (p<0.01), CHEW<MIX (p<0.01), CHEW<MISS (p<0.01), SNUFF<MIX (p<0.01), SNUFF<MISS (p<0.01), GUM<MIX (p=0.03), MIX>MISS (p<0.01)	NSM<CIGS (p<0.01), NSM<CGRS (p<0.01), NSM<CHEW (p=0.02), NSM<MIX (p=0.03), CIGS>CGRS (p=0.01), CIGS>CHEW (p<0.01), CIGS>MISS (p<0.01), PIPES>CGRS (p<0.01), PIPES>MISS (p<0.01), CGRS>MISS (p=0.04), MIX>MISS (p=0.04)	

Table 4: Unadjusted geometric means with 95% confidence intervals for urinary thiocyanate in ng/mL by smoking and exposure to second hand smoke (SHS) at home status. Data from National Health and Nutrition Examination Survey 2005-2012.

Those aged 20-64 years had statistically significantly higher USCN levels than those who were aged 65+ years old (1390.0 *vs.* 855.9 ng/mL, p<0.01), (Table 5). Males had higher UGMs than females (1385.7 *vs.* 1185.6 ng/mL, p<0.01, Table 5). The order in which UGMs for USCN by race/ethnicity was observed was NHW>NHB>OTH> MA and all

pairwise differences except those between OTH and MA were statistically significant (p<0.01), (Table 5).

Demographic Group	UGM (95% CI)	Statistically Significant Differences
20-64 Years (A20)	1390 (1306.6 - 1478.6)	A20>A65 (p<0.01)
65+Years (A65)	855.9 (795 - 921.5)	
Male (M)	1385.7 (1287.7 - 1491.1)	M>F (p<0.01)
Female (F)	1185.6 (1113 - 1263)	
Non-Hispanic White (NHW)	1426.3 (1344.3 - 1513.3)	NHW>NHB (p<0.01), NHW>MA (p<0.01), NHW>OTH (p<0.01)
Non-Hispanic Black (NHB)	1270.9 (1185.9 - 1361.9)	NHB>MA (p<0.01), NHB>OTH (p<0.01)
Mexican American (MA)	877 (801.3 - 959.8)	
Other race/ethnicities (OTH)	893.1 (806.4 - 989.2)	

Table 5: Model based creatinine unadjusted geometric means with 95% confidence intervals for urinary thiocyanate in ng/mL by age, gender, and race/ethnicity. Data from National Health and Nutrition Examination Survey 2005-2012.

Percentile distributions

Every percentile for those aged 20-64 years was substantially higher than for those who were aged ≥ 65 years. Thus, the distribution for those aged 20-64 years was not only shifted to the right of the distribution for those aged 65+years but also the rightward shift became larger and larger as percentiles moved from lower to higher percentiles. For example, while the difference for the 25th percentile was 217.6 ng/mL, the difference for 95th percentile was 5777.2 ng/mL (Table 6). Or, the skewness of the distribution for those aged 20-64 years was higher than the skewness of the distribution for those who were aged ≥ 65 years. A similar pattern of distribution was observed for percentile distributions of males and females. While for 25th percentile, the distribution of male was to the right of the distribution of females by 238 ng/mL, this difference for 95th percentile was 1027 ng/mL. Except for the 5th percentile, all other percentiles for NHW were higher than the percentiles for NHB and the differences increased with increased percentiles. For example, while the difference for the 25th percentile was 95.9 ng/mL, the difference for 95th percentile was 2880.2 ng/mL (Table 6). Or, the skewness of the distribution for NHB was higher than the skewness of the distribution for NHW. Except for the 95th percentile, all other percentiles were smaller for OTH than for MA.

	Percentile				
Demographic Group	**5th**	**25th**	**Median**	**75th**	**95th**
Total	179.8 (169.3-190.2)	567.6 (540.5-594.6)	1189.2 (1148.2-1230.2)	2738.5 (2566.4-2910.5)	9291.3 (8766.2-9816.4)
20-64 Years (A20)	194.1 (175.5-212.7)	623.3 (585.7-660.9)	1328.2 (1270.6-1385.8)	3109.7 (2912.1-3307.3)	9911.1 (9183.8-10638.5)
65+ Years (A65)	130.3 (112.6-148)	405.7 (381.6-429.7)	787 (728-846.1)	1420 (1308.6-1531.3)	4133.9 (3519.3-4748.5)
Male (M)	228.5 (207.1-249.8)	711.4 (674.2-748.5)	1435.3 (1348.6-1522)	3272.1 (3013-3531.1)	9773 (9078.9-10467.1)
Female (F)	159.4 (144.6-174.2)	473.4 (437.4-509.3)	1009.5 (943.1-1075.9)	2203 (2027.6-2378.4)	8746 (8047.7-9444.3)
Non-Hispanic White (NHW)	204.2 (179.3-229)	612.5 (577.7-647.3)	1280.6 (1212.8-1348.3)	2933 (2734.4-3131.6)	9553.8 (8920.4-10187.3)
Non-Hispanic Black (NHB)	173.1 (139.7-206.5)	708.4 (634.4-782.4)	1498.1 (1406-1590.2)	3560.2 (3111 - 4009.4)	12434 (10928.9-13939.1)
Mexican American (MA)	151.5 (129.6-173.4)	453.5 (420.5-486.5)	878.6 (801-956.1)	1670.6 (1550.7-1790.4)	5009 (4504.9-5513.1)
Other race/ethnicities (OTH)	133.6 (110.9-156.3)	402.6 (363.9-441.3)	800.3 (719.1 - 881.5)	1658.4 (1396.2-1920.6)	6637 (5598.9-7675.1)

Table 6: Selected percentile points with 95% confidence intervals for urinary thiocyanate in ng/mL by age, gender, and race/ethnicity. Data from National Health and Nutrition Examination Survey 2005-2012.

Discussion

Serum cotinine has been traditionally used as a biomarker of exposure to tobacco smoke. In the last few years, the use of urinary NNAL as the biomarkers of exposure to tobacco smoke has been proposed. Serum cotinine has a half-life of about 15 hours and as such is suitable as a biomarker of recent exposure to tobacco smoke. Thus, those who may not have been exposed to tobacco smoke recently but may have been exposed to tobacco smoke prior to the last 3-4 days may be incorrectly classified as nonsmokers based on their serum cotinine levels.

Urinary NNAL, a metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific pulmonary carcinogen [9-12] has been reported to have a half-life of as much as 10-18 days by [13] and from 10 days to three weeks by [14] and as such, is suitable to detect exposure to tobacco smoke for 6-12 weeks after cessation of exposure [13]. Jain [15] used NNAL as an independent biomarker of exposure to tobacco smoke in determining urinary NNAL levels

among several classes of tobacco use statuses and developed cut off points on NNAL scale to differentiate among different classes of exposure to tobacco smoke. For example, author proposed a cut off of 13.4 pg/mL to distinguish smokers from nonsmokers with a sensitivity and specificity of 92%. Jain [16] also proposed cut offs of 0.038 ng/mL, 0.150 ng/mL, 0.036 ng/mL for blood benzene, toluene, and styrene respectively to distinguish smokers from nonsmokers with specificities and sensitivities of 83.6% and 83.7%, 80.0% and 77.8%, and 77.4% and 78.6% respectively.

However, this is the first time that USCN has been investigated as a biomarker of exposure to tobacco smoke based on nationally representative sample of participants.

Variability in specificities and sensitivities

In general, specificities and sensitivities associated with suggested cut off levels to distinguish smokers from nonsmokers for MA were somewhat lower than those for NHW and NHB. For example, for all smokers and nonsmokers, the sensitivities were computed to be 83.9%, 81.5%, and 69.9% for NHW, NHB, and MA respectively. Similarly, for all smokers and nonsmokers, the specificities were computed to be 83.9%, 81.6%, and 70.1% for NHW, NHB, and MA respectively. Relatively lower levels of sensitivities and specificities for MA as compared to NHW and NHB may be due to racial/ethnic differences in accuracy of self-reported smoking status and differences in exposure to thiocyanates from sources other than smoking, for example, diet. In addition, some of MAs may have recently migrated to US from Mexico and thus, may have been exposed to substantially different levels of thiocyanates in Mexico from sources like traffic emissions. All these factors may have contributed to relatively lower levels of specificities and sensitivities for MA when compared with NHW and NHB.

Adequacy and applicability of cut offs

In order for a cut off to be reliably and validly applicable for the population for which it was developed, it should have certain properties. First, it should be developed for a representative sample of the population of interest. For example, for the cut off to be applicable for the entire U.S. population, it should be developed for a representative sample of the U.S. population, or the sample should adequately represent U.S. population as far as the age, racial/ethnic and gender mix of the population is concerned. NHANES data 2005-2010 used to develop cut offs for USCN do meet these requirements. Second, it should be a representative sample of the characteristic of interest for which the cut off is developed. Smoking behavior was the characteristics of interest for this study. If the data from several NHANES cycles are aggregated to develop the cut off, as was done for this study, it is safer to assume that the cut off was developed from an approximately representative sample of smokers and nonsmokers.

Aggregating data from several cycles also provide for changes in characteristic of interest over time. Cut off for the characteristic of interest is developed based on the data for a surrogate marker of the characteristic of interest. USCN was considered to be a surrogate marker for smoking in this study. Unless the sample to be used to develop cut off represents full variability for the surrogate marker as may be found in the target population, cut off is not likely to be sensitive and specific enough to be of use. NHANES data if aggregated over several cycles are likely to meet this requirement. Cut off so developed may be used to classify individuals or for a smaller sample with specific set of properties, for example, a sample of NHB females. Specificities and sensitivities for these subsamples should not always be expected to be as high as they were for the original sample used to develop cut off for the reasons explained in the next paragraph.

Subsamples are usually smaller in sizes; do not necessarily represent the full range of variability for both the characteristic of interest (i. e., smoking) and the surrogate marker (i.e. USCN) that represents the characteristic of interest. For this reason, the observed sensitivities and specificities for the subsamples may be substantially different than those observed for the original sample used to generate the cut off. For this study, we did not apply the developed cut offs for subsamples, for example, for NHB males or males aged 20-64 years; however, we did apply the developed cut offs for sample of sizes substantially smaller (NHANES 2011-2012) then the ones used to generate cut offs (NHANES 2005-2010).

Sample size for NHANES 2011-2012 was about 15% of what it was for NHANES 2005-2010, namely, 1474 *vs.* 10454. In most cases, the observed sensitivities and specificities for NHANES 2011-2012 data were almost as good as they were for NHANES 2005-2010 (Tables 2 *vs.* Table 3) but in some cases, smaller sample sizes of NHANES 2011-2012 did affect the observed specificities and/or sensitivities. For example, for the total population specificity to distinguish smokers from nonsmokers with exposure to SHS was 82.1% for NHANES 2005-2010 data, it was 72% for NHANES 2011-2012 data. However, the confidence interval for this, i. e. 48.6%-95.5% still contained 82.1%. Consequently, the adequacy and applicability of the originally developed cut offs for smaller samples sizes should be based on the confidence intervals, not the point estimates.

Levels of USCN

Ko et al. [7] used data from 2005-2006 NHANES for those aged ≥ 20 years old and reported UGM levels for total population to be 1129 ng/mL, and 1361 ng/mL for males (as compared to 1385.7 ng/mL for this study), 948 ng/mL for females (as compared to 1185.6 ng/mL), 1247 ng/mL for NHW (as compared 1426.3 ng/mL for this study), 1469 ng/mL for NHB (as compared to 1270.9 ng/mL for this study), 714 ng/mL for MA (as compared to 877 ng/mL for this study), and 922 ng/mL for OTH (as compared to 893.1 ng/mL for this study). Some of these data are comparable to what was observed in this study but UGMs for females are substantially higher for this study than what was reported by Ko et al. [7]. Also, while USCN levels were higher for NHW in this study than NHB, the reverse relationship was reported by Ko et al. [7]. Authors investigated the reasons for these discrepancies and found the explanation. Ko et al. [7] computed the UGMs for USCN without making any adjustments for urine creatinine and we were able to almost match their UGMs for 2005-2006 data. However, as has been recommended by [17], we used urine creatinine in the models to adjust for urine creatinine. Barr et al. [17] reported NHB to have substantially higher levels of urine creatinine than NHW and for that reason the direction of differences between NHW and NHB was reversed. This was an accidental finding by us and researchers should use necessary adjustments for the role of urine creatinine beyond dilution. More work with many other chemicals will be needed to study and fully understand the role of variability in urine creatinine levels by age, gender, and race/ethnicity.

Effect of smoking and exposure to SHS on the levels of USCN

Ko et al. [7] reported USCN levels at 4044 ng/mL for current smokers (as compared to 4102.6 ng/mL for this study), 819 ng/mL for

former smokers, and 771 ng/mL for never smokers (as compared to 890.6 ng/mL for this study). These data from the two studies are comparable. Thus, for all smokers and nonsmokers with or without exposure to SHS, USCN levels for smokers were 4.6 times of what they were for nonsmokers.

The contribution of the exposure to SHS towards USCN was also notable. For smokers and nonsmokers with exposure to SHS, USCN levels for smokers were five times of what they were for nonsmokers (5789.7 *vs.* 1160.9 ng/mL). For smokers and nonsmokers without exposure to SHS, USCN levels for smokers were 3.5 times of what they were for nonsmokers (3049.0 *vs.* 874.2 ng/mL). Based on NHANES 2005-2008 data, Jain [6] reported smokers to have USCN levels that were six times of what they were for nonsmokers (4712.76 *vs.* 769.86 ng/mL) among females aged 15-44 years.

Use of self-reported smoking status

ROC analysis uses a floating point on a continuous scale to convert a continuous measure into a binary measure usually labeled as positive and negative and compares these percent positives and negatives with the percent positives and negatives obtained by a confirmatory test. For example, optical density is used as a floating point in an ELISA test to assess the presence of HIV virus. The accuracy of which can then be assessed by Western Blot test for HIV virus. The confirmatory test is usually called the Gold Standard. In the present context, urinary thiocyanate as a continuous measure is like the optical density measured on an ELISA test.

However, a Gold Standard to differentiate smokers from nonsmokers does not exist. Instead, self-reported smoking status is used as a Gold Standard. Self-reported smoking status is subject to error of reporting and may lead to underestimation of the prevalence of smoking. For this reason, cut off points reported here are subject to the error of self-reporting. Actual sensitivity associated with the specific cut off points reported here may be somewhat lower and specificity may be somewhat higher than reported here. However, Benowitz et al. [18] also used self-reported smoking status as a pseudo-Gold Standard to develop revised serum cotinine cut offs by age, gender, and race/ethnicity.

As also reported [18,19], the smoking questionnaire used to self-report smoking status in NHANES is administered in a Mobile Examination Center in a private and nonjudgmental environment where NHANES participants aged ≥ 20 years, respond to trained interviewer who use questions from a Computer Assisted personal Interviewing System (http://wwwn.cdc.gov/nchs/nhanes/2011-2012/SMQRTU_G.htm#Component_Description). Hence, while misreporting of smoking status cannot be eliminated, it is minimized.

Summary and Conclusion

It should be noted that environmental exposure to cyanides and thiocyanates also lead to thiocyanate in the urine. Sources of exposure to cyanides and/or thiocyanates include traffic emissions, from consuming foods such as almonds, nuts, pulses and cruciferous vegetables, for example, brassica, cabbage, and broccoli etc., and endogenous generation in the colon by bacteria. They can all act as potential confounders. It is unknown to what degree, this may have affected the observed levels of SCN in urine and as such the cut offs developed in this study. It should also be realized that the use of USCN as a biomarker of tobacco smoke as proposed in this study is not intended to replace other biomarkers of tobacco smoke, for example, serum cotinine and urinary NNAL, but as an alternate and/or concurrent biomarker of tobacco smoke.

In summary, (i) a cut off of 1840 ng/mL for USCN was able to distinguish smokers from nonsmokers with a sensitivity of 80.7% and a specificity of 80.8%, (ii) a cut off of 2630 ng/mL for USCN was able to distinguish smokers from nonsmokers with exposure to SHS at home with a sensitivity of 82.0% and a specificity of 82.1%, (iii) a cut off of 1550 ng/mL for USCN was able to distinguish smokers from nonsmokers without exposure to SHS at home with a sensitivity of 75.7% and a specificity of 75.6%, (iv) USCN levels for smokers were 4.6 times of what they were for nonsmokers, (v) those aged 20-64 years had higher levels of USCN than those aged 65+ years ($p<0.01$), (vi) males had higher levels of USCN than females ($p<0.01$), and (vii) order of USCN levels by race/ethnicity was NHW>NHB>OTH>MA.

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