HHS Public Access

Author manuscript

Biomarkers. Author manuscript; available in PMC 2024 August 14.

Published in final edited form as:

Biomarkers. 2023 December ; 28(7): 637–642. doi:10.1080/1354750X.2023.2276030.

Smoke Exposure Associated with Higher Urinary Benzene Biomarker Muconic Acid (MUCA) in Golestan Cohort Study Participants

Deepak Bhandari[1,*], **Yuyang Zhu**[1], **Can Zhang**[1], **Wanzhe Zhu**[1], **Apostolos Alexandridis**[2], **Arash Etemadi**[3,4], **Neal D. Freedman**[3], **Cindy Chang**[2], **Christian C. Abnet**[3], **Sanford M. Dawsey**[3], **Maki Inoue-Choi**[3], **Hossein Poustchi**[4], **Akram Pourshams**[4], **Paolo Boffetta**[5,6], **Reza Malekzadeh**[4], **Benjamin Blount**[1]

[1]Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Tobacco and Volatiles Branch, Atlanta, GA 30341

[2]Center for Tobacco Products, Food and Drug Administration, Silver Spring, MD 20993, USA

[3]Metabolic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

[4]Digestive Oncology Research Center, Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran 1411713135

[5]Stony Brook Cancer Center, Stony Brook University, Stony Brook, NY 11794, USA

[6]Department of Medical and Surgical Sciences, University of Bologna, 40138 Bologna, Italy

Abstract

Background.—Benzene is a known human carcinogen. Human exposure to benzene can be assessed by measuring *trans,trans*-muconic acid (MUCA) in urine. Golestan Province in northeastern Iran has been reported to have high incidence of esophageal cancer linked to the use of tobacco products. This manuscript evaluates the urinary MUCA concentrations among the participants of the Golestan Cohort Study (GCS).

Methods.—We analyzed MUCA concentration in 177 GCS participants' urine samples and performed nonparametric pairwise multiple comparisons to determine statistically significant difference among six different product use groups. Mixed effects model was fitted on 22 participants who exclusively smoked cigarette and 51 participants who were classified as nonusers. The urinary MUCA data were collected at the baseline and approximately five years later, and intraclass correlation coefficient (ICC) was calculated from the model.

[*]Corresponding Author: Deepak Bhandari, PhD, Centers for Disease Control and Prevention, National Center for Environmental Health, Division of Laboratory Sciences, Tobacco and Volatiles Branch, Atlanta, GA 30341, dbhandari@cdc.gov.

Disclosure statement

The authors report no declarations of interest.

Results.—Compared with nonusers, tobacco smoking was associated with higher urinary MUCA concentrations. Based on the nonparametric test of pairwise multiple comparisons, MUCA concentrations among participants who smoked combusted tobacco products were statistically significantly higher compared to nonusers. Urinary MUCA collected five years apart from the same individuals showed moderate reliability (ICC = 0.41), which was expected given the relatively short half-life (~6 hrs) of MUCA.

Conclusion.—Our study revealed that tobacco smoke was positively associated with increased levels of urinary MUCA concentration, indicating that it is a significant source of benzene exposure among GCS participants.

Keywords

trans,trans-muconic Acid; benzene exposure; Golestan Cohort Study; tobacco smoke exposure

Introduction

Benzene is a colorless and highly volatile liquid (CDC, 2007). It is among the top 20 most widely used chemicals in the United States (CDC, ACS). As a natural constituent of crude petroleum, human activities related to some petroleum products can lead to benzene exposure in humans. Benzene is also present in indoor air from building materials and tobacco smoke (WHO, 2010). Smoking 14 cigarettes a day (average for US daily smokers) leads to inhalation of ~790 μg of benzene per day (CDC, 2007, CDC, 2018). Exposure to benzene has been linked with acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin lymphoma (IARC, 2018). Benzene is listed as a group 1 human carcinogen by the International Agency for Research on Cancer (IARC, 2012). It is also listed in the U.S. Food and Drug Administration's established list of Harmful and Potentially Harmful Constituents in Tobacco Products and Smoke (FDA, 2012). Numerous approaches have been implemented to lower benzene exposure in humans, such as by decreasing benzene content in gasoline, reducing automobile emissions, removing benzene from consumer products, and strict tobacco smoke regulation (IARC, 2018, Arnold et al., 2013).

Human exposure to benzene can be monitored by measuring benzene or its urinary metabolites in biofluids (Weisel, 2010). Benzene is primarily metabolized in the liver by cytochrome P450E1 (CYP2E1) to benzene oxide. Benzene oxide undergoes various metabolic pathways to form catechol, hydroquionone, phenylmercapturic acid (PhMA), and *trans*,*trans*-muconic acid (MUCA) (Snyder et al., 1996) (Lau et al., 2010). In particular, MUCA is formed via ring cleavage of benzene oxide to *trans*,*trans*-muconaldehyde, which is further oxidized to MUCA. Urinary MUCA is an effective biomarker for monitoring occupational benzene exposure (Lauwerys, Buchet and Andrien, 1994, Koh, Lee, Chung, Jang and Park, 2018, Chaiklieng, Suggaravetsiri, Kaminski and Autrup, 2019, 2021), as well as benzene exposure from tobacco smoke (Wiwanitkit, Suwansaksri and Soogarun, 2005, Bhandari, McCarthy, Biren, Movassaghi, Blount and De Jesús, 2019, Melikian, Prahalad and Secker-Walker, 1994), although urinary MUCA can also result from ingesting foods containing sorbic acid, one of the most commonly used food preservatives (Feng et al., 2006, Weaver, Buckley and Groopman, 2000).

In this study, we aimed to assess benzene exposure among selected Golestan Cohort Study (GCS) participants by measuring urinary MUCA levels. The GCS is a population-based longitudinal cohort study in the Golestan province in northeastern Iran (Etemadi et al., 2017). The people living in the Golestan Province have an increased risk of overall cancer mortality, including esophageal cancer, associated with cigarette smoking, waterpipe smoking, and smokeless tobacco, nass, use (Etemadi et al., 2017, Pourshams, Khademi, Malekshah, Islami, Nouraei, Sadjadi, Jafari, Rakhshani, Salahi, Semnani, Kamangar, Abnet, Ponder, Day, Dawsey, Boffetta and Malekzadeh, 2010). Therefore, we measured MUCA in urine collected from cancer-free study participants in December 2016 and stratify these results by tobacco smoke exposure.

Materials and methods

Study design

The GCS is a longitudinal study that involves 50,045 individuals living in Golestan Province, Northeast Iran. Baseline urine samples were collected from all 50,045 participants at the enrollment time. Additional urine samples were collected approximately five years later from a subset of 11,418 enrolled participants. Detailed self-reported tobacco use, and other demographic and lifestyle information were collected via questionnaire at both urine sample collection visits. This study was approved by the appropriate ethics committees at Tehran University of Medical Sciences, the U.S. National Cancer Institute NCI, and the International Agency for Research on Cancer (IARC). The involvement of the CDC laboratory did not constitute an engagement in human subjects research.

From 2004 to 2008, this cohort study recruited participants between 40 and 75 years old. Among the participants of GCS who were both alive and cancer-free in December 2016, a total of 225 individuals were randomly chosen. These selected participants were then divided into six groups according to the information they provided about their tobacco use during enrollment(Etemadi et al., 2019, Etemadi et al., 2020). One hundred seventy seven of these individuals had available urine samples for the current analysis; 27 exclusively smoked cigarette, 31 exclusively smoked hookah, 18 exclusively used nass, 25 exclusively used opium, 22 dual used cigarette & opium, and 54 never used any tobacco product during their lifetime (nonusers), (Table 1). Among 177 baseline participants, additional urine samples were collected from 73 participants (22 who exclusively smoked cigarette, and 51 never users of any tobacco product) approximately five years later.

Measurement of urinary MUCA and creatinine

Urine samples from the GCS were stored at −70 °C freezer prior the analysis. The samples were thawed at room temperature and analyzed for urinary MUCA concentration using ultrahigh-performance liquid chromatography (UPLC; I-Classic Acquity, Waters Inc., Milford, MA) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS; Sciex 5500 Triple quad, Sciex, Framingham, MA) according to the method published elsewhere (Bhandari, McCarthy, Biren, Movassaghi, Blount and De Jesús, 2019). Chromatographic separation was achieved using Waters HSS PFP, 1.8 μm, 2.1 mm × 100 mm column (Waters Inc., Milford, MA) with a Waters HSS-PFP VanGuard

precolumn (Waters Inc., Milford, MA). The mass spectrometer was operated in negative ion ESI scheduled multiple reaction monitoring mode. MUCA was monitored using ion transitions *m/z* 141.1→97.1 (quantifier), *m/z* 141.1→53.2 (qualifier), and *m/z* 147→102 (MUCA-[$^{13}C_6$], internal standard). Sample concentrations were determined based on its relative response ratio (ratio of native analyte to stable isotope-labeled internal standard) against a calibration curve with known standard concentrations. The internal standard concentration, i.e., 7.5 ng/mL, was kept constant for both calibrants and sample vials. The limit of detection (LOD) was 1.20 ng/mL for MUCA. Urinary creatinine was measured using Enzymatic Roche Cobas 6000 Analyzer with a LOD of 1.1 mg/dL, and a detailed description of the laboratory procedure can be found elsewhere (CDC, 2014).

Statistical analysis

SAS statistical software application version 9.4 (SAS Institute, Cary, NC) was used to carry out the statistical analyses. Urinary MUCA concentration was reported in 82.3% of the urine samples analyzed. All data that did not meet our quality control standards were excluded from the analysis. In addition, data calculated below the LOD were imputed as LOD divided by the square root of 2. Descriptive statistics including median (and 25th and 75th percentiles) and geometric mean (and 95% confidence interval of the geometric mean) of urinary MUCA concentrations (ng/mL) and creatinine-adjusted urinary MUCA (μg/g creatinine) by tobacco or opium use were summarized. Given the small subgroup sizes, normality assumptions might not hold for all groups. If the data were normally distributed, then analysis of variance (ANOVA) would be used to evaluate whether geometric mean MUCA concentrations were equal across all groups. When normality assumptions did not hold even after log transformation, Kruskal-Wallis test, a nonparametric test, would be used to evaluate whether median MUCA concentrations were equal across groups. If the null hypothesis of the Kruskal-Wallis test was rejected, then Dunn's test was used to determine which use groups were different from each other. Kruskal-Wallis test and Dunn's test were performed for urinary MUCA concentration as well as creatinine-adjusted MUCA level.

We combined the urinary MUCA concentration of the baseline samples with the second urine samples collected after, on average, five years for participants in exclusive cigarette use and nonusers groups. Since the normality assumption holds after log-transformation, log-transformed, creatinine-adjusted data were used to calculate intraclass correlation coefficients (ICCs) for each group. We also estimated the association between MUCA level and smoking status among participants who exclusively smoked cigarettes and nonusers by another mixed model, using nonusers as a reference group.

Results

Table 2 shows the summary descriptive statistics including median with the interquartile range and geometric mean with its 95% confidence interval. Summary statistics were categorized by tobacco product and opium use status. The dual use of cigarettes & opium resulted in the highest median urinary MUCA concentration at 117 ng/mL, while exclusive cigarette use had a median concentration of 81.6 ng/mL. Exclusive hookah use had a median

concentration of 77.1 ng/mL, followed by exclusive nass use at 54.6 ng/mL. Nonusers had the lowest median concentration at 41.6 ng/mL.

To adjust for hydration, we also summarized the creatinine-adjusted MUCA level in Table 2. Median MUCA levels were 112 μg/g creatinine for dual cigarette & opium use, 82.4 μg/for exclusive hookah use, 65.6 μg/g for exclusive cigarette use, 42.1 μg/g for exclusive opium use, 40.6 μg/g for exclusive nass use, and 30.2 μg/g for nonusers. As normality assumptions were not met for any tobacco or opium user group, nonparametric tests were conducted to compare MUCA concentrations between groups. Our analysis using the Kruskal-Wallis test revealed significant differences in MUCA concentrations among certain groups ($p <$ .0001). To further investigate these differences, we conducted a Dunn's test, a nonparametric pairwise multiple comparison test (Dinno et al., 2015). The statistical significance of the Dunn's test at 0.05 significance level was determined to be 0.0033 (n = 6, r = 2). Results from Dunn's test found higher urinary MUCA among exclusive cigarette, exclusive hookah, and cigarette & opium dual use groups ($p \leq 0.00013$) compared to nonusers (Table 3a). In addition, exclusive nass use was associated with lower MUCA ($p = 0.00242$) compared to cigarette & opium dual use. When the test was performed for creatinine-adjusted MUCA level (Table 3b), exclusive cigarette, exclusive hookah, and cigarette & opium dual use groups again exhibited higher urinary MUCA compared to nonusers ($p < 0.003$). In addition, the use of nass only and opium only had statistically lower MUCA ($p \leq 0.00125$) compared to the use of hookah only and cigarette & opium dual use.

We also fitted mixed effects models on baseline urine samples and samples collected after five years for 73 selected participants. The selected participants were either nonusers (N = 51) or individuals who exclusively smoked cigarettes (N = 22). The results indicated exclusive cigarette use was associated with 111% higher creatinine-adjusted MUCA levels than in the nonuser group. The intraclass correlation coefficients (ICC) between baseline samples and the second sample sets for creatinine-adjusted MUCA were 0.41, 0.40, and 0.12 for overall, exclusive cigarette, and nonuser groups, respectively.

Discussion

This report characterized the urinary concentration of the benzene metabolite, MUCA, in Golestan Cohort Study (GCS) participants of Golestan province, Iran. Of the six groups of participants, tobacco smoking (cigarette & opium, exclusive cigarette, and exclusive hookah) was associated with higher median MUCA levels compared to other groups. More specifically, participants who used both cigarette & opium had the highest median urinary MUCA concentration, which is attributed to the multiple sources of smoke exposure (cigarette smoke and opium smoke) and the higher consumption rate in the former, as explained by Etemadi et al. elsewhere (Etemadi et al., 2019, Etemadi et al., 2020). Etemadi et al. reported that the use of both cigarettes & opium in the GCS study was associated with a higher concentration of VOC biomarkers compared to the use of either exclusive cigarettes or opium(Etemadi et al., 2019, Etemadi et al., 2020). While the study did not particularly look into urinary MUCA, the reported analytes included 2-cyanoethyl mercapturic acid (2CyEMA), a smoke exposure biomarker for smoking status classification (Bhandari, Zhang, Zhu, De Jesús and Blount, 2022). The study found that the dual use of

cigarettes & opium resulted in more than two-fold higher urinary 2CyEMA concentration than exclusive cigarette use (190.6 vs 86.4 ug/g creatinine), indicating that smoke exposure was relatively high in the dual use group.

Also, we performed nonparametric pairwise multiple comparisons among six different groups using Dunn's test. The Dunn test was useful for determining the statistical significance between the participant groups. The multiple pairwise comparisons of median urinary MUCA concentration showed that the use of combusted tobacco products (cigarette & opium, cigarette, and hookah) had significantly higher MUCA levels compared to nonusers (Figure 1a). Neither exclusive opium nor exclusive nass use groups were significantly different from nonusers indicating that tobacco smoke is the primary source of urinary MUCA in GCS participants. Similar to the urinary MUCA concentration, the use of smoked tobacco had statistically higher creatinine adjusted MUCA level compared to nonusers. Furthermore, those who exclusively smoked hookah had statistically higher creatine adjusted MUCA level compared to the opium only or nass only groups. This result is anticipated because hookah smoke is one of the primary sources of benzene exposure and exclusive hookah users in the GCS study were predominantly female (Table 1). Connell et al., reported that creatinine excretion was 55% higher in men than women (Connell et al., 1994). Therefore, the exclusive hookah group, which was predominantly female, would have higher creatinine adjusted MUCA concentration compared to groups where the products were predominantly or exclusively used by males (Table 1). Additionally, creatinine-adjusted MUCA levels were significantly higher with dual use of cigarette & opium compared to either opium only or nass only use (Table 3b).

Overall, participants using combusted tobacco had higher urinary MUCA levels compared with nonusers. Also, we had 73 participants whose urine samples were collected on average five years after the baseline sample collection. We performed linear regression analysis of creatinine-adjusted MUCA for exclusive cigarette and nonuser groups and observed 111% higher urinary MUCA level in participants who exclusively smoked cigarettes. This indicates that the use of combusted tobacco results in higher benzene exposure compared to nonusers. In addition, the calculated ICC based on creatinine-adjusted MUCA level was 0.41 for the exclusive cigarette use group. The ICC is the ratio of between-person variance to the total variance and ranges from 0 to 1, with values nearer to 0 signifying poor reproducibility and closer to 1 signifying excellent reproducibility. The low ICC for participants who exclusively smoked cigarettes, regardless of the relatively higher MUCA level, was due to the high within-individual variation calculated from the two samples collected several years apart. Many factors affect within-individual urinary MUCA levels, including changes in cigarette smoking frequency and smoking behavior in the last five years. Also, because of the relatively short half-life of urinary MUCA (~6 hours) (Jalai, 2017), the time of the spot sample collection and the interval between the smoking events can affect MUCA levels. Finally, urinary MUCA is also derived from episodic ingestion of sorbic acid preserved food (Weaver, Buckley and Groopman, 2000).

Conclusions

This study assessed benzene exposure in selected GCS participants based on the analysis of its urinary biomarker MUCA. Tobacco smoke (cigarette, hookah, and cigarette & opium) was a significant source of benzene exposure in the Golestan population and was associated with higher MUCA levels when compared with nonusers. This report demonstrated important biomonitoring data associated with benzene exposure in the Golestan Province of Iran, an area known for its elevated cancer mortality rates. This study characterizes benzene exposure in the targeted populations and thereby improves understanding of potential health harm and advises public health management efforts to mitigate these risks.

References

CDC. (2007). Agency for Toxic Substances and Disease Registry. Toxicological Profile for Benzene. https://www.atsdr.cdc.gov/ToxProfiles/tp3.pdf.

CDC. Facts About Benzene. https://emergency.cdc.gov/agent/benzene/basics/facts.asp. In: Editior, editorêditors.

ACS. American Cancer Society. Benzene and Cancer Risk. https://www.cancer.org/cancer/cancer-causes/benzene.html.

WHO. (2010). World Health Organization. Exposure to Benzene: A Major Public Health Concern. https://www.who.int/ipcs/features/benzene.pdf.

CDC. (2018). Smoking is down, but almost 38 million American adults still smoke. In: Editior, editorêditors.

IARC. (2018). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Benzene, Volume 120. In: Editior, editorêditors. Lyon, France, 105–74.

IARC. (2012). Chemical Agents and Related Occupations. A Review of Human Carcinogens. Chemical Agents and Related Occupations A Review of Human Carcinogens 100 F:249–94.

FDA. (2012). US Food and Drug Administration. Harmful and potentially harmful constituents in tobacco products and tobacco smoke: established list. https://www.fda.gov/tobacco-products/rules-regulations-and-guidance/harmful-and-potentially-harmful-constituents-tobacco-products-and-tobacco-smoke-established-list.

Arnold et al. (2013). The use of biomonitoring data in exposure and human health risk assessment: benzene case study. Crit Rev Toxicol 43:119–53. [PubMed: 23346981]

Weisel. (2010). Benzene exposure: an overview of monitoring methods and their findings. Chem Biol Interact 184:58–66. [PubMed: 20056112]

Snyder et al. (1996). An overview of benzene metabolism. Environ Health Perspect 104 Suppl 6:1165–71. [PubMed: 9118888]

Lau et al. (2010). Role of hydroquinone-thiol conjugates in benzene-mediated toxicity. Chem Biol Interact 184:212–7. [PubMed: 20034486]

Lauwerys RR, Buchet JP and Andrien F. (1994). Muconic acid in urine: a reliable indicator of occupational exposure to benzene. Am J Ind Med 25:297–300. [PubMed: 8147402]

Koh DH, Lee MY, Chung EK, Jang JK and Park DU. (2018). Comparison of personal air benzene and urine t, t-muconic acid as a benzene exposure surrogate during turnaround maintenance in petrochemical plants. Ind Health 56:346–55. [PubMed: 29643270]

Chaiklieng S, Suggaravetsiri P, Kaminski N and Autrup H. (2019). Factors Affecting Urinary tt-Muconic Acid Detection among Benzene Exposed Workers at Gasoline Stations. Int J Environ Res Public Health 16.

(2021). Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Wiwanitkit V, Suwansaksri J and Soogarun S. (2005). Monitoring of urine trans,trans-muconic acid level among smokers and non-smokers. Respir Med 99:788–91. [PubMed: 15878497]

Bhandari D, McCarthy D, Biren C, Movassaghi C, Blount BC and De Jesús VR. (2019). Development of a UPLC-ESI-MS/MS method to measure urinary metabolites of selected VOCs: Benzene, cyanide, furfural, furfuryl alcohol, 5-hydroxymethylfurfural, and N-methyl-2-pyrrolidone. J Chromatogr B Analyt Technol Biomed Life Sci 1126–1127:121746.

Melikian AA, Prahalad AK and Secker-Walker RH. (1994). Comparison of the levels of the urinary benzene metabolite trans,trans-muconic acid in smokers and nonsmokers, and the effects of pregnancy. Cancer Epidemiol Biomarkers Prev 3:239–44. [PubMed: 8019374]

Feng et al. (2006). Evaluation of Urinary 1-Hydroxypyrene, Sphenylmercapturic Acid, *trans*,*trans*-Muconic Acid, 3-Methyladenine, 3-Ethyladenine, 8-Hydroxy-2′-Deoxyguanosine and Thioethers as Biomarkers of Exposure to Cigarette Smoke. Biomarkers 11:28–52. [PubMed: 16484135]

Weaver VM, Buckley T and Groopman JD. (2000). Lack of specificity of trans,trans-muconic acid as a benzene biomarker after ingestion of sorbic acid-preserved foods. Cancer Epidemiol Biomarkers Prev 9:749–55. [PubMed: 10919747]

Etemadi et al. (2017). Hazards of cigarettes, smokeless tobacco and waterpipe in a Middle Eastern Population: a Cohort Study of 50 000 individuals from Iran. Tob Control 26:674–82. [PubMed: 27872345]

Pourshams A, Khademi H, Malekshah AF, Islami F, Nouraei M, Sadjadi AR, Jafari E, Rakhshani N, Salahi R, Semnani S, Kamangar F, Abnet CC, Ponder B, Day N, Dawsey SM, Boffetta P and Malekzadeh R. (2010). Cohort Profile: The Golestan Cohort Study--a prospective study of oesophageal cancer in northern Iran. Int J Epidemiol 39:52–9. [PubMed: 19332502]

Etemadi et al. (2019). Urinary Biomarkers of Carcinogenic Exposure among Cigarette, Waterpipe, and Smokeless Tobacco Users and Never Users of Tobacco in the Golestan Cohort Study. Cancer Epidemiol Biomarkers Prev 28:337–47. [PubMed: 30622099]

Etemadi et al. (2020). Opiate and Tobacco Use and Exposure to Carcinogens and Toxicants in the Golestan Cohort Study. Cancer Epidemiology Biomarkers & Prevention 29:650–58.

CDC. (2014). Urine Creatinine: Laboratory Procedure Manual. In: Editior, editorêditors. cdc.gov: Centers for Disease Control and Prevention

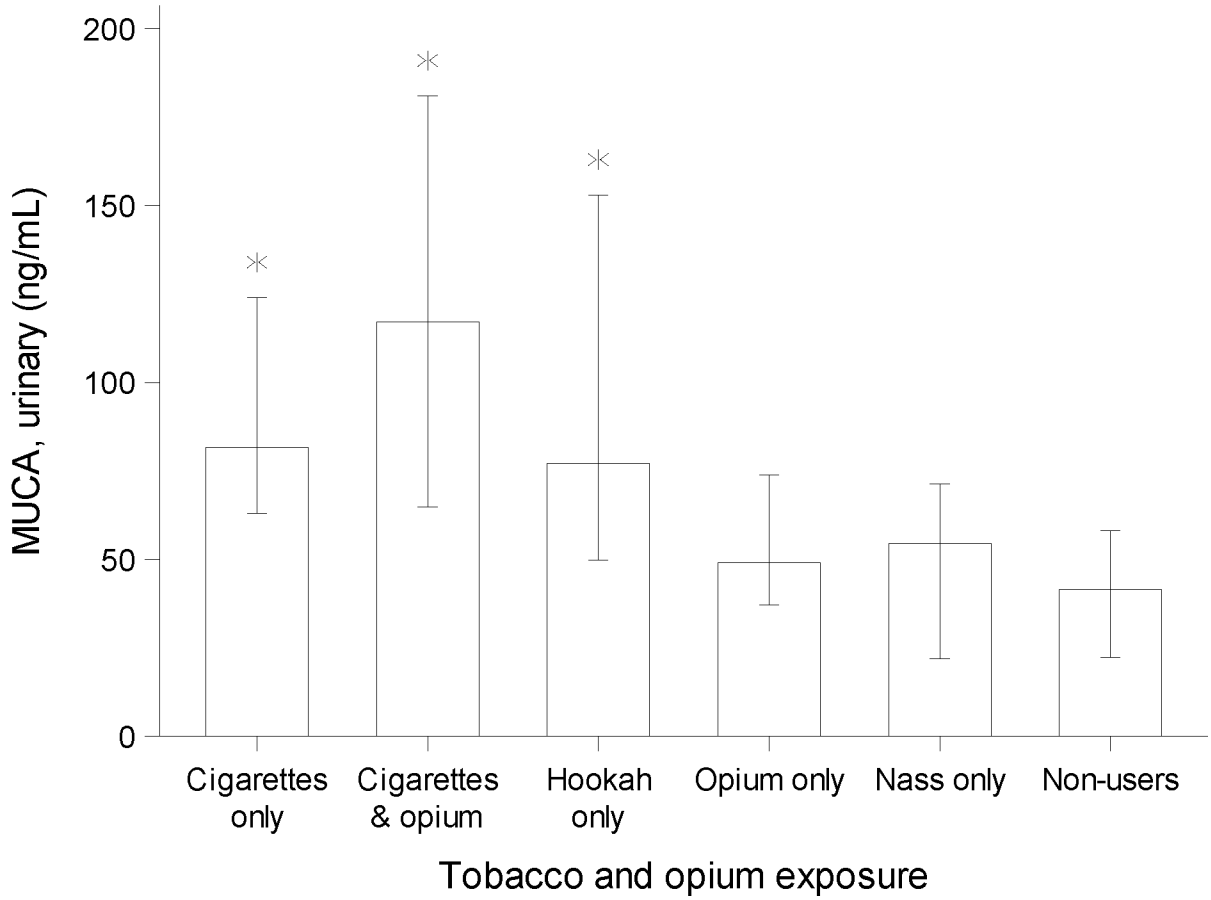
Dinno et al. (2015). Nonparametric Pairwise Multiple Comparisons in Independent Groups using Dunn's Test. The Stata Journal 15:292–300.

Bhandari D, Zhang L, Zhu W, De Jesús VR and Blount BC. (2022). Optimal Cutoff Concentration of Urinary Cyanoethyl Mercapturic Acid for Differentiating Cigarette Smokers From Nonsmokers. Nicotine and Tobacco Research 24:761–67. [PubMed: 34718769]

Connell et al. (1994). Gender and the clinical usefulness of the albumin: creatinine ratio. Diabet Med 11:32–6. [PubMed: 8181249]

Aea Jalai. (2017). Urinary Trans,Trans-Muconic Acid is Not a Reliable Biomarker for Low-level Environmental and Occupational Benzene Exposures. Saf Health Work 8:220–25. [PubMed: 28593080]

a)



b)

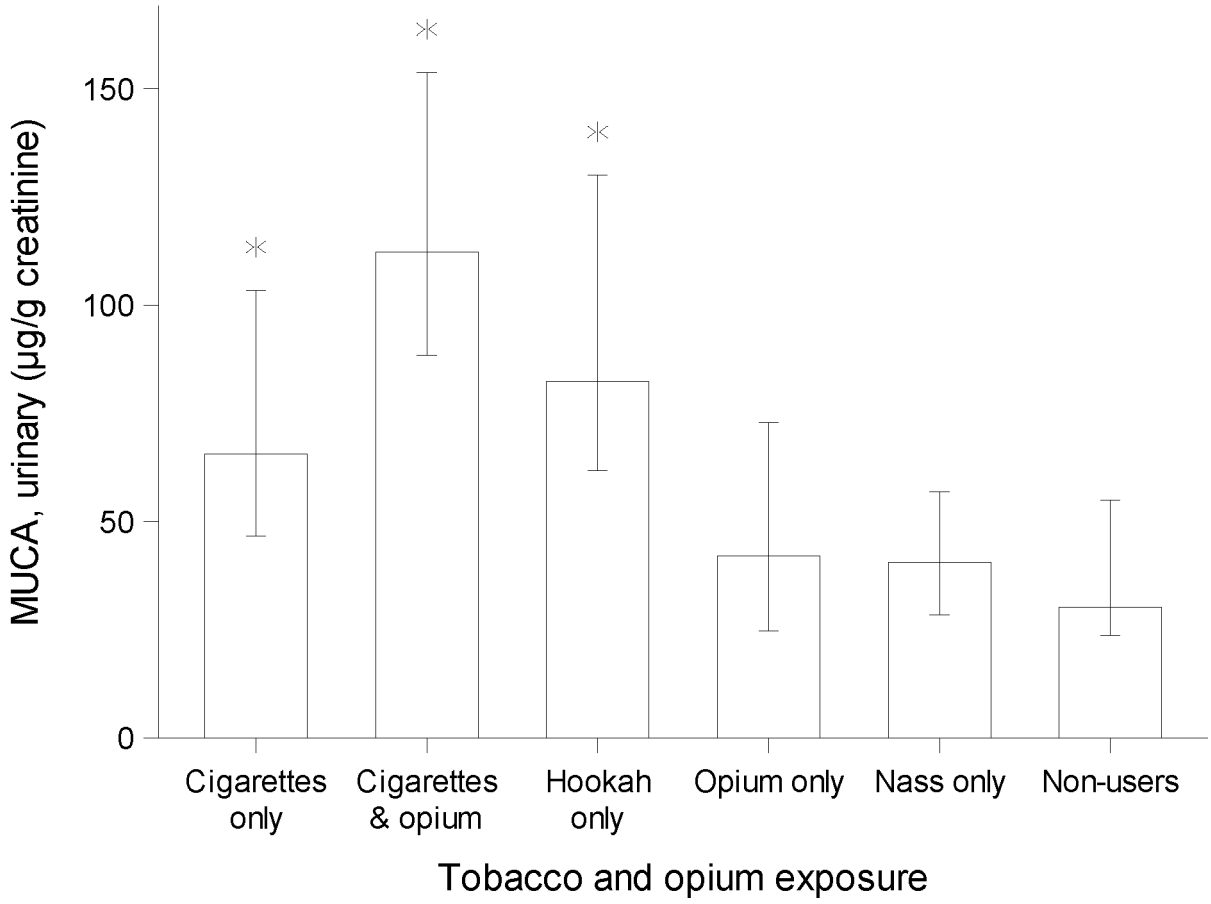


Figure 1.

Bar charts of six different groups. a) urinary MUCA distribution; b) MUCA-creat distribution. Bar charts represent the median and whiskers represent 25th and 75th percentiles. Significant differences between smoking and nonuser groups are indicated by *. * p <0.0033.

Table 1.

Participants information by users' group. Cigarette smoking in GCS is almost exclusive to male participants (Etemadi et al., 2019, Etemadi et al., 2020).

Users' Group	Gender	
	Male	Female
Overall	121	56
Cigarette only	27	0
Cigarette & opium	22	0
Hookah only	4	27
Opium only	25	0
Nass only	15	3
Nonusers	28	26

Table 2.

Urinary MUCA median [25th, 75th percentile] concentrations (ng/mL), and creatinine adjusted urinary MUCA median [25th, 75th percentile] concentrations (μg/g creatinine) categorized by tobacco and opium use status among subset of GCS participants, 2004–2008 (n = 177).

Group	Cigarette Only			Cigarette & Opium			Hookah Only			Opium Only			Nass Only			Nonusers		
	N	Median [25th %ile, 75th %ile]	GM [95%CI]	N	Median [25th %ile, 75th %ile]	GM [95%CI]	N	Median [25th %ile, 75th %ile]	GM [95%CI]	N	Median [25th %ile, 75th %ile]	GM [95%CI]	N	Median [25th %ile, 75th %ile]	GM [95%CI]	N	Median [25th %ile, 75th %ile]	GM [95%CI]
MUCA	27	81.6 [63.1, 124]	90.9 [70.5, 117]	22	117 [64.9, 181]	106 [77.8, 144]	31	77.1 [49.8, 153]	76.1 [57.0, 102]	25	49.1 [37.1, 73.8]	54.4 [39.9, 74.1]	18	54.6 [22.0, 71.4]	49.6 [31.3, 78.6]	54	41.6 [22.3, 58.1]	39.5 [33.0, 47.4]
Creatinine adjusted MUCA		65.6 [46.6, 103]	73.4 [57.5, 93.7]		112 [88.3, 154]	107 [85.7, 133]		82.4 [61.7, 130]	92.3 [72.3, 118]		42.1 [24.7, 72.8]	44.3 [30.8, 63.9]		40.6 [28.4, 56.8]	44.7 [33.9, 58.9]		30.2 [23.7, 54.8]	36.2 [31.1, 42.3]

Table 3a

Pairwise comparisons of median MUCA concentrations.

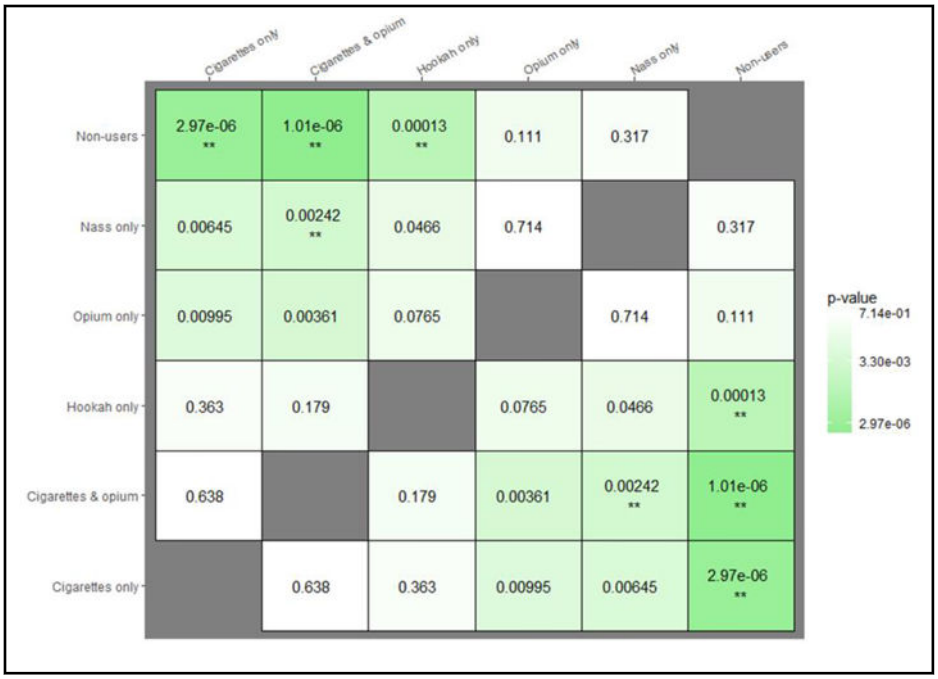


Table 3b.

Pairwise comparisons of median creatinine-ratioed MUCA levels