Research Article

Cancer Epidemiology, Biomarkers & Prevention

Urinary Biomarkers of Carcinogenic Exposure among Cigarette, Waterpipe, and Smokeless Tobacco Users and Never Users of Tobacco in the Golestan Cohort Study

Arash Etemadi[1,2], Hossein Poustchi[2,3], Cindy M. Chang[4], Benjamin C. Blount[5], Antonia M. Calafat[5], Lanqing Wang[5], Victor R. De Jesus[5], Akram Pourshams[2], Ramin Shakeri[2], Meredith S. Shiels[1], Maki Inoue-Choi[1], Bridget K. Ambrose[4], Carol H. Christensen[4], Baoguang Wang[4], Gwen Murphy[1], Xiaoyun Ye[5], Deepak Bhandari[5], Jun Feng[5], Baoyun Xia[5], Connie S. Sosnoff[5], Farin Kamangar[6], Paul Brennan[7], Paolo Boffetta[8], Sanford M. Dawsey[1], Christian C. Abnet[1], Reza Malekzadeh[2], and Neal D. Freedman[1]

Abstract

Background: How carcinogen exposure varies across users of different, particularly noncigarette, tobacco products remains poorly understood.

Methods: We randomly selected 165 participants of the Golestan Cohort Study from northeastern Iran: 60 never users of any tobacco, 35 exclusive cigarette, 40 exclusive (78% daily) waterpipe, and 30 exclusive smokeless tobacco (nass) users. We measured concentrations of 39 biomarkers of exposure in 4 chemical classes in baseline urine samples: tobacco alkaloids, tobacco-specific nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), and volatile organic compounds (VOC). We also quantified the same biomarkers in a second urine sample, obtained 5 years later, among continuing cigarette smokers and never tobacco users.

Results: Nass users had the highest concentrations of tobacco alkaloids. All tobacco users had elevated TSNA concentrations, which correlated with nicotine dose. In both cigarette and waterpipe smokers, PAH and VOC biomarkers were higher than never tobacco users and nass users, and highly correlated with nicotine dose. PAH biomarkers of phenanthrene and pyrene and two VOC metabolites (phenylmercapturic acid and phenylglyoxylic acid) were higher in waterpipe smokers than in all other groups. PAH biomarkers among Golestan never tobacco users were comparable to those in U.S. cigarette smokers. All biomarkers had moderate to good correlations over 5 years, particularly in continuing cigarette smokers.

Conclusions: We observed two patterns of exposure biomarkers that differentiated the use of the combustible products (cigarettes and waterpipe) from the smokeless product. Environmental exposure from nontobacco sources appeared to contribute to the presence of high levels of PAH metabolites in the Golestan Cohort.

Impact: Most of these biomarkers would be useful for exposure assessment in a longitudinal study.

Introduction

In recent years, there has been an increasing trend of popularity for noncigarette forms of tobacco, particularly among young people (1, 2). Noncigarette tobacco products come in many different forms and preparations. Waterpipe (also known as hookah, shisha, hubbly bubbly, narghile, or qualyan) is a global concern, with high rates of use in the Middle East and North Africa as well as in young people in the USA, Europe, and elsewhere (3). Different forms of smokeless tobacco are used globally by over 300 million people, especially in South Asia (4). One of these products is nass, sometimes known as naswar, a chewable mixture of tobacco, ash, and slaked lime that is commonly used in South and Central Asia and the former Soviet Union. Although causally

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland. [2]Digestive Oncology Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran. [3]Liver and Pancreaticobiliary Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran. [4]Center for Tobacco Products, Food and Drug Administration, Silver Spring, Maryland. [5]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia. [6]Department of Biology, School of Computer, Mathematical, and Natural Sciences, Morgan State University, Baltimore, Maryland. [7]International Agency for Research on Cancer, Lyon, France. [8]The Tisch Cancer Institute, Mount Sinai School of Medicine and Institute for Translational Epidemiology, New York, New York.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Authors: Arash Etemadi, NCI, 9609 Medical Center Drive, Bethesda, MD 20892. Phone: 240-276-7197; Fax: 240-276-7837; E-mail: arash.etemadi@nih.gov; and Reza Malekzadeh, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran. Phone: 98-21-82415300; Fax: 98-21-82415400; E-mail: malek@ams.ac.ir

doi: 10.1158/1055-9965.EPI-18-0743

linked to cancers of the oral cavity, esophagus, and pancreas (5, 6), the carcinogenic content of many types of smokeless tobacco is not well understood.

Compared with cigarettes, relatively little is known about chemical exposures in users of other combustible and noncombustible tobacco products (7). Cigarette smoke is known to contain more than 60 carcinogens and cause at least 20 different forms of cancer (8). Other forms of tobacco will also expose users to carcinogens (9), but the specific relationships between these carcinogens and particular cancers are less well understood (10). The FDA has published a list of 93 harmful and potentially harmful tobacco constituents (11). Tobacco manufacturers and distributors must test for 20 of these constituents and report test results to FDA (12). Understanding how specific carcinogens and toxicants, such as tobacco alkaloids (e.g., nicotine), tobacco-specific nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), and volatile organic compounds (VOC) vary across the range of different tobacco products is informative for etiologic understanding, risk prediction, and evidence-based tobacco regulation (7).

In the Golestan Cohort Study (GCS), conducted in Golestan Province, in northeastern Iran, we have previously shown increased risk of overall and cancer mortality associated with cigarette smoking, waterpipe smoking, and nass use (13). This cohort has collected baseline urine samples from all 50,000 subjects enrolled, and collected additional urine 5 years later in a subset of about 11,000 study participants. In the present study, we tested urine samples from a group of GCS participants for a comprehensive array of urinary exposure biomarkers developed at the Centers for Disease Control and Prevention (CDC) National Center for Environmental Health laboratory. These assays have been used previously in the National Health and Nutrition Examination Survey (NHANES; ref. 14) and Population Assessment of Tobacco and Health (PATH; ref. 15) studies. We compared the concentrations of biomarkers belonging to different chemical classes (tobacco alkaloids, TSNAs, PAHs, and VOCs) in self-reported never users of tobacco products, exclusive cigarette users, exclusive waterpipe users, and exclusive nass users in the GCS. Although some of these biomarkers are not direct metabolites of known carcinogens and toxicants (11), they correlate well with other biomarkers in the same chemical class, and thus probably reflect the exposure to harmful compounds in that class. We also examined the consistency of these biomarkers over five years among continuing cigarette smokers and never tobacco users, using the repeated sample collection in the cohort.

Materials and Methods

In the GCS, 50,045 individuals from the general population ages $\geq$ 40 years who lived in Golestan Province, in the northeast of Iran, were recruited between 2004 and 2008 (16). At the time of enrollment, a spot urine sample was collected, along with a baseline questionnaire on detailed self-reported tobacco use [cigarettes, waterpipe, and chewed tobacco (nass)], and other demographic and lifestyle information. About 20% of the cohort (11,418) provided a second spot urine sample and completed a repeat questionnaire, on average, 5 years after the baseline. The GCS was approved by appropriate ethics committees at Tehran University of Medical Sciences, the NCI, and the International Agency for Research on Cancer (IARC). The involvement of the CDC laboratory did not constitute engagement in human subjects research.

For this study, from among GCS participants who were alive and cancer-free in December 2016, we randomly selected 4 groups based on self-reported tobacco use at enrollment: 60 never users of any tobacco product during their life, 35 exclusive current cigarette smokers, 40 exclusive current waterpipe smokers, and 30 exclusive current nass users. Never tobacco users reported they had never used any tobacco product during their lifetime. Exclusive current users of each product reported regular daily or non-daily use of that product (at least 6 months of the year) until the date of enrollment, but reported never using any other type of tobacco product. Opioids are used by about 17% of the cohort subjects, often smoked. To avoid the need to separate the exposures, we selected only nonopioid users for this study. Because cigarette smoking was almost exclusive to men, we restricted this group to male participants, but the other groups included a random sample of men and women. For never tobacco users and cigarette smokers, we restricted the selection to participants who provided both a baseline and a second urine sample 5 years later, and consistently reported being either a never tobacco user or an exclusive cigarette smoker at both time points.

Laboratory measurements

The assays were conducted at the Division of Laboratory Sciences of the National Center for Environmental Health at the CDC. The panel of biomarkers used in this study consisted of 4 general classes of compounds (Table 1). These included tobacco alkaloids (7 nicotine metabolites and 2 minor tobacco alkaloids), TSNAs (4 compounds), metabolites of PAHs (7 compounds), and VOCs (19 compounds). All urines, regardless of self-reported tobacco use, were tested for nicotine metabolites and subsequently categorized as collected from active or inactive tobacco users based on urinary cotinine concentration. Because the concentrations of tobacco-specific metabolites (tobacco alkaloids and TSNAs) were below the limits of detection (LOD) in individuals with very low or undetectable concentrations of urinary cotinine, we only tested TSNAs on individuals with a tested cotinine concentration above 20 ng/mL (17), regardless of self-reported use. However, we performed a more sensitive assay that measures only cotinine and hydroxycotinine in individuals testing below 20 ng/mL to confirm participants' secondhand tobacco exposure. PAHs and VOCs, two classes of combustion products that are expected to be found among both tobacco users and nonusers, were tested in all samples. Details of the assay methodology are presented in Supplementary Material.

Statistical analysis

We compared self-reported tobacco use against urinary cotinine concentrations, using a value of 50 ng/mL or greater to define active tobacco use (18). For the analyses in this report, we excluded any discordant urine specimen (self-reported never tobacco users with cotinine values greater than 50 ng/mL, and current tobacco users with cotinine values below 50 ng/mL). These included 7 specimens at baseline (2 never tobacco users, 2 cigarette smokers, and 3 waterpipe smokers) and 7 repeat samples (1 never tobacco user and 6 cigarette smokers).

For each biomarker, concentrations below the LOD were replaced by the LOD divided by the square root of 2 (19). For most assays, less than 10% of the values were below LOD, and none of the assays had 20% or more below-LOD values. All biomarker concentrations were then divided by urinary creatinine to adjust for urinary concentration and log-transformed to

Table 1. Metabolites used in the biomarker panel developed by the CDC National Center for Environmental Health

Biomarker class	Full compound name	Parent compound	Abbreviation	CV (%)
Nicotine and its metabolites	Cotinine	Nicotine	COTT[a]	4.6
	Trans-3′-hydroxycotinine	Nicotine	HCTT[a]	4.3
	Cotinine N-oxide	Nicotine	COXT[b]	7.3
	Norcotinine	Nicotine	NCTT[b]	6.7
	Nicotine	Nicotine	NICT[b]	2.5
	Nicotine 1′-oxide	Nicotine	NOXT[b]	5.0
	Nornicotine	Nicotine	NNCT[b]	3.7
Other tobacco alkaloids	Anabasine	Anabasine	ANBT[b]	3.1
	Anatabine	Anatabine	ANTT[b]	3.9
TSNAs	*N*′-Nitrosoanabasine	NAB	NABT[b]	4.1
	N′-Nitrosoanatabine	NAT	NATT[b]	4.6
	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol	NNK	NNAL[b]	1.4
	N′-Nitrosonornicotine	NNN	NNNT[b]	13.7
Metabolites of PAHs	1-Hydroxynaphthalene	Naphthalene/carbaryl[c]	1-nap[a]	2.2
	2-Hydroxynaphthalene	Naphthalene	2-nap[a]	2.9
	1-Hydroxyphenanthrene	Phenanthrene	1-phe[a]	7.5
	Sum of 2- and 3-hydroxyphenanthrene	Phenanthrene	$\sum_{2,3}$phe[a]	6.9
	2-Hydroxyfluorene	Fluorene	2-flu[a]	3.1
	3-Hydroxyfluorene	Fluorene	3-flu[a]	5.7
	1-Hydroxypyrene	Pyrene	1-pyr[a]	20.1
Metabolites of VOCs	2-Methylhippuric acid	Xylene	2MHA[a]	7.4
	3-Methylhippuric acid + 4 methylhippuric acid	Xylene	34MH[a]	10.8
	N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	Acrylamide	AAMA[a]	13.5
	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	Acrylamide	GAMA[a]	11.0
	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	Acrylonitrile	CYHA[a]	16.5
	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	Acrylonitrile	CYMA[a]	12.3
	N-Acetyl-S-(2-carboxyethyl)-L-cysteine	Acrolein	CEMA[a]	12.8
	N-Acetyl-S-(3-hydroxypropyl)-L-cysteine	Acrolein	HPMA[a]	15.1
	N-Acetyl-S-(benzyl)-L-cysteine	Toluene[c]	BMA[a]	12.2
	Mandelic acid	Styrene	MADA[a]	21.4
	Phenylglyoxylic acid	Ethylbenzene/styrene	PHGA[a]	13.6
	N-Acetyl-S-(phenyl)-L-cysteine	Benzene	PMA[a]	17.3
	N-Acetyl-S- (2-hydroxypropyl)-L-cysteine	Propylene oxide	HPM2[a]	10.1
	N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	Dimethylformamide[c]	AMCA[a]	11.8
	N-Acetyl-S-(3,4-dihydroxybutyl)-L-cysteine	1,3-Butadiene	DHBM[a]	11.5
	N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	1,3-Butadiene	MHB3[a]	15.3
	N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	Crotonaldehyde	HPMM[a]	11.3
	N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	Isoprene	IPM3[a]	17.1
	2-Thioxothiazolidine-4-carboxylic acid	Carbon disulfide	TTCA[a]	10.1

Abbreviation: CV: Coefficient of variation.
[a]Measured in all individuals.
[b]Measured only among those with a cotinine above 20 ng/mL.
[c]Multiple other parent chemicals can also be metabolized to these compounds.

conform to a normal distribution. We calculated geometric means (GM) and 95% confidence intervals (95% CI) of these creatinine-corrected values. GM and 95% CI were calculated in men and women separately, but because there were no significant differences between them for any biomarker, we reported them together. As the gold standard of nicotine dose (20), we calculated the total nicotine equivalent (TNE) as the molar sum of nicotine metabolites. Because some of these metabolites could only be measured in active tobacco users, we calculated two types of TNE: TNE2 (the molar sum of cotinine and hydroxycotinine available for everyone) and TNE7 (the molar sum of all 7 nicotine metabolites in tobacco users). To test biomarker differences by tobacco use groups, we used linear regression. Correlations among biomarkers were calculated using Pearson correlation of the creatinine-corrected log-transformed values, and regression lines were fitted using predicted values from the regression models. To further characterize our results, we compared our results with PAH and VOC biomarkers in US cigarette smokers and nonusers of any tobacco products in the NHANES 2011–2012 Special Sample, published in the National Report on Human Exposure to Environmental Chemicals (14). The reporting and analytical methodology was identical to this study and was similarly conducted by the Division of Laboratory Sciences of the National Center for Environmental Health at the CDC. Intraclass correlation coefficients (ICC) were calculated for each biomarker measured at two time points in GCS (baseline and after 5 years), among cigarette smokers and never tobacco users, to assess the consistency of the biomarker over time.

We used principal-component factor analysis (with orthogonal varimax rotation) and heat maps to depict the underlying patterns of biomarker concentrations across tobacco products, and to explore which products had similar exposure biomarker patterns (i.e., they clustered together). Heat maps were created for the creatinine-corrected log-transformed values of biomarkers against the tobacco use group, using *heatmap.2* function from the *gplots* R package. Dendrograms were based on complete hierarchical clustering using the Euclidian distance metric. Other analyses were conducted using the STATA 14.0 package (StataCorp Inc.).

Table 2. GM and 95% confidence intervals of metabolites of polycyclic aromatic hydrocarbons and volatile organic compounds across study groups from the GCS and the U.S. NHANES 2011–2012 Special Sample population (14)

	Never tobacco users ($n = 58$)[a]	Exclusive cigarette smokers ($n = 33$)[a]	Exclusive waterpipe smokers ($n = 37$)[a]	Exclusive nass users ($n = 30$)	U.S. tobacco nonusers	U.S. cigarette smokers
Nicotine and other tobacco alkaloids (ng/mg creatinine)						
COTT	1.3 (0.9, 1.8)	1,799.9 (1,200.7–2,698.3)	1,093.5 (723.7–1,652.2)	4,744.3 (3,726.7–6,039.8)	NA	NA
HCTT	2.8 (2.1–3.8)	2,661.6 (1,742.6–4,065.3)	1,790.8 (1,079.9–2,969.9)	8,261.4 (6,434.5–10,606.9)	NA	NA
TNE2[b]	0.02 (0.02–0.03)	24.9 (16.6–37.4)	16.3 (10.5–25.2)	71.2 (56.4–89.8)	NA	NA
COXT	—	214.9 (142.7–323.8)	141.9 (91.9–219.0)	510.3 (394.2–660.6)	NA	NA
NCTT	—	56 (37.1–84.4)	44.1 (27.6–70.4)	153.4 (119.6–196.8)	NA	NA
NICT	—	676.8 (382.1–1,198.8)	313.5 (172.8–568.5)	2,809.1 (1,979.5–3,986.5)	NA	NA
NOXT	—	182.6 (109.2–305.6)	76.1 (46.7–124.4)	581.1 (436.3–774.1)	NA	NA
NNCT	—	42.5 (27.0–67.0)	20.9 (13.3–32.9)	145.7 (105.6–201.1)	NA	NA
TNE7[b]	—	33.4 (21.9–50.8)	21.3 (14.0–32.4)	100.3 (79.6–126.4)	NA	NA
ANBT	—	4.6 (3.0–7.1)	1.8 (1.1–2.9)	45.9 (33.1–63.7)	NA	NA
ANTT	—	6.5 (3.9–10.7)	1.2 (0.7–2.0)	46.3 (30.0–71.5)	NA	NA
Tobacco-specific nitrosamines (pg/mg creatinine)						
NABT	—	9.1 (6.2–13.5)	9.8 (5.7–17.0)	9.9 (6.9–14.2)	NA	NA
NATT	—	53.7 (34.5–83.6)	20.6 (12.4–34.3)	43.2 (28.4–65.6)	NA	NA
NNAL	—	130.9 (92.6–185.1)	166.1 (88.6–311.1)	114.8 (79.8–165.0)	NA	NA
NNNT	—	10.4 (6.9–15.8)	5.0 (3.4–7.3)	14.1 (9.7–20.5)	NA	NA
Polycyclic aromatic hydrocarbons (ng/g creatinine)						
1-nap	10,872 (8,064–14,657)	14,637 (11,511–18,613)	15,392 (11,268–21,026)	14,344 (9,116–22,569)	1,380 (1,250–1,520)	9,950 (8,940–11,100)
2-nap	2,299.7 (1,835.7–2,881.0)	9,048.9 (6,864.2–11,929.0)	9,042.8 (6,806.8–12,013.3)	2,150.5 (1,596.2–2,897.3)	3,670 (3,440–3,920)	13,200 (12,400–14,000)
1-phe	247.1 (213.9–285.5)	264.5 (227.4–307.8)	392.0 (309.6–496.4)	223.6 (181.6–275.2)	134 (123–146)	218 (204–233)
$\sum_{2,3}$phe	300.3 (250.4–360.3)	482.9 (371.5–627.6)	753.0 (530.0–1,069.8)	407.7 (275.2–604.1)	118.8 (109.7–128.9)	276 (260–293)
2-flu	396.0 (330.6–474.3)	1,238.6 (973.2–1,576.5)	1,355.1 (984.0–1,866.0)	435.1 (335.3–564.5)	190 (177–204)	1,240 (1,160–1,330)
3-flu	168.1 (133.3–212.0)	755.7 (566.1–1,008.9)	644.9 (442.2–940.7)	225.8 (166.9–305.4)	66.8 (61.6–72.6)	634 (575–700)
1-pyr	412.0 (344.2–493.1)	636.0 (504.0–802.4)	960.8 (725.1–1,273.0)	441.9 (340.2–574.0)	96.7 (90.8–103)	259 (240–280)
Volatile organic compounds (μg/g creatinine)						
2MHA	66.4 (50.0–88.3)	148.2 (113.5–193.5)	129.4 (91.0–184.2)	72 (48.2–107.4)	30.4 (27.2–34.0)	107 (97.2–119)
34MH	320.7 (252.5–407.2)	839.4 (650.3–1,083.5)	679.9 (495.5–933.0)	371 (262.0–525.2)	201 (187–217)	745 (671–827)
AAMA	47.7 (41.3–55.2)	124.7 (97.2–159.9)	107.9 (84.8–137.4)	58.9 (45.5–76.2)	42.5 (40.1–44.9)	120 (107–135)
GAMA	8.5 (7.2–10.1)	15.2 (12.4–18.7)	17.3 (14.2–21.2)	11.7 (9.1–14.9)	15.2 (14.3–16.0)	30.3 (27.4–33.5)
CYHA	0.7 (0.6–0.9)	14.2 (9.1–22.1)	9.0 (5.7–14.1)	1.1 (0.8–1.6)	0.7 (0.6–0.9)	14.2 (9.1–22.1)
CYMA	1.1 (0.9–1.4)	86.4 (58.3–127.9)	32.4 (20.3–51.5)	1.8 (1.2–2.9)	1.71 (1.55–1.88)	122 (103–145)
CEMA	77.8 (65.4–92.6)	186.2 (153.9–225.3)	116.5 (96.7–140.4)	78.3 (61.9–99.0)	85.6 (79.9–91.8)	227 (209–247)
HPMA	188.1 (154.5–228.9)	881.8 (661.8–1,174.9)	337.4 (266.3–427.6)	185 (148.6–230.4)	260 (244–277)	1,190 (1,100–1,290)
BMA	5.3 (4.0–7.0)	6.4 (4.4–9.2)	7.4 (5.9–9.4)	5.2 (3.7–7.2)	7.63 (7.06–8.24)	6.78 (6.35–7.24)
MADA	186.8 (161.9–215.5)	274.2 (225.7–333.2)	313.9 (257.1–383.4)	205.6 (175.6–240.7)	150 (140–160)	313 (286–341)
PHGA	86.5 (65.1–115.0)	101.3 (68.8–149.3)	162.8 (123.5–214.6)	83.5 (61.5–113.4)	186 (173–201)	339 (307–375)
PMA	1.3 (1.1–1.6)	1.5 (1.2–1.8)	2.1 (1.7–2.5)	1.4 (1.1–1.7)	Below LOD[b]	Below LOD[b]
HPM2	25.3 (21.5–29.8)	53.9 (42.9–67.7)	48.5 (41.1–57.2)	23.0 (19.4–27.4)	34.4 (30.9–38.3)	63.4 (56.6–71.1)
AMCA	92.1 (74.6–113.6)	296.3 (233.1–376.7)	284.8 (229.8–351.8)	115.7 (91.2–146.9)	145 (137–155)	507 (446–577)
DHBM	280.0 (235.4–333.1)	384.5 (337.3–438.4)	383.0 (326.3–449.7)	281.7 (243.3–326.1)	279 (267–292)	387 (363–412)
MHB3	4.3 (3.6–5.2)	23.3 (16.5–32.8)	8.2 (6.6–10.2)	4.8 (4.1–5.7)	8.14 (7.44–8.91)	61.3 (55.8–67.4)
HPMM	373.4 (311.9–446.9)	1,359.7 (980.2–1,886.0)	586.6 (492.5–698.6)	341.8 (286.8–407.4)	392 (362–424)	1,910 (1,730–2,100)
IPM3	2.0 (1.7–2.4)	21.6 (12.9–36.2)	8.2 (5.4–12.4)	2.8 (2.2–3.7)	NA	NA
TTCA	12.4 (9.3–16.4)	12 (9.3–15.4)	16.7 (11.9–23.8)	13.1 (9.4–18.3)	Below LOD[b]	Below LOD[b]

Abbreviation: NA: not available.

[a]Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine concentrations.

[b]Proportion of results below limit of detection was too high (>40%) to provide a valid result.

Results

There was excellent agreement (kappa = 0.84–0.90) between self-reported tobacco use and urinary cotinine concentrations, particularly at baseline (Supplementary Table S1). Cigarette smokers started tobacco use earlier than waterpipe smokers or nass users. All nass users, 97% of cigarette smokers, and 78% of waterpipe smokers used tobacco every day (Supplementary Table S2).

Tobacco alkaloids and TSNAs

As expected, the concentrations of cotinine and hydroxycotinine were much higher in tobacco users compared with nonusers (Table 2). Among tobacco users, nass users had the highest concentrations of alkaloid (nicotine and nonnicotine) metabolites and waterpipe users had the lowest concentrations.

TSNAs showed a somewhat different pattern: NABT and NNAL were similar across the tobacco use groups. NATT and NNNT were lower in waterpipe smokers than in cigarette smokers and nass users. TNE7 had strong correlations with all nitrosamines ($r = 0.77$–0.86) among cigarette smokers (Supplementary Table S3). These correlations were slightly weaker in waterpipe smokers ($r = 0.51$–0.78) and nass users ($r = 0.42$–0.72), but remained statistically significant, except for NNAL in nass users.

Figure 1A shows the heat map for all these tobacco-specific compounds among tobacco users. As the figure shows, nass users had a different pattern from cigarette and waterpipe smokers, who clustered together; the main difference was the high concentrations of nicotine and other alkaloid metabolites in nass users.

PAHs and VOCs

Both cigarette and waterpipe smokers had elevated concentrations of PAH biomarkers (except for 1-hydroxynaphthalene) compared with nass users and never tobacco users (Table 2). The concentrations of phenanthrene biomarkers and 1-hydroxypyrene were particularly high in waterpipe smokers. In Fig. 2 (and

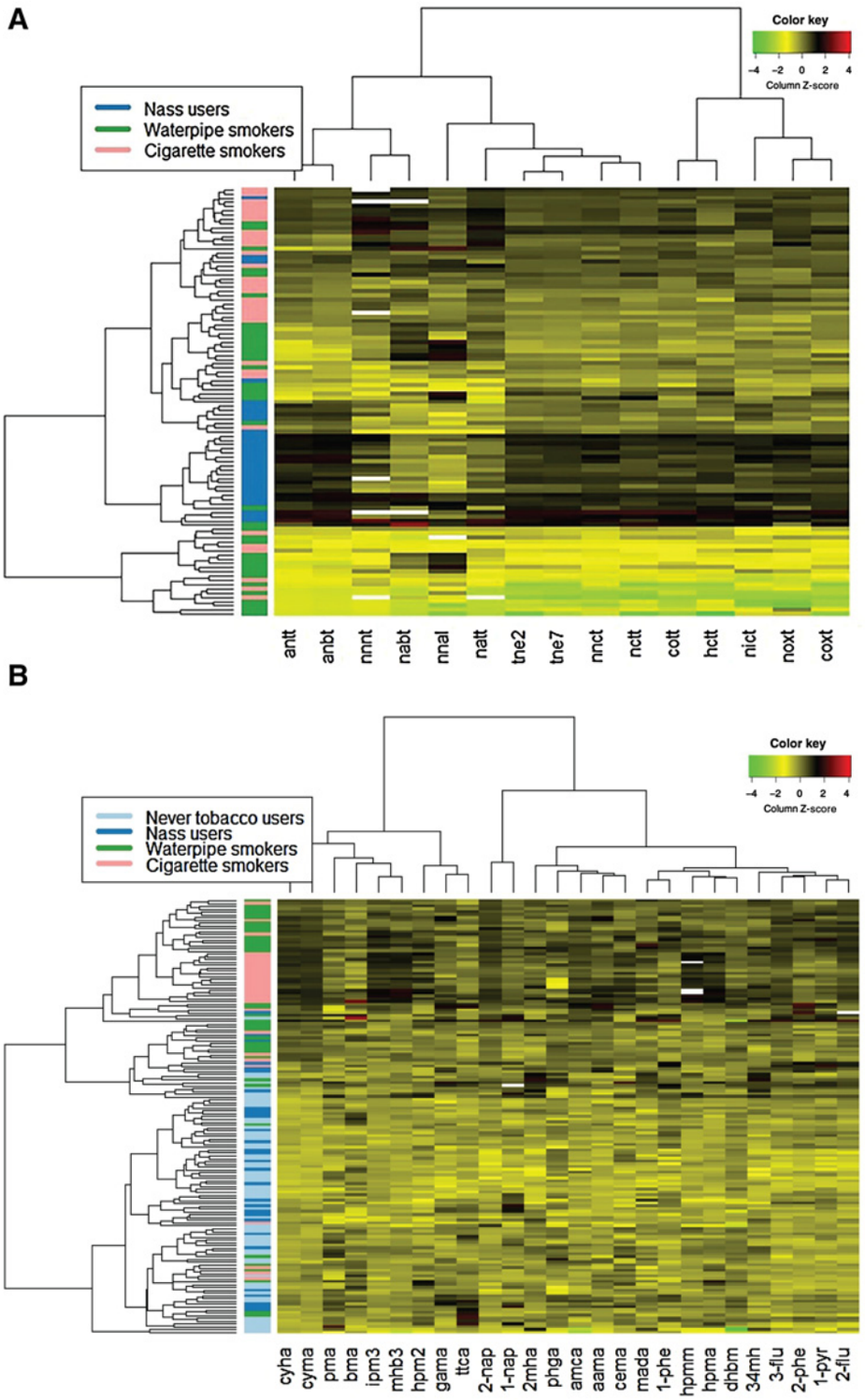

Figure 1.
Heat maps of **A,** nicotine metabolites, other tobacco alkaloids, and nitrosamines; **B,** biomarkers of PAHs and VOCs among different study groups. Each row shows biomarker concentrations in one individual, and the colors represent the concentrations standardized for each biomarker (z scores shown in the Color Key).

Supplementary Table S4), we show the correlations between TNE2 and each PAH biomarker. Two patterns can be seen in these correlations: TNE2 was highly correlated with PAH biomarkers in waterpipe smokers ($r = 0.65–0.90$), and, to a lesser extent, in cigarette smokers ($r = 0.28–0.88$). However, in never tobacco users and nass users, TNE2 did not correlate with the concentrations of PAH biomarkers.

VOC biomarker concentrations (except for BMA and TTCA) were higher in combustible product (i.e., cigarette and waterpipe) users compared with both never tobacco users and exclusive nass

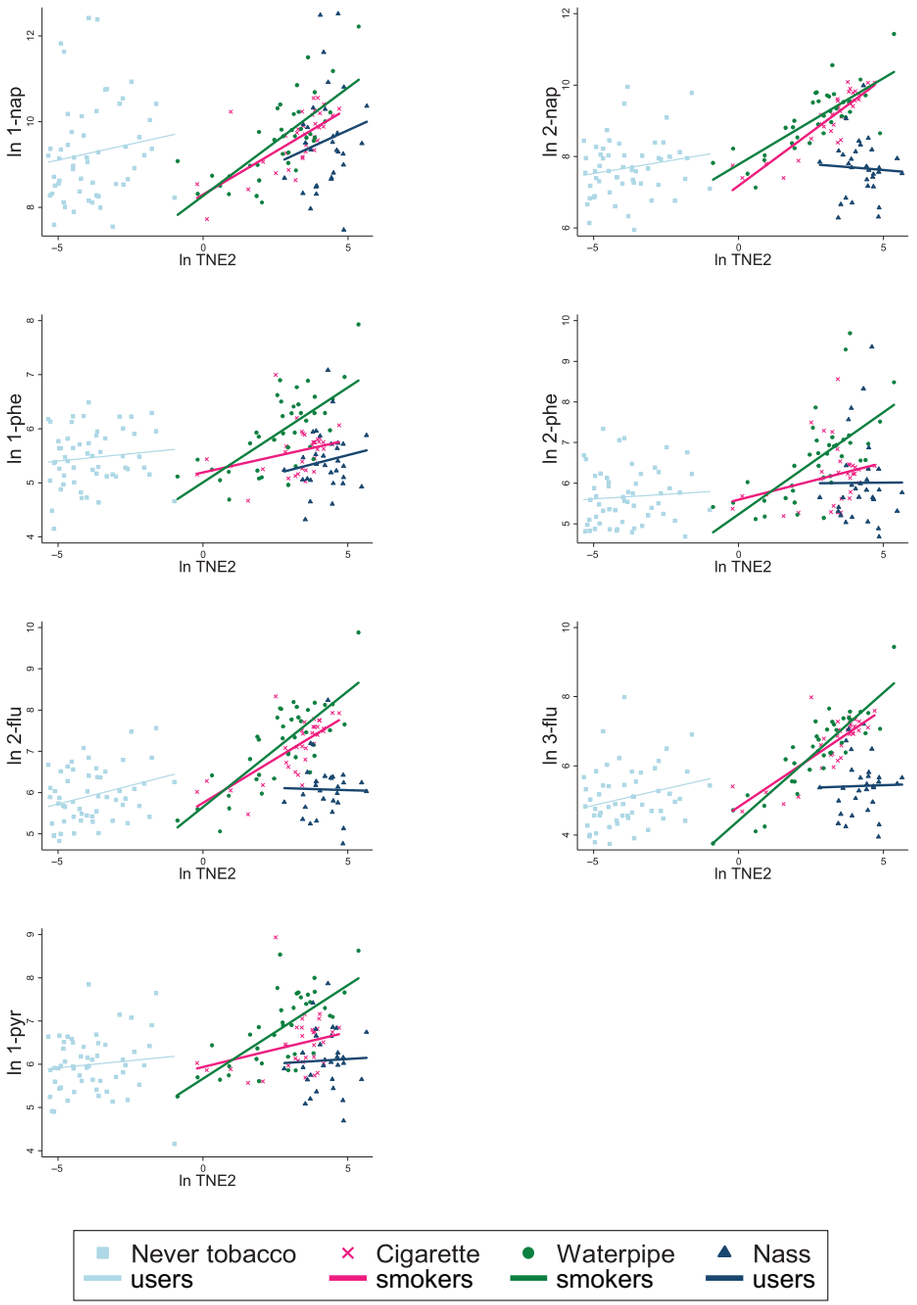

Figure 2.
Correlation between TNE and PAH biomarkers among never tobacco users, cigarette smokers, waterpipe smokers, and nass users.

users (Table 2). The highest VOC biomarker concentrations were typically found in cigarette smokers; however, the benzene biomarker phenylmercapturic acid (PMA) and the ethylbenzene/styrene biomarker phenylglyoxylic acid (PHGA) were significantly higher in waterpipe smokers than in other groups (including cigarette smokers). In cigarette smokers, TNE2 was strongly correlated with nearly all VOC biomarkers except BMA, PHGA, and TTCA. Waterpipe smokers had significant correlations between TNE2 and 2MHA, 34MH, AAMA, GAMA, CYMA, CYHA, AMCA, HPMM, IPM3, MADA, and MHB3. In contrast, in never tobacco users and nass users, no correlations between TNE2 and VOCs were observed (Fig. 3; Supplementary Table S5).

Figure 1B summarizes the concentrations of PAH biomarkers and VOC biomarkers in a heat map across the four tobacco use groups. As the figure shows, waterpipe and cigarette smokers had similar concentration patterns for both PAHs and VOC biomarkers, whereas nass users clustered mainly with nontobacco users in these two classes of biomarkers.

Factor analysis

Only the first three factors had eigenvalues above 3 and, together, explained 66% of the variance in biomarker concentrations. Supplementary Table S6 shows rotated factor loadings for these 3 factors. As the factor loadings show, in each

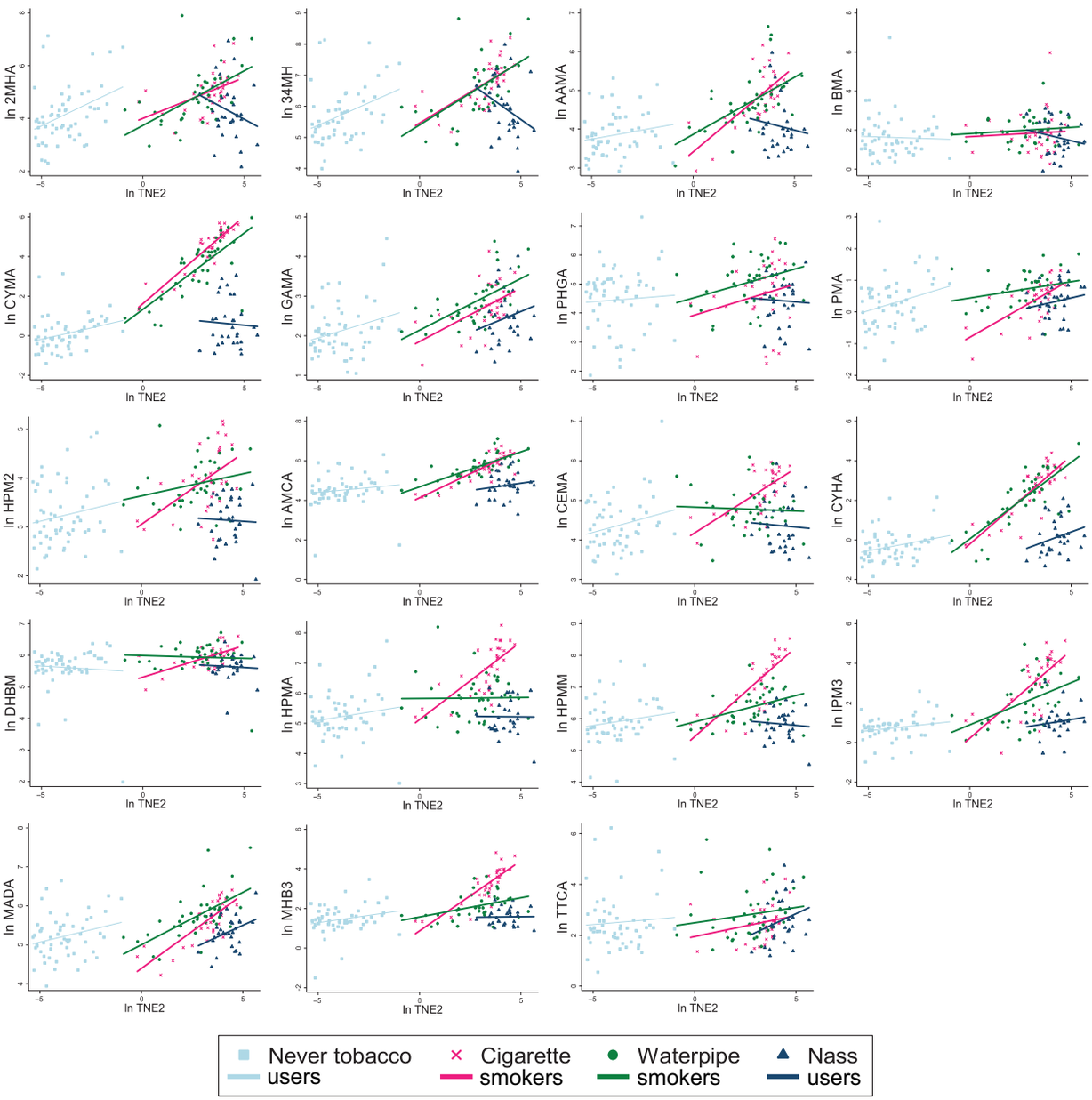

Figure 3.
Correlation between TNE and VOC metabolites among never tobacco users, cigarette smokers, waterpipe smokers, and nass users.

factor, one group of biomarkers had higher loadings than the others: tobacco-specific biomarkers (tobacco alkaloids and TSNAs) in the first factor, PAHs in the second factor, and VOCs in the third factor. The scores generated based on these factors were thus called the tobacco score, the PAH score and the VOC score, respectively. Nass users had the highest tobacco score and the lowest PAH and VOC scores (all $P < 0.01$). Compared with cigarette smokers, waterpipe smokers had a significantly higher PAH score and a significantly lower VOC score.

Comparison with the U.S. population

Table 2 also shows concentrations of PAH and VOC biomarkers in US cigarette smokers and nonusers of tobacco products, based on analyses of the NHANES 2011–2012 Special Sample. The concentrations of PAHs among Golestan nonsmokers were not only much higher than those seen in the US NHANES tobacco nonuser population, sometimes they were even higher than the average for US cigarette smokers.

The concentrations of VOC biomarkers were broadly similar between the Golestan and US populations. Some VOC

Table 3. ICCs between the two measurements, 5 years apart, in cigarette smokers and never tobacco users

		Never tobacco users		Cigarette smokers	
Biomarker class	**Biomarker**	**ICC (95% CI)**	***P* value**	**ICC (95% CI)**	***P* value**
Nicotine metabolites	COTT	0.64 (0.45–0.77)	0.0001	0.58 (0.27–0.78)	0.0001
	HCTT	0.51 (0.29–0.68)	0.0001	0.44 (0.09–0.7)	0.007
	COXT	NA		0.45 (0.11–0.7)	0.006
	NCTT	NA		0.51 (0.18–0.74)	0.002
	NICT	NA		0.41 (0.06–0.68)	0.012
	NOXT	NA		0.48 (0.14–0.72)	0.004
	NNCT	NA		0.5 (0.17–0.73)	0.002
Other tobacco alkaloids	ANBT	NA		0.57 (0.26–0.77)	0.001
	ANTT	NA		0.61 (0.31–0.8)	0.0001
TSNAs	NABT	NA		0.63 (0.34–0.81)	0.0001
	NATT	NA		0.64 (0.36–0.81)	0.0001
	NNAL	NA		0.62 (0.34–0.81)	0.0001
	NNNT	NA		0.49 (0.14–0.73)	0.004
Metabolites of PAHs	1-nap	0.08 (−0.18 to 0.32)	0.283	0.51 (0.18–0.74)	0.002
	2-nap	0.18 (−0.08 to 0.42)	0.087	0.43 (0.08–0.69)	0.009
	1-phe	0.31 (0.06–0.53)	0.003	0.09 (−0.28 to 0.44)	0.315
	$\sum_{2,3}$phe	0.37 (0.13–0.57)	0.001	0.34 (−0.03 to 0.63)	0.034
	2-flu	0.36 (0.12–0.57)	0.002	0.31 (−0.06 to 0.61)	0.049
	3-flu	0.44 (0.21–0.62)	0.0001	0.46 (0.12–0.71)	0.005
	1-pyr	0.34 (0.10–0.55)	0.003	0.37 (0.01–0.65)	0.021
Metabolites of VOCs	2MHA	0.14 (−0.12 to 0.38)	0.15	0.48 (0.15–0.72)	0.004
	34MH	0.08 (−0.18 to 0.33)	0.28	0.34 (−0.03 to 0.63)	0.034
	AAMA	0.19 (−0.07 to 0.43)	0.073	0.27 (−0.11 to 0.58)	0.079
	GAMA	0.73 (0.58–0.83)	0.0001	0.24 (−0.13 to 0.56)	0.099
	CYHA	0.76 (0.63–0.85)	0.0001	0.65 (0.37–0.82)	0.0001
	CYMA	0.42 (0.18–0.61)	0.0001	0.66 (0.4–0.83)	0.0001
	CEMA	0.49 (0.27–0.66)	0.0001	0.39 (0.04–0.66)	0.016
	HPMA	0.27 (0.01–0.49)	0.021	0.39 (0.03–0.66)	0.016
	BMA	0.25 (0.00–0.48)	0.025	0.42 (0.07–0.68)	0.011
	MADA	0.37 (0.13–0.58)	0.002	0.38 (0.02–0.65)	0.021
	PHGA	0.23 (−0.02 to 0.46)	0.036	−0.5 (−0.73 to 0.17)	0.997
	PMA	0.07 (−0.19 to 0.32)	0.29	0.18 (−0.2 to 0.51)	0.176
	HPM2	0.27 (0.02–0.49)	0.018	0.41 (0.05–0.67)	0.013
	AMCA	0.31 (0.05–0.52)	0.009	0.43 (0.07–0.68)	0.01
	DHBM	0.13 (−0.13 to 0.37)	0.167	−0.01 (−0.37 to 0.36)	0.51
	MHB3	0.11 (−0.15 to 0.36)	0.197	0.45 (0.11–0.7)	0.006
	HPMM	0.11 (−0.15 to 0.36)	0.204	0.45 (0.08–0.71)	0.009
	IPM3	0.11 (−0.16 to 0.35)	0.215	0.2 (−0.17 to 0.53)	0.142
	TTCA	0.32 (0.07–0.53)	0.007	0.39 (0.03–0.66)	0.017

biomarkers (e.g., 2MHA, 34MH, and PMA) were higher in Golestan, and some (GAMA, HPMA, PHGA, HPM2, AMCA, and MHB3) were lower compared with the U.S. population.

Repeated measurement

ICCs between two specimens collected, on average, 5 years apart from the same never tobacco users and cigarette smokers are shown in Table 3. Most biomarkers showed statistically significant ICCs, particularly in cigarette smokers. Most ICCs were higher in cigarette smokers than in never tobacco users, showing a more consistent underlying exposure over 5 years. TSNAs (0.49–0.64) and tobacco alkaloid biomarkers (0.50–0.64) had the highest ICCs among the biomarkers studied.

Discussion

Users of cigarettes, waterpipe, and nass all showed higher concentrations of at least one class of the studied biomarkers than the never tobacco users. All tobacco users had high levels of TSNAs. Smokeless tobacco (nass) users had the highest concentrations of nicotine and minor tobacco alkaloids. In contrast, concentrations of PAH and VOC biomarkers were markedly higher in cigarette and waterpipe smokers, and correlated with nicotine dose. Although some differences existed for individual biomarkers, the similarities in the overall concentration patterns between cigarette and waterpipe smokers were more striking than their differences. In addition, all four exposure groups in the Golestan Cohort population (including the never tobacco users) had remarkably high concentrations of PAH biomarkers compared with the NHANES populations of US smokers and nonusers of tobacco products.

Similar concentrations of examined biomarkers in cigarette and waterpipe smokers (as demonstrated in the heat maps) are important findings, although not a complete surprise. The tobacco used in a waterpipe is heated by burning charcoal directly above it, which would be expected to produce PAHs, VOCs, and other combustion products from both the tobacco and the charcoal. Waterpipe smokers are also known to be exposed to nicotine and TSNAs (21). Jacob and colleagues studied 13 individuals who crossed over from cigarettes to waterpipe and observed some similarities and differences in exposure biomarkers between the two products (22). The biggest differences were greater urinary concentrations of a high-molecular-weight PAH (1-hydroxypyrene) and higher VOC metabolite concentrations

suggestive of benzene exposure (phenylmercapturic acid or PMA) in the waterpipe period, accompanied by lower concentrations of NNAL, metabolites of low-molecular-weight PAHs (naphthalene and fluorene) and a few other metabolites of VOCs (1,3-butadiene, acrolein, acrylonitrile, propylene oxide, and ethylene oxide) compared with the cigarette smoking period. Many of our results replicated these findings, including higher concentrations of pyrene and benzene biomarkers and lower concentrations of several other VOC metabolites in waterpipe users compared with cigarette smokers. However, we also observed similarly increased NNAL and metabolites of low-molecular-weight PAHs in waterpipe smokers and cigarette smokers. It is not surprising that some results differed between our study and this previous one, because the populations (and their customs of tobacco use) differed, the previous study evaluated dual smokers of both cigarettes and waterpipe, and their waterpipe sessions were done in lab settings under specific instructions.

In waterpipe smokers, like cigarette smokers, we saw high correlations between the nicotine dose and almost all assessed biomarkers, showing a dose–response association between the amount of tobacco used and the potential carcinogenic exposure. In nass users, these correlations were only present with TSNAs. TSNAs, which are produced during tobacco curing and processing, may be the most important carcinogens in unburned tobacco (8).

Tobacco alkaloids and TSNAs

Metabolites of nicotine and nonnicotine tobacco alkaloids are direct measures of the degree of exposure to tobacco (20). The fact that even never tobacco users had some concentrations of two of the most commonly measured nicotine metabolites (cotinine and 3-hydroxycotinine) suggests substantial exposure to secondhand smoke in GCS. However, by far the largest exposure to tobacco alkaloids was seen among nass users. Similar results have also been observed among users of other smokeless tobacco products (23), and may be due to the enhanced absorption of the hydrophilic alkaloids in the mouth. Though not considered carcinogenic by itself, nicotine is pharmacologically responsible for tobacco dependence, and the resulting exposure to carcinogens found in tobacco products (24). In addition, nicotine and other tobacco alkaloids are readily transformed during curing and processing to nitrosamines, many of which are potent carcinogens causing DNA adduct formation, mutation, and tumorigenesis (8).

NNAL is the major metabolite of a strong carcinogen, nicotine-derived nitrosamine ketone (NNK), known to be involved in the development of cancers of the lung, nasal and oral cavity, liver, pancreas, and cervix (25). NNK is a systemic lung carcinogen in animal studies, independent of its route of administration (26). NNN, another TSNA, is thought to be a major carcinogen for cancers of the esophagus (the most common cancer type in our study population), and also for cancers of the oral and nasal cavity (25). The concentrations of NNAL in cigarette smokers in Golestan were lower than those found in US cigarette smokers (23). This difference may be due to lower cigarette smoking intensity (fewer cigarettes smoked per day) in this population relative to the US (13), or perhaps differences in the commonly used tobacco brands. As Wu and colleagues have shown, TSNAs in the mainstream smoke from US brand cigarettes may be higher than that from non-US brand cigarettes (27). In all tobacco users, we observed strong correlations between TNE and TSNAs, i.e., a dose–response association between the amount of tobacco people were exposed to and the level of carcinogens in the body.

PAHs

PAHs have been implicated in the etiology of different cancers, including lung, larynx, oral cavity, skin, and esophagus (8). Previous studies have shown that adults in the Golestan region, including nonsmokers, have high urinary concentrations of 1-hydroxypyrene glucuronide (a PAH metabolite; ref. 28), and high levels of blood PAH-DNA adducts even among nonsmokers (29). 1-Hydroxypyrene is often used as a biomarker of exposure to benzo-a-pyrene, one of the carcinogens detected in cigarette smoke. We similarly observed high concentrations of 1-hydroxypyrene and other PAH urinary biomarkers among both tobacco users and nonusers in Golestan. Indeed, the concentrations of PAH biomarkers among never tobacco users in Golestan were comparable with, and sometimes greater than, those in the US cigarette smokers (14). Also, concentrations of 1-hydroxynaphthalane, a metabolite not only of naphthalene, but also of several pesticides (e.g., carbaryl; refs. 30, 31), were so high among never tobacco users that we could not detect any statistically significant increases with smoking. The fact that these high PAH biomarker concentrations in nass users and never tobacco users did not correlate with TNE suggests a nontobacco-related exposure source in these individuals. Previous studies have also shown that only about 15% of the variance in PAH biomarkers can be explained by known exposures such as place of residence and tobacco or opium use (32). Although the exact source of such high PAH biomarkers in this population is not clear, exposure through food and water (33), and the presence of genetic variants affecting PAH metabolic pathways in the body (29), have been proposed. We hypothesize that this exposure may contribute to the high rates of esophageal squamous cell carcinoma (ESCC) in this population (34), which are not driven by tobacco use alone (35). High levels of PAH biomarkers have been reported from Linxian in China, another high-risk area for ESCC (36).

Two other groups of PAH biomarkers were particularly increased in waterpipe smokers: the metabolites of phenanthrene (1-hydroxyphenanthrene and $\sum_{2,3}$hydroxyphenanthrene) and pyrene (1-hydroxypyrene). Sepetdjian and colleagues have showed that the concentrations of biomarkers of phenanthrene and pyrene were particularly high in the smoke generated by waterpipe compared with cigarettes (37), in part due to the charcoal used to heat the tobacco in the waterpipe (38).

VOCs

There are a number of VOCs among US FDA's list of harmful and potentially harmful tobacco constituents (11), and these include known human toxicants (like acrolein) and carcinogens (like benzene, ethylbenzene, styrene, 1,3-butadiene, and ethylene oxide; ref. 39).

Like US smokers, Golestan cigarette and waterpipe smokers had higher concentrations of almost all VOC metabolites compared with never tobacco users and nass users, which correlated with their nicotine dose. Among the VOCs, we saw the highest concentrations of a benzene-related metabolite (PMA) and ethylbenzene/styrene-related PHGA in waterpipe smokers. Previous studies have also shown benzene to be a constituent of waterpipe smoke (40), and high concentrations of its biomarkers have been found in waterpipe smokers (22) and in waterpipe cafés (41). The charcoal used in the waterpipe has been shown to release benzene when heated (42). Kassem and colleagues observed higher concentrations of PMA among waterpipe smokers compared with never smokers, which increased by 2.9 to 4.2 times after each

"social hookah event" (43). In addition to waterpipe smokers, the concentrations of PMA among all groups in our study (even never tobacco users) were higher than those in the NHANES US samples. These high PMA concentrations did not correlate well with TNE, suggesting a nontobacco source in addition to waterpipe smoking in this region, which warrants further investigation. Benzene is an important cause of leukemia in smokers (8), and the relatively high concentrations of benzene biomarkers both in the general population and waterpipe smokers in Golestan, compared with the NHANES study, warrant further investigation.

We tested the consistency of our exposure assessment over time, using another set of spot urine specimens collected after five years in the same individuals. This comparison showed statistically significant ICCs for most biomarkers. As one could expect, the consistency of the exposure assessment (quantified by the ICC) was higher in cigarette smokers, suggesting that this source of the biomarkers was consistently present after 5 years. The ICCs were also higher when the biomarker was highly correlated with tobacco dose (tobacco alkaloids and TSNAs). This reproducibility decreased, but was still moderate to good, when other nontobacco sources may have contributed to the biomarker level (PAHs and VOCs), and were more likely to change over the course of 5 years. These findings show that most of these biomarkers would be useful for exposure assessment in a longitudinal study, particularly among tobacco users.

All urinary tobacco metabolites are markers of relatively recent exposure. We did not have information on the most recent tobacco use before the spot urine collection, but the cotinine test results showed excellent agreement with our self-reported general tobacco use questionnaire. Because we excluded people whose self-reported tobacco use and cotinine concentrations were discordant, we reduced the chance that our exposure estimates were altered by inclusion of intermittent smokers. We had a relatively small sample size, which was limited by the cost and urine volume requirements of the analytical methods. However, the large number of assays conducted provided the opportunity to paint a relatively broad picture of the potentially carcinogenic exposure associated with each tobacco product in the study. Most of the assays for tobacco exposure biomarkers have been optimized for urine (20), which is available only in a small number of cohorts. By using the same state-of-the-art analytical methods as those used for the NHANES study urine samples, we directly compared biomarker concentrations in the cohort with those among tobacco users and nonusers in the US general population.

In conclusion, participants of the Golestan Cohort are exposed to high levels of PAHs even among nonsmokers, an exposure that increased even further with tobacco smoking, particularly by waterpipe. Eighty percent of waterpipe smokers were daily users, which is ideal for assessing relatively short-term urinary biomarkers. We found two general patterns of exposure biomarkers differentiating the use of two combustible tobacco products (cigarettes and waterpipe) from the smokeless tobacco product (nass). We also showed that the biomarkers used in our study, the same as those used in the NHANES and PATH studies, are relatively reliable for exposure assessment in a longitudinal study, particularly among tobacco users. These findings warrant further investigation in the cohort, including studies to determine the environmental sources of specific exposures among never tobacco users and nested studies that evaluate associations between specific chemical biomarker classes and the incidence of specific types of cancer and other outcomes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The views and opinions expressed in this article are those of the authors only and do not necessarily represent the views, official policy, or position of the U.S. Department of Health and Human Services or any of its affiliated institutions or agencies. Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the U.S. Department of Health and Human Services.

Authors' Contributions

Conception and design: A. Etemadi, H. Poustchi, C.M. Chang, L. Wang, A. Pourshams, M.S. Shiels, M. Inoue-Choi, B. Wang, F. Kamangar, P. Brennan, P. Boffetta, S.M. Dawsey, C.C. Abnet, R. Malekzadeh, N.D. Freedman

Development of methodology: A. Etemadi, H. Poustchi, B.C. Blount, L. Wang, A. Pourshams, M. Inoue-Choi, B. Wang, X. Ye, D. Bhandari, P. Brennan, R. Malekzadeh

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Etemadi, H. Poustchi, B.C. Blount, L. Wang, V.R. De Jesus, A. Pourshams, R. Shakeri, M. Inoue-Choi, G. Murphy, X. Ye, D. Bhandari, J. Feng, B. Xia, C.S. Sosnoff, F. Kamangar, P. Brennan, P. Boffetta, R. Malekzadeh, N.D. Freedman

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Etemadi, C.M. Chang, B.C. Blount, A.M. Calafat, L. Wang, V.R. De Jesus, M.S. Shiels, M. Inoue-Choi, B.K. Ambrose, C.H. Christensen, B. Wang, J. Feng, P. Brennan, C.C. Abnet, R. Malekzadeh

Writing, review, and/or revision of the manuscript: A. Etemadi, C.M. Chang, B.C. Blount, A.M. Calafat, L. Wang, V.R. De Jesus, R. Shakeri, M.S. Shiels, M. Inoue-Choi, B.K. Ambrose, C.H. Christensen, B. Wang, G. Murphy, X. Ye, D. Bhandari, F. Kamangar, P. Brennan, P. Boffetta, S.M. Dawsey, C.C. Abnet, N.D. Freedman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Poustchi, A.M. Calafat, G. Murphy, D. Bhandari, P. Brennan, R. Malekzadeh, N.D. Freedman

Study supervision: H. Poustchi, A.M. Calafat, D. Bhandari, F. Kamangar, P. Boffetta, C.C. Abnet, R. Malekzadeh, N.D. Freedman

Acknowledgments

We thank the study participants, the Behvarz (community health workers) in the study areas for their help, and the Social Security Organization of Iran Golestan Branch. We also thank the general physicians, nurses, and nutritionists in the enrollment teams for their collaboration and assistance, and Golestan University of Medical Sciences (Gorgan, Iran), the Golestan health deputies, and the chiefs of the Gonbad and Kalaleh health districts for their close collaboration and support. The authors also wish to thank Yuesong Wang for the quantification of the PAH biomarkers.

The Golestan Cohort Study was supported by Tehran University of Medical Sciences (grant no: 81/15); Cancer Research UK (grant no: C20/A5860); the Intramural Research Program of the NCI, NIH; and various collaborative research agreements with IARC.

The current project was supported with federal funds from the Center for Tobacco Products, FDA, Department of Health and Human Services, through interagency agreements among the Center for Tobacco Products, FDA, and the Centers for Disease Control and Prevention and NCI, NIH.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 2, 2018; revised September 27, 2018; accepted October 17, 2018; published first January 8, 2019.

References

1. Giovino GA, Mirza SA, Samet JM, Gupta PC, Jarvis MJ, Bhala N, et al. Tobacco use in 3 billion individuals from 16 countries: an analysis of nationally representative cross-sectional household surveys. Lancet 2012;380:668–79.
2. Bilano V, Gilmour S, Moffiet T, d'Espaignet ET, Stevens GA, Commar A, et al. Global trends and projections for tobacco use, 1990–2025: an analysis of smoking indicators from the WHO Comprehensive Information Systems for Tobacco Control. Lancet 2015;385:966–76.
3. Maziak W. Rise of waterpipe smoking. BMJ 2015;350:h1991.
4. Eriksen MP, Mackay J, Schluger NW, Islami F, Drope J. The tobacco atlas. Atlanta, GA: American Cancer Society; 2015.
5. Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol 2008;9:667–75.
6. Hatsukami DK, Stepanov I, Severson H, Jensen JA, Lindgren BR, Horn K, et al. Evidence supporting product standards for carcinogens in smokeless tobacco products. Cancer Prev Res (Phila) 2015;8:20–6.
7. Hecht SS. Research opportunities related to establishing standards for tobacco products under the Family Smoking Prevention and Tobacco Control Act. Nicotine Tob Res 2012;14:18–28.
8. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nat Rev Cancer 2003;3:733–44.
9. Shihadeh A, Schubert J, Klaiany J, El Sabban M, Luch A, Saliba NA. Toxicant content, physical properties and biological activity of waterpipe tobacco smoke and its tobacco-free alternatives. Tob Control 2015;24 Suppl 1:i22–i30.
10. Hecht SS. Progress and challenges in selected areas of tobacco carcinogenesis. Chem Res Toxicol 2008;21:160–71.
11. FDA. (U.S. Food and Drug Administration). Harmful and potentially harmful constituents in tobacco products and tobacco smoke: established list. Fed Regist 2012;20034–7.
12. Center for Tobacco Products/U.S. Food and Drug Administration. Reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke under section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act—Draft Guidance; 2012. Available from: http://www.fda.gov/downloads/TobaccoProducts/Labeling/RulesRegulationsGuidance/ucm297828.pdf.
13. Etemadi A, Khademi H, Kamangar F, Freedman ND, Abnet CC, Brennan P, et al. Hazards of cigarettes, smokeless tobacco and waterpipe in a Middle Eastern Population: a cohort study of 50 000 individuals from Iran. Tob Control 2017;26:674–82.
14. CDC (Centers for Disease Control and Prevention). Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables (January 2017), Vol. 2. 2017. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/exposurereport/. Accessed Dec 18, 2017.
15. Hyland A, Ambrose BK, Conway KP, Borek N, Lambert E, Carusi C, et al. Design and methods of the Population Assessment of Tobacco and Health (PATH) study. Tob Control 2017;26:371–8.
16. Pourshams A, Khademi H, Malekshah AF, Islami F, Nouraei M, Sadjadi AR, et al. Cohort Profile: The Golestan Cohort Study–a prospective study of oesophageal cancer in northern Iran. Int J Epidemiol 2010;39:52–9.
17. Goniewicz ML, Eisner MD, Lazcano-Ponce E, Zielinska-Danch W, Koszowski B, Sobczak A, et al. Comparison of urine cotinine and the tobacco-specific nitrosamine metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and their ratio to discriminate active from passive smoking. Nicotine Tob Res 2011;13:202–8.
18. Kim S. Overview of cotinine cutoff values for smoking status classification. Int J Environ Res Public Health 2016;13:1236.
19. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990;5:46–51.
20. Schick SF, Blount BC, Jacob PR, Saliba NA, Bernert JT, El Hellani A, et al. Biomarkers of exposure to new and emerging tobacco delivery products. Am J Physiol Lung Cell Mol Physiol 2017;313:L425–L52.
21. Jacob P 3rd, Abu Raddaha AH, Dempsey D, Havel C, Peng M, Yu L, et al. Nicotine, carbon monoxide, and carcinogen exposure after a single use of a water pipe. Cancer Epidemiol Biomarkers Prev 2011;20:2345–53.
22. Jacob P 3rd, Abu Raddaha AH, Dempsey D, Havel C, Peng M, Yu L, et al. Comparison of nicotine and carcinogen exposure with water pipe and cigarette smoking. Cancer Epidemiol Biomarkers Prev 2013;22:765–72.
23. Rostron BL, Chang CM, van Bemmel DM, Xia Y, Blount BC. Nicotine and toxicant exposure among U.S. smokeless tobacco users: results from 1999 to 2012 National Health and Nutrition Examination Survey data. Cancer Epidemiol Biomarkers Prev 2015;24:1829–37.
24. DHHS (U.S. Department of Health and Human Services). The health consequences of smoking; nicotine addiction: a report of the Surgeon General. Rockville, MD: U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, Center for Health Promotion and Education, Office on Smoking and Health; 1988.
25. IARC (International Agency for Research on Cancer). Working Group on the Evaluation of Carcinogenic Risks to Humans. Personal habits and indoor combustions. Volume 100 E. A review of human carcinogens. IARC monographs on the evaluation of carcinogenic risks to humans. Lyon, France: World Health Organization, International Agency for Research on Cancer; 2012. p 1–538.
26. Yuan JM, Butler LM, Stepanov I, Hecht SS. Urinary tobacco smoke-constituent biomarkers for assessing risk of lung cancer. Cancer Res 2014;74:401–11.
27. Wu W, Zhang L, Jain RB, Ashley DL, Watson CH. Determination of carcinogenic tobacco-specific nitrosamines in mainstream smoke from U.S.-brand and non-U.S.-brand cigarettes from 14 countries. Nicotine Tob Res 2005;7:443–51.
28. Islami F, Boffetta P, van Schooten FJ, Strickland P, Phillips DH, Pourshams A, et al. Exposure to polycyclic aromatic hydrocarbons among never smokers in Golestan Province, Iran, an area of high incidence of esophageal cancer – a cross-sectional study with repeated measurement of urinary 1-OHPG in two seasons. Front Oncol 2012;2:14.
29. Etemadi A, Islami F, Phillips DH, Godschalk R, Golozar A, Kamangar F, et al. Variation in PAH-related DNA adduct levels among non-smokers: the role of multiple genetic polymorphisms and nucleotide excision repair phenotype. Int J Cancer 2013;132:2738–47.
30. Meeker JD, Barr DB, Serdar B, Rappaport SM, Hauser R. Utility of urinary 1-naphthol and 2-naphthol levels to assess environmental carbaryl and naphthalene exposure in an epidemiology study. J Expo Sci Environ Epidemiol 2007;17:314–20.
31. Maroni M, Colosio C, Ferioli A, Fait A. Biological monitoring of pesticide exposure: a review. Introduction. Toxicology 2000;143:1–118.
32. Kamangar F, Strickland PT, Pourshams A, Malekzadeh R, Boffetta P, Roth MJ, et al. High exposure to polycyclic aromatic hydrocarbons may contribute to high risk of esophageal cancer in northeastern Iran. Anticancer Res 2005;25:425–8.
33. Hakami R, Mohtadinia J, Etemadi A, Kamangar F, Nemati M, Pourshams A, et al. Dietary intake of benzo(a)pyrene and risk of esophageal cancer in north of Iran. Nutr Cancer 2008;60:216–21.
34. Abedi-Ardekani B, Kamangar F, Hewitt SM, Hainaut P, Sotoudeh M, Abnet CC, et al. Polycyclic aromatic hydrocarbon exposure in oesophageal tissue and risk of oesophageal squamous cell carcinoma in north-eastern Iran. Gut 2010;59:1178–83.
35. Nasrollahzadeh D, Kamangar F, Aghcheli K, Sotoudeh M, Islami F, Abnet CC, et al. Opium, tobacco, and alcohol use in relation to oesophageal squamous cell carcinoma in a high-risk area of Iran. Brit J Cancer 2008;98:1857–63.
36. Roth MJ, Qiao YL, Rothman N, Tangrea JA, Dawsey SM, et al. High urine 1-hydroxypyrene glucuronide concentrations in Linxian, China, an area of high risk for squamous oesophageal cancer. Biomarkers 2001;6:381–6.
37. Sepetdjian E, Shihadeh A, Saliba NA. Measurement of 16 polycyclic aromatic hydrocarbons in narghile waterpipe tobacco smoke. Food Chem Toxicol 2008;46:1582–90.
38. Sepetdjian E, Saliba N, Shihadeh A. Carcinogenic PAH in waterpipe charcoal products. Food Chem Toxicol 2010;48:3242–5.
39. Baan R, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V, et al. A review of human carcinogens—part F: chemical agents and related occupations. Lancet Oncol 2009;10:1143–4.
40. Schubert J, Muller FD, Schmidt R, Luch A, Schulz TG. Waterpipe smoke: source of toxic and carcinogenic VOCs, phenols and heavy metals? Arch Toxicol 2015;89:2129–39.
41. Hazrati S, Rostami R, Fazlzadeh M. BTEX in indoor air of waterpipe cafes: levels and factors influencing their concentrations. Sci Total Environ 2015;524–525:347–53.
42. Olsson M, Petersson G. Benzene emitted from glowing charcoal. Sci Total Environ 2003;303:215–20.
43. Kassem NO, Kassem NO, Jackson SR, Liles S, Daffa RM, Zarth AT, et al. Benzene uptake in Hookah smokers and non-smokers attending Hookah social events: regulatory implications. Cancer Epidemiol Biomarkers Prev 2014;23:2793–809.