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Opiate use and exposure to toxicants and carcinogens in Golestan Cohort Study

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Abstract

Background: Over 19.5 million people worldwide abuse natural opiates, such as opium-derived products common in Central Asia and Middle East, and many of them also smoke cigarettes.

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However, there is little information on human exposure to carcinogens and other toxicants related to opiate use, alone or in combination with tobacco use.

Methods: Based on self-reported information, we randomly selected four groups of participants of the Golestan Cohort Study in Northeast Iran: 60 never users of either opiates or tobacco, 35 exclusive current cigarette smokers, 30 exclusive current opiate users, and 30 current opiate users who also smoked cigarettes (dual users; 21 smoked opiates and 9 took them by mouth). We quantified urinary concentrations of 39 exposure biomarkers in four chemical classes: tobacco alkaloids, tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), and volatile organic compounds (VOCs). Total nicotine equivalent (TNE) was used as a measure of nicotine dose. We used Oaxaca-Blinder decomposition to parse out the share of the biomarker concentrations explained by opiate use and nicotine dose.

Results: Exclusive opiate users and exclusive cigarette smokers had substantially higher concentrations of PAH and VOC biomarkers than never users of either product, but dual users had the highest concentrations. Decomposition analysis showed that opiate use contributed a larger part of the PAH concentrations than nicotine dose, and the sum of 2- and 3-hydroxyphenanthrene ($\sum_{2,3}$-phe) resulted almost completely (92%) from opiate use. Concentrations of most VOC biomarkers were explained by both nicotine dose and opiate use, but nicotine dose contributed more. Two acrylamide metabolites (AAMA: 90%, GAMA: 91%), the 1,3-butadiene metabolite (DHBM: 73%), and the dimethylformamide metabolite (AMCA: 72%) were more strongly explained by opiate use. Acrylamide metabolites and $\sum_{2,3}$-phe were significantly higher in opiate smokers than opiate eaters; other biomarkers did not vary by the route of opiate intake.

Conclusion: Both opiate users and cigarette smokers are exposed to several toxicants and carcinogens. Most biomarkers in opiate users were independent of exposure route, but a few were higher among opiate smokers than eaters. As opiates are widely used worldwide, exposure to some of these toxicants, including PAHs and VOCs, may have substantial global public health impact.

Introduction

Opiates are structurally related to compounds found in the resin of the opium poppy, *Papaver somniferum*. Opiate products (opium, morphine and heroin) are derived from this resin, and an opioid is any agent (synthetic or natural) with the functional and pharmacological properties of an opiate. (1) Over 35 million people worldwide are estimated to abuse opioids. (2) Among them, an estimated 19.5 million people abuse non-prescription opiates, including opium-derived products common in Central Asia and the Middle East. In 2017, 86% of the total estimated global opium production originated from Afghanistan, and about 90% of this production was processed into heroin in this country. Naturally, most of the seizures of opiates are made close to the production, mainly in the Near and Middle East/ Southwest Asia (83%), and these countries constitute important targets for global illicit drug markets. (2)

Opiate use is associated with the risk of mental disorders, infections, and overdose death. (3) While considerable attention has been directed, due to the recent opioid epidemic, towards these acute risks, chronic opiate use can also have long-lasting effects on health. There is accumulating evidence about the potential carcinogenicity of opiate use (4), and associations

have been reported between chronic opiate use and the risk of esophageal (5), gastric (6), pancreatic (7), and bladder cancers.(8) Chronic opiate use has also been associated with increased risk of cardiovascular disease (9) and all-cause premature mortality (10). Furthermore, opiate dependence is closely related to heavy smoking and increased nicotine dependence, (11, 12) and opioid users have more difficulty quitting smoking than non-users. (13) As such, potential synergistic effects of opiates and tobacco on health are important. One of the best ways to investigate these effects is to study biomarkers of exposure to toxicants and carcinogens, such as those included in the U.S. Food and Drug Administration's (FDA) list of "Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke", (14) among exclusive and dual users of opiates and tobacco.

Population-based studies of biomarkers among opiate users are complex because of challenges in obtaining detailed and reliable exposure data. Besides, most of these biomarkers are only measured in urine, which is not available in most large-scale population-based studies. (15) The Golestan Cohort Study (GCS) in Iran (neighboring Afghanistan and the major route of opiate transit to the rest of the world) provides a unique opportunity for such studies. About 17% of the participants in this cohort are chronic opiate users (16), with detailed and validated self-reported tobacco and opiate use information, (17) along with concurrent urine samples collected at the time of recruitment. We have previously shown that several biomarkers of toxicant and carcinogen exposure developed at the Centers for Disease Control and Prevention (CDC) National Center for Environmental Health (NCEH) Laboratory, (18, 19) can be successfully measured in the urine samples from this cohort to evaluate toxicant and carcinogen exposures. (20) In the present study, we evaluated such exposures among chronic opiate users, with or without concomitant cigarette smoking.

Methods

The Golestan Cohort Study (21) includes 50,045 individuals aged ≥ 40 years who live in Golestan Province, in the northeast of Iran. A baseline questionnaire collected information on self-reported use of opiates and tobacco, the ages of starting and stopping each product, the frequency of use, and daily consumption amount, along with other demographic and lifestyle information. All GCS participants gave a spot urine sample between 2004 and 2008, when they enrolled in the Cohort. These samples were stored at −20°C until 2015 when they were transferred on dry ice to the US National Cancer Institute (NCI) Biorepository and stored at −80°C. The GCS was approved by appropriate ethics committees at Tehran University of Medical Sciences, (NCI, and the International Agency for Research on Cancer (IARC). The involvement of the CDC laboratory did not constitute engagement in human subjects research.

From GCS participants who were alive and cancer-free in December 2016, we randomly selected 4 groups of participants based on self-reported opiate and tobacco use at enrollment: 60 never users of any tobacco or opiate product during their life, 35 exclusive current cigarette smokers, 30 exclusive current opiate users who never used tobacco, and 30 current opiate users who currently smoked cigarettes (dual users). Users of non-cigarette tobacco products were excluded from the study. We restricted the last three groups to men as cigarette smoking in GCS is almost exclusive to men, and a relatively small group of women

used opiates. The most common opiates used by GCS participants are raw opium and opium-derived juice (Shireh), and the two most common routes are smoking (68%) and oral ingestion (26%).(10)

Laboratory measurements

The analytical measurements were conducted at the Division of Laboratory Sciences of NCEH at CDC. We have previously described the panel of 39 biomarkers used in this study (Table 1), which were the same used in the National Health and Nutrition Examination Survey (NHANES) and The Population Assessment of Tobacco and Health (PATH) studies (18). These included 9 metabolites of tobacco alkaloids (nicotine and its six metabolites and 2 minor tobacco alkaloids), 4 tobacco-specific nitrosamines (TSNAs), 7 metabolites of polycyclic aromatic hydrocarbons (PAHs), and 19 metabolites of volatile organic compounds (VOCs). Nicotine metabolites were tested in all urine samples, regardless of opiate or cigarette use, to check whether study participants were active cigarette smokers or not. Tobacco-specific metabolites (tobacco alkaloids and TSNAs) would fall below the limits of detection (LODs) in samples with very low or undetectable concentrations of urinary cotinine; these biomarkers were only tested in samples with a cotinine concentration above 20 ng/mL (22) regardless of self-reported tobacco use. However, if the cotinine concentration was below 20 ng/mL, we used a more sensitive cotinine and hydroxycotinine assay to evaluate the participants' secondhand tobacco smoke exposure. PAHs and VOCs are not tobacco-specific and were tested on all samples.

Urinary tobacco alkaloids were measured by an isotope dilution high-performance liquid chromatography/tandem mass spectrometric method.(23) The LODs ranged from 0.39 to 10.5 ng/mL, depending on the analyte. The highly-sensitive assays for cotinine and trans-3'-hydroxycotinine used a modified version of isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry as described by Bernert et al.(24) The LOD was considerably lower than the usual assay (0.030 ng/mL) for both analytes in this sensitive assay. TSNAs were measured by isotope dilution high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry using a modified version of the method described by Xia et al.(25) The LOD for urinary TSNAs ranged from 0.0006 to 0.0042 ng/mL. The 7 PAH metabolites were quantified by online solid phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as previously described.(26) The LODs for PAHs ranged from 0.008 to 0.09 ng/mL. Urinary VOC metabolite concentrations were measured using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry according to a published procedure.(27) LODs for VOC metabolites ranged from 0.500 to 15.0 ng/mL. Last, creatinine was measured by a commercial automated, colorimetric enzymatic (creatinase) method implemented on a Roche/Hitachi Cobas 6000 Analyzer. Table 1 shows the coefficients of variation (CVs) of the tests calculated on blind pooled samples from GCS; all but two were below 20%, and 19 were below 10%, showing excellent assay performance.

Validity of self-reported cigarette and opiate use

Self-reported opiate use has been previously tested against urinary codeine and morphine in a random sample of 150 participants of the GCS and proven to be a valid indicator of ever use (k statistics >0.9) with 93% sensitivity and 89% specificity.(17)

To validate self-reported cigarette smoking, in the present study, we defined active cigarette smoking as urinary cotinine concentrations of 50 ng/mL or greater.(28) There was excellent agreement between urinary cotinine and self-reported cigarette smoking (Supplementary Table 1). The only group with a relatively high number of discordant results (7 out of 30) were the exclusive opiate users. To reduce any potential error from incorrect reporting of cigarette smoking, we excluded individuals with discordant questionnaire data and urine specimens (self-reported never tobacco users with high cotinine concentrations, and self-reported current cigarette smokers with cotinine values below 50 ng/mL), because self-report was deemed unreliable for these participants.

Statistical Analysis

For most biomarkers, fewer than 10% of the values were below the LOD, and none of the biomarkers had 20% or more below-LOD values. Concentrations below the LOD were replaced by the LOD divided by the square root of 2.(29) All biomarker concentrations were adjusted for urinary dilution by dividing by urinary creatinine, and were log-transformed. We calculated geometric means (GM) and 95% confidence intervals (95%CI) of these creatinine-corrected values. The total nicotine equivalent (TNE) is a standard method of estimating nicotine exposure and was calculated as the molar sum of nicotine metabolites. (15) Depending on the number of metabolites measured in each person, we calculated TNE2 (the molar sum of cotinine and hydroxycotinine) for everyone, and TNE7 (the molar sum of all 7 nicotine metabolites) for cigarette smokers.

To establish the proportion of each biomarker concentration explained by opiate use and nicotine dose we used a decomposition method called the Oaxaca-Blinder method. (30, 31) Oaxaca-Blinder decomposition uses stratified linear regression to segregate the observed differences among study groups into the differences in observed characteristics of individual participants (termed “endowments”) and unexplained differences due to their group membership (coefficients). We stratified the study participants to opiate users and non-users, and then decomposed the differences in biomarker concentrations between these two groups into the proportion explained by “endowments” (nicotine dose, age, ethnicity, place of residence, education, and BMI) and the unexplained part due to being either an opiate user or non-user. Because nicotine dose was, by far, the strongest component of those “endowments,” we report this endowment part as the share of nicotine dose and (a much smaller share of) all other variables combined. Decomposition was done using the Oaxaca ado file for Stata (StataCorp Inc., College Station, TX).

Results

Table 2 shows the baseline characteristics of the opiate non-users and opiate users. Both groups were stratified by cigarette smoking (never tobacco users and current cigarette

smokers). Opiate users were more likely to be of Turkmen ethnicity and live in a village, and they had lower BMI. Opiate users who also smoked cigarettes (dual users) were both heavier smokers and heavier opiate users than exclusive users of either cigarettes or opiates. Among dual users 90% used opiates every day, compared with 74% of exclusive opiate users.

In Table 3, we compare the geometric means and 95% CI of the 39 study biomarkers across the opiate users and non-users subdivided by cigarette smoking. Exclusive opiate users (i.e. those who did not smoke cigarettes) had high concentrations of most biomarkers, relative to non-opiate users who also did not smoke cigarettes. Except for one PAH (sum of 2- and 3-hydroxyphenanthrene or $\sum_{2,3}$-phe) and two VOC (BMA and PHGA) biomarkers, opiate users who also smoked cigarettes had the highest concentrations of all biomarkers.

Opiate users had higher nicotine doses (i.e. TNE2) than non-opiate users with a similar cigarette smoking history (Table 3). We used Oaxaca-Blinder decomposition to explore the proportion of the biomarker differences between opiate users and non-users that could be explained by the amount of exposure to tobacco (their nicotine dose) vs. the proportion explained by opiate use. As seen in Table 4, differences between opiate users and non-users in PAH concentrations were explained by both the nicotine dose and opiate use, but opiates seemed to contribute a larger part of the differences in PAHs. The most striking of these was for $\sum_{2,3}$-phe which was almost completely explained by opiate use. Among the VOCs, nicotine dose (compared with opiate use) seemed to explain a bigger share of the observed biomarker differences between opiate users and non-users. However, a few VOC biomarkers were strongly explained by opiate use, including acrylamide metabolites (AAMA: 90%, GAMA: 91%), the 1,3-butadiene metabolite (DHBM: 73%), and the dimethylformamide metabolite (AMCA: 72%). Two other VOCs (BMA and TTCA) had a high percentage of the difference explained by opiate use, but because the difference between opiate users and non-users was low, these differences were not statistically significant. All other variables in the model (age, ethnicity, place of residence, education, and BMI) contributed a much smaller share of biomarker differences than nicotine dose and opiate use.

Last, we examined whether the observed higher concentrations of PAH and VOC biomarkers in opiate users came from the opiates themselves or their combustion. To this end, we compared nine dual users who only took opiates by mouth vs. the larger group of dual users (n=21) who only smoked them (Table 5). Concentrations of examined PAH and VOC biomarkers were largely similar between opiate eaters and smokers. Among PAHs, the only significant difference was in $\sum_{2,3}$-phe concentration which was almost five times higher in opiate smokers (geometric mean: 3477.4 ng/g creatinine; 95%CI: 1891.3–6393.6) than opiate eaters (geometric mean: 771.4; 95% CI: 436.4–1363.4). Among VOCs, two acrylamide biomarkers (AAMA and GAMA) were substantially higher in opiate smokers than eaters.

Discussion

Opiate users in our study were exposed to toxicants and carcinogens, with biomarker concentrations comparable to those among cigarette smokers. Although a relatively high percentage of biomarker concentrations among opiate users could be explained by their

higher nicotine dose, opiate use itself contributed substantially to exposure to PAHs. Among toxicant biomarkers with relatively high concentrations in opiate users, most had similar concentrations across opiate eaters and smokers, although three biomarkers ($\sum_{2,3}$-phe, AAMA and GAMA) were higher among opiate smokers.

To the best of our knowledge, this is the first study to specifically compare several tobacco-related biomarkers in the urine from opiate users and non-users. Based on our results, the biomarkers we studied can be broadly categorized into 4 groups. Group 1 were tobacco-specific biomarkers which exclusively (or almost exclusively) originated from cigarette smoking. This group included tobacco alkaloids, and TSNAs, which are usually below LOD in tobacco non-users and exclusive opiate users. There were also two VOC metabolites (HPM2 and MHB3) in this group, which are normally detectable among tobacco non-users, but the role of non-tobacco exposure (including opiate use) was small compared to cigarette smoking. Group 2 included biomarkers moderately (≤60%) associated with opiate use: this group of biomarkers included three PAHs and most of the VOCs. The contribution of opiate use to these biomarkers ranged between 16% (IPM3) and 60% (MADA) and was independent of the route of opiate use. Group 3 biomarkers were strongly associated with opiate use, irrespective of route of use: three PAHs (1-hydroxyphenanthrene, 3-hydroxyfluorene, and 1-hydroxypyrene), and two VOCs (AMCA and DHBM) fell into this category. Opiate use contributed almost 70% of these biomarkers, and their concentrations were comparable between opiate eaters and smokers. Group 4 biomarkers were specifically associated with the combustion of opiates: these included the sum of two phenanthrene metabolites $\sum_{2,3}$-phe, and acrylamide metabolites (AAMA and GAMA). More than 90% of these biomarkers came from opiate use, and concentrations were much higher in opiate smokers than eaters. It is noteworthy that many (but not all) of the parent compounds for the biomarkers in our study (such as acrylamide) are on the FDA's list of Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke. (14) In addition to these four groups, three VOCs did not show a remarkable contribution from either opiate use or cigarette smoking (BMA, PHGA, TTCA).

The concentration of $\sum_{2,3}$-phe among opiate users in our study reached almost 10 times the concentration reported among U.S. cigarette smokers participating in NHANES. (18) Opiates contain different alkaloids, and among them morphine, codeine and thebaine are the most abundant. These alkaloids are usually called phenanthrene-type alkaloids and contain a similar heterocyclic ring (32). Previous studies have shown that compounds resulting from high-temperature treatment of these alkaloids, either by burning of opiate products, or the direct pyrolysis of alkaloids (particularly morphine) shared a common 3-hydroxyphenanthrene moiety (33). The pyrolysates of phenanthrene-type alkaloids showed strong mutagenic capacity against *Salmonella typhimurium*, which was strongest when morphine itself was pyrolysated, and correlated with their nitrogen content (34), as described for other PAHs such as benzo[a]pyrene.(35) Phenanthrene-based species are also produced as a result of pyrolysis of heroin, the most common illicit opiate used worldwide. (36) Because smoked drugs bypass first-order metabolism in the liver, many other drugs of abuse, even synthetic opioids, are being increasingly used in this way. A study of smoked fentanyl has shown significant amounts of VOCs such as styrene and benzene in the pyrolysis

products of this synthetic opioid. (37) However, the information on the toxicity of these opioid products, and biomarkers associated with their use are not available.

Pyrolysis does not seem to be the only factor contributing to toxicant exposure among opiate users in our study. Some relatively high PAH and VOC metabolite concentrations were associated with eating opium, and to the best of our knowledge, these opium products were not processed using high temperature. (36) Eating opium has also been associated with the risk of some cancers (5) and death from different causes. (10, 38) Together, these findings suggest that VOCs and PAHs are present in the opiate products even without exposure to high temperatures. As there are little prior data, we are currently conducting additional field studies to evaluate this hypothesis.

Combined use of opiates and tobacco have been more strongly associated with increased risk of bladder (39) and esophageal cancer (5), than with either exposure alone. In the present study, opiate users who also smoked cigarettes had the highest concentrations of all biomarkers across different groups. Two factors can contribute to these high concentrations: these individuals were receiving PAHs and VOCs from two sources (cigarettes and opiates), and they were heavier users of both cigarettes and opium than individuals who suede only one of these products. Previous studies have also shown that opioid use is associated with greater nicotine dependence and poorer smoking cessation outcomes.(12)

This exploratory study is the first to use the same state-of-the-art analytical methods used for large population-based studies, such as NHANES, among opiate users. Urinary metabolites are markers of relatively recent exposure, but we have previously shown acceptable correlations between biomarker concentrations in baseline urine and repeated samples taken after several years, (20) particularly in the presence of a strong source of exposure (such as cigarette smoking). It should be noted that study participants used raw opium or opium-derived non-prescription opiates which were smoked or eaten. Further studies should be directed towards identifying biomarkers of toxicant exposures among people who use other types of opiates, and/or use them by other routes (such as injection).

In conclusion, the current study provides evidence of opiate users' exposure to toxicants and carcinogens from opiate use, and, in dual users, from concomitant cigarette smoking. Given the large number of people using different forms of opiates across the world, these exposures may have a substantial global public health impact. Future studies examining the chronic effects of such opiate uses, as well as the underlying physiologic mechanisms of such effects, are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1-

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

Biomarker Class	Full compound name	Parent compound	Abbreviation	CV (%)
Nicotine and its metabolites	cotinine	Nicotine	COTT[1]	4.6
	trans-3'-hydroxycotinine	Nicotine	HCTT[1]	4.3
	cotinine N-oxide	Nicotine	COXT[2]	7.3
	norcotinine	Nicotine	NCTT[2]	6.7
	nicotine	Nicotine	NICT[2]	2.5
	nicotine 1'-oxide	Nicotine	NOXT[2]	5.0
	nornicotine	Nicotine	NNCT[2]	3.7
Other tobacco alkaloids	anabasine	Anabasine	ANBT[2]	3.1
	anatabine	Anatabine	ANTT[2]	3.9
Tobacco specific nitrosamines (TSNAs)	*N*′-nitrosoanabasine	NAB	NABT[2]	4.1
	N′-nitrosoanatabine	NAT	NATT[2]	4.6
	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	NNK	NNAL[2]	1.4
	N′-nitrosonornicotine	NNN	NNNT[2]	13.7
Metabolites of polycyclic aromatic hydrocarbons (PAHs)	1 -Hydroxynaphthalene	Naphthalene/carbaryl*	1-nap[1]	2.2
	2-Hydroxynaphthalene	Naphthalene	2-nap[1]	2.9
	1 -Hydroxyphenanthrene	Phenanthrene	1-phe[1]	7.5
	Sum of 2- and 3-hydroxyphenanthrene	Phenanthrene	$\sum_{2,3}$phe[1]	6.9
	2-Hydroxyfluorene	Fluorene	2-flu[1]	3.1
	3-Hydroxyfluorene	Fluorene	3-flu[1]	5.7
	1-Hydroxypyrene	Pyrene	1-pyr[1]	20.1
Metabolites of volatile organic compounds (VOCs)	2-Methylhippuric acid	Xylene	2MHA[1]	7.4
	3-Methylhippuric acid + 4 Methylhippuric acid	Xylene	34MH[1]	10.8
	N-Acetyl-S-(2-carbamoylethyl)-L-cysteine	Acrylamide	AAMA[1]	13.5
	N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine	Acrylamide	GAMA[1]	11.0
	N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	Acrylonitrile	CYHA[1]	16.5
	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	Acrylonitrile	CYMA[1]	12.3
	N-Acetyl-S- (2-carboxyethyl)-L-cysteine	Acrolein	CEMA[1]	12.8
	N-Acetyl-S- (3-hydroxypropyl)-L-cysteine	Acrolein	HPMA[1]	15.1
	N-Acetyl-S-(benzyl)-L-cysteine	Toluene*	BMA[1]	12.2

Biomarker Class	Full compound name	Parent compound	Abbreviation	CV (%)
	Mandelic acid	Styrene	MADA[1]	21.4
	Phenylglyoxylic acid	Ethylbenzene/styrene	PHGA[1]	13.6
	N-Acetyl-S-(phenyl)-L-cysteine	Benzene	PMA[1]	17.3
	N-Acetyl-S- (2-hydroxypropyl)-L-cysteine	Propylene oxide	HPM2[1]	10.1
	N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine	Dimethylformamide[*]	AMCA[1]	11.8
	N-Acetyl-S- (3,4-dihydroxybutyl)-L-cysteine	1,3-Butadiene	DHBM[1]	11.5
	N-Acetyl-S-(4-hydroxy-2-buten-1-yl)-L-cysteine	1,3-Butadiene	MHB3[1]	15.3
	N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine	Crotonaldehyde	HPMM[1]	11.3
	N-Acetyl-S-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine	Isoprene	IPM3[1]	17.1
	2-Thioxothiazolidine-4-carboxylic acid	Carbon disulfide	TTCA[1]	10.1

CV: Coefficient of variation

[*] Multiple other parent chemicals can also be metabolized to these compounds.

[1] Measured in all individuals

[2] Measured only among those with cotinine above 20 ng/mL.

Table 2.

Baseline and demographic characteristics of the study population selected from the Golestan Cohort participants

	Opiate non-users			Opiate users		
	Never tobacco users (n=58)*	Cigarette smokers (n=33)*	Total (n=91)	Exclusive opiate users (n=23)*	Users of opiate and cigarette (n=30)	Total (n=53)
Age: mean (SD)	51.2(8.4)	50.7(7.6)	51.0 (8.1)	50.4(5.3)	48.2(6.9)	49.2 (6.3)
Sex: m/f	28/30	33/0	61/30	23/0	30/0	53/0
Turkmen ethnicity %	70.7	72.7	71.4	100	86.7	92.5
Residence						
Urban: %	19.0	42.4	27.5	4.3	20	13.2
Rural: %	81.0	57.6	72.5	95.7	80	86.8
education						
None: %	63.8	39.4	55.0	52.2	56.7	54.7
1–8 years: %	25.9	30.3	27.5	34.8	23.3	28.3
> 8 years: %	10.3	30.3	17.5	13.0	20.0	17.0
BMI						
Underweight: %	1.8	0	1.1	4.3	16.7	11.3
Normal: %	31.0	42.4	35.2	26.1	70	51.0
Overweight: %	36.2	39.4	37.4	60.9	13.3	34.0
Obese: %	31.0	18.2	26.4	8.7	0	3.7
Age when use started: mean (SD)						
Tobacco	NA	25.2(7.4)	25.2(7.4)	NA	27.2(11.1)	27.2(11.1)
Opiate	NA	NA	NA	45.2(7.2)	35.4(8.0)	39.7 (9.0)
Use intensity: mean per day (SD)						
Cigarette	NA	10.9(7.3)	NA	NA	16.4(8.9)	NA
Opiate	NA	NA	NA	3.5(2.7)	4.7 (3.4)	4.2 (3.2)

*Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine concentrations

Table 3.

Geometric means and 95% confidence intervals of several urinary biomarkers across groups of Golestan Cohort participants

	Opiate non-users		Opiate users	
	Never tobacco users (n=58)*	Cigarette smokers (n=33)*	Exclusive opiate users (n=23)*	Users of opiate and cigarette (n=30)
Nicotine and other tobacco alkaloids (ng/mg creatinine)				
COTT	1.3(0.9,1.8)	1799.9(1200.7,2698.3)	3.5(1.9,6.4)	4238.2(3420.8,5250.9)
HCTT	2.8(2.1,3.8)	2661.6(1742.6,4065.3)	5.4(2.9,10.0)	7201.6(5768.9,8990.0)
TNE2**	0.02(0.02,0.03)	24.9(16.6,37.4)	0.05(0.03,0.09)	62.6(51.0,77.0)
COXT	-	214.9(142.7,323.8)	-	437.3(353.8,540.4)
NCTT	-	56(37.1,84.4)	-	136.5(109.2,170.7)
NICT	-	676.8(382.1,1198.8)	-	1872.9(1329.1,2639.4)
NOXT	-	182.6(109.2,305.6)	-	307.6(234.7,403.1)
NNCT	-	42.5(27.0,67.0)	-	101(79.4,128.4)
TNE7**	-	33.4(21.9,50.8)	-	82.1(66.2,101.8)
ANBT	-	4.6(3.0,7.1)	-	12(9.0,16.0)
ANTT	-	6.5(3.9,10.7)	-	16.6(12.3,22.5)
Tobacco-specific nitrosamines (pg/mg creatinine)				
NABT	-	9.1(6.2,13.5)	-	19.5(15.1,25.1)
NATT	-	53.7(34.5,83.6)	-	107.9(83.9,138.6)
NNAL	-	130.9(92.6,185.1)	-	230(181.9,290.7)
NNNT	-	10.4(6.9,15.8)	-	13.3(8.7,20.1)
Polycyclic aromatic hydrocarbons (ng/g creatinine)				
1-nap	10872(8064,14657)	14637(11511,18613)	14443.8(9310.7,22406.9)	28993.2(21459.5,39171.8)
2-nap	2299.7(1835.7,2881.0)	9048.9(6864.2,11929.0)	3544.7(2475.5,5075.7)	18857.0(15123.1,23512.8)
1-phe	247.1(213.9,285.5)	264.5(227.4,307.8)	290.6(228.2,370.1)	491.9(410.3,589.8)
$\sum_{2,3}$-phe	300.3(250.4,360.3)	482.9(371.5,627.6)	2561(1290.9,5080.7)	2213.4(1326.8,3692.6)
2-flu	396.0(330.6,474.3)	1238.6(973.2,1576.5)	538(371.8,778.4)	2044.2(1656.6,2522.4)
3-flu	168.1(133.3,212.0)	755.7(566.1,1008.9)	775.8(452.9,1328.9)	2467.5(1835.1,3317.8)
1-pyr	412.0(344.2,493.1)	636.0(504.0,802.4)	966.3(665.2,1403.7)	1421.9(1111.6,1819.0)
Volatile organic compounds (ng/mg creatinine)				
2MHA	66.4(50.0,88.3)	148.2(113.5,193.5)	95.1(53.5,168.9)	190.3(133.3,271.8)
34MH	320.7(252.5,407.2)	839.4(650.3,1083.5)	450.4(264.7,766.3)	1231.0(875.6,1730.7)
AAMA	47.7(41.3,55.2)	124.7(97.2,159.9)	243.5(171.4,346.0)	281.0(214.0,368.8)
GAMA	8.5(7.2,10.1)	15.2(12.4,18.7)	28.8(19.3,42.9)	31.5(23.8,41.8)
CYHA	0.7(0.6,0.9)	14.2(9.1,22.1)	4.7(2.7,8.4)	35.5(26.9,46.8)
CYMA	1.1(0.9,1.4)	86.4(58.3,127.9)	17.4(9.7,31.0)	190.6(148.6,244.4)
CEMA	77.8(65.4,92.6)	186.2(153.9,225.3)	105.4(81.6,136.1)	244.2(193.1,308.9)
HPMA	188.1(154.5,228.9)	881.8(661.8,1174.9)	300.5(211.7,426.5)	1243.6(855.6,1807.7)
BMA	5.3(4.0,7.0)	6.4(4.4,9.2)	6.9(4.5,10.5)	6.1(4.5,8.2)

	Opiate non-users		Opiate users	
	Never tobacco users (n=58)*	Cigarette smokers (n=33)*	Exclusive opiate users (n=23)*	Users of opiate and cigarette (n=30)
MADA	186.8(161.9,215.5)	274.2(225.7,333.2)	248.4(201.2,306.8)	464.9(375.9,575.0)
PHGA	86.5(65.1,115.0)	101.3(68.8,149.3)	60(35.6,101.0)	97.9(65.1,147.2)
PMA	1.3(1.1,1.6)	1.5(1.2,1.8)	1.3(0.8,2.0)	2.0(1.6,2.5)
HPM2	25.3(21.5,29.8)	53.9(42.9,67.7)	22.4(17.3,29.0)	70.9(56.7,88.7)
AMCA	92.1(74.6,113.6)	296.3(233.1,376.7)	283.3(187.7,427.4)	771.1(631.1,942.3)
DHBM	280.0(235.4,333.1)	384.5(337.3,438.4)	326.5(259.8,410.3)	443.0(371.5,528.4)
MHB3	4.3(3.6,5.2)	23.3(16.5,32.8)	5.3(4.1,6.8)	32.9(23.0,46.9)
HPMM	373.4(311.9,446.9)	1359.7(980.2,1886.0)	422.7(304.2,587.5)	2068.9(1434.0,2985.0)
IPM3	2.0(1.7,2.4)	21.6(12.9,36.2)	3.4(2.3,4.9)	39.0(23.8,64.0)
TTCA	12.4(9.3,16.4)	12(9.3,15.4)	13(8.3,20.4)	19.5(12.5,30.4)

*Numbers exclude individuals who had self-reported tobacco status discordant with measured cotinine levels

**TNE: total nicotine equivalent in nmol/mg creatinine. TNE2 was calculated based on cotinine and hydroxycotinine only, TNE7 based on all 7 nicotine metabolites.

Table 4.

Geometric means and 95% confidence intervals of different metabolites across opiate users and non-users in Golestan Cohort participants and Oaxaca- Blinder decomposition of the percent explained by different factors

			% of the difference[†]:		
	All opiate non-users (n=91)	**All opiate users (n=53)**	**explained by nicotine dose**	**explained by opiate use**	**explained by other factors**
Polycyclic aromatic hydrocarbons (ng/g creatinine)					
1-nap	12103.3(9826.0,14908.4)	21427.6(16572.0,27705.8)	26.3 *	59.6 *	14.0
2-nap	3776.0(3028.2,4708.5)	9129.8(6814.5,12231.6)	55.7 **	43.2 *	1.1
1-phe	249.4(225.0,276.5)	391.5(335.3,457.0)	22.2 **	68.9 *	8.9
$\sum_{2,3}$-phe	354.1(303.6,413.0)	2358.0(1597.3,3481.1)	4.2	91.6 **	4.2
2-flu	596.4(496.9,716.0)	1218.2(942.7,1574.2)	66.2 **	21.1	12.7
3-flu	291.1(230.3,367.9)	1493.4(1094.8,2037.1)	28.7 **	66.5 **	4.9
1-pyr	479.8(414.7,555.2)	1202.5(978.0,1478.6)	13.0 *	70.7 **	16.3
Volatile organic compounds (ng/mg creatinine)					
2MHA	87.4(70.3,108.6)	140.8(102.8,192.8)	68.8 **	47.9	−16.7
34MH	448.7(367.5,547.9)	795.7(582.2,1087.4)	63.2 **	42.1	−5.3
AAMA	67.7(57.6,79.4)	264.0(215.3,323.8)	17.6 **	90.4 **	−8.1
GAMA	10.5(9.1,12.1)	30.3(24.3,37.8)	17.0 **	90.6 **	−7.5
CYHA	2.2(1.5,3.1)	14.8(10.1,21.8)	43.2 **	59.9 **	−3.1
CYMA	5.6(3.4,9.0)	67.4(44.3,102.6)	45.0 **	59.8 **	−4.8
CEMA	106.6(91.2,124.7)	169.6(139.1,206.7)	65.2 **	45.7	−10.9
HPMA	331.2(264.6,414.5)	671.4(492.0,916.3)	69.0 **	32.4	−1.4
BMA	5.7(4.6,7.1)	6.4(5.1,8.1)	23.1	107.7	−30.8
MADA	214.4(190.1,241.8)	356.6(302.1,421.0)	41.6 **	60.4 *	−2.0
PHGA	92.2(73.6,115.5)	75.6(56.8,100.6)	NA	NA	NA
PMA	1.4(1.2,1.6)	1.6(1.3,2.0)	57.9 *	36.8	5.3
HPM2	33.5(28.8,39.0)	43.0(34.4,53.7)	120.0 *	−24.0	4.0
AMCA	141.1(115.8,171.9)	499.3(393.2,634.2)	27.0 **	72.2 **	0.8
DHBM	312(275.8,353.0)	388.1(338.0,445.5)	50.0 **	72.7	−22.7
MHB3	8.0(6.3,10.1)	14.9(10.7,20.7)	91.9 **	8.1	0.0
HPMM	578.4(470.2,711.6)	1038.6(754.8,1429.1)	89.8 **	23.7	−13.6
IPM3	4.9(3.6,6.7)	13.5(8.6,21.1)	77.2 **	16.8	5.9
TTCA	12.2(10.0,14.9)	16.4(12.1,22.2)	16.7	113.3	−30.0

NA: Opiate and nicotine contributions could not be calculated since biomarker concentration was lower among opiate users.

[†]Using Oaxaca- Blinder decomposition; negative percentages mean that the factors were in favor of a lower level in opiate users, percentages above 100 show that the concentrations due to nicotine/opiate could have been higher if not counter-acted by other factors acting in the opposite direction.

* $p<0.05$

** $p<0.001$ for the association between each factor and the biomarker concentration in linear regression models including TNE2 and opiate use, in addition to age, residence, ethnicity, education, and BMI.

Table 5.

Geometric means and 95% confidence intervals of different tobacco-related metabolites among dual opiate and cigarette smokers by their route of opiate use

	Opiate smokers (n=21)	Opiate eaters (n=9)
Nicotine and other tobacco alkaloids (ng/mg creatinine)		
COTT	4448.7(3445.8,5743.3)	3785(2348.1,6101.3)
HCTT	6938.9(5212.8,9236.7)	7853.8(5229.4,11795.2)
COXT	456.3(352.4,590.8)	396.0(252.1,621.9)
NCTT	126.5(97.9,163.4)	163.2(97.1,274.4)
NICT	1992.3(1296.9,3060.5)	1621.5(814.4,3228.3)
NOXT	344.3(252.3,469.8)	236.5(128.9,434.0)
NNCT	104.5(77.7,140.3)	93.3(56.1,155.1)
TNE7 **	83.1(63.4,108.9)	79.8(51.8,122.9)
ANBT	14.0(10.2,19.2)	8.5(4.3,16.5)
ANTT	19.3(14.1,26.3)	11.8(5.5,25.5)
Tobacco-specific nitrosamines (pg/mg creatinine)		
NABT	21.7(15.8,29.8)	15.0(9.4,24.1)
NATT	115.7(83.7,160.0)	91.6(59.1,142.1)
NNAL	235.5(178.3,311.2)	217.5(127.2,372.0)
NNNT	16.2(9.6,27.4)	9.1(4.2,19.5)
Polycyclic aromatic hydrocarbons (ng/g creatinine)		
1-nap	27434.6(18469.6,40751.3)	32982.9(19830.3,54858.9)
2-nap	18544.3(13979.5,24599.7)	19607.2(12869.4,29872.5)
1-phe	486.3(392.9,601.7)	505.4(330.2,773.6)
$\sum_{2,3}$-phe	3477.4(1891.36393.6)	771.4(436.4,1363.4)
2-flu	1961.4(1510.0,2547.7)	2251.2(1468.8,3450.5)
3-flu	2668.2(1827.2,3896.1)	2056.0(1195.9,3534.5)
1-pyr	1488.2(1128.1,1963.2)	1278.6(693.9,2356.0)
Volatile organic compounds (ng/mg creatinine)		
2MHA	166.9(107.7,258.6)	258.5(127.9,522.2)
34MH	1150.2(753.8,1755.1)	1442.1(718.1,2896.0)
AAMA	341.4(243.7,478.2)	178.3(124.0,256.5)
GAMA	40.9(30.2,55.3)	17.2(10.9,27.2)
CYHA	38.7(28.0,53.5)	28.9(15.4,54.5)
CYMA	183.2(134.7,249.3)	209.0(124.7,350.3)
CEMA	219.1(164.7,291.5)	314.5(199.7,495.4)
HPMA	1211.3(743.5,1973.5)	1322.4(677.8,2579.9)
BMA	5.3(3.8,7.4)	8.4(4.4,16.2)
MADA	441.1(335.9,579.3)	525.6(356.7,774.5)
PHGA	80.9(49.3,132.5)	152.8(69.0,338.2)
PMA	2.0(1.6,2.5)	2.0(1.2,3.4)

	Opiate smokers (n=21)	Opiate eaters (n=9)
HPM2	71.4(53.6,95.1)	69.6(45.4,106.8)
AMCA	818.9(627.3,1069.1)	670.2(495.0,907.5)
DHBM	415.7(328.7,525.7)	514(400.0,660.6)
MHB3	30.7(19.2,49.2)	38.4(21.3,69.3)
HPMM	1945.9(1213.2,3121.2)	2386.8(1217.3,4680.1)
IPM3	35.9(18.5,69.8)	47.2(22.1,100.7)
TTCA	15.3(10.0,23.4)	34.3(10.4,113.5)

** TNE7: total nicotine equivalent in nmol/mg creatinine based on all 7 nicotine metabolites.