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Original investigation

Biomarkers of Secondhand Smoke Exposure in Waterpipe Tobacco Venue Employees in Istanbul, Moscow, and Cairo

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Abstract

Background: Most smoke-free legislation to reduce secondhand smoke (SHS) exposure exempts waterpipe (hookah) smoking venues. Few studies have examined SHS exposure in waterpipe venues and their employees.

Methods: We surveyed 276 employees of 46 waterpipe tobacco venues in Istanbul, Moscow, and Cairo. We interviewed venue managers and employees and collected biological samples from employees to measure exhaled carbon monoxide (CO), hair nicotine, saliva cotinine, urine cotinine, urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and urine 1-hydroxypyrene glucuronide (1-OHPG). We estimated adjusted geometric mean ratios (GMR) of each SHS biomarker by employee characteristics and indoor air SHS measures.

Results: There were 73 nonsmoking employees and 203 current smokers of cigarettes or waterpipe. In nonsmokers, the median (interquartile) range concentrations of SHS biomarkers were 1.1 (0.2, 40.9) µg/g creatinine urine cotinine, 5.5 (2, 15) ng/mL saliva cotinine, 0.95 (0.36, 5.02) ng/mg hair nicotine, 1.48 (0.98, 3.97) pg/mg creatinine urine NNAL, 0.54 (0.25, 0.97) pmol/mg creatinine urine 1-OHPG, and 1.67 (1.33, 2.33) ppm exhaled CO. An 8-hour increase in work hours was associated with higher urine cotinine (GMR: 1.68, 95% CI: 1.20, 2.37) and hair nicotine (GMR: 1.22, 95% CI: 1.05, 1.43). Lighting waterpipes was associated with higher saliva cotinine (GMR: 2.83, 95% CI: 1.05, 7.62).

Conclusions: Nonsmoking employees of waterpipe tobacco venues were exposed to high levels of SHS, including measurable levels of carcinogenic biomarkers (tobacco-specific nitrosamines and PAHs).
Implications: Smoke-free regulation should be extended to waterpipe venues to protect nonsmoking employees and patrons from the adverse health effects of SHS.

Introduction

Secondhand smoke (SHS) is a complex mixture of carcinogenic and toxic chemicals that is composed of exhaled mainstream tobacco smoke and sidestream smoke emitted directly from the burning source.[1] Smoke-free and clean indoor air legislation has successfully reduced exposure to SHS, which causes and exacerbates numerous adverse health outcomes,[2] in public places around the world.[3] Most of these policies, however, explicitly or implicitly exempt waterpipe tobacco venues[4] and leave employees and patrons of these venues at risk of exposure to the harmful effects of SHS.

Waterpipe, commonly known as hookah, nargile, calean, goza, or shisha, is a centuries-old traditional method of smoking tobacco in the Eastern Mediterranean and parts of Asia and Africa.[5] Popularity of waterpipe tobacco smoking has been rising and it is commonly perceived as less harmful than cigarette smoking.[6] Active waterpipe smoking, however, has been associated with similar acute and chronic health effects as cigarette smoking.[7,8] Smoking machine studies suggest that waterpipe SHS contains similar or higher concentrations of many carcinogens and toxic chemicals as compared to cigarette SHS, including carcinogenic polycyclic aromatic hydrocarbons (PAHs), fine particulate matter ($PM_{2.5}$), volatile aldehydes, and carbon monoxide (CO).[9,10] Waterpipe SHS is derived from combustion of both tobacco and the burning source (usually charcoal),[5,11] and some studies have reported a lower burning temperature compared to cigarettes.[12] As reviewed recently,[13] a growing number of studies have found that waterpipe tobacco venues have elevated indoor air SHS,[14–18] and suggest that exposure to SHS in waterpipe tobacco venues is associated with higher levels of biological indicators of exposure to SHS in nonsmokers[19–22] and acute respiratory symptoms.[19]

We previously reported high concentrations of indoor air markers of SHS, including $PM_{2.5}$, CO, PAHs, the tobacco specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and air nicotine, in indoor air of waterpipe venues in Istanbul, Moscow, and Cairo.[16] In the same study, but not previously reported, we collected samples of exhaled breath, hair saliva, and urine from employees of these waterpipe tobacco venues and measured five biomarkers of SHS exposure (exhaled CO, hair nicotine, saliva cotinine, urine cotinine, urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, a metabolite of the tobacco-specific nitrosamine NNK), and urine 1-hydroxypyrene glucuronide (1-OHPG), a biomarker of exposure to PAHs). In this study, we aimed to characterize the concentrations of biomarkers of exposure to SHS by employee smoking status and to evaluate the association of SHS biomarker concentrations in nonsmoking waterpipe tobacco venue employees with self-reported measures of SHS exposure and measured indoor air SHS constituents.

Methods

Study Population

We conducted a study of waterpipe tobacco venues and their employees in Istanbul, Turkey, Moscow, Russia, and Cairo, Egypt, using a convenience sampling strategy to select waterpipe venues.[16] The three countries were selected based on high waterpipe consumption data from the Global Adult Tobacco Survey.[23–25] To be eligible to participate, venue owners/managers had to provide oral informed consent to conduct air sampling in the venue and at least one nonsmoking adult employee (⩾18 years of age) had to provide oral informed consent and be willing to provide hair, saliva, urine, or exhaled breath samples. Data were collected between January and May 2013 in Istanbul, December 2013 to May 2014 in Moscow, and November 2013 to April 2014 in Cairo. Field staff fluent in the local language conducted all communications with venues and participants. The Johns Hopkins Bloomberg School of Public Health (JHSPH) Institutional Review Board and the ethics committees at the local coinvestigators' institutions approved the study protocol.

Questionnaire Data Collection

A total of 46 venues (9 in Istanbul, 17 in Moscow, and 20 in Cairo) participated (response rates were 30% in Cairo, 32% in Istanbul, and 34% in Moscow). A total of 283 employees (mean six per venue) participated (96% response rate in Istanbul, 95% in Moscow, and 76% in Cairo). The participants answered an interviewer-administered questionnaire assessing information on sociodemographic and occupational factors, smoking status, exposure to SHS at work, home, and other places, and opinions about SHS. We categorized participant smoking status using data on self-reported tobacco use (cigarette, waterpipe, pipe without water, and chewing tobacco).

Never-smokers must have either never tried any kind of tobacco, or have smoked fewer than 100 cigarettes and smoked waterpipe for no more than one 20-minute session in their lifetime. Former smokers reported past tobacco use but did not report smoking cigarette, waterpipe, or other types of tobacco within the past three months. Current smokers reported smoking cigarettes or waterpipe within the past three months either "daily", "less than daily", or "just a few puffs". Employees were asked about self-reported physician diagnoses of asthma and other respiratory disease, and respiratory and sensory symptoms, using questions applied in previous studies of SHS in hospitality venues.[26] After excluding seven participants missing data on age, hours of work per week, education, job title, and whether the employee lived with a smoker, there were 276 employees included in the analysis.

Biomarkers of Secondhand Smoke

Fieldworkers collected samples of exhaled breath (CO), hair (nicotine), saliva (cotinine), and urine (cotinine, NNAL, and 1-OHPG) from each employee. Samples were obtained during work hours but at times of low business activity for convenience. The analytical measurement methods and characteristics and half-lives of the biomarkers are summarized in Supplementary Table 1. Exhaled CO was available in all employees in all cities (N = 276) and hair nicotine was available from 87% (N = 241) of employees. In Cairo, only exhaled breath samples and hair samples were analyzed due to administrative hurdles transporting urine and saliva samples to JHSPH for analysis. For participants from Istanbul and Moscow, saliva was available in 98% (N = 168) and urine cotinine was available in 96% (N = 165). Urine NNAL, 1-OHPG, and cotinine were measured in 95% to 96% of samples (N = 163, 164, and 165,

Table 1. Characteristics of Employees of Waterpipe Tobacco Venues in Istanbul, Moscow, and Cairo in 2013–2014

	Overall *N* = 276	Istanbul *N* = 70	Moscow *N* = 102	Cairo *N* = 104	*p*-value[a]
Number of employees per venue	6 (5, 9)	11 (5, 17)	6 (5, 9)	6 (5, 6)	**<0.001**
Age, years	30 (24, 40)	30 (24, 41)	30 (26, 48)	28 (24, 35)	**0.010**
Men, %	78%	90%	50%	98%	**<0.001**
Average time at work, hours/week	60 (48, 84)	60 (54, 72)	40 (40, 48)	84 (72, 84)	**<0.001**
Education, %					**<0.001**
Less than high school	62%	87%	52%	56%	
High school	28%	13%	28%	38%	
College/university	9%	0%	20%	6%	
Smoking status, %					**<0.001**
Never-smoker	21%	1%	42%	14%	
Former smoker	5%	7%	5%	4%	
Current cigarette only smoker	19%	17%	27%	12%	
Current waterpipe only smoker	12%	23%	7%	9%	
Current cigarette and waterpipe smoker	43%	51%	19%	62%	
Primary job, %					**<0.001**
Owner/Manager	13%	15%	11%	15%	
Bartender/Waiter	65%	61%	49%	83%	
Cook/kitchen staff	11%	7%	26%	0%	
Other[b]	10%	17%	14%	2%	
Chemical hair treatment, % yes[c]	17%	12%	31%	6%	**<0.001**
Light waterpipes for customers, % yes	54%	57%	26%	79%	**<0.001**
Lives with a smoker, % yes[d]	50%	64%	34%	56%	**<0.001**
Physician-diagnosed asthma or other respiratory disease, % yes	10%	16%	5%	11%	0.063
⩾ 1 sensory symptom, % yes[e]	49%	59%	34%	58%	**0.001**
⩾ 1 respiratory symptom, % yes[e]	61%	63%	38%	80%	**<0.001**
Urine creatinine, mg/dL	210 (134, 290)	180 (116, 250)	220 (181, 309)	n/a[f]	**<0.001**

Categorical variables are percentages of total sample (*N*). Continuous variables are median (25th percentile, 75th percentile).

[a]*p*-values are Pearson's chi-square test of independence for categorical variables and one-way ANOVA for continuous variables. Bold values indicate differences were significant at $p < 0.05$.

[b]"Other" jobs included security (*N* = 12), waterpipe preparation (*N* = 6), cleaning (*N* = 6), and other miscellaneous positions.

[c]Color or dye, bleach, highlighted, perm, or straightened.

[d]Of the employees with a household member that smokes, the majority (83%) of these household members smoked cigarettes.

[e]Number (percentage) reporting at least one sensory symptom (ie, red or irritated eyes; runny nose, sneezing or nose irritation; sore or scratchy throat) or at least one respiratory symptom (ie, wheezing or whistling in chest; feeling short of breath, coughing in morning, coughing during the rest of the day or at night, or bringing up any phlegm).

[f]Urine samples were not available from Cairo.

respectively). Among the 73 nonsmoking employees (both never and former smokers) in Istanbul, Moscow, and Cairo, all had measured exhaled CO and 72 had hair nicotine measurements (99%). Among 54 nonsmoking employees in Istanbul and Moscow, 53 (98%) had measured urine cotinine, 52 (96%) had measured 1-OHPG, and 51 (94%) had measured urine NNAL.

Exhaled CO

Exhaled CO was measured using a breath CO monitor fitted with a sampling-T connector and a single-use mouthpiece (Vitalograph BreathCO, Vitalograph, Lenexa, KS).

We took the average of three repeated samples. Thirty-six (13%) samples were below the limit of detection (LOD) (3 ppm) and were recorded as half the LOD.

Hair Nicotine

Hair samples were collected from the back of each participant's scalp. A total of 30–50 strands (~30 mg) of hair with a median (10th, 90th percentile) length of 3 (2, 3) cm were collected. Three centimeters of hair represent approximately 3 months of hair growth.[27] Hair samples were stored in a smoke-free environment until analysis and prepared as previously described.[28] We analyzed samples from Istanbul and Moscow by gas chromatography/mass spectrometry (GC-17/MS-QP5000, Shimadzu, Canby, OR)[28] and samples from Cairo by gas chromatography and triple quadrupole mass spectrometry (TG-5MS, Thermo Scientific, Waltham, MA). Of the 105 available hair samples, 19 (18%) were too small to analyze. For quality control, 29 duplicate samples (8–10 in each city) were collected and the duplicate measurements were averaged. Sixteen (7%) samples were below the batch-specific LOD (1.17 ng in Istanbul, 2.90 ng in Moscow, and 5.54 ng in Cairo) and replaced by half the LOD.

Saliva Cotinine

To collect saliva, employees chewed on a cotton swab for at least 45 seconds, or until saturated, and expelled the swab directly into a plastic collection tube (Salivette, Sarstedt, Numbrecht, Germany). Samples were stored in a cooler with ice until the end of the day and stored at –20°C before being shipped on dry ice to JHSPH. Samples were thawed, vortexed, and centrifuged to remove mucins and particulate matter. Salivary cotinine was measured using a high sensitivity quantitative enzyme linked immunoassay kit (Salimetrics LLC, State College, PA). Ten (5.8%) samples were below the LOD (1 ng/mL) and recorded as half the LOD.

Urine Cotinine, NNAL, and 1-OHPG

Employees collected a urine sample (spot urine or first morning void, whichever was possible) using the provided collection cups. Employees brought the sample with them to work and the samples were refrigerated until picked up by fieldworkers. Samples were stored at −20°C before being shipped on dry ice to JHSPH. Urine creatinine, to correct for variability of urine dilution, was measured using a creatinine colorimetric assay kit (Cayman Chemicals, Ann Arbor, MI). No samples were below the LOD (2 mg/dL).

We measured urine cotinine using a direct enzyme linked immunoassay kit (Calbiotech, Spring Valley, CA). Thirty (17.9%) samples, all from Moscow, were below the LOD (1 µg/L) and replaced with half the LOD. Urinary 1-OHPG concentrations were measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy (Perkin-Elmer LS50, Waltham, MA) as previously described.[29,30] In our laboratory, the inter-batch coefficient of variation for 1-OHPG is typically 8%–10%. Three (1.8%) samples were below the LOD (0.02 ng/mL) and recorded as half the LOD. We measured urine NNAL adapting the protocol from Shimelis *et al.*[31] and analyzed using gas chromatography and triple quadrupole mass spectrometry (GC-MS/MS, Thermo Scientific) in selected reaction monitoring mode with capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) (TG-5MS, Thermo Scientific). Fifty-eight (35%) samples (50 samples in Moscow and eight samples in Istanbul) were below the LOD (5.9 pg/mL) and recorded as half the LOD.

Indoor Air Secondhand Smoke Markers

As described previously,[16] we measured $PM_{2.5}$, CO, particle-bound PAHs (p-PAHs), NNK, and nicotine in indoor air in each of the venues. Real-time measurements of $PM_{2.5}$ and CO were collected for up to 4 hours during peak business hours and p-PAHs were collected for 1–2 hours during peak business hours. $PM_{2.5}$ concentrations were corrected for relative humidity and we applied a waterpipe-specific gravimetric correction factor of 0.6.[32] NNK was collected for up to 23 hours on $PM_{2.5}$ filters and nicotine filters were left in the venues for approximately 3–4 days.

Statistical Analysis

SHS biomarker concentrations were right-skewed; therefore, medians or geometric means were used for analysis. After examining the distribution of each biomarker by smoking status and city, we restricted further analyses to nonsmoking employees (never and former smokers) to assess the influence of venue and employee characteristics on biomarker concentrations derived from SHS, rather than active smoking. We divided the concentrations of urine biomarkers by urine creatinine in order to correct for variability in urine dilution. We assessed the correlation between each biomarker within employees using Spearman rank correlation coefficients.

In a separate model for each biomarker, we calculated geometric mean ratios (GMR) by employee and venue characteristics related to SHS exposure using multivariable linear regression with generalized estimating equations (GEE) with robust variance and an independent correlation structure within venues. Sensitivity analyses assuming exchangeable correlation structure produced similar results (data not shown). GMR were adjusted in sequential models for city, age, sex, and living with a smoker. Adjustment for age, sex, and living with a smoker in sequentially adjusted models did not substantially change the associations and we present only fully-adjusted models. Models of hair nicotine were additionally adjusted for self-reported hair chemical treatment (eg, color or dye, bleach, highlights, perm, or straightened). Venue mean indoor air nicotine, NNK, and p-PAHs were categorized into tertiles excluding 10 venues without nonsmoking employees and separately for each biomarker because of variable sample sizes. For indoor air SHS markers, we calculated the GMR of each biomarker comparing the 75th to the 25th percentile of log-transformed venue mean indoor air SHS concentrations. Sensitivity analyses using urine biomarkers without dividing by creatinine and adjusting in models for urine creatinine found similar results.

Statistical analyses were performed with Stata Version 12.1 (StataCorp, College Station, TX) and R Version 3.2.2 (R Foundation for Statistical Computing, www.r-project.org, Vienna, Austria). All statistical tests were two-sided and *p*-values less than .05 were considered statistically significant.

Results

Employee Characteristics

Most waterpipe tobacco venue employees in Istanbul and Cairo were men (90% and 98%, respectively), whereas there were equal proportions of men and women employees in Moscow (Table 1). The employee median (interquartile range [IQR]) age was 30 (24, 40) years and most employees had less than a high school education (62%). The most common primary job descriptions were bartender/waiter (65%), owner/manager (13%), and cook/kitchen staff (11%). Overall, 54% of employees light waterpipes for customers, ranging from 26% in Moscow to 79% in Cairo. Seventy-three (26%) employees were nonsmokers, including former and never-smokers (6 in Istanbul, 48 in Moscow, and 19 in Cairo). The prevalence of current smoking, either cigarette or waterpipe, was 53%, 82%, and 91% among employees in Moscow, Istanbul, and Cairo, respectively. Smoking both cigarette and waterpipe was more common in Cairo (62%) and Istanbul (51%), compared to Moscow (19%). Smoking waterpipe exclusively was more common in Istanbul (23%), compared to Moscow (7%) and Cairo (9%). Between 34% and 64% of employees lived with at least one smoker in their household. Self-reported data on employee opinions about their workplace air quality are presented in Supplementary Table 2.

SHS Biomarker Concentrations by Smoking Status

In nonsmoking employees, the median (IQR) concentrations of SHS biomarkers were 1.1 (0.2, 40.9) µg/g creatinine for urine cotinine, 5.5 (2.0, 15.0) ng/mL for saliva cotinine, 0.95 (0.36, 5.02) ng/mg for hair nicotine, 1.48 (0.98, 3.97) pg/mg creatinine for urine NNAL, 0.54 (0.25, 0.97) pmol/mg creatinine for urine 1-OHPG, and 1.67 (1.33, 2.33) ppm for exhaled CO (Supplementary Figure 1, Supplementary Table 3). Median (IQR) concentrations of urine biomarkers not divided by urine creatinine are presented in Supplementary Table 4. Among nonsmoking employees, we found moderate correlations among the tobacco-specific biomarkers hair nicotine, urine cotinine, saliva cotinine, and urine NNAL (Spearman ρ range from 0.36 to 0.61, *N* range from 51 to 53) (Table 2). Concentrations of urine cotinine and urine 1-OHPG were also moderately correlated (Spearman $\rho = 0.33$, $N = 52$). We found similar results in a sensitivity analysis stratified by city (data not shown).

SHS Biomarkers by Employee Characteristics in Nonsmoking Employees

Among nonsmoking employees, an 8-hour increase in the average number of hours spent at work per week was associated with higher urine cotinine (GMR: 1.68, 95% CI: 1.20, 2.37) and hair nicotine

Table 2. Correlations Between Secondhand Smoke Biomarker Concentrations Among Nonsmoking Employees of Waterpipe Tobacco Venues in Istanbul, Moscow, and Cairo

	Urine Cotinine (µg/g creatinine)		Saliva Cotinine (ng/mL)		Hair Nicotine (ng/mg)		Urine NNAL (pg/mg creatinine)		1-OHPG `(µg/g creatinine)		Exhaled CO (ppm)	
	N	P	*N*	P	*N*	P	*N*	P	*N*	P	*N*	P
Urine cotinine (µg/g creatinine)	52	1.00										
Saliva Cotinine (ng/mL)	51	**0.54**	53	1.00								
Hair Nicotine (ng/mg)	52	**0.53**	53	**0.53**	72	1.00						
Urine NNAL (pg/mg creatinine)	51	**0.61**	51	**0.36**	51	**0.53**	53	1.00				
Urine 1-OHPG (µg/g creatinine)	52	**0.33**	51	0.14	52	0.04	51	0.11	52	1.00		
Exhaled CO (ppm)	52	-0.08	**53**	**0.17**	72	0.05	51	0.09	52	0.12	73	1.00

N = Number of employees; NNAL = urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 1-OHPG = urine 1-hydroxypyrene glucuronide; ρ = Spearman rho correlation coefficient.

Bold values indicate correlation was significant at $p < .05$.

Urine and saliva samples were only available from Istanbul and Moscow.

(GMR: 1.22, 95% CI: 1.05, 1.43) after adjustment for city, age, sex, and living with a smoker (Table 3). Compared to owners and managers, employees working as cooks or kitchen staff had significantly lower geometric mean concentrations of urine cotinine (GMR: 0.05, 95% CI: 0.003, 0.76) and saliva cotinine (GMR: 0.18, 95% CI: 0.05, 0.62). Employees that lit waterpipes for customers had higher geometric mean saliva cotinine (GMR: 2.83, 95% CI: 1.05, 7.62). Living with a smoker was associated with higher geometric mean saliva cotinine (GMR: 3.54, 95% CI: 1.07, 4.23) and hair nicotine (GMR: 2.13, 95% CI: 1.07, 4.23). There were no statistically significant associations with urine NNAL, urine 1-OHPG, or exhaled CO.

SHS Biomarkers by Venue Characteristics in Nonsmoking Employees

Among nonsmoking employees, working in a venue with a mean nicotine concentration in the highest tertile was associated with higher geometric mean urine cotinine (GMR: 95% CI: 6.43, 95% CI: 1.50, 27.66) compared to the lowest tertile in adjusted models (Table 4). Compared to employees in venues in the lowest tertile of mean indoor air p-PAHs, employees working in venues with mean p-PAHs in the highest two tertiles had significantly higher urine 1-OHPG (GMR: 2.64, 95% CI: 1.54, 4.55 and GMR: 2.15, 95% CI: 1.36, 3.40, respectively). Venue concentrations of air NNK were not significantly related to SHS biomarkers. There were no statistically significant associations with saliva cotinine, hair nicotine, urine NNAL, and exhaled CO concentrations. Supplementary Table 5 presents the association between ventilation or air condition, venue volume, number of smokers, and smoker density. While a higher number of waterpipe smokers was positively associated with higher CO levels, for other biomarkers the associations were inverse.

Discussion

We observed that nonsmoking employees of waterpipe tobacco venues in Istanbul, Moscow, and Cairo had high levels of SHS biomarkers, including measurable levels of two biomarkers of carcinogens (urine NNAL and 1-OHPG). Although the associations between biomarker concentrations and venue and employee characteristics were inconsistent, measures of higher occupational exposure to SHS, such as the number of hours spent at work and whether the employee was responsible for lighting waterpipes, were associated with higher levels of the tobacco-specific biomarkers, urine and saliva cotinine and hair nicotine. In addition, higher indoor air nicotine concentrations were associated with higher urine cotinine, and higher indoor p-PAHs were associated with higher 1-OHPG concentrations. Studying real-world exposure to SHS in waterpipe venues and biomarkers of SHS exposure, particularly those related to early adverse health effects, is important to demonstrate that SHS in waterpipe tobacco venues may be harmful to the health of employees and the public. Employees who spend a substantial number of hours working in the venues, as in this study, may be especially vulnerable.

Compared to our study, the few studies that have previously measured biomarkers of SHS among persons exposed to SHS in waterpipe venues have generally had small sample sizes and measured a limited number of biomarkers of SHS.[19–22] Mean concentrations of the inflammatory cytokine interferon-γ and exhaled CO in 10 employees of waterpipe venues in New York City were significantly higher at the end of a shift compared to samples taken pre-shift, whereas concentrations of saliva cotinine were not statistically different.[20] In exhaled breath samples collected from employees in 12 waterpipe venues in Toronto, the mean exhaled CO was six times higher in high indoor air nicotine compared to venues with low air nicotine.[21] In 50 waterpipe venue employees in Beirut, mean exhaled

Table 3. Ratio of Geometric Means of Secondhand Smoke Biomarker Concentrations by Employee Characteristics in Nonsmoking Employees of Waterpipe Tobacco Venues in Istanbul, Moscow, and Cairo in 2013–2014

	Urine Cotinine *N* = 52		Saliva Cotinine *N* = 53		Hair Nicotine *N* = 71		Urine NNAL *N* = 51		Urine 1-OHPG *N* = 52		Exhaled CO *N* = 73	
	N	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)
Time spent at work												
<50 h/week	38	1.00 (reference)	38	1.00 (reference)	39	1.00 (reference)	38	1.00 (reference)	38	1.00 (reference)	39	1.00 (reference)
⩾ 50 h/week	14	**5.94 (1.56, 22.54)**	15	0.95 (0.40, 2.28)	33	**2.90 (1.71, 4.90)**	13	1.57 (0.70, 3.50)	14	1.42 (0.80, 2.50)	34	0.64 (0.37, 1.11)
Per 8 h/week	52	**1.68 (1.20, 2.37)**	53	0.86 (0.63, 1.16)	71	**1.22 (1.05, 1.43)**	51	0.99 (0.75, 1.31)	52	0.97 (0.83, 1.14)	73	0.92 (0.85, 1.01)
Job												
Owner/Manager	9	1.00 (reference)	9	1.00 (reference)	11	1.00 (reference)	9	1.00 (reference)	9	1.00 (reference)	11	1.00 (reference)
Bartender/Waiter	21	0.54 (0.02, 12.81)	22	0.73 (0.13, 4.17)	37	0.99 (0.41, 2.39)	21	1.12 (0.30, 4.09)	21	0.92 (0.49, 1.73)	38	0.94 (0.66, 1.33)
Cook/kitchen staff	15	**0.05 (0.003, 0.76)**	14	**0.18 (0.05, 0.62)**	15	0.56 (0.16, 2.01)	14	0.44 (0.13, 1.42)	15	0.73 (0.04, 1.24)	15	0.77 (0.46, 1.27)
Other[a]	7	1.38 (0.06, 34.44)	8	0.92 (0.14, 6.04)	9	1.06 (0.40, 2.82)	7	0.84 (0.28, 2.48)	7	1.22 (0.68, 2.20)	9	0.64 (0.39, 1.05)
Light waterpipes for customers												
No	41	1.00 (reference)	42	1.00 (reference)	48	1.00 (reference)	40	1.00 (reference)	41	1.00 (reference)	49	1.00 (reference)
Yes	11	0.67 (0.08, 5.77)	11	**2.83 (1.05, 7.62)**	24	0.93 (0.30, 2.86)	11	1.17 (0.46, 3.00)	11	1.62 (0.73, 3.59)	24	0.86 (0.58, 1.29)
Lives with a smoker												
No	36	1.00 (reference)	36	1.00 (reference)	45	1.00 (reference)	36	1.00 (reference)	36	1.00 (reference)	46	1.00 (reference)
Yes	16	2.49 (0.26, 23.79)	17	**3.54 (1.78, 7.02)**	27	**2.13 (1.07, 4.23)**	15	1.40 (0.70, 2.80)	16	1.18 (0.56, 2.50)	27	1.22 (0.93, 1.61)

Geometric mean ratios were adjusted by city, age, sex, and lives with a smoker (yes/no). Models of hair nicotine were also adjusted for chemical hair treatment (yes/no). Bold values indicate associations were significant at $p < 0.05$.

CI = Confidence interval; GMR = Geometric mean ratio; *N*, Number of employees; NNAL = urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 1-OHPG = urine 1-hydroxypyrene glucuronide.

[a]Other includes security, waterpipe preparation, cleaning, and other miscellaneous job duties.

Table 4. Ratio of Geometric Means of Secondhand Smoke Biomarker Concentrations by Venue Indoor Air Secondhand Smoke Measurements in Nonsmoking Employees of Waterpipe Tobacco Venues in Istanbul, Moscow, and Cairo in 2013–2014

	Urine Cotinine *N* = 52		Saliva Cotinine *N* = 53		Hair Nicotine *N* = 71		Urine NNAL *N* = 51		Urine 1-OHPG *N* = 52		Exhaled CO *N* = 73	
	N	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)	*N*	GMR (95% CI)
Venue Mean Air Nicotine (μg/m^3)												
Tertile 1[a]	19	1.00 (reference)	21	1.00 (reference)	25	1.00 (reference)	19	1.00 (reference)	19	1.00 (reference)	27	1.00 (reference)
Tertile 2[a]	21	0.23 (0.05, 1.11)	16	0.56 (0.21, 1.46)	33	1.37 (0.65, 2.89)	21	**0.39 (0.28, 0.56)**	21	0.84 (0.43, 1.64)	33	0.82 (0.54, 1.24)
Tertile 3[a]	12	**6.43 (1.50, 27.66)**	14	0.79 (0.34, 1.82)	11	1.29 (0.40, 4.16)	11	1.08 (0.53, 2.18)	12	1.08 (0.54, 2.18)	13	0.69 (0.43, 1.10)
75th vs. 25th percentile[b]	52	2.23 (0.13, 39.7)	53	0.54 (0.15, 1.93)	71	1.40 (0.65, 3.04)	51	0.71 (0.32, 1.57)	52	0.82 (0.40, 1.70)	73	0.60 (0.33, 1.11)
Venue Mean Air NNK (ng/m^3)												
Tertile 1[a]	17	1.00 (reference)	15	1.00 (reference)	25	1.00 (reference)	16	1.00 (reference)	17	1.00 (reference)	25	1.00 (reference)
Tertile 2[a]	16	0.45 (0.05, 4.10)	17	1.69 (0.48, 5.99)	22	0.74 (0.31, 1.76)	16	0.80 (0.33, 1.93)	16	0.58 (0.32, 1.03)	26	0.92 (0.62, 1.37)
Tertile 3[a]	17	1.37 (0.12, 16.28)	17	0.97 (0.33, 2.89)	20	1.30 (0.57, 2.99)	17	1.38 (0.54, 3.52)	17	1.50 (0.72, 3.09)	20	0.75 (0.44, 1.26)
75th vs. 25th percentile[b]	50	1.41 (0.43, 4.70)	51	0.92 (0.51, 1.66)	69	0.98 (0.59, 1.61)	49	1.15 (0.68, 1.94)	50	1.23 (0.85, 1.78)	71	0.91 (0.73, 1.14)
Venue Mean Air p-PAHs (ng/m^3)												
Tertile 1[a]	14	1.00 (reference)	15	1.00 (reference)	21	1.00 (reference)	14	1.00 (reference)	14	1.00 (reference)	24	1.00 (reference)
Tertile 2[a]	7	1.45 (0.17, 12.09)	6	0.83 (0.32, 2.16)	11	1.58 (0.68, 3.70)	6	**0.31 (0.11, 0.88)**	7	**2.64 (1.54, 4.55)**	11	0.95 (0.60, 1.50)
Tertile 3[a]	7	11.33 (0.25, 506.6)	6	1.56 (0.29, 8.29)	11	2.45 (0.81, 7.42)	7	1.79 (0.69, 4.63)	7	**2.15 (1.36, 3.40)**	12	1.30 (0.93, 1.81)
75th vs. 25th percentile[b]	28	7.31 (0.84, 63.32)	29	1.96 (0.72, 5.34)	46	1.00 (0.60, 1.68)	27	0.99 (0.48, 2.03)	28	**2.15 (1.74, 2.65)**	47	1.14 (0.99, 1.31)

Geometric mean ratios were adjusted by city, age, sex, and lives with a smoker (yes/no). Bold values indicate associations were significant at $p < 0.05$.

CI = Confidence interval; GMR = Geometric mean ratio; *N* = Number of employees; NNAL = urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; 1-OHPG = urine 1-hydroxypyrene glucuronide.

[a]Tertiles of each marker of indoor air SHS (nicotine, NNK, and p-PAHs) were created at the venue level, among venues with at least one nonsmoker. Samples sizes for each tertile vary because not every venue had measured air NNK and p-PAHs (97% [*N* = 35] of venues had NNK, and 69% [*N* = 25] of venues had p-PAHs), and because the numbers of nonsmoking employees with complete employee data varied by venue (mean 2; range: 1–7). All venues had measured air nicotine.

[b]75th vs. 25th percentile of log-transformed venue mean concentrations of indoor air SHS markers. Interquartile ranges (25th percentile, 75th percentile) of air nicotine, NNK, and mean p-PAHs were 22 (0.05, 22) μg/m^3, 1.2 (0.5, 1.7) ng/m^3, and 83 (25, 108) ng/m^3, respectively.

CO concentrations were significantly associated with the number of hours of weekly exposure to waterpipe smoke.[19] In 103 nonsmokers with who attended a social event at a venue or home event with waterpipe smoking, there was a nonstatistically significant increase in the median concentration of urine S-phenylmercapturic acid (SPMA, a metabolic of benzene, a hemotoxicant and carcinogen)[22] comparing samples collected before and after the event.

Higher exhaled CO has been associated with an increased risk of respiratory diseases[33] and there are case reports of acute CO poisoning after waterpipe smoking.[7] Mean exhaled CO concentrations among fieldworkers after two hours inside a waterpipe venue were 3.4 ppm in low air nicotine venues (mean air CO < 1 μg/m^3) and 22.2 ppm in high air nicotine venues (mean CO ⩾ 1 μg/m^3) in Toronto,[21] 49.4 ppm in employees at the end of a work shift in New York City waterpipe venue,[20] and 26.8 ppm in nonsmoking employees of waterpipe venues in Beirut.[19] Compared to those studies, the exhaled CO concentrations (median 1.67 ppm in nonsmokers) in this study were relatively low, which could be explained by the short half-life of CO and our sampling when business activity was low for convenience.

Urine 1-OHPG is a biomarker of exposure to PAHs, many of which are carcinogenic.[34] PAHs are formed by incomplete combustion of organic material and are not specific to tobacco. We did not have information on other environmental exposures and diet that can also contribute to PAH exposure.[34] No previous studies of SHS in waterpipe venues or waterpipe SHS have measured urine 1-OHPG; however, several studies have demonstrated that urine 1-OHPG concentrations were associated with SHS exposure.[35,36] In a study of California teachers, the median concentration of 1-OHPG in nonsmokers exposed to SHS was 0.25 pmol/mL compared to 1.61 pmol/mL in smokers.[35] Among nonsmoking military cooks in Taiwan, pre- and postshift concentrations of 1-OHPG were 1.2 and 1.8 pmol/mL, respectively.[37] The median concentrations of 1-OHPG in nonsmokers were 0.48 pmol/mL (0.35 pmol/mg creatinine) in Istanbul and 1.14 pmol/mL (0.61 pmol/mg creatinine) in Moscow. Thus, concentrations of 1-OHPG in employees in this study were higher than some other studies of individuals exposed to SHS and were even similar to active smokers in some groups. In this study, we also observed an adjusted association between venue mean air p-PAH concentrations and geometric mean concentrations of urine 1-OHPG.

In nonsmokers, the presence of NNAL, a metabolite of the tobacco-specific nitrosamine NNK, a lung carcinogen found in indoor air SHS, provides a biochemical link between SHS exposure and risk of lung cancer.[34] With a half-life of 10–18 days, urine NNAL is likely to be a good biomarker of long-term exposure over the past 6–12 weeks.[38] In a small sample of nonsmokers in Syria, the mean (95% CI) urine NNAL was 3.6 (1.6, 7.9) pg/mg creatinine; however, no information was reported about exposure to SHS.[39] In the U.S. general population, NNAL was detectable in 41% of nonsmokers; the 75th percentile was 2.3 pg/mg creatinine and 90th percentile was 6.8 pg/mg creatinine in adults over 20 years.[40] Concentrations of urine NNAL were higher in nonsmokers in Istanbul (median 6.3 pg/mg creatinine) compared to Moscow (median 1.5 pg/mg creatinine).

Hair nicotine is a marker of past long-term exposure to tobacco and cotinine is the major metabolite of nicotine.[27] Although the half-lives of urine and saliva cotinine are similar, urine cotinine is more sensitive for measuring low levels of cotinine, and the interpretation of saliva cotinine can be limited by variability across individuals.[27] In comparison, nonsmokers in the general population of Israel (geometric mean 1.3 μg/g creatinine),[41] the urine cotinine concentrations among nonsmoking employees were higher in Istanbul (median 5.2 μg/g creatinine) and lower in Moscow (0.6 μg/g creatinine). The mean concentration of saliva cotinine was 27.9 ng/mL in New York City waterpipe venue employees,[20] compared to a median of 2 ng/mL in nonsmokers in Istanbul and 6 ng/mL in Moscow. In bars or restaurants that allowed cigarette smoking in Chile, the median hair nicotine concentration in 28 nonsmoking employees was 1.9 ng/mg.[42] In our study, concentrations of hair nicotine varied considerably by city: median concentrations of hair nicotine in nonsmoking employees were 2.3 ng/mg in Istanbul, 0.6 mg/mg in Moscow, and 5.8 ng/mg in Cairo. Lower hair nicotine concentrations despite similar or even higher environmental SHS exposure concentrations in Russia compared to Middle Eastern and Asian countries have also been found in studies of SHS exposure in mothers and children[43] and in bar and nightclub employees.[44] In this study, urine cotinine and hair nicotine concentrations were associated with higher number of work hours per week and saliva cotinine was associated with lighting waterpipes for customers, suggesting that waterpipe employees have substantial occupational exposure to tobacco SHS.

A major strength of this study was the breadth of objective measures of SHS exposure, using both indoor air markers of SHS and biomarkers of exposure to SHS in employee breath, urine, saliva, and hair. We selected these biomarkers to characterize exposure to different components of the complex mixture of SHS (tobacco-specific or related to combustion), to represent a range of different exposure time periods (from short-term biomarkers with a half-life of 2–4 hours, like exhaled CO, to long-term biomarkers that represent the past three months of exposure, like hair nicotine), and to allow comparisons to previous studies which often measured only one or a few biomarkers of SHS exposure. Although nicotine and cotinine in urine, saliva, and hair tend to be highly correlated, we measured this important tobacco-specific biomarker in multiple media for several reasons. The longer half-life of hair nicotine compared to urine and saliva cotinine allowed us to capture temporal variation in the employee's SHS exposure. Accurate measurement of urine biomarkers requires correction for urine dilution, saliva measurements may be biased by age, gender, race, oral pH, diet, dehydration, or drug treatment, and chemical treatment of hair can lower concentrations.[27] Finally, compared to saliva cotinine, urine cotinine is a more sensitive assay and is able to detect lower levels of exposure.[27] To our knowledge, this is the first study of SHS in waterpipe tobacco venues to measure concentrations of urine NNAL, urine 1-OHPG, or hair nicotine. Other strengths included the multi-city design, high employee participation rate, and self-reported data on both cigarette and waterpipe smoking status and on exposure to SHS in the home.

This study also had some limitations. Our sample of waterpipe tobacco venues in each city was selected by convenience; therefore, these venues may not be representative of all waterpipe venues in each city. Further, fear of regulation by less compliant venues may have played a role in the low venue participation rate (ranging from 32%–34%) in each city, which may indicate that the magnitude of the indoor air SHS in waterpipe venues is larger than we observed. Most of the sampling was conducted during colder months when ventilation would be limited, especially in Istanbul and Moscow, which could have resulted in higher indoor air concentrations of SHS constituents and biomarker levels. While this may reflect higher exposures than other seasons, it represents real worker exposure. To isolate the effects of occupational SHS exposure, we restricted our analyses to a small group of employees who self-reported as never or former smokers.

The same biomarkers that we used to assess SHS exposure are also found in cigarette and waterpipe smokers.[39,45–48] We hypothesized that the exposure of nonsmoking employees would best reflect occupational exposure, rather than personal tobacco use, and would be more strongly related to employee and venue characteristics. Although the tobacco-specific biomarkers urine cotinine or saliva cotinine are often used as objective markers, identifying a threshold to differentiate active versus passive exposure to tobacco is difficult in this setting due to the substantial occupational SHS exposure. We used whether the employee lived with a smoker as a proxy for secondhand smoke exposure in the home; however, we could not account for SHS exposures in other places and residual confounding is possible. This study was conducted among employees of waterpipe venues in the real world, and we may not have been able to account for important sources of variability that could explain some of the heterogeneity of our findings across biomarkers. We collected breath, saliva, and hair samples at times of low business activity for convenience and employees may have been at work for varying amounts of time before sampling. The biomarkers measured in this study characterize different exposure windows depending on the half-lives and concentrations could reflect exposure at work, in public places, or in the home. However, employees reported spending a substantial number of hours at work and therefore, we believe that occupational exposure should not be discounted. For indoor air SHS concentrations, the average concentrations measured in the venues may not represent the relevant time period captured by the biomarkers of exposure. Finally, both cigarette and waterpipe smoking were observed in waterpipe venues[16] and we could not differentiate between sources of SHS.

Turkey, Russia, and Egypt ratified the current global regulatory standard for tobacco products, the World Health Organization Framework Convention on Tobacco Control (WHO FCTC), between 2004 and 2008.[49] Unfortunately, waterpipe-specific regulation in global tobacco control frameworks is rare, and smoke-free clean air regulation of waterpipe tobacco has been hampered by lack of enforcement and noncompliance in many countries,[4] including Turkey.[50] Smoke-free legislation banning cigarette smoking in public places in Turkey was extended to include waterpipe venues in early 2013, at the time of this study's data collection.[4] In Russia, regulations related to advertising tobacco products specifically mention waterpipe ("hookah") but there is no smoke-free legislation for public places.[4]

In summary, we found high levels of SHS biomarkers in nonsmoking employees of waterpipe venues in Istanbul, Moscow, and Cairo, including markers of burning tobacco, combustion by-products, and detectable levels of biomarkers that indicate potentially carcinogenic exposures to tobacco-specific nitrosamines and PAHs. Nonsmoking employees of waterpipe tobacco venues and patrons may be at an increased risk of the health problems associated of SHS exposure. Smoke-free legislation has been tremendously successful in reducing exposure to SHS from cigarettes.[2] Policy makers should ensure that waterpipe tobacco smoking and waterpipe venues are not excluded from clean air regulations.[4] Additional policy and research goals should include reducing exposure to SHS in waterpipe tobacco venues by working with venues and their employees to identify and remove barriers to compliance and conducting periodic surveillance to ensure continuing compliance with smoke-free legislation. As called for in a recent article,[4] the unique aspects of waterpipe smoking and the waterpipe industry may require the creation of a complementary regulatory framework to the WHO FCTC that is specific to waterpipe smoking.

Supplementary Material

Supplementary data are available at *Nicotine & Tobacco Research* online.

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Declaration of Interests

The authors declare no potential conflicts of interest.

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