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A comparative study of systemic carcinogen exposure in waterpipe smokers, cigarette smokers and non-smokers

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ABSTRACT

Background In the past decade, waterpipe smoking—also known as hookah, shisha, narghileh—has increased among youth. The scarcity of rigorous studies linking waterpipe smoking to smoking-related diseases has hindered policy and regulatory efforts to confront the waterpipe epidemic. This study compares systemic carcinogen exposure between independent groups of exclusive waterpipe smokers, cigarette smokers and non-smokers.

Methods This study was conducted at the Syrian Center for Tobacco Studies (SCTS) in Aleppo, Syria, between 2010 and 2011. First morning urinary samples were collected from three groups of subjects; exclusive daily waterpipe smokers (n=24), exclusive daily cigarette smokers (n=23), and non-smokers (n=28). These samples were analysed for carcinogenic tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) using liquid chromatography—tandem mass spectrometry (LC-MS/MS).

Results Our results show that waterpipe smokers are exposed to about 5–10 times greater NNAL than non-smokers. Mean (95% CI) free and total NNAL was 0.7 (0.3 to 1. 4) and 3.9 (1.6 to 9.5) pg/mL urine for non-smokers, 8.4 (4.8 to 14.8) and 33.0 (21.6 to 50.6) pg/mL urine for waterpipe smokers, and 10.7 (5.0 to 22.6) and 46.8 (27.6 to 79.3) pg/mL urine for cigarette smokers ($p<0.001$ for all comparisons). Daily waterpipe smokers were less exposed to NNAL than daily cigarette smokers, although the difference did not reach statistical significance for all measurements.

Conclusions These results provide the clearest indication to date about systemic exposure to harmful carcinogens associated with long-term waterpipe smoking. Such evidence can support policy and regulatory efforts designed to confront the emerging global waterpipe epidemic, as well as drive interventions aimed at increasing the public awareness about the cancer risk associated with waterpipe smoking.

INTRODUCTION

Available data from several Arab societies reveal a worrisome picture of the tobacco epidemic, where about half the men are daily smokers, and most of those are heavy smokers (ie, average 20 cigarettes/day).[1] Moreover, in the past decade or so, waterpipe smoking (also known as hookah, shisha, narghileh) has become a worldwide epidemic among youth.[2] Prevalence estimates of waterpipe smoking in the Middle East have already surpassed those of cigarette smoking in youth, and the rest of the world is quickly catching up.[1] For example, a multicountry study involving a representative sample of 13–15-year-old school children in several Arab Gulf countries (Bahrain, Oman, Qatar, UAE, Kuwait, Yemen) showed a prevalence of waterpipe smoking ranging from 9% to 15%, and mostly surpassing that of cigarette smoking.[3] The trend in waterpipe smoking is catching up around the world. For example, among college students in the USA, a recent survey involving more than 100 000 students in 152 colleges, the prevalence of waterpipe smoking was 8.4%, second only to cigarettes (16.8%).[4] Many factors have been implicated as the drivers behind this epidemic, including the salient belief that waterpipe smoking is free of most of the harms associated with cigarette smoking.[5 6] Although such belief is not supported by empirical evidence, the lack of rigorous studies linking waterpipe smoking with smoking-related diseases has hindered challenging such beliefs in a systematic evidence-based way.[7]

One of the barriers to developing such evidence lies in the relative novelty of the current waterpipe epidemic compared with the long latency of smoking-related morbidity and mortality.[8] Researchers therefore, have resorted to looking at waterpipe-associated exposure to common tobacco-related toxicants, such as tobacco-specific nitrosamines derivative 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and polycyclic aromatic hydrocarbons. Tobacco-specific nitrosamines play a major role in the development of lung cancer, and the dose of this carcinogen delivered to a lifetime smoker is remarkably close to the lowest total dose shown to induce lung tumours in rats.[9] Several studies following that line of enquiry showed elevated levels of these carcinogens in waterpipe smoke, as well as in smokers following a single waterpipe smoking session.[10–12] For example, in two pioneering studies, Jacob *et al*[11 12] demonstrated that urinary excretion of NNAL is increased significantly following waterpipe smoking. However, given the terminal half-life of NNAL of 10–18 days, this biomarker is perhaps a better indicator of long-term exposure to tobacco smoke (6–12 weeks) rather than acute exposure.[13] Moreover, no study to date has compared NNAL exposure between independent groups of exclusive waterpipe smokers, cigarette smokers and non-smokers. Such comparison is very important to tease out waterpipe-specific risks and provide an exposure gradient orientation to carcinogens associated with waterpipe smoking compared to cigarette smoking and non-smoking.

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Table 1 Main characteristics of study participants according to their smoking status

	Non-smokers	Waterpipe smokers	Cigarette smokers
Gender			
Male (n)	5	24	20
Female (n)	23	0	3
	Mean±SD		
Age	31.0±12.9	24.3±4.5	29.0±12.1
Smoking frequency	–	15.2±7.6*	26.7±9.8†
Years of smoking	–	8.0±3.2	13.4±11.2
Years of daily smoking	–	4.7±2.9	11.5±11.8
Breath CO ppm	0.8±0.5	25.1±16.9	17.0±7.8
Average hours/day spent around smokers of any type in the past week	3.48±2.46	6.13±4.03	7.39±3.60

*Waterpipes smoked per week.
†Cigarettes smoked per day.

METHODS

This study was conducted at the Syrian Center for Tobacco Studies (SCTS) in Aleppo, Syria, between 2010 and 2011. Through advertisement and word of mouth, we recruited smokers (waterpipe, cigarette) and non-smokers to the clinical laboratory of the SCTS. Of those who consented, 27 did not provide sufficient urine sample, and one had invalid NNAL measurements due to ion suppression and were thus excluded. This left us with 24 participants who smoked waterpipe daily and exclusively (reported daily waterpipe smoking in the past year and no smoking of any other tobacco product in the past month), 23 who smoked cigarettes daily and exclusively (reported daily cigarette smoking in the past year and no smoking of any other tobacco product in the past month), and 28 who have never smoked (reported not being a user of any tobacco product in the past, and had measured breath CO<7 ppm). Information on participants' smoking habits, exposure to secondhand smoke, and CO levels in the breath (Breath CO Monitor, Vitalograph, Lenexa, KS) were collected. First morning urine samples were collected from all subjects and stored at −20°C until their transfer in liquid ice from Aleppo, Syria, to the Environment Core Lab at the American University of Beirut (AUB), and immediately stored at −30°C until day of analysis. Frozen urine samples were thawed overnight in the refrigerator (4°C) prior to sample processing and analysis. Urinary-free NNAL (ie, non-conjugated) and total NNAL (ie, non-conjugated and conjugated) after hydrolysis, were both extracted from urine using solid-phase column and measured using Micromass Quattro LC triple quadrupole mass spectrometer (LC MS/MS). Sample preparation and NNAL measurement were done according to the method previously described.[14 15] The lower limit of quantitation (LLOQ) for this test is 0.25 pg/mL (0.0012 pmol/mL); in consideration of NNAL exposure from environmental sources, we used the LLOQ/square root 2 for values below the LLOQ for data analysis.[11] Comparison of free and total NNAL between groups was conducted on the crude measurements, as well as those normalised by urine creatinine clearance to correct for variation in urine concentration.[16 17] Because the distribution of NNAL measures was skewed, mean levels of NNAL of the three groups were compared using the Kruskal–Wallis test, and between-groups comparisons were conducted using the Mann–Whitney U test. The study protocol and informed consent documents were approved by the Institutional Review Boards of the SCTS, and AUB. All participants provided written informed consent prior to start of the study.

RESULTS

The study involved 49 men and 26 women, with mean age±SD of 31±12.9 years for non-smokers, 24±4.5 years for waterpipe smokers, and 29±12.1 years for cigarette smokers. Waterpipe smokers smoked, on average, 15.2±7.6 waterpipes per week, and had an 8.0±3.2 years of smoking history, while cigarette smokers smoked, on average, 26.7±9.8 cigarettes per day, and had 13.4±11.2 years of smoking history (details of the study groups are shown in table 1).

Results of comparison of NNAL urinary levels between the three groups showed that NNAL exposure of waterpipe smokers and cigarette smokers was higher than non-smokers. NNAL exposure in waterpipe smokers generally showed a lower level compared with cigarette smokers, but the difference did not reach statistical significance for all the measurements. The differences between NNAL levels among the three study groups were generally consistent across all measurements and preserved after creatinine normalisation. Details of the NNAL analysis results are provided in table 2.

DISCUSSION

This is the first study to show an exposure gradient for NNAL in independent samples of exclusive waterpipe smokers, cigarette smokers and non-smokers. Since NNAL is a marker of extended exposure to tobacco smoke (6–12 weeks), our study design provides a more robust indicator of carcinogen exposure associated with long-term waterpipe smoking, than the pre/post design adopted previously.[11 12] Levels of NNAL found among waterpipe smokers in our study were similar to those measured in a group of 13 waterpipe users who smoked two waterpipes per day,[12] and significantly greater than those reported when

Table 2 Mean (95% CI) urinary NNAL among exclusive waterpipe, cigarette and non-smokers

	Smoking pattern			p Value			
	None (n=28)	WP (n=24)	Cig (n=23)	All groups*	None vs WP†	None vs Cig†	WP vs Cig†
Free NNAL pg/mL urine	0.7 (0.3 to 1. 4)	8.4 (4.8 to 14.8)	10.7 (5.0 to 22.6)	<0.001	<0.001	<0.001	0.25
Total NNAL pg/mL urine	3.9 (1.6 to 9.5)	33.0 (21.6 to 50.6)	46.8 (27.6 to 79.3)	<0.001	<0.001	<0.001	0.17
Free NNAL ng/g creatinine	0.6 (0.3 to 1.3)	4.1 (2.2 to 7.9)	8.9 (5.2 to 15.2)	<0.001	<0.001	<0.001	0.026
Total NNAL ng/g creatinine	3.6 (1.6 to 7.9)	16.6 (10.5 to 26.2)	39.1 (26.6 to 57.4)	<0.001	<0.001	<0.001	0.004

Data are shown as geometric mean (95% CI).
*Three-group comparison using Kruskal–Wallis test.
†Between-groups comparison using Mann–Whitney U test.
Cig, cigarette smokers; NNAL, nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; None, non-smokers; WP, waterpipe smokers.

NNAL levels were measured in the 24 h period following a single waterpipe use episode in a population of waterpipe users who smoked two to three waterpipes per month.[18] These differences likely reflect accumulation due to the slow clearance kinetics of NNAL. Levels of NNAL in cigarette smokers in our study were similar to those reported by Jacob *et al*.[12] A recent study comparing NNAL of waterpipe smokers, cigarette smokers, and their non-smoking wives (passively exposed by definition), also detected higher levels of NNAL in cigarette and waterpipe smokers when compared with their wives.[19] However, the selection of the non-smoking group (spouses), and the daily smoking frequency of waterpipe smokers (approximately 12 waterpipes/day) in that study limits its generalisability in terms of differentiating exposure gradients between waterpipe smokers and non-smokers.[19] In our study, we recruited the three groups independent of each other; although our recruitment strategy did not guarantee that the enrolled participants were representative of waterpipe, cigarette and non-smokers in the general population. This is particularly true for the waterpipe smokers, where intermittent (ie, less than daily) smoking patterns predominate among the general population.[20] We also did not collect information about when they last smoked prior to giving the urine sample, although with an approximately 10–18 day half-life for NNAL in the body,[13] it is unlikely that this factor influenced the results of the study. Finally, because of the smoking patterns in Syrian society, the gender distribution of our study participants differed between the study groups, which precluded accounting for this important factor in the analysis. However, because we normalised our results by individual creatinine clearance, which is closely related to gender and age, we believe that our comparisons are not greatly affected by this factor.

Our results show that regular waterpipe smokers are exposed to about 5–10 times greater quantities of nitrosamines than non-smokers, and to similar quantities as pack-a-day cigarette smokers. Given our adequate sample size, selection of groups of exclusive smokers and non-smokers, and study design suitable for a metabolite with slow pharmacodynamics, our results provide the clearest indication to date about systemic exposure to potent carcinogens associated with long-term waterpipe smoking. Such evidence can support policy and regulatory efforts designed to confront the emerging global waterpipe epidemic, as well as drive interventions aimed at increasing the public awareness about the cancer risk associated with waterpipe smoking. Future studies need to examine exposure to carcinogens among waterpipe smokers in the general population, where different smoking levels and heterogeneous smoking patterns exist.

What this paper adds

- Waterpipe smoking is increasing among youth around the globe and is associated with misperception of reduced harm.
- This study, the first to examine exposure to tobacco-specific nitrosamines (NNAL) in independent samples of exclusive waterpipe smokers, cigarette smokers and non-smokers, shows that waterpipe smokers are exposed to about 5–10 times greater NNAL than non-smokers.
- Such evidence can support policy and regulatory efforts designed to confront the emerging global waterpipe epidemic, as well as help increase public awareness about the cancer risk associated with waterpipe smoking.

Correction notice This article has been corrected since it was published Online First. The author's name 'Sana Fayyad' has been corrected to 'Sanaa Fayad'.

Contributors WM, ALS and GSZ designed the study. RAA, SR and II conducted the study. AB, GSZ, SF did the lab analysis. RAA and SR did the data management and analysis. WM and RAA wrote the initial draft, and all other coauthors contributed significantly to the writing and revision of the study manuscript.

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