

Smoking patterns in waterpipe smokers compared with cigarette smokers: an exploratory study of puffing dynamics and smoke exposure

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Abstract

Aim: It is estimated that globally there are about 100 million people who smoke waterpipes daily yet exposure resulting from smoking waterpipes is under-researched relative to cigarette smoking. This exploratory study assesses smoke exposure and puffing profiles among waterpipe smokers in comparison with cigarette smokers in Turkey, where there is a concern that waterpipe smoking prevalence is increasing. **Method:** A convenience sample of waterpipe (n=20) and cigarette smokers (n=110) was recruited from Istanbul, Turkey. Both waterpipe and cigarette smokers followed broadly a similar protocol with two visits to the laboratory where puff recordings, measurements of expired air carbon monoxide (CO) as well as saliva and urine samples for measurements of cotinine and 1-hydroxypyrene (1-HOP) were taken. Both group of smokers used specially designed puff recording devices to establish puffing profiles unique for cigarettes and waterpipes. **Results:** On average, the volume of smoke inhaled from each puff of a waterpipe was almost 20 times greater than that from a manufactured cigarette: 1077.63 ml (± 486.03) versus 55.96 ml (± 15.12) respectively. Waterpipe smokers experienced significantly higher boosts of expired air CO following smoking a waterpipe in the laboratory compared with those smoking a cigarette, (means 42.9 versus 4.2; $p < 0.001$). Lower levels of cotinine and 1-HOP were observed in waterpipe smokers compared with cigarette smokers ($p < 0.001$). **Conclusion:** Waterpipe smokers in Turkey are exposed to larger volumes of smoke and higher CO levels, but less frequent nature of their smoking seems to be reflected in lower levels of exposure to other smoke constituents. The high smoke intake during waterpipe smoking is of public health concern.

Keywords: Smoking, waterpipe, carbon monoxide, cotinine, 1-Hydroxypyrene

Nargile ve sigara içim şekillerinin ve maruz kalım oranlarının karşılaştırılması

Özet

Amaç: Küresel olarak nargile kullanıcılarının sayısı yaklaşık 100 milyon kişi olmasına karşın, sigara içimi ile kıyaslandığında nargile ile ilgili daha az bilimsel çalışma bulunmaktadır. Bu araştırmanın amacı, nargile kullanımının giderek arttığı düşünülen Türkiye’de, nargile kullanıcılarının sigara kullanıcılarına kıyasla dumana maruz kalım ve içim dinamiklerinin değerlendirilmesidir.

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Received date: 26.08.2013, Accepted date: 01.04.2014

Yöntem: İstanbul'dan olasılıklı olmayan örnekleme yöntemi ile nargile (n=20) ve sigara içicisi (n=110) kişiler çalışmaya dahil edilmiştir. Gerek nargile gerekse sigara içicileri benzer protokollerle takip edilerek, iki kez laboratuvar testine tabi tutulmuş, içim dinamikleri ve nefeslerindeki karbon monoksit (CO) oranının yanı sıra tükürük ve idrar örneklerinde sırasıyla kotinin ve 1-hidroksipiren (1-HOP) ölçümleri yapılmıştır. Sigara içenler iki laboratuvar izlemi sırasında ve arada geçen 24 saatlik süre zarfında içtikleri bütün sigaralar için, nargile içenler ise özel tasarlanmış benzer bir içim kayıt cihazını iki kez sadece laboratuvar ortamında kullanmışlardır. **Bulgular:** Ortalama olarak, her bir nargile içiminde inhale edilen duman hacmi sigara içicilerine göre 20 kat daha fazla olup, ölçümler sırasıyla 1077.63 ml (\pm 486.03)' ye karşı 55.96 ml (\pm 15.12)'dir. Nargile içicilerinde sigara içicilerine kıyasla nargile içimini takiben anlamlı olarak daha yüksek CO artışı görülmüştür (ortalamalar: 42.9'a karşı 4.2; $p < 0.001$). Sigara içicilerine kıyasla nargile içicilerinde daha düşük kotinin ve 1-Hidroksipiren (1-HOP) düzeyleri gözlenmiştir ($p < 0.001$). **Sonuç:** Türkiye'de nargile içimi sırasında kullanıcılar daha fazla miktarda dumana ve daha yüksek CO düzeylerine maruz kalmaktadır. Ancak nargilenin kullanıcılar tarafından rapor edilen daha az sıklıkta içimine bağlı olarak, kullanıcıların diğer duman bileşenlerine sigaraya göre daha az düzeyde maruz kaldığı gözlenmektedir. Nargile içimi esnasında maruz kalınan yüksek duman hacmi toplum sağlığı açısından endişe vericidir.

Anahtar Kelimeler: sigara içme, nargile karbon monoksit, kotinin, 1- hidroksipiren

Introduction

Turkey is the fifth largest tobacco manufacturing country in the world¹ and smoking prevalence is high, with approximately 16 million smokers, including 31% of adults (aged 15 years and over), with higher numbers among men (48%) than women (15%).² However, in the last few years, Turkey has greatly increased its efforts to control tobacco use. For example, Turkey now has the best provisions for health warning labels in Europe with a pictorial health warning covering 65% of the surface area on the front of the pack; and from 2009 onwards, Turkey successfully introduced comprehensive smoke-free legislation with no exceptions and no designated "smoking rooms". The price of cigarettes has doubled from 2005 to 2010 by taxation.³ A recent report has shown that these measures contributed to positive health outcomes in Turkey, and overall consumption rates decreased by 15% and positive attitudes towards quitting smoking are increasing.^{4,5} The most recent update to smoking prevalence in Turkey comes from the Global Adult Tobacco Survey (GATS) 2012, which showed that tobacco use is on a consistent decline across genders as well as across places where tobacco consumption is not restricted legally (e.g. designated areas in

workplaces and restaurants), from the previous update in 2008.

Of concern however, is the use of waterpipes (also known as hookah, narghile, shisha) globally as well as in Turkey.⁶⁻⁸ Waterpipes are traditional smoking devices often smoked socially by two or more people. In a waterpipe, smoke is drawn through water and then through a tube. There are two components in the head of a waterpipe: tobacco (either flavoured or unflavoured) and charcoal (traditional or quick-lighting). The most recent data in Turkey indicate that 2.3% of the population smoke waterpipes and that the prevalence is higher among younger age groups (4.3% 15-24 year olds vs. 0.9% in the 45-64 year olds) and highest among male adolescents living in urban areas (10%).¹ Furthermore, around a third of students in one university were found to smoke waterpipes.⁹ There are misconceptions in Turkey that waterpipe smoking is not harmful to health, similar attitudes also exist elsewhere in other countries.¹⁰⁻¹²

There are fewer studies on the health effects of waterpipe smoking, in comparison with those of cigarette smoking, but very similar health effects have been found. A recent systematic review of the literature on the health effects of waterpipe

smoking found significant associations with lung cancer, respiratory illness, lower birth weight and periodontal disease.¹³ There is some evidence to suggest that waterpipe smoking has greater negative respiratory effects compared with cigarette smoking.^{14,15} Carcinoembryonic antigen (CEA) is a known marker for a variety of cancers and its levels have been demonstrated to be very high for heavy waterpipe smokers.^{13,16,17} Previous studies have also shown increased risk of coronary heart disease as well as atherosclerosis and some association between hepatitis C and waterpipe smoking. Waterpipe smoking also constitutes a potential risk factor for infectious diseases if a single pipe is shared in a group.^{13, 18-21}

We could find no previous research carried out in Turkey comparing exposure patterns between waterpipe and cigarette smokers despite concerns about the increase in the number of people smoking waterpipes in Turkey. This study therefore assesses smoke related exposure and associated puffing patterns among smokers of cigarettes and waterpipes in Turkey. Throughout this paper, smoking dynamics will refer to behavioural aspects of tobacco smoking including the number of puffs taken, elapsed puff duration, puff volume and total smoke volume.

Methods

This study obtained ethical approval for the use of human subjects and tissue samples from Marmara University School of Medicine.

Participant eligibility criteria

Waterpipe and cigarette smokers were recruited through advertisements in the local press and flyers & posters on bulletin boards in Istanbul in 2008. The adverts, posters and flyers contained the same information about the study, summarising procedures and the number of laboratory visits. The posters were placed on bulletin boards across the University campus and a smaller version of these posters (i.e. the flyers) were distributed to off-licence stores

(i.e. TEKEL shops) around Acibadem, Istanbul from where the majority of the subjects were recruited. The recruitment and data collection for the present study was completed over 13 months.

Eligibility criteria for waterpipe smokers were that they had to be regular smokers of waterpipes (at least three times a month; for example see frequencies of waterpipe smoking in young adults) and cigarette smokers were required to be regular smokers (at least five cigarettes daily for at least three months continuously, replicating the recruitment strategy of a previous publication from our research group; see Shahab et al.) of one of six named brands, chosen on the basis of their popularity.^{9,35} The popular brands were chosen in order to speed up the recruitment process as well as to increase the generalizability of our results, so that they may be relevant to a higher percentage of the smoking population in Turkey. Exclusion criteria for both cigarette and waterpipe smokers included self-reported lung and heart disease and pregnancy. We also excluded people who were younger than 18 and older than 65, as well as people who did not have a predominant preference of one of the cigarette brands that was included in the study protocol. The brand information was not included in any of the study advertisements in order to avoid recruiting false-positives for the purposes of the present study (referring to those smokers who smoked more than one brand interchangeably, see below). We wanted to recruit cigarette smokers who smoked one brand consistently so that the biomedical exposure measures could be attributed to the constituents of that particular cigarette brand (brand specific exposure patterns are not reported here). We used a convenience sampling approach; without necessarily aiming to reflect gender distributions of tobacco smokers based on information coming from published surveys of nationwide tobacco use.

Smoking procedures in the laboratory for both waterpipe and cigarette smokers, as well as the times and frequencies of the biomedical exposure

assessments were identical apart from the arrangement of the second laboratory visits. For the cigarette smokers, the second visits were arranged exactly 24 hours later, whereas for the waterpipe smokers the second visits were arranged for the fourth day at the same hour as the first visit. This interval was used, in order not to force waterpipe smokers to smoke a waterpipe more frequently than they would do otherwise in their weekly routines, and on the basis of the perception that waterpipes are mostly smoked recreationally only about once or twice a week. This interval also allowed those users who smoke waterpipes more frequently than the arranged visits not to be affected by the experimental procedures.

Procedures

A brief telephone survey, lasting 5 to 10 minutes, was conducted among people who responded to the advertisements, flyers and brochures, to assess eligibility. In total, around 180 individuals were screened for recruitment of cigarette smokers, and approximately 50 people were screened for

recruitment of waterpipe smokers. Eligible subjects (cigarette smokers n=110; waterpipe smokers n=20) were then invited to the laboratory for two visits, 24 hours apart for cigarette smokers and four days apart for waterpipe smokers (see Figure 1 for the timeline of the laboratory testing protocols). All the participants who attended the first visit also attended the second visit; there were no dropouts from the study. Two laboratory visits were arranged mainly because cigarette smokers needed to return the CreSSmicro (Clinical Research Support System, see Figure 2a) topography device after using it for 24 hours. This device records puffing dynamics from a smoker whilst smoking a cigarette. We wanted to use the second laboratory visits as an opportunity to collect more information on smoking behaviour and to investigate whether exposure patterns were comparable across two visits. We adopted a similar approach to test the waterpipe smokers and investigated whether smoking behaviour and exposure patterns are comparable across the two visits.

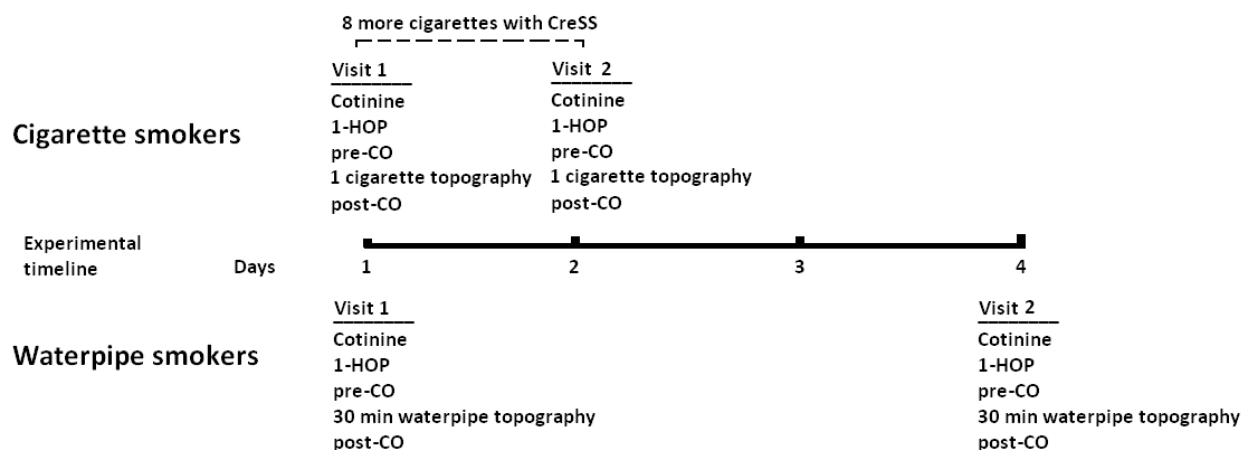


Figure 1. Schematic timeline of the experimental protocols

Schematic diagram of the experimental protocols based on an example comparing a cigarette smoker smoking 10 cigarettes per day (smoking one cigarette each in both visits in the lab and 8 cigarettes in the 24 hours between Visit 1 and 2 on his/her own; i.e. 1+8+1=10 cigarettes per day) to a waterpipe smoker smoking waterpipes twice per week.

At the beginning of each visit, both waterpipe and cigarette smoking subjects completed a brief questionnaire about their

smoking behaviour assessing their smoking history, frequency, health concerns associated with smoking and considerations

to quit smoking. Secondly, baseline exposure measurements related to expired air carbon monoxide (CO), saliva cotinine and urinary 1-hydroxypyrene (1-HOP) were taken. After that, the researcher (EP) provided information about how to use the relevant puff recording devices by a brief demonstration and any remaining questions were answered to ensure that the data collection would not be disrupted. Research subjects then smoked either one of their cigarettes or a waterpipe which had been purchased by the researchers solely for this work in the laboratory and puffing measures were recorded (see below). Within the context of this paper, puffing dynamics/topography refers to behavioural aspects of smoking such as number of puffs, time between puffs, puff duration and puff volume for both cigarette and waterpipe smokers. Finally, post-smoking CO measurements were taken in order to assess CO boost from the pre-smoking assessments. Salivary cotinine and urinary 1-HOP measurements were taken only once per visit and always before smoking in order not to compromise the quality of samples by immediate exposure to constituents (particularly applicable to salivary cotinine). We acknowledge that not doing so would have an immediate confounding effect on saliva samples but may not have immediate effect on urinary samples. Nevertheless, the urine samples were also collected at the same time in order to make the study procedures easier to administer and standardise across subjects.

Thus the biomedical exposure levels (i.e. CO, cotinine and 1-HOP) were assessed exactly in the same manner across two visits and for each group. However, smoking measures related to puffing dynamics were collected from cigarette smokers for only one cigarette in the first interview to make sure that our participants learned how to use the recording device and were able to use it on their own, so that data capturing the average smoking behaviour over 24 hours could be collected accurately, until the end of the second visit. On the other hand, waterpipe smokers had two identical

30 minute smoking sessions in the laboratory. Administration of an identical protocol for investigating smoking for both lab visits was achieved for waterpipe but not for the cigarette smokers, mainly because the waterpipe puff recording device was immobile and large (see the details below for the puff recording devices). Therefore, waterpipe smokers did not need to have a practice session to learn how to use the device. Consequently, we report smoking behaviour for cigarette smokers averaged over 24 hours (as a representative of average cigarette smoking behaviour of any participant), whereas we report puffing dynamics for waterpipe smokers individually for each visit. However, because exposure patterns were assessed in the same way for each group, we were able to compare them directly. We used the exposure patterns from Visit 1 for between-group comparisons as the most unbiased recording of exposure patterns, considering the possibility that exposure information obtained in the second visit may be confounded by the use of the puff recording devices.

Waterpipe features

The waterpipe used was the most common type found in Turkey, of medium size with a glass jar and copper top and a hose made of sheep skin. The type of charcoal used was of a quick-lighting type for ease of use in the laboratory. This type of charcoal is known to be more convenient for regular waterpipe smokers who smoke in their homes. The tobacco used was one of two types (strawberry or apple flavoured); the most commonly used in shisha cafes in Istanbul at the time, and were weighed carefully using a sensitive scale before and after burning.

Puff recording devices

Recordings for puffing dynamics included measurements of puff number, puff volume, puff duration, average puff flow, inter-puff interval, time and date. The puff dynamics of waterpipe smokers were measured using a first generation and digitally programmable smoking machine that had been developed specifically for this purpose.²²⁻²⁵ This machine utilized a pressure drop transducer

attached to a laptop computer which runs the software to convert pressure drops into volumetric readings. Subjects smoked the waterpipe through the machine in the laboratory during each visit. The puff recording machine used for the waterpipe smoking session was immobile due to its size and weight (see Figure 2b). Therefore, waterpipe participants only used the machine in the laboratory. The puffing dynamics of cigarette smokers was measured through a CReSSmicro device

(Plowshare Technologies Inc., Baltimore MD, US) which is a hand held, battery-operated portable device that is mass-produced (see Figure 2 for comparisons).

On visit one, the cigarette smokers were given instructions on how to use the CReSSmicro device and they were asked to smoke all their cigarettes (during, and in between, the two visits) through the machine. All recording devices were calibrated daily prior to participants use.

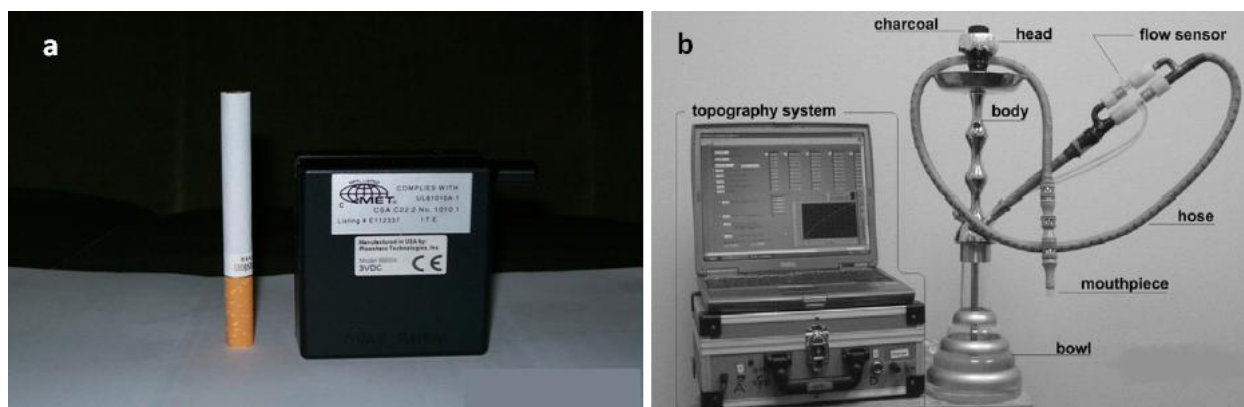


Figure 2. Comparisons of puff recording devices

Figure showing comparisons of the two devices. Panel (a) the CReSS micro device is a handheld device shorter than the length of a cigarette (b) the waterpipe topography device is a prototype attached to a laptop computer, developed by Shihadeh and colleagues.²² The descriptive figure is adapted from Eissenberg and Shihadeh, 2009²².

Biomarkers of smoke exposure

A Bedfont hand held monitor was used to measure expired air CO levels, as an instantaneous measure of exposure to smoke before and after each smoking session (twice on each occasion, as also used by Shahab el al.).^{26,35,36} As explained above, saliva and urine samples were collected from both cigarette and waterpipe smokers at the start of each session, before any smoking, for the measurement of cotinine, a metabolite of nicotine; and 1-HOP levels, as an accepted biomarker of carcinogenic hydrocarbons, respectively.²⁷ Saliva samples were taken using a dental roll with a cotton swab that the participants were asked to hold in their mouth and lightly chew for two minutes, until fully saturated. Urine samples were collected in a sterilized sealable cup.

Analysis of salivary cotinine and 1-HOP samples were conducted by a laboratory at the Acibadem Hospital in Istanbul (a partner of the internationally accredited Labmed, Germany), in a single-blind manner. In addition, urine was assayed for creatinine to correct for variable urine flow rates in 1-HOP analysis.

Statistical Analysis

The analysis was carried out using SPSS 20.0. We used the independent samples t-test in order to compare the results between waterpipe and cigarette smokers. A paired samples t-test was used for within group comparisons.

Results

Twenty waterpipe smokers and 110 cigarette smokers were included in the final

analysis. The between group comparisons for overlapping socio-demographical variables suggested that the groups were comparable for the distribution of gender ($p=0.179$), but the waterpipe smoking sample was significantly younger ($p<0.001$).

Waterpipe smoking sample

Three quarters ($n=15$) of the sample were male, the average age was 21.15 years (± 2.08). Four participants also smoked cigarettes daily (mean 14 cigarettes ± 4.96). Three quarters reported normally sharing their waterpipes with others. Participants reported smoking their last waterpipe on an average of 4.4 days prior to attending the study (± 4.19 days).

Each session of waterpipe smoking

lasted approximately 30 minutes. The average tobacco consumption during each smoking session was 2.17 g (± 0.63); the average charcoal consumption 6.16 g (± 1.56). There were no significant differences in the amounts of tobacco consumed across the two sessions or in the puff measures from the two visits, apart from the number of puffs which increased significantly from visit 1 to 2 (Table 1). In order to investigate whether these patterns remained the same for exclusive waterpipe users, we repeated the analyses for the 16 waterpipe users who were not cigarette smokers. The results did not change significantly from the total waterpipe smoking sample [data not shown].

Table 1. Puff measures for waterpipe and cigarette smokers (mean \pm SD)

Puff measures	Waterpipe smokers (n=20)				Cigarette smokers (n= 110)			
	Visit 1	Visit 2	t-value**	Average	Visit 1	Visit 2	t-value**	Average
Total smoke volume/session (ml)	102583.00 ± 48101.00	114936.00 ± 47834.00	t=-1.04 ns	108759.00 ± 42044.00	908.00 ± 61.20	761.00 ± 64.70	3.51 p<0.001	783.10 ± 60.8
Number of puffs/session	96.00 ± 27.99	119.74 ± 47.00	t=-2.38 p<0.05	107.87 ± 31.29	14.30 ± 4.60	14.45 ± 4.01	-0.19 ns	14.42 ± 4.02
Puff volume/session (ml)	1115.79 ± 526.90	1039.47 ± 469.70	t= 0.61 ns	1077.63 ± 486.03	63.51 ± 17.30	55.92 ± 15.10	3.39 p<0.001	55.96 ± 15.12
Puff duration/session (secs)	3.36 ± 0.94	3.37 ± 1.19	t=-1.00 ns	3.37 ± 1.02	1.56 ± 0.40	1.43 ± 0.40	1.71 ns	1.47 ± 0.37
Weight of tobacco consumed/session (gr)	2.18 ± 0.63	2.26 ± 0.59	t=-0.82 ns	2.22 ± 0.61	N/A*	N/A*	N/A*	N/A*
Weight of charcoal consumed/session (gr)	6.16 ± 1.56	6.57 ± 1.81	t=-1.51 ns	6.37 ± 1.68	N/A*	N/A*	N/A*	N/A*

* N/A: Not Applicable. ** Paired samples t-test. ns: Not significant.

Table 2. Summary of exposure patterns between waterpipe and cigarette smokers (mean± SD)

Exposure patterns	Waterpipe smokers			Cigarette smokers		
	Visit 1	Visit 2	t-value*	Visit 1	Visit 2	t-value*
CO pre smoking (particles/10 ⁶)	6.50 ± 7.11	7.00 ± 7.62	t=-0.38 ns	18.36±8.80	19.76±9.00	t=-2.03 p<0.05
CO post smoking (particles/10 ⁶)	49.28 ± 41.53	53.13±44.15	t=-0.81 ns	22.58±8.78	24.81±9.88	t=-2.18 p<0.05
CO boost (particles/10 ⁶)	42.90±43.17	42.65±41.62	t=-0.89 ns	4.20±3.40	4.21 ± 3.25	t=0.47 ns
Cotinine (ng/ml)	119.08±129.30	121.40±147.2 1	t=-0.11 ns	304.10±218.7 3	519.45±322.1 7	t=-8.73 p<0.001
1-HOP (ug/creatinine gr)	0.08±0.08	0.09 ± 0.13	t=-0.22 ns	0.29 ± 0.26	0.32± 0.32	t=-1.11 ns

ns: Not significant. *Paired samples t-test.

Exposure measures are shown in Table 2 - there were no significant differences within the waterpipe smoking group across the two visits.

Cigarette smoking sample

Fifty nine per cent (n=65) were male and the average age was 28.15 years (±9.40). Average daily cigarette consumption was 15.8 (±6.2). First, we compared the puffing dynamics associated with the first cigarette, which was smoked under the observation of the researcher, with the average puff recordings over 24 hour period. There were significant differences between the first cigarette and the average over 24 hours for average puff volume (p<0.001), average puff flow (p<0.05), average peak puff flow (p<0.01), and average inter-puff interval (p<0.01), but not in average puff number and average puff duration. We therefore averaged the puff topography measures across the 24 hour period including the first and the second laboratory sessions as a more accurate representation of overall smoking behaviour, in order to compare

them with the average waterpipe puff measurements (also see Table 1 above).

Exposure measures for the cigarette smokers are reported in Table 2 (above). Some of the measures were significantly different on visits 1 and 2. In particular, the cotinine levels for the second visit were significantly higher than those from visit 1 (p<0.001). This rise is hard to explain but, in line with the changes in puff measurements, indicating that the use of the puff recording device over the 24 hour period increased the amount of smoke inhaled during the study. Pre and post CO values were also significantly higher at the time of visit 2 than visit 1.

Comparison between cigarette and waterpipe smokers

There were clear differences between waterpipe and cigarette smokers. The average number of puffs taken by waterpipe smokers was between seven and eight times greater than the average number of puffs taken by cigarette smokers and puffing duration of waterpipe smokers was longer and larger in volume than that of cigarette smokers. Hence, the total smoke volume inhaled on average per any waterpipe

smoking session was nearly 14 times higher relative to the average per cigarette. All comparisons between the average measures of waterpipe and cigarette smokers were significant ($p < 0.001$).

For the biomarker comparisons we have only relied on the baseline data (visit 1) because of the differences at visit 2 which might be due to the influence of using the puff recording machines. There were significant differences in the pre and post cigarette/waterpipe CO levels between cigarette and waterpipe smokers. Pre

smoking CO levels for waterpipe smokers were significantly lower than those of cigarette smokers ($t = 9.28$, $p < 0.001$), post smoking CO levels were significantly higher than those of cigarette smokers ($t = -4.11$, $p < 0.001$) and CO boosts were significantly greater for waterpipe smokers ($t = -5.8$, $p < 0.001$). The cotinine levels were significantly lower for waterpipe smokers than cigarette smokers ($t = 5.2$, $p < 0.001$) as were the 1-HOP levels ($t = 6.9$, $p < 0.001$; Table 3).

Table 3. Between group differences for toxicant exposures in Visit 1 (mean \pm SD)

Toxicant exposures	Waterpipe smokers	Cigarette smokers	t-value*	Significance
CO pre smoking (particles/ 10^6)	6.50 \pm 7.11	18.36 \pm 8.80	9.28	$p < .001$
CO post smoking (particles/ 10^6)	49.28 \pm 41.53	22.58 \pm 8.78	-4.11	$p < .001$
CO boost (particles/ 10^6)	42.90 \pm 43.17	4.20 \pm 3.40	-5.80	$p < .001$
Cotinine (ng/ml)	119.08 \pm 129.30	304.10 \pm 218.73	5.20	$p < .001$
1-HOP (ug/ Creatinine gr)	0.08 \pm 0.08	0.29 \pm 0.26	6.90	$p < .001$

*Independent samples t-test.

Discussion

CO levels in the expired air after smoking a waterpipe in a laboratory setting increased between seven and eight fold in a sample of waterpipe smokers, compared to cigarette smokers for whom these levels increased only by 25%. This finding was consistent across two separate visits to the laboratory and reflected that greater smoke volume was inhaled during the waterpipe sessions than a single cigarette smoking session. Hence, although the baseline CO levels of the waterpipe smokers were lower than those of cigarette smokers, they were significantly higher after smoking. Two

other measures of smoke exposure were used, cotinine and 1-HOP levels, which measure exposure to nicotine and polycyclic aromatic hydrocarbons (PAHs) respectively. These biomarkers have longer half-lives (approximately 16 and 18 hours respectively) than CO (half life of 6-9 hours).^{22,28,29} Therefore, these reflect exposure over a longer period of time and they were significantly lower in waterpipe than cigarette smokers.

Whilst our samples of smokers were not randomly selected, and the sample of waterpipe smokers was small, best to our knowledge this is the first study in the

literature to examine a number of different biomarkers (specifically 1-HOP) and smoking topography in near exclusive waterpipe and cigarette smokers using a similar methodology. These findings contribute to understanding of differences between waterpipe and cigarette smoking reported by previous studies, which investigated toxicant exposures in mixed populations, and near exclusive populations of waterpipe smokers, but mainly considered CO and nicotine exposure.^{22,30,31} Our modest sample size reflects the exploratory aspects of this research. Although the biomarker data were collected using similar procedures, there were differences with respect to how the puffing dynamics were measured. Cigarette smokers used a portable CreSS device for measuring smoking behaviour across the 24 hour interval between and including the cigarettes smoked during two visits. Smoking behaviour for waterpipe smokers was only measured in the laboratory because the recording device was an immobile one, but it was the only one that was available to us. Hence, the tools for measurements differed, but we do not expect this to make a significant impact on the accuracy of the puff recordings. The puff recording machine used for waterpipe smoking was unobtrusive; and during the smoking sessions magazines, tea and coffee were offered to the participants in order to simulate a natural smoking environment. Future studies may be designed to record puffing measures in waterpipe smoking cafes so that more accurate data may be collected. However, this approach may also have limitations as it might attract interest and attention from other people present in the cafe which could in return influence participants' smoking patterns. The type of charcoal used in our study was of the quick lighting type rather than traditional charcoal and one study found significantly higher levels of PAHs in quick burning types of charcoal compared to the traditional type.³² However, best to our knowledge, whether quick lighting charcoal produces more CO than other traditional types has not been subject to any investigation although use of quick lighting charcoal disks

has found to be responsible for most of the CO boost when compared with an electric heater.^{32,33}

Waterpipe smoking participants reported smoking waterpipes once per week with their last waterpipe being smoked around an average of just over four days prior to testing, whereas smokers of manufactured cigarettes reported smoking daily with an average consumption of 15.8 cigarettes per day. This difference may explain the lower exposure to nicotine and 1-HOP in waterpipe smokers, despite the fact that waterpipes emit more PAHs than cigarettes.³⁴ A previous study showed that both nicotine and 1-HOP reach their peak plasma concentrations within two hours following a waterpipe smoking session, but fall to their baseline values within 24 hours.³¹ It is also worth noting that 1-HOP levels for cigarette smokers are considerably lower than those observed in smokers in the United Kingdom, which may reflect differences between the cigarette brands.³⁵ The post smoking measure of CO was obtained in the same way for both waterpipe and cigarette smokers and gives a direct comparison of exposure during the smoking sessions. Various other studies carried out in a wide range of countries, also showed a rapid increase in the CO boost following a waterpipe smoking session (e.g. United States, Syria).²²⁻²⁴ A recent study showed that smoking a tobacco-free (nicotine less than 0.01 mg) product through a waterpipe produces similar levels of CO with that of smoking waterpipe tobacco, corroborating the findings above (i.e. the CO boost is not tobacco-related) and highlights the dangers of smoking herbal preparations with a waterpipe. In some cases this rapid CO boost may lead to CO poisoning as previously observed following waterpipe smoking.^{33,34}

Our findings suggest that although smoke intake from a single waterpipe smoking session is greater than the total puff volume from a manufactured cigarette, the lower levels of cotinine and 1-HOP indicate a lower frequency of waterpipe smoking perhaps related to a less chronic exposure to certain smoke constituents.

Nevertheless, our results give cause for concern as the volume of smoke intake and related CO rise are likely to have serious health consequences even if chronic exposure to other constituents of tobacco smoke is lower over a sustained period of time.

This exploratory study has found significant differences in a range of biomarkers between cigarette and waterpipe smokers. However, our study had a few limitations. Firstly, we took a non-randomised sampling approach in recruitment (mainly recruiting healthy volunteers who responded to the study's adverts). Therefore, the present cohort do not fully represent characteristics of the cigarette and/or waterpipe smoking population in Turkey. We have to acknowledge that our waterpipe smoking sample was much younger than the cigarette smoking sample, which is another limitation. However, using age as a covariate while directly comparing exposure parameters between waterpipe and cigarette smokers did not produce any changes in the level of statistical significance, as between group comparisons were vastly different from each other. Nevertheless, this age difference is in line with previous studies showing that waterpipe use is a recreational activity among youth. This could mean that if such a recreational activity becomes habitual it might elevate health risks exponentially, accumulating by repeated exposure over the years and potentially increasing the risks for cardiovascular and respiratory illnesses as suggested by the previous literature. Another limitation of our study is that we were unable to report more detailed socio-demographic information in our cigarette smoking sample as the protocol was an international one which mainly focused on exposure parameters. Therefore, although unlikely, there may be demographical differences between our groups which contributed to overall smoking intensity. Finally, our waterpipe smoking sample did not smoke waterpipes exclusively, but included four individuals who also smoked cigarettes occasionally. However, repeating

the between group comparisons for the subsample smoking waterpipes only (n=16) showed that between group differences remained significant, suggesting that the four individuals who also smoked cigarettes displayed similar smoking behaviour and had comparable exposure characteristics with the rest of the waterpipe smokers.

In summary, our study shows that acute exposure was much greater following a single waterpipe smoking session relative to smoking a single cigarette. However, chronic smoke exposure, as indicated by cotinine and 1-HOP levels, was significantly lower among waterpipe smokers perhaps reflecting the more infrequent nature of waterpipe smoking. These results merit further research with more controlled experimental protocols and particularly with larger populations of waterpipe smokers.

Acknowledgments

This study is funded by Cancer Research UK, grant number C25586/A8324. The authors also thank Professor Alan Shihadeh from the American University of Beirut, who constructed the prototype smoking topography device and Professor Tom Eissenberg and Dr Asli Carkoglu for their helpful advice during the study, and Berna Kalkan for her help in carrying out the recruitment of subjects. The study obtained ethical approval for the use of human subjects and tissue samples from Marmara University School of Medicine.

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