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Assessment of genotoxicity of waterpipe smoking using 8-OHdG biomarker

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ABSTRACT. Waterpipe tobacco smoking is increasing in popularity, particularly among young adults. This popularity is related to the lack knowledge regarding the health effects of waterpipe smoking. In this study, we examined the genotoxicity of waterpipe smoking using an 8-hydroxy deoxyguanosine (8-OHdG) assay. Genotoxicity was evaluated in the saliva, urine, and serum of 66 waterpipe adult smokers and 46 healthy nonsmokers. The level of addiction to waterpipe smoking was evaluated using the Lebanon Waterpipe Dependence Scale-11. Levels of 8-OHdG in the samples were measured using commercially available enzyme-linked immunosorbent assays. Levels of 8-OHdG in the saliva (52,430 ± 2923 *vs* 48,430 ± 4189 pg/mL), urine (2815 ± 312 *vs* 2608 ± 180 pg/mL), and serum (19,720 ± 202 *vs* 19,670 ± 254 pg/mL) were similar between waterpipe smokers and nonsmokers ($P > 0.05$). In addition, no correlations were found between dependence

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score and levels of 8-OHdG in all sample types. In conclusion, 8-OHdG is not a good biomarker for genotoxic effect of waterpipe smoking.

Key words: DNA damage; 8-Hydroxy deoxyguanosine; Smoking; Tobacco; Waterpipe

INTRODUCTION

Tobacco smoking is a major world health concern that contributes to millions of deaths each year (WHO, 2009). While tobacco is commonly consumed in the form of cigarettes, waterpipe tobacco smoking is a different method of tobacco use that has become increasingly popular worldwide, including in the United States (Barnett et al., 2009; Cobb et al., 2010; Dugas et al., 2010; Primack et al., 2008, 2010), Europe (Pärna et al., 2008; Døssing, 2009; Jensen et al., 2010; Poyrazoğlu et al., 2010), and countries in the Eastern Mediterranean region (Maziak et al., 2004; Azab et al., 2010; Khabour et al., 2012b), particularly among youths (Eissenberg et al., 2008; Maziak, 2008; Primack et al., 2008; Warren et al., 2009). A waterpipe (also referred to as a hookah or shisha) is constructed of a hose, water bowl, body, and a "head" filled with tobacco that is heated over charcoal. Users inhale through the mouthpiece and hose, drawing air over the charcoal. The heated air, that now also contains charcoal combustion products, passes through the tobacco and a mainstream smoke aerosol is produced. Smoke passes through the body, bubbles through the water in the bowl, and is carried through the hose to the user (Shihadeh, 2003).

Previous studies have suggested that the health effects of waterpipe tobacco smoking are comparable or even worse than with cigarette tobacco use. For example, compared to cigarette smoke, waterpipe smoke contains toxicants such as polycyclic aromatic hydrocarbons that cause cancer, volatile aldehydes that cause lung disease, carbon monoxide (CO) that contributes to cardiovascular disease, and heavy metals that causes tissue damage (Shihadeh and Saleh, 2005). Recent studies found that acute exposure to waterpipe smoke caused lung inflammation and oxidative stress to same degree to that induced by cigarette smoking (Khabour et al., 2012a). Moreover, the genotoxicity associated with waterpipe use is significantly higher in heavy waterpipe users than in heavy cigarette smokers (Khabour et al., 2011; Alsatari et al., 2012).

8-Hydroxy deoxyguanosine (8-OHdG) is thought to be a good marker of oxidative DNA damage and can be measured easily in the laboratory (Kasai, 1997; Pilger and Rüdiger, 2006). 8-OHdG levels were high in the lungs, liver, and heart of mice exposed to cigarette smoke (Howard et al., 1998; Thaiparambil et al., 2007). In this study, we examined the genotoxicity of waterpipe smoking using 8-OHdG as a marker in the saliva, urine, and serum of users.

MATERIAL AND METHODS

Subjects

This cross-sectional study included 66 adult and apparently healthy waterpipe smokers and 46 age-matched healthy nonsmokers in the year 2013. A full description of the study and its goals were offered to participants prior to providing informed consent. The study was approved by the institutional review board. Participants' demographics were obtained using a self-administered questionnaire. The level of dependence on waterpipe smoking was mea-

sured using the Lebanon Waterpipe Dependence Scale-11 as previously described (Salameh et al., 2008; Alzoubi et al., 2014a). Investigators were available to answer participants' questions when the questionnaires were filled out and the scaling was conducted.

Samples collection

First, 5 mL blood was drawn from an antecubital vein and collected into tubes. Tubes were incubated at room temperature for 30 min and were then centrifuged at 1500 *g* for 15 min for serum isolation. Urine samples were collected in urine containers. Before collection of approximately 1 mL saliva, the subject's mouths were rinsed 3 times with distilled water. Aliquots of the samples were stored at -20°C until use.

8-OHdG assay

Levels of 8-OHdG in the samples were determined using an enzyme-linked immunosorbent assay (ELISA) (8-OH-dG EIA kit; Abcam, Cambridge, UK) according to the manufacturer instructions and as previously described (Alzoubi et al., 2013, 2014a,b). Samples were assayed in duplicate. Absorbance was measured at 405 nm using an automated ELISA reader (ELx 800/universal microplate reader, Bio-Tek, Winooski, VT, USA). Urine levels of 8-OHdG were normalized to the amount of creatinine in the samples.

Statistical analysis

Data were analyzed using SPSS ver. 19 (SPSS, Inc., Chicago, IL, USA). Values are reported as means ± SE. Comparisons were performed using 2-tailed Student *t*-test. Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

This study included a total of 66 waterpipe smokers (34 men and 32 women) and 46 nonsmokers (23 men and 23 women). The mean age of smokers was 30.2 ± 10.3 years, while the average age was 31.9 ± 11.1 years in the nonsmoker group (Table 1, $P > 0.05$). The 2 groups were did not differ in terms of income and gender ($P > 0.05$).

Table 1. Demographics of waterpipe smokers and controls.

Variable	Smokers (N = 66)	Control (N = 46)
Age (mean ± SD)	30.2 ± 10.3	31.9 ± 11.1
Gender ((N)%)		
Male	34 (30.4)	23 (20.5)
Female	32 (27.7)	23 (20.5)
Family income		
<990 JD	26 (23.2)	32 (27.1)
≥990 JD	35 (31.3)	14 (12.6)

Table 2 shows characteristics of waterpipe smokers. Approximately 27% initiated waterpipe smoking before the age of 17 years. In addition, 59% of waterpipe users were daily smokers, 68% smoked less than 1 h per session, and approximately half of the subjects smoked 2 heads per session. Most of the waterpipe smokers owned at least 1 waterpipe.

Table 2. Parameters used to evaluate waterpipe smoking behavior among participants.

Variable	Waterpipe smokers (N = 66)
Age at waterpipe initiation	
<17	18 (27.3)
≥17	48 (72.8)
Smoke waterpipe	
Daily	39 (59.1)
Weekly	14 (21.2)
Monthly	9 (13.6)
Waterpipe session duration	
≤1h	45 (68.2)
1-2h	15 (22.7)
≥2h	6 (9.1)
Number of heads/session	
≤2	127 (52.3)
2-5	114 (46.9)
≥5	2 (0.8)
Owns waterpipe (%)	56 (86.1)
LWDS score (mean ± SD)	11.7 ± 5.9

Figure 1 shows levels of 8-OHdG in the different samples as measured using ELISA. The levels of 8-OHdG in the saliva (52,430 ± 2923 *vs* 48,430 ± 4189 pg/mL) urine (2815 ± 312 *vs* 2608 ± 180 pg/mL), and serum (19,720 ± 202 *vs* 19,670 ± 254 pg/mL) were similar between waterpipe smokers and nonsmokers, respectively (P > 0.05). Table 3 shows the correlation between the addiction score of the participants measured using the Lebanon Waterpipe Dependence Scale-11 and 8-OHdG in the samples. No correlations between dependence score and levels of 8-OHdG in all sample types were detected (P > 0.05).

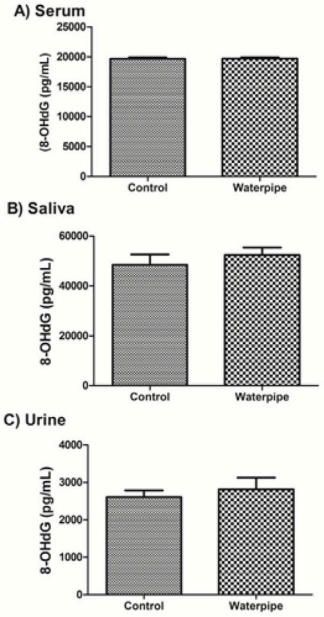

Figure 1. Changes in saliva, urine, and serum 8-OHdG in waterpipe smokers. Waterpipe smokers (N = 66) and control nonsmokers (N = 46) were included in the study. No significant differences in the levels of 8-OHdg were detected between the 2 groups in serum (**A**), saliva (**B**), and urine (**C**), (P > 0.05). Data are reported as means ± SE.

Table 3. Correlation between 8-OHdG levels and waterpipe addiction score.

Sample	Addiction score (correlation coefficient)	P value
Saliva	-0.041	0.756
Urine	0.079	0.545
Plasma	-0.021	0.873

DISCUSSION

In this study, we examined the genotoxic effect of waterpipe smoking using an 8-OHdG assay in urine, saliva, and serum samples. The 8-OHdG assay is a good biomarker for oxidative DNA damage (Kasai, 1997; Pilger and Rüdiger, 2006). The results showed that waterpipe smoking is not associated with significant increases in 8-OHdG in smokers.

Waterpipe smoke has been shown to contain a wide variety of toxic compounds similar to those detected in cigarette smoke (Shihadeh et al., 2004; Shihadeh and Saleh, 2005). For example, "tar" (volatile aldehydes) of a single waterpipe smoking session is approximately 2-fold more concentrated than that produced from a single cigarette (Al Rashidi et al., 2008). Because of use of charcoal, CO and polycyclic aromatic hydrocarbon levels are several magnitudes higher in waterpipe smoke compared to cigarette smoke (Shihadeh et al., 2004; Shihadeh and Saleh, 2005; Bacha et al., 2007; Monzer et al., 2008; Sepetdjian et al., 2008; Eissenberg and Shihadeh, 2009; Maziak et al., 2009). Moreover, a study by Eissenberg and Shihadeh (2009) showed that carboxyhemoglobin after waterpipe smoking is 3-fold higher that observed after cigarette smoking (Eissenberg and Shihadeh, 2009).

The genotoxicity of waterpipe smoking was previously examined in cells derived from smokers. For example, waterpipe smoking has been shown to be associated with increases in the frequencies of sister chromatid exchanges and chromosomal aberrations in lymphocytes obtained from smokers (Yadav and Thakur, 2000; Khabour et al., 2011; Alsatari et al., 2012). In addition, the level chromosomal damage was found to be strongly correlated with the magnitude of waterpipe use (Khabour et al., 2011; Alsatari et al., 2012). Moreover, waterpipe smoking has been reported to induce micronuclei in buccal mucosa cells (Boulos et al., 2009). However, our results showed that waterpipe smoking was not associated with increased 8-OHdG levels as measured in samples of urine, saliva, and serum obtained from smokers. This may have been because of the high background levels of this DNA damage biomarker in healthy subjects. Data regarding cigarette smoking is controversial. While some studies showed an association between cigarette smoking and 8-OHdG levels (Campos et al., 2011; Chiang et al., 2012), others failed to show such an association (Feng et al., 2006). Previous studies revealed higher levels of 8-OHdG in the lung, liver, and heart of mice exposed to cigarette smoke (Howard et al., 1998; Thaiparambil et al., 2007). However, other studies did not show this effect (Arif et al., 2001). In conclusion, we found that 8-OHdG in the urine, saliva, and serum was not a good biomarker for waterpipe tobacco smoking.

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