Comparison of Cotinine Salivary Levels in Hookah Smokers, Passive Smokers, and Non-Smokers

Tahereh Nosratzehi¹, Fateme Arbabi-Kalati², Ebrahim Alijani PhD³, Hassan Tajdari⁴

Abstract

Background: At present smoking is considered a great health-related problem. Smoking cigarettes and use of tobacco are on the rise in the Middle East countries; therefore, the number of people exposed to passive cigarette smoke is increasing, too. The aim of the present study was to determine and compare salivary cotinine levels in hookah smokers, individuals exposed to passive cigarette smoke and non-smoker (passive smokers).

Methods: In the present cross-sectional study, unstimulated salivary samples were collected from 150 subjects, including 50 hookah smokers, 50 passive smokers, and 50 non-smokers. Bioassay Technology Laboratory cotinine kit was used to determine salivary levels of cotinine using the enzyme-linked immunosorbent assay (ELISA) technique at a sensitivity rate of 0.019 pg/ml. Data were analyzed with SPSS software using t-test and Pearson’s correlation coefficient.

Findings: The highest salivary cotinine levels were recorded in hookah users (20.24 ± 5.62 ng/ml), followed by passive smokers (16.09 ± 3.51 ng/ml), in descending order. No detectable cotinine levels were observed in non-smokers. Pearson’s correlation coefficient showed a strong and positive correlation between use of hookah and salivary cotinine levels (r = 0.932, P = 0.001).

Conclusion: Based on the results of the present study, salivary cotinine levels were higher in hookah smokers compared with passive smokers and non-smokers, in descending order.

Keywords: Tobacco use, Smoking, Saliva, Cotinine

Citation: Nosratzehi T, Arbabi-Kalati F, Alijani E, Tajdari H. Comparison of Cotinine Salivary Levels in Hookah Smokers, Passive Smokers, and Non-Smokers. Addict Health 2015; 7(3-4): 184-91.

Received: 28.06.2015
Accepted: 11.09.2015
Introduction

Smoking is considered a health problem at present all over the world. Based on estimates, smoking will be the cause of 1 death in every 3 deaths by 2020.\(^1\) Research has shown that cigarette, and recently hookah, is the most important etiologic factors for oral squamous cell carcinomas.\(^2\)

Use of hookah is increasing in the Middle East countries and many people believe it is harmless. Traditional coffee shops have become widespread, and an integral part of recreation centers. The reasons for the popularity of hookah in recent years might include attractive fruit flavors added to tobacco, the traditional aspects of hookah, an increase in the number of coffee shops, low knowledge level in the community and inadequate official supervision. The World Health Organization (WHO) has declared tobacco use, especially hookah and cigarette smoking, a universal threat to health.\(^3\) Contrary to cigarette, no supervision is exercised for the packaging and quality of tobacco used in hookah and only “tartar-free” labels are used on the packages of flavored hookah tobacco.\(^4\)

It has been estimated that deaths due to tobacco smoke will increase to 10 million a year by 2020, with more deaths in developing countries compared to developed countries. Countries and health officials spend large amounts of money to treat smokers, to combat smoking and to adopt preventive measures each year. If the resources expended had been diverted to development of countries many problems of these countries would have been solved.\(^3,4\)

Another concern in relation to tobacco use is the exposure of non-smokers to tobacco smoke. These individuals suffer from the complications of tobacco smoke indirectly. In recent years, passive smoking has been reported to be a strong risk factor for cardiovascular diseases (CVD).\(^5\) The number of tobacco users increased to 1.4 billion individuals worldwide from 2000 to 2020; therefore, everyone is possible to be exposed to tobacco smoke.\(^5,7\)

Hookah was invented in the 15th century in India and rapidly became very popular in the Middle East (in countries like Iran, Syria and Yemen).\(^8\) The initial coconut design was converted to a glass container with a tube attached to it.\(^2,3\)

In 1990, tobacco was flavored with fruits and flavors and was termed “maasel,” a derivative of “maa’selm,” which means honey.\(^4\)

Studies have shown that tobacco use leads to various diseases, including lung cancer, pulmonary diseases, low birth weight (LBW), and periodontal diseases. It also increases the risks of some conditions, albeit non-significant, including urinary bladder cancer, oral dysplasias, and infertility.\(^9\) The term “smoking” indicates an active habit of smoking cigarettes, i.e., a smoker smokes cigarettes on purpose. The term “passive smoking” indicates involuntary inhalation of tobacco smoke by non-smokers. The term was introduced in 1970, which resulted in more attention to the relationship between diseases and individuals exposed to tobacco smoke.\(^6,7\) Environmental tobacco smoke (ETS) indicates evaluation of individuals exposed to cigarette smoke and is scored based on a questionnaire filled out by the individual himself/herself.\(^8\)

Cotinine is an alkaloid in tobacco and is one of the metabolites of nicotine. It can be measured using different techniques, including immunoassay, radioimmunoassay, fluid gas chromatography, enzyme-linked immunosorbent assay (ELISA), and fluid chromatography.\(^6\)

Saliva is a proper alternative diagnostic tool for other body fluids because salivary tests are cost-effective, simple and non-invasive. A correlation has been demonstrated between salivary and plasma cotinine levels.

Because of the high prevalence use of water pipe and passive smokers in Zahedan, Iran, and adverse effects of smoking and according to a new study of the issue and the lack of it in this area, the aim of the present study was to compare the salivary cotinine levels in hookah smokers, passive smokers and non-smokers.

A serum cotinine level of 10 ng/ml is considered a cut-off point between smokers and non-smokers. This cut-off point is 200 mg/ml for urine and 5 ng/ml for saliva.\(^10\)

Methods

The present comparative/cross-sectional study was carried out in Zahedan. After gaining approval of the University Ethics Committee, 150 subjects were included in the study in three groups: Hookah smokers, passive smoker, and
non-smokers. The inclusion criteria consisted of submitting an informed consent form, an age range of 15-65 years, body mass index (BMI) of 18.5-25, a history of smoking a hookah at least one session a day for 20-80 minutes for 3-5 years, use of tobacco same brand with a nicotine level of 0.5 mg/g with medium-sized hookah (50 subjects); individuals exposed to tobacco or cigarette smoke at home or workplace (50 subjects based on a score of > 2 on ETS questionnaire) and non-smokers who did not smoke cigarettes or hookah (50 subjects).

Participants smoked hookah 20 hours prior to salivary sampling. Participants were asked to refrain from smoking or exposure to smoke for the next 20 hours until the salivary sampling smokers smoked hookah at the same place under the same conditions. At the time of sampling, subjects were asked again if they had smoked or been exposed to smoke in the past 20 hours (half-life of salivary cotinine is 17 hours). This was done to exclude those who answered positively.

An unstimulated salivary sample was collected from each subject. Exclusion criteria consisted of patients with any oral lesion, systemic diseases or use of medications, individuals who smoked cigarettes and hookah or any other tobacco at the same time, and individuals with 18.5 > BMI > 20. The unexposed group subjects were matched with those in other groups in relation to age, gender and BMI. The spitting technique was used to collect unstimulated salivary samples from all the case and control subjects. After collecting the salivary samples in test tubes, the test tubes were sealed with Parafilm and coded. The samples were stored at -20 °C until used for the purpose of the study in the Laboratory of Zahedan University of Medical Sciences. The cotinine kit of Bioassay Technology Laboratory (2014, China) was used to determine salivary cotinine levels using ELISA. The sensitivity of the kit was 0.019 pg/ml, with a normal range of 0.5-80 pg/ml. The kit had been stored under a temperature of 2-8 °C. Data were analyzed with SPSS software (version 20, SPSS Inc., Chicago, IL, USA) t-test was used to reveal significant differences in frequencies, means a distribution parameters.

Results

The present study was carried out to compare salivary cotinine levels in individuals referring to the School of Dentistry, Zahedan University of Medical Sciences in three groups of hookah smokers (n = 50), passive smokers (n = 50), and non-smokers (n = 50).

Kolmogorov-Smirnov test showed normal distribution of age, weight, height and cotinine level data in all the three study groups (P > 0.050). The results showed that hookah smokers used hookah 5.3 ± 4.9 sessions weekly. The results of Pearson’s correlation test showed a strong and positive relationship between use of hookah (r = 0.93, P = 0.001) and salivary cotinine levels of the subjects. In this context, an increase in smoking hookah resulted in an increase is salivary cotinine levels (Figure 1).

![Figure 1. The linear graph depicting an increase in salivary cotinine levels with an increase in the number of hookah sessions daily](http://ahj.kmu.ac.ir)
Table 1. The means and standard deviation of salivary cotinine levels in the subjects separately in each group

| Group       | Mean ± SD  | 95% CI Lower | 95% CI Upper | Min   | Max   | One-way ANOVA |
|-------------|------------|--------------|--------------|-------|-------|---------------|
| Hookah      | 20.24 ± 5.26 | 17.75        | 22.73        | 10.70 | 38.30 | P = 0.001     |
| Passive smoker | 16.098 ± 3.51 | 14.45        | 17.73        | 11.00 | 25.30 |               |
| Non-smoker  | 0.66 ± 0.26  | 0.54         | 0.78         | 0.03  | 1.09  |               |
| Total       | 14.37 ± 8.80 | 12.46        | 16.28        | 0.03  | 38.30 |               |

SD: Standard deviation; CI: Confidence interval

Table 1 shows that the differences in the mean salivary cotinine levels of the subjects were statistically significant between different groups (P = 0.001). Two-by-two comparisons of the groups with post-hoc least significant difference tests showed statistically significant differences between hookah smokers on one hand and passive smokers (P = 0.001) and non-smokers (P = 0.001) on the other hand, and between non-smokers on one hand hookah smokers (P = 0.001) and passive smokers (P = 0.001).

Independent t-test did not reveal any significant differences in mean salivary cotinine levels between males and females in any of the groups (P > 0.050).

Pearson’s correlation coefficient showed no significant correlation between age and BMI and salivary cotinine levels in any of the groups (P > 0.050).

Discussion

Cotinine is an alkaloid in tobacco and is one of the metabolites of nicotine. The term “cotinine” is an anagram of the term “nicotine” and is used to evaluate exposure to tobacco smoke as a biologic marker. Since cotinine has longer durability (almost 20 hours) compared to nicotine (almost 2 hours) and remains in the body for longer hours, it can be used as the most sensitive and routine biomarker to identify cigarette smokers, hookah smokers, and passive smokers.¹ ⁵

The results of the present study showed the highest salivary cotinine levels in hookah smokers, followed passive smokers in descending order, with no salivary cotinine levels in non-smokers.

A higher level of salivary cotinine levels in hookah smokers in comparison to cigarette smokers has been confirmed in other studies. However, no study is available to compare the results of the present study in relation to salivary cotinine levels in passive smokers, hookah smokers, cigarette smokers, and non-smokers.

Hookah is a tool to smoke tobacco, which is very popular in the Middle East and Far East. However, a large number of studies have shown that the tobacco smoke produced by a hookah contains toxic materials, including carbon monoxide, heavy metals, nicotine, and carcinogens.²

A study by Martinasek et al.¹¹ showed higher urinary levels of cotinine in hookah smokers compared to cigarette smokers; however, the differences were not significant (5.980 µg/ml in cigarette smokers vs. 6.080 µg/ml in hookah smokers). A study by Pascale et al.¹² on salivary levels of cotinine and carbon monoxide in cigarette smokers, hookah smokers and non-smokers showed lower salivary cotinine levels in the hookah smokers compared to cigarette smokers, but they were significantly higher than those in non-smokers. They concluded that height and weight of hookah smokers affect the salivary cotinine levels; therefore, in the present study attempts were made to include subjects with normal BMI (18.5-25.0) in the study with similar conditions.

Bacha et al.¹³ reported very similar mean levels of salivary cotinine in cigarette smokers and hookah smokers. In addition, they concluded that the hookah size can affect salivary cotinine levels. Therefore, in the present study only subjects who used moderate-size hookah were included.

Jaakkola et al.¹⁴ determined the salivary cotinine levels in cigarette smokers and evaluated factors affecting these levels using a simple linear regression model. In individuals who smoked more than 20 cigarettes during a 24-hour period, the salivary cotinine levels increased almost 5.5 ng/ml when they smoked 1 extra cigarette; in individuals who smoked 20 cigarettes or less than that during a 24-hour period, the salivary cotinine levels increased almost 7.3 ng/ml when they smoked 1 extra cigarette. They conclude that it was possible that with the lower number of cigarettes smoked an increase in salivary cotinine levels occurs...
faster than that with more cigarettes smoked. Of course, other variables, including the cigarette filter, the cigarette brand, differences in the rate of cigarette paper burning, the cigarette design and the amount of nicotine levels might be effective.

In relation to hookah use, it should be pointed out that hookah tobacco smoke contains polycyclic aromatic hydrocarbons 20 times that of cigarette smoke and heavy polycyclic aromatic hydrocarbons 50 times that of cigarette smoke. A study by Martinasek et al.11 showed that 45 minutes of hookah smoking creates smoke 40 times that of cigarette and 50-200 puffs of hookah in a period of 20-80 minutes will result in inhalation of 0.15-1 l of smoke; however, 8-12 puffs of cigarette in a period of 5-7 minutes will result in inhalation of 50 ml of smoke. In the present study, the duration of smoking was the same and all the individuals who had a history of hookah use at least once a day for a session continuing 20-80 minutes for 3-5 years were included in the study. The charcoal used was the same by all the samples, and the hookah tobacco brand was a specific brand with 0.5% of nicotine per each gram of tobacco. Hookah was smoked in an open environment so that the subjects were not exposed to passive smoke.

The amount of smoke produced by a hookah is much more than that produced by a cigarette. The smoke produced by a hookah is 500000 ml but a cigarette produces 500-600 ml of smoke. The blood levels of nicotine in daily hookah smokers are similar to those in individuals who smoke 10 cigarettes a day. The detrimental effects of hookah smoking on cancer, CVD, and addiction to nicotine are obvious; however, further epidemiological studies are required.15 The term “passive smoker” refers to those who are exposed to cigarette smoke of others. A study by Nuca et al.5 on 286 subjects showed that 113 (39.5%) of the subjects were passive smokers.

Lack of accurate estimates about the exposure of individuals to tobacco smoke is a major concern in epidemiological studies. Evaluation of passive exposure to tobacco or cigarette smoke is difficult than reporting hookah or cigarette smoking. In this context, a proper evaluation for the extent of recent exposure to tobacco smoke is to analyze cotinine levels in human body fluids such as blood, urine, and saliva. Even if individual differences in relation to the cotinine levels in body fluids and cotinine use affect conversion of nicotine to cotinine (routine levels of 55-95%) and the cotinine metabolism, the presence of cotinine in a physiologic body fluid is a definite sign of exposure to nicotine. In the present study, ETS index was used, which is an indicator to evaluate individuals exposed to tobacco smoke and all the individuals with ETS > 2 were included in the study.8

The results of the present study did not show a relationship between salivary cotinine levels in subjects smoking hookah and exposed to tobacco smoke and age. Other studies, too, have shown that cotinine metabolism is not affected by age.

The mean salivary cotinine levels were a higher in males; however, the differences were not significant (P = 0.050). Previous studies on the relationship between cotinine and gender are contradictory. A study by Etter et al.16 showed higher mean salivary cotinine levels in males compared to females. Benowitz et al.17 reported a higher metabolism of cotinine to nicotine in females compared to males, which might explain lower cotinine and nicotine levels in females. However, some other studies have reported similar nicotine and cotinine levels in males and females.18

Cotinine levels were higher in subjects with lower BMI, but the differences were not significant (P = 0.050). The cotinine levels in the body are different in terms of the daily intake of nicotine, the conversion rate of cotinine to nicotine and the metabolic rate of cotinine. The metabolic rate of cotinine is slower in individuals with lower BMI, which might explain the higher cotinine levels in subjects with lower BMI.19

The results of the present study, consistent with those of Rabiei et al.4 and Primack et al.15 showed a positive and strong correlation between hookah smoking and salivary cotinine levels. In this context, an increase in the use of tobacco resulted in an increase in salivary cotinine levels. In addition, a significant difference was found between non-smokers and passive smokers, indicating the importance of this problem and avoiding environments that might expose individuals to tobacco smoke, which might increase cotinine and nicotine levels in the body, resulting in dangerous complications.

Therefore, factors that endanger the health of the community should be further and accurately
evaluated. Tobacco and cigarettes, and hookah in more recent research have been shown to be the most important etiologic factors for oral squamous cell carcinoma and dysplastic lesions. Some believe that filtration with water decreases nicotine levels. However, this idea is true in relation to the solubility of nicotine in water: Only 5% of nicotine is dissolved in water. A misunderstanding in this respect becomes evident by long smoking duration, higher volumes of puffs, and an increase in salivary nicotine levels required to reach an enjoyable level of nicotine, compared to smoking cigarettes.4 In addition, smoking is a harmful habit that has a detrimental effect on the oral health and has the most important role in inducing cancerous and precancerous lesion.7

One of the limitations of the present study was relying on the reports of subjects in relation to smoking during the week or the day. In addition term “passive smoking” indicates involuntary inhalation of tobacco smoke by non-smokers and EST indicates evaluation of individuals exposed to cigarette smoke and is scored based on a questionnaire filled out by the individual himself/herself, and smoke and tobacco smoke are not isolated.

Further paucity of studies on passive smokers prevented us from making comparisons with individuals smoking hookahs. There is misunderstanding in modern societies, especially among young adults, in relation to tobacco use because its use is rooted in the tradition of Asian communities, including that of India and the Middle East.2,14

### Conclusion
Based on the results of the present study, salivary cotinine levels were higher in hookah smokers compared to passive smokers and non-smokers, in descending order. It is necessary to increase the awareness of the family and the youth and combat the wrong opinion that hookah smoking is a recreation and also combat its trading and its tobacco with different flavors.

### Conflict of Interests
The Authors have no conflict of interest.

### Acknowledgements
The authors thank the Zahedan University of Medical Sciences for financial support of this research project.

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مقایسه سطح کوتینین پزاقی در افراد استعمال کننده قیان و در معرض دود با
افراد غیر سیگاری

دکتر طاهره نصرت‌نژاد، دکتر فاطمه اردبیلی کلانی، دکتر ابراهیم علی‌نژاد، دکتر حسن تاج‌نژاد، دکتر بهروشی

چکیده
مقدمه: ارزش استعمال دخانیات به عنوان یک معضل بهداشتی فراگیر مطرح شده است و کشورهای خاورمیانه در حال گسترش می‌باشد. به طور طبیعی، تعادل افرادی که مصرف دود سیگاری می‌کنند، نتایج حاصل از قیان و فلزات افزوده‌شده به آن می‌تواند نتایج منتشر شده باشد. در این مطالعه، نتایج حاصل از مصرف قیان و فلزات افزوده‌شده به آن می‌تواند نتایج منتشر شده باشد.

روش‌ها: در این مطالعه مقطعی (Cross-sectional) از ۱۵۰ نمونه شامل ۵۰ نفر قیانی، ۵۰ نفر سیگاری، ۵۰ نفر دود حاصل از مصرف قیان و فلزات افزوده به آن می‌تواند نتایج منتشر شده باشد. در این مطالعه، نتایج حاصل از مصرف قیان و فلزات افزوده به آن می‌تواند نتایج منتشر شده باشد.

نتایج: نتایج نشان داد که قیان و فلزات افزوده به آن می‌تواند نتایج منتشر شده باشد. در این مطالعه، نتایج حاصل از مصرف قیان و فلزات افزوده به آن می‌تواند نتایج منتشر شده باشد.

واژگان کلیدی: تنبækو، استعمال دخانیات، قیان، کوتینین

ارجاع: نصیری‌نژاد، دکتر ابراهیم کلانی، دکتر علی‌نژاد، دکتر حسن تاج‌نژاد. مقایسه سطح کوتینین پزاقی در افراد استعمال کننده قیان و در معرض دود با افراد غیر سیگاری. مجله اعتیاد و سلامت ۷(۲)، ۸۵-۹۱، ۱۳۸۴.

تاریخ پذیرش: ۹۹/۹/۱۱

Email: arbab@zaums.ac.ir

Addict Health, Summer & Autumn 2015; Vol 7, No 3-4
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http://ahj.kmu.ac.ir, 7 October