

# Comparison of Salivary Cotinine Concentration in Cigarette Smokers, Water Pipe Smokers and Non-Smokers

M. Rabiei <sup>1</sup>, M. Rahbar Taramsar <sup>2</sup>, M. Sadegh Kanjani <sup>3</sup>, E. Kazemnezhad Leyli <sup>4</sup>, H. Masoudi Rad <sup>5</sup>, M. Jafari Farshami <sup>6</sup>.

<sup>1</sup>Associate Professor, Department of Oral and Maxillofacial Medicine, School of Dentistry, Guilan University of Medical Sciences, Guilan, Iran

<sup>2</sup>Assistant Professor, Department of Legal Medicine and Toxicology, School of Medicine, Guilan University of Medical Sciences, Guilan, Iran

<sup>3</sup>PHD Reference laboratory, Guilan University of Medical Sciences, Guilan, Iran

<sup>4</sup>Assistant Professor, Department of Biostatistics, School of Medicine, Guilan University of Medical Sciences, Guilan, Iran

<sup>5</sup>Assistant Professor, Department of Endodontics, School of Dentistry, Guilan University of Medical Sciences, Guilan, Iran

<sup>6</sup>Dentist

## Abstract

**Background and Aim:** Water pipe smoking has become a public health hazard. The aim of this study was to measure and compare the concentration of salivary cotinine in cigarette smokers, water pipe smokers and nonsmokers.

**Materials and Methods:** Forty-eight volunteers (16 cigarette smokers, 16 water pipe smokers and 16 nonsmokers) participated in this cross-sectional study. High sensitivity Salivary Cotinine Quantitative enzyme immunoassay kit was used to measure the salivary cotinine concentration. Non-parametric Kruskal Wallis test, Pearson's correlation coefficient and linear regression were used to determine the correlation between number of cigarettes smoked and concentration of cotinine.

**Results:** The mean concentration of salivary cotinine was 223.74(±181.56) ng/ml (the highest) in cigarette smokers, 106.24 (±135.23) ng/ml in water pipe smokers and 0.73(±1.24) ng/ml in nonsmokers. The difference in this respect among the 3 groups was statistically significant ( $p<0.0001$ ). In smokers, the level of salivary cotinine increased by 1.84 ng/ml per each time of cigarette smoking per week. This increase was 14.57 ng/ml per each time of water pipe consumption per week.

**Conclusion:** The mean concentration of salivary cotinine was significantly higher among cigarette smokers compared to water pipe smokers and nonsmokers. However, one time consumption of water pipe caused a greater rise in salivary cotinine level compared to cigarette smoking.

**Key Words:** Cotinine, Saliva, Smoking, Tobacco

✉ Corresponding author:  
M. Rabiei, Associate Professor,  
Department of Oral and  
Maxillofacial Medicine, School  
of Dentistry, Guilan University  
of Medical Sciences. Guilan,  
Iran  
rabiei@gums.ac.ir

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## Introduction

Hookah was invented in the 15<sup>th</sup> century in India and quickly became popular in the Middle Eastern countries like Iran, Syria, Yemen, and Egypt [1]. It underwent a transformation from its rudimentary coconut-and-straw look to its current form, a glass vessel with hoses attached to the body [2,3]. In 1990, Arabic countries added floral and fruity flavors to tobacco and made it milder called "Maasel"

derived from the word "Mua' sel" meaning honey [2-3]. At present, hookah smoking is popular worldwide mostly attributed to misconceptions about its safety. Its popularity among the youth is due to its social acceptance, novel design, availability in numerous appealing flavors, and low cost [4]. In a study conducted in Bandar Abbas, Iran, the prevalence of smoking was 11.7% and mainly in males; whereas 9.1% mentioned hookah smoking

including 6.8% of females and 2.3% of males [5]. Hookah smoking is not considered as bad as cigarette smoking in many countries and young adults in the age of 5-18 yrs. are even invited to smoke hookah by their family members [4].

Hookah smoking is becoming popular in developed countries as well. Unlike cigarette smoking, the American Food and Drug Administration (FDA) has no supervision over hookah tobacco packaging and only a "no tar" label is displayed on hookah tobacco packs [4].

It is estimated that smoking will cause 10 million deaths annually by the year 2020. This rate will be much higher in developing countries compared to developed ones. Moreover, it is predicted that by the year 2030, 70% of annual deaths from smoking worldwide will occur in developing countries [5].

Tobacco leaf combustion is an incomplete process producing a gaseous phase consisting of carbon monoxide, nitrosamine, acetaldehyde, formaldehyde, volatile hydrocarbons, and hydrogen cyanide and particulate matter phase that is essentially unfiltered consisting of tar and nicotine. The gaseous phase contains more carcinogens [6,7]. Cigarette smoke contains free radicals that can cause tissue damage by reacting with unsaturated fatty acids in cell membranes and DNA nucleotides [8].

Studies have shown that smoking hookah significantly increases the prevalence of many diseases, such as lung cancer, lung disease, weight loss and periodontal disease. In some cases this increase has not been significant, but an increased risk of bladder cancer, esophageal cancer, oral dysplasia and infertility as the result of hookah smoking has been noticed [9].

Cotinine is an alkaloid found in tobacco and also a nicotine metabolite. Cotinine is also an anagram of "nicotine" and is used as a biomarker for measurement of exposure to tobacco smoke [10]. Also, due to its relatively long half-life of approximately 20 hours (compared to 2 hours for nicotine) in body fluids, it has optimal sensitivity and specificity for measuring tobacco exposure instead of nicotine [11]. Cotinine serum level of 10 ng/ml is considered as the breakpoint between smokers and non-smokers. This rate is 200 ng/ml and 5 ng/ml in urine and saliva, respectively [12,13].

At present, saliva is considered a reliable alternative to other body fluids for measurement of coti-

nine concentration. Measurement of salivary cotinine is affordable, convenient and non-invasive requiring no expertise in sample collection. The correlation between salivary and serum cotinine levels has been confirmed. Thus, saliva seems to be an ideal alternative to serum for this purpose [14,15].

This study sought to compare the mean level of salivary cotinine in hookah smokers, cigarette smokers and nonsmokers.

## Materials and Methods

This comparative cross-sectional study was conducted in Rasht, Iran in 2012. The study design was approved in the Ethics Committee of Gilan University. After obtaining written informed consent, 48 volunteers, divided into 3 groups of cigarette smokers, hookah smokers and nonsmokers were evaluated. The inclusion criteria were age between 18 to 25 yrs. and Body Mass Index (BMI) of 18.5 to 25 (normal range). All participants were males and matched in terms of age ( $3 \pm$  yrs). The cigarette used by cigarette smokers was Winston Light with a nicotine level of 0.6 mg per cigarette. The tobacco used by hookah smokers was Nakhla Tobacco with 0.5%/g nicotine level. Medium size waterpipes were used in this study. The type and amount of charcoals were also the same. The experiment was done in an open-air environment in order to eliminate the effect of secondhand smoke on participants. The smoker groups were requested not to use any other tobacco products during the course of study or they will be excluded from the study.

Before the experiment, participants were thoroughly informed about the process of study and matching the conditions among hookah smokers. The experiment was carried out in an outdoor cafe in the city of Rasht. The number of tobacco consumptions per week was also recorded.

Participants smoked hookah 20 hours prior to salivary sampling. For each hookah smoking, 20 grams of tobacco was used containing 100 mg of nicotine. The mean time of smoking was approximately 45 minutes and during this time period smokers had different numbers of puffs. Participants were asked to refrain from smoking or exposure to smoke for the next 20h until the salivary sampling.

Cigarette smokers smoked a cigarette at the same place under the same conditions. Smokers were asked to refrain from smoking or exposure to smoke for the next 20h until the salivary sampling. 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 based on the Salimetrics™ cotinine kit manual is 17 hours). This was done to exclude those who answered positively. Before sampling, hookah and cigarette smokers were asked to write down the average number of Hookah smoking sessions and cigarettes smoked per week, respectively. Salivary samples were then obtained from the two smoker groups and the nonsmokers.

Salivary samples were collected by spitting. Volunteers were first asked to wash their mouths, hold their saliva for at least 5 minutes and then spit into a test tube and funnel glass. All subjects had a minimum of 5 ml of salivary samples taken. All salivary samples were collected between 11 am to 1 pm, and sent to the Reference Laboratory of Rasht by cold chain.

In the laboratory, using a 1000µL pipette, each salivary sample was divided into at least 2 Eppendorf tubes of 1 ml volume. The tubes containing salivary samples were then frozen in an ultra-low temperature freezer at -75°C. Salivary cotinine analysis was conducted by using a Sail metrics®(USA, PA) high sensitivity salivary cotinine quantitative enzyme immunoassay kit and enzyme-linked immunosorbent assay (ELISA). First, all salivary samples were removed from the freezer and kept at room temperature for 30 minutes (for liquefaction). Samples were centrifuged at 3000 rpm for 15 minutes using a Tadghiz Gostar®microcentrifuger. Samples were the prepared following the kit instructions.

The normality of cotinine distribution was studied using One Sample Kolmogorov-Smirnov test. Considering the normality of cotinine changes in smoker groups, the independent t-test was applied to compare the mean cotinine changes. Due to the lack of normality of cotinine distribution, the mean changes of cotinine between cigarette smokers and nonsmokers and also hookah smokers and nonsmokers were compared using the Mann-Whitney test. Due to the lack of normality, nonparametric

Kruskal-Wallis test was applied to compare salivary cotinine levels among the 3 groups. Pearson's correlation coefficient was used to assess the relationship between salivary cotinine level, duration and frequency of cigarette smoking and hookah consumption. IBM SPSS version16 software was used for statistical analysis with a significance level of  $p < 0.05$ .

## Results

In this study, 48 subjects were divided into three groups of 16 hookah smokers, 16 cigarette smokers and 16 nonsmokers. Each group was evaluated in terms of the salivary cotinine level. The mean age was  $23.50 \pm 1.26$  years,  $24.18 \pm 0.91$  years and  $21.68 \pm 1.2$  years in hookah smokers, cigarette smokers and nonsmokers, respectively. Results showed that the mean and standard deviation of cotinine was  $106.24 \pm 135.23$  ng/ml in hookah smokers,  $223.74 \pm 181.56$  ng/ml in cigarette smokers and  $0.73 \pm 1.24$  in the control group with 95% CI.

Comparison of cotinine levels among the 3 groups using the nonparametric Kruskal-Wallis test revealed significant differences among the three groups ( $p < 0.0001$ ). The mean cotinine level was significantly different between the cigarette and hookah smokers and the nonsmoker group ( $p < 0.0001$ ).

Independent t-test demonstrated a significantly higher cotinine level in cigarette smokers compared to hookah smokers ( $p < 0.04$ ) (Table 1).

Cigarette smokers averagely smoked  $101.12 (\pm 92.93)$  cigarettes per week while hookah smokers smoked hookah averagely  $10.87 (\pm 15.79)$  times a week. A correlation existed between the salivary cotinine level and frequency of smoking in both smoking groups ( $p < 0.0001$ ,  $r = 0.943$  for cigarette smokers and  $p < 0.033$ ,  $r = 0.535$  for hookah smokers – both statistically significant).

Table 2 shows the regression coefficients of the effect of tobacco consumption on level of salivary cotinine in hookah smokers and cigarette smokers.

Linear regression model showed the significant effect of the frequency of tobacco consumption on salivary cotinine level in hookah smokers and cigarette smokers ( $p < 0.033$  for hookah and  $p < 0.0001$  for cigarette smokers). In cigarette smokers, smoking one cigarette per week increased the cotinine level by averagely 1.84 ng/ml (95%

**Table 1.** Level of salivary cotinine in the 3 groups with 95% CI

| Variables         | Number | Mean  | Standard deviation | Minimum | Maximum |
|-------------------|--------|-------|--------------------|---------|---------|
| Hookah smokers    | 16     | 1/062 | 135/23             | 7.00    | 480.00  |
| Cigarette smokers | 16     | 2/237 | 181/56             | 36.40   | 657.00  |
| Nonsmokers        | 16     | 0/73  | 1/24               | 00      | 36.60   |
| Total             | 48     | 1/102 | 157/58             | 00      | 657.00  |

**Table 2.** Regression Coefficients of the effect of smoking frequency on salivary cotinine levels in cigarette and hookah smokers

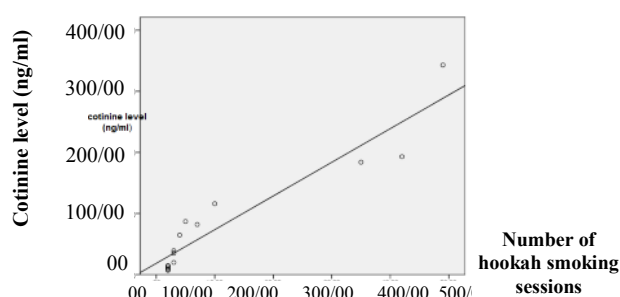
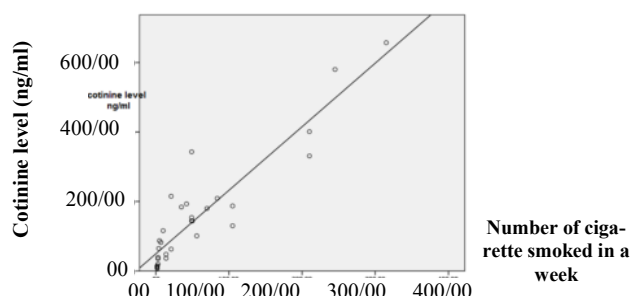
| Variable                    | $\beta$ regression coefficient | Standard error | P value | 95% confidence interval for $\beta$ regression coefficient |         |
|-----------------------------|--------------------------------|----------------|---------|------------------------------------------------------------|---------|
|                             |                                |                |         | Minimum                                                    | Maximum |
| Hookah smokers              | 91.123                         | 39.524         | 0.029   | 10.161                                                     | 172.085 |
| Cigarette smokers           | -31.959                        | 28.096         | 0.265   | -89.510                                                    | 25.592  |
| Number of cigarettes smoked | 1.944                          | 0.177          | 0.00    | 1.581                                                      | 2.306   |

\*Effect of frequency of tobacco use on the salivary cotinine level

\*\*Effect of confounding factors (fixed amount)

CI; 1.46, 2.22) while one time hookah smoking per week increased salivary cotinine by averagely 4.57 ng/ml (95% CI; 0.428, 8.72).

Figures 1 and 2 show the linear regression model of correlation between salivary cotinine level and frequency of smoking in hookah and cigarette smokers.

**Diagram 1.** Linear regression model of the correlation of cotinine with frequency of hookah smoking**Diagram 2.** Linear regression model of the correlation of cotinine with frequency of cigarette smoking

## Discussion

The highest level of salivary cotinine was observed in cigarette smokers followed by hookah smokers. Salivary cotinine was negative in nonsmokers. The higher mean cotinine level in cigarette smokers compared to hookah smokers has been reported in several studies. However, due to global concerns regarding the use of hookah, level of cotinine in urine and other body fluids such as plasma, saliva and nasal secretions has been assessed with laboratory methods namely liquid chromatography GM, HPLC and ELISA (Table 3).

Previous relevant studies have been mostly conducted in countries with a high prevalence of hookah smoking. High rate of hookah smoking among families has raised some concerns. In most cases cotinine levels were higher in cigarette smokers than hookah smokers. Macaron et al. found urinary cotinine levels to be higher in hookah smokers. However, this difference was not statistically significant (5.980  $\mu\text{g/ml}$  in cigarette smokers versus 6.080  $\mu\text{g/ml}$  in hookah smokers) [4]. Bacha et al. reported salivary cotinine to be 87 ng/ml in cigarette smokers and 78 ng/ml in hookah smokers. They also found that the height and weight of smokers and size of waterpipe affected the level of cotinine [16]. Behera et al. found higher cotinine levels among cigarette smokers, although this difference was not significant [17].

**Table 3.** Salivary cotinine concentration in cigarette smokers versus waterpipe smokers

| Authors and Country                            | Method                           | Cotinine in water pipe smokers | Cotinine in cigarette smokers |
|------------------------------------------------|----------------------------------|--------------------------------|-------------------------------|
| <b>Bacha et al<sup>16</sup></b><br>(Lebanon)   | Salivary cotinine                | 78 ng/mL                       | 87 ng/mL                      |
| <b>Shafagoj et al<sup>2</sup></b><br>(Lebanon) | Plasma cotinine-3 h post smoking | 51.95 13.58ng/mL               | Not measured                  |
| <b>Shafagoj et al<sup>4</sup></b><br>(Lebanon) | Saliva-45 min post-smoking       | 283.49 75.04ng/mL              | Not measured                  |
| <b>Macaron et al<sup>4</sup></b><br>(Lebanon)  | Urinary cotinine                 | 6.080 g/mL (6080 ng/mL)        | 5.980 g/mL (5980 ng/mL)       |
| <b>Behera et al<sup>17</sup></b><br>(India)    | Urinary cotinine                 | 2.379 g/mL (2379 ng/mL)        | 2.739 g/mL (2739 ng/mL)       |
| <b>Al Muntari et al<sup>6</sup></b> (Kuwait)   | Urinary cotinine                 | 0.678 g/mL (678 ng/mL)         | 1.321 g/mL (1321 ng/mL)       |
| <b>Rabiei et al</b><br>(Iran)                  | Salivary cotinine                | 106.24 ng/mL                   | 223.74 ng/mL                  |

However, in our study salivary cotinine level was significantly higher among cigarette smokers compared to hookah smokers.

On the other hand, results showed that one time hookah smoking per week increased cotinine levels more than twice the rate by smoking one cigarette (4.57 ng/ml increase by hookah compared to 1.84 ng/ml increase by cigarette smoking). It should be noted that we tried our best to match the conditions in order to eliminate the effect of confounding factors such as BMI, age, gender and size of water pipe. Shafagoj et al. reported that 3 hours after smoking serum cotinine level increased by 0.79-51.95 ng/ml compared to the baseline level before smoking. This rate was 0.79-283.49 for salivary cotinine level [4]. Al-Muntari et al. stated that smoking 30 cigarettes is equivalent to 168 mg of nicotine while one time smoking of maassal is equivalent to 25 mg of nicotine [6]. In the study by Maritta S. Jaakkola et al, using a simple linear regression model, cotinine level increased by 5.5 ng/ml for each additional cigarette smoked by subjects smoking more than 20 cigarettes per 24 hours [18]. This value was 7.3 ng/ml in those smoking less than 20 cigarettes during 24 hours. This finding indicated that the rise in nicotine level is greater in those with less frequency of smoking. The

habit of smoking, cigarette filter and more importantly cigarette brand may also affect the results. In our study, we used Winston Light with 0.6 mg nicotine per cigarette while in Shafagoj's study participants smoked regular cigarettes with twice the nicotine amount.

Hookah smoking produces 20 times more polycyclic aromatic hydrocarbons and 50 times more heavy polycyclic aromatic hydrocarbons compared to a cigarette. Level of produced carbon monoxide by water pipe is also 5 times higher. One study showed that 45 minutes of waterpipe smoking produced 40 times the smoke volume produced by cigarette smoking. Cigarette smoking averages 8-12 puffs yielding a volume of around 50 mL over a 5- to 7-minute period; whereas, waterpipe gives 50-200 puffs over 20-80 minutes resulting in 0.15-1 L of smoke inhalation [4].

Monn and colleagues reported that particles produced by hookah smoke are ultrafine measuring 0.02-1 micron attributed to the combustion of wood charcoal. Particles ranging in size from 5 to 10 µm deposit primarily in the large airways. Particles that are 2-5 µm deposit in the lower respiratory tract and 0.8-3 µm particles deposit in the terminal airways and alveoli [19]. Lung cancer, respiratory diseases and periodontal disease occur

significantly more in relation to hookah smoking. The correlation of cigarette smoking and oral cancer has been confirmed in many studies. Systematic reviews have reported increased prevalence of oral cancer as the result of hookah smoking but have not mentioned a definite correlation in this respect. Increasing the number of cigarettes smoked results in an increased risk of oral cancer while the number of hookah smoking sessions is usually low about 2-3 times per week [5].

Many people believe that filtration of smoke through water decreases the amount of nicotine. In contrast to this view, research demonstrates that only about 5% of the nicotine is dissolved in water. Moreover, waterpipe smokers may increase the duration of smoking and the volume of puffs to obtain the nicotine level necessary to reach the pleasurable level of nicotine [4].

This study had some limitations. Participants had to quit smoking for 3 to 4 days prior to sampling to better find the difference in level of salivary cotinine following smoking one cigarette or one time hookah consumption. However, this was very difficult for participants.

This study showed an increase in cotinine level as the result of hookah smoking; which totally contradicts the public misconception about hookah smoking even among families denouncing cigarette smoking. Reports show that hookah, traditionally popular in the Middle East, is making its way to developed countries where smoking cigarettes and drinking alcohol are banned for people under 18-21 years of age; however, smoking hookah is allowed in specific places. Based on these facts a strong worldwide reaction by World Health Organization (WHO) is required. It should be taken into account that the ability to smoke a high number of cigarettes in a short time and in almost everywhere are the reasons behind the higher salivary cotinine level in cigarette smokers compared to hookah smokers. Although smoking hookah is rooted in the traditions of Asian countries, India and the Middle East and considering that making hookah for decoration and business purposes is quite popular nowadays, awareness in this respect and fighting this false tradition seem to be essential.

This study compared salivary cotinine levels of cigarette and hookah smokers. Both cigarette and

hookah smoke contain toxic materials such as methanol, carbon monoxide, arsenic, tar and etc. and should be evaluated in further studies.

Future studies are required to evaluate the incidence of oral lesions, nasopharynx cancer and oral cancer due to hookah smoking.

## Conclusion

The highest level of salivary cotinine in this study was observed in cigarette smokers; but one time hookah smoking caused greater increase in cotinine level compared to smoking one cigarette.

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