

RESEARCH ARTICLE

Editorial Process: Submission:01/06/2018 Acceptance:05/03/2018

Comparison of Salivary Cotinine Concentrations in Male Smokers and Smokeless Tobacco Users

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Abstract

Objective: Smoking cigarettes and smokeless tobacco are one of the causes of oral cancer. This study compared the salivary level of cotinine in male smokeless tobacco users and smokers. **Methods:** In this cross-sectional (descriptive-analytical) study, stimulated saliva samples from 30 male smokers and 30 male smokeless tobacco consumers were collected and their cotinine contents were measured using the competitive ELISA method according the standard curve. The data was analyzed with independent t-test and linear regression using SPSS-19, and $P < 0.05$ was considered significant. **Result:** Among the 60 subjects with the mean age of 21.27 ± 2.6 years, the average level of cotinine in smokers (12.32 ± 7.5 ng/ml) had no significant difference with that of smokeless tobacco consumers (11.23 ± 4.4 ng/ml) ($p = 0.49$). **Conclusion:** Salivary levels of cotinine were not significantly different in smokeless tobacco users and cigarette smokers. In addition, increases in the number of cigarettes smoked and in pack of smokeless tobacco used, were associated with increased salivary levels of cotinine. The increase was higher in smokeless tobacco consumers.

Keywords: Saliva- cotinine- smokeless tobacco

Asian Pac J Cancer Prev, **19** (5), 1363-1366

Introduction

Tobacco use in any form, smoked or smokeless products, is one of the main causes of mortality (Vendhan et al., 2015), and it is estimated that by 2030, it will lead to 10 million deaths, 70% of which will be in the developing world. This figure is higher compared to those for AIDS, drug abuse, road accidents, murder and suicide (sardar et al., 2007).

Oral tobacco products also contain carcinogenic substances like tobacco-specific N-nitrosamines (TSNAs) leading to an increased risk of cancers of oral cavity, pharynx, and oesophagus (Stanfill et al., 2011; Boffetta et al., 2008).

Tobacco can be consumed in different forms including smoking cigarettes, with a hookan, and as smokeless tobacco, and its composition varies according to the region in which it is grown (sardar et al., 2007). Approximately 300 million people consume smokeless tobacco worldwide, of which about 90% are in Southeast Asia (NCI&CDC., 2014). No tangible plan has been carried out to control the use of smokeless tobacco (SLT) despite its high consumption in Southeast Asia region (Bhawna et al., 2013).

The vicinity Sistan and Baluchestan province with Afghanistan, Pakistan, and India, illegal import of smokeless products in deluxe packages and their distribution on the market and grocery stores, and the unawareness of many students about the nature

and complications of these materials could lead to the increase in the consumption of these materials (Honarmand et al., 2013).

Nicotine is the substance that causes addiction to cigarettes and smokeless tobacco. It has a half-life of approximately 2–3 hours in the blood. Nicotine is metabolized in the liver. The major pathway of nicotine metabolism is C-oxidation to cotinine. About 80% of nicotine is changed to cotinine. Cotinine is a metabolite of nicotine, and can be used as a marker of exposure to tobacco. Cotinine in vivo has a half-life of about 20 hours, Due to its longer half-life, cotinine levels accumulate throughout the day. In addition, cotinine is eliminated over a longer period of time than nicotine. (Florescu et al., 2009).

Cotinine can be measured in body fluids such as serum, saliva, and urine (Binnie et al., 2004; Hyun-Suk et al., 2012). Measuring cotinine in saliva is a safe and convenient method for assessment of exposure to nicotine (Aliva-tang et al., 2013). Robson et al. studied the concentrations of nicotine and cotinine in stimulated and unstimulated saliva. The level of nicotine and cotinine in stimulated saliva was considerably higher compared to that of the unstimulated saliva (Robson et al., 2010).

Considering the various oral complications of smoking and smokeless tobacco consumption, and since nicotine contained in these materials is one of the causes of these complications, the present study aimed at evaluating

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salivary cotinine levels in smokers and in smokeless tobacco users.

Materials and Methods

The study population

In this descriptive-analytical study, 30 male smokers and 30 male smokeless tobacco consumers referred to the Zahedan Dental School in 2015 were enrolled after obtaining informed consent from them. This project was approved in the Ethics Committee of Zahedan University of Medical Sciences, code 6859. Inclusion criteria were smokers with 5-20 cigarettes per day (the Bahman brand) for at least past one year (in smoking group), consumers of smokeless tobacco (the BT brand) with consumption of at least 3 packs per day for at least past one year (in smokeless tobacco group), age of 18-25 years, and BMI of 18.5-25. Exclusion criteria were systemic diseases, consumption of drugs affecting the amount of saliva, and use of other nicotine products or exposure to tobacco smoke (passive smoker). The two groups were matched in terms of age and BMI.

Measurement of salivary cotinine

Prior to any dentistry intervention, stimulated saliva samples were collected from all subjects in the morning (9-11 AM). First, the subjects' mouths were rinsed with water, and they were asked to chew flavor-free gum for one minute and collect their saliva in disposable cups for 5 minutes. The collected saliva samples were then transferred to test tubes and they were then sent to the biochemistry lab of the Medical School where they were kept at -70 °C until tested. Cotinine contents of all samples were measured by the Human Cotinine ELISA Kit and using the competitive ELISA method according to the protocol of the manufacturer (the Hangzhou Eastbiopharm Co.CK-E90354).

Data analysis

Data analysis was performed using SPSS-19. Kolmogorov-Smirnov test was used to evaluate normal distribution of the variables. The independent t-test was used to compare salivary cotinine levels between the two groups. The linear regression was employed to determine the relationship between salivary cotinine levels and

tobacco consumption. P<0.05 was considered significant.

Results

Salivary samples of 60 male including 30 smokers and 30 smokeless tobacco consumers were tested in this study. The mean age of the smokers was 21.83±2.5 years and that of the smokeless tobacco consumers was 21.6±2.37 years. The Kolmogorov-Smirnov test was used to evaluate normal distribution of the variables.

The cotinine variable had a normal distribution in both groups. Therefore, the independent samples t-test was used to compare salivary cotinine levels between the two groups. Results of the comparison are shown in Table 1.

In terms of consumption frequency, the smokers smoked 10.43 cigarettes per day and the smokeless tobacco consumers used 3.17 packs per day on average. There was a correlation between the frequency of smoking and smokeless tobacco consumption and the levels of salivary cotinine. This relationship was significant for both the smokers (p=0.001, r=0.951) and the smokeless tobacco consumers (p<0.001, r=0.929).

Table 2 shows the regression coefficients of the effect the consumption frequency on salivary cotinine levels in both groups. In smokers, an increase of one cigarette per day increased the cotinine level by 1.59 ng/ml, while an increase of one pack per day in smokeless tobacco consumption raised the cotinine content by 4.005 ng/ml.

Discussion

In this study, 60 men referred to the Zahedan Dental School clinic in 2015 (with an average age of 21.27±2.6 years) were evaluated in terms of salivary cotinine in smokers and smokeless tobacco users. The mean levels of cotinine in smokers and smokeless tobacco consumers were 12.32±7.5 ng/ml and 11.23±4.4 ng/ml, respectively. Increases in the number of cigarettes smoked and in pack of smokeless tobacco used, were associated with increased salivary levels of cotinine. The increase was higher in smokeless tobacco consumers.

In study performed by Singh et al., (2013) cotinine levels for daily betel quid use among women were similar to cotinine levels for daily cigarette smoking in men. They reported that the typical betel quid habit among women supports the same level of nicotine addiction as the typical

Table 1. Saliva Cotinine Concentrations According to Type of Tobacco Use

Variable	Cigarette smoker Mean±SD	Smokeless tobacco user Mean±SD	P-value
Cotinine Concentration (ng/mL)	12.32±7.5	11.23±4.4	0.49

Table 2. Regression Coefficients According to Tobacco Consumption

Group	Variable	Unstandardized coefficients (Std. Error)	Standardized coefficient (Beta)	P-value	R
Cigarette smoker	Constant	1.11	-4.334	0.000	0.951
	Cigarette*	0.098	1.597	0.001	
Smokeless tobacco user	Constant	0.005	-1.446	0.161	0.929
	Smokeless* tobacco	0.303	4.005	0.000	

* The effect of tobacco use on the salivary cotinine

cigarette habit in men.

In a study conducted by Patel et al., (2017) has shown that participants who consume smokeless tobacco show high level of salivary cotinine as compared to participants who consume tobacco in smoking form and this difference is statistically significant.

The difference in cotinine level between this study and the present study may be due to age differences in the study populations, differences in the nicotine content of cigarettes tobacco and smokeless tobacco products, sampling methods, and different methods of cotinine measurement.

In this study, the level of cotinine increased more than twice for an increase of a pack per day in smokeless tobacco consumers compared to an increase of one cigarette per day in smokers (1.59 and 4.005 ng/ml, respectively).

Jaakkola et al., (2003) measured the salivary levels of cotinine in smokers and found that in people who smoked more than 20 cigarettes in 24 hours, per each more cigarette, the concentration of cotinine increased about 5.5ng/ml.

In a study by Rabiei et al., (2013) the level of cotinine increased 1.84ng/mL for each extra cigarette, which is almost similar to the finding in the present study. Huque et al., (2016) suggested that salivary cotinine concentration increased with each additional dip of ST per day while holding the effect of other factors constant. They reported that high cotinine concentration among ST users and increased prevalence of ST use in Bangladesh indicates that ST products are highly addictive and ST users need adequate support to leaving addiction.

Factors such as the way of cigarette smoking, the type of cigarette, its nicotine content and type of measurement method can affect the level of salivary cotinine.

One limitation of this study was the potential lack of generality to other populations. This study was conducted in one city. In addition, we trusted to reporting people about the amount and type of tobacco use.

In conclusion, in this study was observed that increased smokeless tobacco consumption resulted in greater increase in the level of salivary cotinine than smoking. Since the use of this compound is increasing among youth, especially among educated people (Honarmand et al., 2013); and, furthermore, as many studies have shown a relationship between smokeless tobacco consumption and mouth cancer (Amtha et al., 2014; Jayalekshmi et al., 2011), it should be a top priority for healthcare organizations to raise awareness of people, especially of young people, about the misunderstanding that smokeless tobacco use is safe.

Acknowledgments

The authors acknowledge their gratitude to the re-search deputy of Zahedan University of Medical Sciences for approval and financial support of the current study (code 6859).

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