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The Determinants of Salivary Cotinine Concentration in Smokeless Tobacco Users

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Abstract

BACKGROUND: Smokeless tobacco products due to their high nicotine content are highly addictive and ultimately lead to an increased risk of oral cavity, laryngeal and oesophagal cancer.

AIM: This research was conducted with the aim of assessing the relationship between the salivary cotinine concentration and demographic characteristics and smokeless tobacco use for the first time in tradespeople in Chabahar, Iran.

METHODS: This descriptive cross-sectional study was conducted on 150 different tradespeople using smokeless tobacco in Chabahar who were selected through simple random sampling in 2018. In addition to the salivary cotinine measurement, data were collected using a researcher-made questionnaire with demographic and behavioural items. The data obtained were analysed in SPSS-16 using descriptive and inferential statistics.

RESULTS: The mean salivary cotinine score was 887.7 ± 180.7 in men and 611.2 ± 139.7 in women, making for a significant intergroup difference (P = 0.043). The mean salivary cotinine score was higher in those who used two or more smokeless tobacco products compared to those who used one type of tobacco, and a significant difference was observed between the type of smokeless tobacco used and the salivary cotinine score in the participants (P = 0.005).

CONCLUSION: Based on the results of the regression analysis, the type of smokeless tobacco used was a strong predictor of the concentration of salivary cotinine in the participants. It is, therefore, necessary for the government to adopt appropriate policies and take educational measures to reduce the vending and use of these substances.

Introduction

Almost one-fifth of the tobacco used in the world is smokeless form [1]. Smokeless tobacco products are highly addictive due to their high nicotine content [2]. These products also contain carcinogenic compounds such as Tobacco-Specific N-nitrosamines (TSNAs), which eventually lead to an increased risk of oral cavity, laryngeal and oesophagal cancer [3]. An increased risk of mortality due to cardiovascular diseases has also been observed among smokeless tobacco users [3]. Around 10 million people are estimated to die of tobacco use in developing countries by 2030, and this figure is higher than the figures estimated for AIDS, drug abuse, road accidents, murder and suicide [4].

There are wide ranges of smokeless tobacco products throughout the world [5]. Naswar, Gutka, Pan Parag, BT and Mawa are some of the common smokeless tobacco products among tradespeople in Chabahar [6]. All brands of smokeless tobacco that are sold for oral or nasal-use have nicotine and nitrosamines as ingredients [7]. Also, smokeless tobacco components consist of tobacco, areca nut, slaked lime, and spices [8]. The proximity of Chabahar to Pakistan and the illicit import of various smokeless tobacco products in attractive packaging has led to the wide availability of these products in the market and stores around this city [9]. The poor knowledge of tradespeople about nature and side-effects and ease of access to these substances have led to their increased use [9]. The nicotine content of a smokeless tobacco product may vary depending on its type [10]. The measurement of nicotine and its metabolite in smokeless tobacco users is therefore important for understanding its addictive potential [10].

Cotinine is a sensitive and special quantitative index for learning of the amount of nicotine absorption over the preceding few days [10]. This concentration is assessed by the rate of nicotine metabolism in body fluids [11], [12]. Saliva currently provides an appropriate diagnostic alternative to other body fluids, as it offers a cost-effective, simple and non-invasive method that does not require any particular expertise for sample collection [13], [14].

Very few studies have investigated the relationship between cotinine concentration and smokeless tobacco use behaviour in the users of these products [3], [13], [15].

The present study was therefore conducted to examine the relationship of salivary cotinine concentration with demographic details and smokeless tobacco use behaviour among tradespeople in Chabahar.

Methods

The present descriptive cross-sectional study was conducted in Chabahar, Iran, in 2018. After obtaining permission from the university ethics committee, 150 different tradespeople in Chabahar entered the study with informed consent. The study inclusion criteria consisted of using at least one type of smokeless tobacco, age 20 to 50 years, the ability to answer the questionnaire items and a trading license from the Guilds Office. The exclusion criteria consisted of age less than 20 or above 50 years, smoking cigarettes, hookah, etc., and being a seasonal tradesman. Simple random sampling was used in the study. To this end, the environmental and occupational health division of the health centre was visited and using the health records, a list of participants was extracted from 18 urban and rural comprehensive health service centres, and those eligible for inclusion in the study were selected through simple random sampling. In addition to measuring the salivary cotinine, data were also collected using a researcher-made questionnaire in two parts, including a part on demographic details and some behavioural items. The demographic questionnaire contained seven items, including age, gender, type of trade, education, marital status, use of smokeless tobacco among family members and use of smokeless tobacco among friends and peers. Behaviour questionnaire contained five items, including the type of smokeless tobacco used, age at the onset of use, the daily frequency of use, the saliva disposal method and the use of tobacco in public places.

The amounts of salivary cotinine in the 150 participants were assessed using the ELISA method and the US-made Salimetrics kit (LOT: 1710502, EXP: 2019-07-11). With the help of some facilitators, the researcher collected participants' saliva samples by spitting method. For this purpose, the candidates were asked to visit the center in a fasting state from one hour before the test and to refrain from alcohol consumption 12 hours before the test and to rinse their mouth properly before the test samples were to be taken (for 10 minutes), and then spit into a test tube via a glass funnel for at least five minutes. At least 5 ml of saliva were collected from each candidate, and the samples were delivered to the laboratory of the comprehensive health service centre of Polan (a village in Chabahar County) with the cold chain maintained and daily. At the laboratory, the salivary samples were frozen at -20°C in an ultra-low temperature freezer. Finally, after these steps were over, all the samples were tested together according to the kit's specific instructions.

The data obtained from the questionnaire were analyzed in SPSS-16 using descriptive statistics (frequency, mean and standard deviation) and inferential statistics (the Kolmogorov-Smirnov test for assessing the normality of the distribution of salivary cotinine, the ANOVA and independent t-test for assessing the relationship of the salivary cotinine concentration with the demographic variables and use behaviors, and the regression analysis for predicting the concentration of salivary cotinine through the variables related to tobacco use). The level of statistical significance was set at $P < 0.05$.

Results

The majority of the participants were male (59.3%), and Most of them were in the 20-30 age group (66.7%). The use of at least one type of smokeless tobacco was reported as 11.3% among the family members and 81.3% among close friends (Table 1).

Table 1: Participants' demographic details

Variable		Count	Percentage
Age (years)	20-30	100	66.7
	30-40	40	26.7
	40-50	10	6.6
Gender	Male	89	59.3
	Female	61	40.7
Marital status	Single	55	36.7
	Married	95	63.3
Trade type	Confectionery	7	4.7
	Drapery	22	14.7
	Supermarket	20	13.3
	Auto repair	17	11.3
	Hairdressing	35	23.3
	Dressmaking and embroidery	9	6
	Wholesale	22	14.6
	Other (hoteling, restaurant, carpentry, etc.)	18	12
Education	Illiterate	19	12.7
	Primary School	25	16.7
	Junior High School	23	15.3
	High School and Above	83	55.3
Use of one type of smokeless tobacco products among family members	Yes	17	11.3
	No	133	88.7
Use of one type of smokeless tobacco products among five close friends	Yes	122	81.3
	No	28	18.7

Dividing the results by gender, Naswar was the most frequent type of smokeless tobacco used by the participating women (83.6%), but most of the participating men (67.42%) used a combination of two or more smokeless tobacco products. A significant relationship was found between the type of smokeless tobacco used and gender (P = 0.000). Dividing the results by age at the onset of use, approximately 86% of the participating men and women had started tobacco use before and after the age of ten. A significant relationship was also observed between age at the onset of use and gender (P = 0.000). Concerning the frequency of use, the majority of the participants (53.33%) used these products more than five times per day. Overall, 70% of the participants spit their saliva into public pathways in an unhygienic manner after consumption. Furthermore, the majority of the participants (62%) used these products in public places. A significant relationship was also found between this variable and gender (P = 0.007) in this study (Table 2).

Table 2: Participants' smokeless tobacco use behaviour

Tobacco Use Behavior		Total Count (percentage)	Male Count (Percentage)	Female Count (Percentage)	P-Value
Type of smokeless tobacco	Pan Parag	2 (1.33)	2 (2.24)	0	0.000
	Gutka	19 (12.66)	11 (12.36	8 (13.12)	
	Naswar	66 (45)	15 (16.86)	51 (83.6)	
	Mawa	1 (0.66)	1 (1.12)	0	
	A combination of two or more	62 (41.34)	60 (67.42)	2 (3.28)	
Age at the onset of use	Below 10	85 (56.67)	77 (86.52)	8 (13.11)	0.000
	Above 10	65 (43.33)	12 (13.48)	53 (86.89)	
Frequency of daily use	Less than 5 times	70 (46.67)	41 (46.07)	29 (47.54)	0.869
	5 times and more	80 (53.33)	48 (53.93)	32 (52.46)	
Disposal of saliva	Hygienic	105 (70)	63 (70.79)	42 (68.85)	0.857
	Unhygienic	45 (30)	26 (29.21)	19 (31.15)	
Use of tobacco in public places	Yes	93 (62)	63 (70.79)	30 (49.18)	0.007
	No	57 (38)	26 (29.21)	31 (50.82)	

The mean score of salivary cotinine was 666.5 ± 119.3 ng/ml in the 30-40 age group, which is higher than that in the other age groups. No significant relationship was observed between the salivary cotinine score and age group (P = 0.295). The mean salivary cotinine score was 887.7 ± 180.7 ng/ml in the women and 611.2 ± 139.7 ng/ml in the men, which makes for a significant difference (P = 0.043). In terms of marital status, the mean salivary cotinine score was higher in the single than the married participants, although not significantly (P = 0.69). The mean salivary cotinine score was the highest in the auto repair business, but the difference was not significant (P = 0.1) between the various businesses (Table 3).

Table 3: The relationship between the demographic variables and the concentration of salivary cotinine in smokeless tobacco users

Variable		Cotinine (ng/ml)		df	F	P-Value
		Mean	SD			
Age (years)	20-30	666.5	119.3	2	1.23	0.295
	30-40	831.5	18.5			
	40-50	112.2	22.7			
Gender	Male	887.7	180.7	148	3.26	0.043
	Female	611.2	139.7			
Marital status	Single	1073.0	132.5	148	0.78	0.69
	Married	642.0	364.0			
Trade type	Confectionery	310.5	150.5	9	1.66	0.1
	Drapery	642.0	364.0			
	Supermarket	831.5	18.5			
	Auto repair	887.7	180.7			
	Hairdressing	660.0	118.3			
	Dressmaking and embroidery	333.5	220.5			
	Wholesale	22.5	528.7			
	Other (hoteling, restaurant, carpentry, etc.)	370.6	220.5			

The mean salivary cotinine score was 1068.5 ± 131.2 ng/ml in the participants who used two or more smokeless tobacco products, which is higher than in those who used other forms of tobacco and a significant difference was thus observed between the type of smokeless tobacco used and the salivary cotinine score (P = 0.005). The mean salivary cotinine score was higher in the participants who had started using smokeless tobacco before the age of ten (760.0 ± 207.6 ng/ml), but no significant relationship was observed between age at the onset of use and the mean salivary cotinine score (P = 0.746). The mean salivary cotinine score was lower in those who had reported their frequency of use as less than five times per day compared to those who had reported their daily use like 5 times and more; however, the difference between these variables was not significant (P = 0.776; Table 4).

Table 4: The relationship between tobacco use behaviours and the concentration of salivary cotinine in smokeless tobacco users

Variable		Cotinine (ng/ml)		df	F	P-Value
		Mean	SD			
Type of smokeless tobacco	Pan Parag	703.5	73.5	4	3.93	0.005
	Gutka	501.0	18.5			
	Naswar	627.0	160.3			
	Mawa	4.0	18.0			
	A combination of two or more	1068.5	131.2			
Age at the onset of use	Below 10	760.0	207.6	148	1.33	0.746
	Above 10	719.3	72.2			
Frequency of daily use	Less than 5 times	790.0	42.8	148	0.118	0.776
	5 times and more	662.0	133.3			

The results of the regression analysis showed that, of the six subscales (gender, trade type, marital status, type of smokeless tobacco used, age at the onset of use and daily frequency of use), only one subscale (type of smokeless tobacco used) predicted the concentration of salivary cotinine significantly (P = 0.001); that is, with every standard deviation of increase in the score of the type of smokeless tobacco used, the score of the concentration of salivary cotinine increased by 0.66 standard deviations (Table 5).

Table 5: The prediction of salivary cotinine concentration through the demographic variables and tobacco use behaviour using the multivariate regression analysis

Predictor Variable	B	SE	Beta	T	P-value
Constant	-47.7	246.1	-	-0.19	0.84
Gender	97.4	90.9	0.13	1.07	0.28
Trade type	6.3	10.4	0.05	0.6	0.54
Marital status	-30.9	57.9	-0.04	-0.53	0.59
Type of smokeless tobacco used	98.4	27.9	0.33	3.53	0.001
Age at the onset of use	-9.3	81.7	0.01	-0.11	0.9
Frequency of daily use	45.2	56.1	0.06	0.8	42

Discussion

The present study was conducted to assess the relationship of the concentration of salivary

cotinine with demographic variables and smokeless tobacco use behaviours for the first time among tradespeople in Chabahar. Another exclusive feature of the study is that it assessed all types of smokeless tobacco products commonly used in Chabahar. The results obtained showed that auto repair businesses had the highest mean salivary cotinine score (888 ± 181ng/ml), while the confectionery trade had the lowest score (311 ± 151 ng/ml); however, the difference between the businesses was not significant in this respect (P > 0.005). In a study conducted by Ferketich et al., [16], the mean salivary cotinine score was 581 ± 364 ng/ml in full-time workers and 389 ± 264 ng/ml in part-time workers.

In this study, the mean salivary cotinine score was very high in both men and women (749.5ng/ml) compared to the other studies on the subject [15], [16], [17], [18], [19].

According to the results, the mean salivary cotinine score was higher in the participants who used two or more types of smokeless tobacco products compared to those who used only one type, and a significant relationship was observed between the type of smokeless tobacco used and the salivary cotinine score (P < 0.005). Naswar was the most commonly-used type of smokeless tobacco (84%) by the women, while the men most commonly used a combination of two or more different types (67%). One of the reasons for this difference between the genders could be the social norms of the society under scrutiny, as women's use of other types of smokeless tobacco products (such as Pan Parag, Gutka, Mawa, BT, etc.) is not acceptable in the cultural fabric of the society. The mean salivary cotinine score in people who used two or more different types of smokeless tobacco was lower in other studies compared to in the present study –revealing an inconsistency between findings [3], [16], [20].

The mean salivary cotinine score was higher in those who had started using smokeless tobacco before the age of ten compared to those who had started after the age of ten (760 ± 208 ng/ml vs 719 ± 72 ng/ml), but there were no significant relationships between the age at the onset of use and the mean salivary cotinine score (P > 0.005). In other words, those who had started using smokeless tobacco from a long time ago had a higher mean salivary cotinine score. The longer duration of use might affect the salivary cotinine concentration in people who use these products. In the study by Ferketich et al., [16], the mean salivary cotinine score in people who had started using smokeless tobacco before the age of ten was 610 ± 387 ng/ml, which is less than that found in the present study.

According to the present findings, the mean salivary cotinine score was higher in people whose daily use of smokeless tobacco products was less than five times compared to in those who used these products more than five times per day (790 ± 43 ng/ml vs 662 ± 133 ng/ml), but the difference between these variables was not significant (P > 0.005). In Ferketich's study [16], the mean salivary cotinine score was higher in those whose daily use of smokeless tobacco was more than 48 times compared to those whose daily use was less (620 ± 400 ng/ml vs. 483 ± 321 ng/ml), which disagrees with the present findings in this regard. This disparity of findings could be due to the self-reporting nature of that study (i.e. false reports of the frequency of use), which is often one of the main challenges in research in various fields of science.

The mean salivary cotinine score was 888 ± 181 ng/ml in men and 611 ± 140 ng/ml in women, which suggests a significant difference between the genders (P < 0.005). One of the reasons for this difference could be the type of product used by the two genders. The most commonly-used product in women was Naswar (84%), while most of the men used a combination of two or more products (67%), which explains the much higher concentration of salivary cotinine in men (who used two or more types of smokeless tobacco products) compared to women (who only used Naswar) in the present study. In a study conducted by Huque et al., [3], the mean salivary cotinine score was 399 ng/ml in men and 361 ng/ml in women, which shows no significant differences between the genders in terms of the concentration of salivary cotinine, as inconsistent with the present findings. The results obtained by Asha et al., [21], however, nearly agree with the present findings.

In the present study, the mean salivary cotinine score was higher in the single compared to the married participants (1073 ± 133 ng/ml vs 642 ± 364 ng/ml); however, the difference between the two groups was not significant (P > 0.005). In Ferketich's study [16], the mean salivary cotinine score was 364 ± 254 ng/ml in the single and 562 ± 319 ng/ml in the married people, which disagrees with the present findings, since it shows a higher mean concentration of salivary cotinine in the married compared to the single people and also a significant relationship between marital status and the concentration of salivary cotinine. In a study conducted by Binnie et al., [22], the mean salivary cotinine score was higher in the married compared to the single people, which disagrees with the present findings. The disparity of findings between the present study and the two cited studies could be due to the false reports of marital status or type of tobacco products used.

The results of the regression analysis in the present study showed that, out of the six subscales of demographic variables and tobacco use behaviors, only one subscale, i.e. the type of smokeless tobacco used, predicted the concentration of salivary cotinine significantly (P < 0.001); that is, with every standard deviation of increase in the score of the type of smokeless tobacco used, the salivary cotinine concentration score increased by 0.66 standard

deviations. In the other studies, too, this variable could predict the concentration of salivary cotinine in the participants [3], [16]. In a study conducted by Lorina et al., [20], subscales including age, the frequency of daily use and age at the onset of use predicted the concentration of salivary cotinine in tobacco users.

One of the limitations of this study is the self-report nature of data collection about smokeless tobacco use behaviours, such as the frequency of use, age at the onset of use, type of smokeless tobacco used, etc.

In conclusion, according to the results of the regression analysis, the type of smokeless tobacco used is a strong predictor of the concentration of salivary cotinine. It is, therefore, necessary for the government to adopt appropriate policies and educational measures to reduce the vending and use of these substances. Similar studies are recommended to be conducted with larger sample sizes on other groups of the users of these products.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethical Approval

The Ethics committee of the Shahid Sadoughi University of Medical Sciences-Yazd approved this study. Ethic code: IR.SSU.SPH.REC.1396.105.

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