



ORIGINAL ARTICLE

The Effect of a Health Education Intervention on Salivary Cotinine Levels among Guilds of Chabahar, Iran

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KEYWORDS

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ABSTRACT: In general, cotinine is taken into account as one of the stable metabolites of nicotine. The most common application of this biomarker is to measure tobacco exposure. The purpose of this study was to determine the effect of a health education intervention on salivary cotinine levels among Chabahar guilds. The present investigation was a quasi-experimental study design with one intervention group. The population study has been performed on the guilds of all classes in Chabahar city in 2019. A total of 320 participants were selected by the simple random sampling method. The baseline cotinine contents were determined for 150 participants by the random sampling technique. The intervention was performed in three training sessions using group discussion methods, lectures, imagery, documentation, brainstorming, and social media (WhatsApp). After six months from the intervention, the consumption behaviors were measured in the same 320 participants. Also, the cotinine levels were measured in the same 150 participants. The obtained data were analyzed using descriptive and analytical tests in SPSS statistical software version 20. In the post-intervention phase, 34.4% of guilds reported that they did not use SLT, and a significant relationship was observed between consumption status before and after the intervention (P 0.001). Also, the mean score of salivary cotinine significantly dropped from $588.02 \pm 344.4 \text{ m m}^{-1}$ to $240.19 \pm 300.8 \text{ m m}^{-1}$ in the post-intervention stage (P 0.001). The cessation of SLT products in the present study and a significant reduction in cotinine levels in the post-intervention phase were compared to the pre-intervention phase. The results confirmed the effect of a health education intervention on the participants. It is suggested that the researchers measure the levels of cotinine in addition to the self-report questionnaire.

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INTRODUCTION

Tobacco use is one of the leading causes of preventable deaths and accounts for more than 6 million deaths worldwide every year [1]. It is estimated that deaths caused by tobacco use will grow to 10 million by 2030 in developing countries. These figures are higher than AIDS, substance abuse, road accidents, homicides, and suicides [2]. In general, tobacco products are divided into two categories: smoking and smokeless products [3]. There are usually a variety of SLT products (at least 40 types) around the world, such as Pan, Pan-Prague, Gutka, Naswar (Nass), Mava, and BT [4]. The consumption of SLT products annually causes the death of more than 250,000 people worldwide. The majority of these deaths (85%) occur in Southeast Asia [3]. The use of SLT products etiologically leads to various types of oral diseases such as periodontal disease, lesions of the oral mucosa, and ultimately tooth loss [5, 6]. SLT products are highly addictive due to the high concentration of nicotine and the presence of more than 3,000 chemicals [7]. Cotinine is one of the stable metabolites of nicotine. Also, the most common use of this biomarker is to measure tobacco exposure [8]. Cotinine is isolated from plasma, urine, saliva, and gingival cervical fluid [8]. Nowadays, saliva is a desirable diagnostic alternative compared to other body fluids [9]. It is a cost-effective, convenient, and non-invasive method without the need for specific expertise in collecting samples [9].

Educational programs play a vital role in empowering people [10]. This procedure is performed by providing the necessary knowledge and awareness. Studies show that effective health education depends on designing appropriate strategies for each event [10, 11]. According to the literature survey, several studies have measured the effect of interventions on salivary cotinine [12-16].

This study investigates the effect of a health education intervention on salivary cotinine levels among Chabahar guilds. This study has been developed due to two reasons: (I) the high prevalence of consumption of these products among guilds in Chabahar city [4] and (II) no study has been conducted to measure the effectiveness of an

intervention on nicotine metabolites in consumers of these products in Iran.

MATERIALS AND METHODS

Study design and setting

The present investigation was a quasi-experimental study design, which has been performed on Chabahar merchants' guilds. This procedure is conducted using one intervention group.

Study participants and sampling

The research population was comprised of guilds consuming SLT products in Chabahar city in 2019. Inclusion criteria included: being 20 to 50 years old, consuming at least one form of SLT products (Pan, Pan-Prague, Gutka, Mava, Naswar, and BT), having the consent to participate in the study, and having a business license in the guild office. Exclusion criteria included: smokers (e.g., cigarettes and hookah) and participation in other programs to reduce tobacco use. The results of a similar study [17] showed that the average behavior score of the participants in the study before the intervention increased from 3.59 to 3.86 in the post-intervention with 95% confidence and 80% test power. The total sample size was considered 310 people. A total of 320 people were ultimately considered to increase the study accuracy and reduce the effects of sample loss.

A simple random sampling method has been used in this study. In the first step, a list of guilds of Chabahar city was requested from the guild chamber, and they were separated by residence and health centers. In the next step, by referring to the environmental and occupational health unit in the Chabahar health center, an office was received from the guild chamber for implementing the analysis processes. In this case, a list of people that consumed SLT products was extracted. A total of 320 people that consumed SLT products (the total number of people that consumed the smokeless tobacco products was 1235) were selected from 18 comprehensive urban and rural health service centers in

the city. This procedure was performed using simple random sampling, which met the inclusion criteria. Also, the tools were distributed and completed among them.

The salivary cotinine levels of participants have been measured to evaluate the influence of the educational intervention on the participants. In this regard, the significance level and test powers were 5% and 80%, respectively. According to the standard deviation of cotinine value, $S = 120$ [18]. To achieve a 20% reduction in the mean salivary cotinine of approximately 30 units, 126 patients were required. In this case, 150 patients were evaluated with a 10% loss.

$$n = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2}{(d)^2}$$

The selection of individuals has been accomplished using simple random sampling. For this purpose, 150 people were randomly selected from 1235 traders that consumed SLT products. In this case, there was 12% disagreement for the sampling process. Also, these 150 participants (salivary cotinine test was taken from them) were selected from 320 people (sample size above).

Samples collection

Dataset was collected from the participants in the study (320 people). The data collection process was performed by a researcher-made questionnaire that included: two background questions (type of guild and consumption of smokeless tobacco products among family members) and one question related to the different consuming forms of SLT products.

Saliva samples were collected by the researcher using the spitting method. This process was completed with the cooperation of facilitators using a salivary cotinine detection kit (Salimetrics) made in the USA (LOT: 1710502, EXP: 2019-07-11). In this way, the volunteers were first asked to fast one hour before the test, refrain from consuming alcohol 12 hours before the test, rinse their mouths thoroughly (for 10 minutes) while taking the test

samples, and finally salivate. Rinse in a glass funnel and test tube for at least 5 minutes. The received samples were transferred to one of the laboratories of the Comprehensive Urban Health Service Center. This procedure has daily been performed to maintain the cold chain. In the laboratory, saliva samples were frozen in a freezer (ultra-low temp freezer) at a temperature of minus 20°C. After completing the work (first and second stages), all samples were tested together. Thus, all saliva samples were first exited from the freezer and placed at room temperature for 30 minutes to liquefy. Then, the samples were tested according to the specific instructions of the kit.

Different intervention strategies have been considered, including group discussion, lectures, imagery, documentaries, brainstorming, and WhatsApp. This procedure has been performed in three sessions. After six months from the intervention, the consumption behaviors and salivary cotinine levels have been measured for the same participants once again.

Statistical analysis

Data were analyzed using SPSS software version 20. The background variables were computed based on the percentage (frequency). Also, the mean and standard deviation were used to determine salivary cotinine scores and various types of SLT products. The McNemar's test was employed to calculate the SLT products before and after the intervention. Also, a Wilcoxon test was utilized to estimate the mean salivary cotinine concentration before and after the intervention. Furthermore, the Kruskal-Wallis test was considered to determine the relationship between the variables of consumption status of SLT products and salivary cotinine concentration. In this case, the significance level was considered 0.05.

RESULTS

In this study, most participants were occupied in the textile store guild. Table 1 summarizes the results of the background variables for the merchants' guilds.

Table 1. Determining the background variables

Variable	Category	Number	Percent
Consumption of smokeless tobacco products among family members	Nobody	203	63.5
	Father	30	9.4
	Mother	2	0.6
	Brother	83	25.9
	Sister	2	0.6
	Confectionery	13	4
	Textile store	62	19.3
Type of guild	Grocery store	46	14.4
	Hairdresser	61	19
	Wholesale	33	10.4
	Auto repair shop	37	11.6
	Other (Carpentry, Tailoring and embroidery, Hotel and restaurant and goldsmith)	68	21.3

The findings demonstrated that Gutka (23.8%) had the highest consumption of SLT products in the pre-intervention stage. In the post-intervention phase, 34.4% of guilds reported that they did not use SLT products. There

was a significant relationship between all SLT products except Pan and Mava before and after the intervention (p-value > 0.001). The frequencies of tobacco use before and after the intervention are expressed in Table 2.

Table 2. Determining the frequency of tobacco use before and after the intervention

Type of SLT products	Before intervention		After intervention		P-value
	Number	Percent	Number	Percent	
Non-consumer	0	0	110	34.4	<0.001
Pan	22	6.9	17	5.3	0.125
Pan-parague	73	22.8	42	13	<0.001
Gutka	76	23.8	44	13.8	<0.001
Naswar and BT	68	21.2	44	13.8	<0.001
Mava	19	5.9	14	4.4	0.219
A combination of 2 items or more	62	19.4	49	15.3	<0.001

Table 3 provides the mean score salivary cotinine concentration before and after the intervention. The mean scores of cotinine concentration before and after the intervention were 588.0 ± 344.44 and 240.19 ± 300.8, respectively. The results of the Wilcoxon test showed that

the mean score of cotinine concentration in the post-intervention stage was significantly lower than the mean score of cotinine concentration before the intervention. The difference between these two situations was statistically significant (p-value < 0.001).

Table 3. Determination and comparison of mean salivary cotinine concentration before and after the intervention

Variable	Before intervention		After intervention		Mean Difference	P-Value
	Mean	SD	Mean	SD		
Cotinine level (m.m ⁻¹)	588.02	344.4	240.19	300.8	-347.83	<0.001

The mean salivary cotinine score was 688 ± 342.5 for the participants who used Gutka. This value was higher than those who used other forms of SLT products. A significant difference was observed between the type of SLT products

and the salivary cotinine score of people who participated in the study (p-value = 0.017). Table 4 represents the relationship between the variables of consumption status of SLT products and salivary cotinine concentration.

Table 4. Relationship between the variables of consumption status of SLT products and salivary cotinine concentration.

Variable	Cotinine level ng ml ⁻¹		DF	F	P-Value
	Mean	Standard Deviation			
Type of SLT products	Pan	658.0	147	3.93	0.017
	Pan-paragur	489.9			
	Gutka	688.0			
	Naswar and BT	582.5			
	Mava	588.9			
	A combination of 2 items and more	564.2			

DISCUSSION

This study determined the effect of a health education intervention on the salivary cotinine levels in Chabahar guilds. The findings revealed that in the post-intervention phase, 34% of guilds did not use SLT products. Also, a significant relationship was observed between consumption status before and after the intervention. SLT product cessation can be related to the intervention strategies considered for the participants consuming these products. The results of the present study are matched with other studies [12, 19-23].

The mean score of salivary cotinine concentration before the intervention in participants consuming these products was 588.02 ± 344.4 m m⁻¹. This value has decreased and reached 240.19 ± 300.8 m m⁻¹ after the intervention. This amount of reduction was statistically significant. The behavioral interventions in the present study showed that in addition to a self-report of participants, it is necessary to compare the salivary cotinine level in the post-intervention phase with the pre-intervention phase. The results of the comparison confirmed the effectiveness of these interventions. The decreased cotinine levels after interventions have been proven in references [12, 24-29]. These results are similar to the results of the present study.

In the present study, the mean salivary cotinine score was 688 ± 342.5 m.m¹ for the participants who used Gutka. This value was higher than participants who used other forms of tobacco. There was a significant difference between all types of SLT products and the score of salivary cotinine. However, the previous studies performed in this field reported different results. For example, in some similar studies, no significant relationship was observed between salivary cotinine level and the type of SLT products [30, 31]. But in other studies, a significant difference was observed between the mean salivary cotinine score and all SLT products [32, 33]. The differences between the outcomes of the present study and other studies can be related to the difference in the sample size, type of product (e.g., Pan, Pan-Prague, Gutka, and Naswar), type of liquid extracted for testing (plasma, urine, saliva, and fluid cervical gingiva), and other individual variables [34].

The limitations of the present study are expressed as follows:

- The authors were faced with a long process in ordering and delivering laboratory kits. This issue was due to the sanctions against Iran.

- There was no available laboratory to test samples taken from participants in Chabahar city. For this purpose, the authors had to transfer the samples to another city (a specialized laboratory in Bouali-Yazd) for the testing process.

It is suggested that health researchers measure the levels of cotinine in participants in addition to the self-report questionnaire.

CONCLUSIONS

The cessation of SLT products in the present study and a significant reduction in cotinine levels in the post-intervention phase were compared to the pre-intervention phase. The results of the comparison confirmed the effect of a health education intervention on the participants.

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ETHICAL CONSIDERATION

Before implementing the sampling process, the researchers explained the study purpose to the participants. Also, participants were asked to sign written informed consent forms to cooperate in the research. They were ensured about the confidentiality of their information. Furthermore, the ethics code of IR.SSU.SPH. REC.1396.105 was obtained from the Ethics Committee of Yazd University of Medical Sciences.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Niaz K., Maqbool F., Khan F., Bahadar H., Ismail Hassan F., Abdollahi M., 2017. Smokeless tobacco (paan and gutkha) consumption, prevalence, and contribution to oral cancer. *Epidemiology and Health*. 39, e2017009.
2. Gajalakshmi V., Kanimozhi V., 2015. Tobacco Chewing and Adult Mortality: a Case-control Analysis of 22,000 Cases and 429,000 Controls, Never Smoking Tobacco and Never Drinking Alcohol, in South India. *Asian Pacific Journal of Cancer Prevention*. 16, 1201-1206.
3. Siddiqi K., Shah S., Abbas S.M., Vidyasagan A., Jawad M., Dogar O., Sheikh A., 2015. Global burden of disease due to smokeless tobacco consumption in adults: analysis of data from 113 countries. *BMC Medicine*. 13, 194-199.
4. Mazloomi Mahmoodabad S.S., Jadgal M.S., Zareban I., Fallahzadeh H., 2019. Smokeless tobacco consumption awareness, attitude and behavior among guilds of Chabahar, Iran. *Medical Science*. 23, 262-268.
5. Asha V., Dhanya M., 2015. Immunochromatographic Assessment of Salivary Cotinine and Its Correlation with Nicotine Dependence in Tobacco Chewers. *Journal of Cancer Prevention*. 20, 159-163.
6. Mahmoodabad S.S.M., Jadgal M.S., Zareban I., Zadeh H.F., 2019. The Determinants of Salivary Cotinine Concentration in Smokeless Tobacco Users. *Open Access Macedonian Journal of Medical Sciences*. 7, 810-815.
7. Mazloomi Mahmoodabad S., Jadgal M., Zareban I., Fallahzadeh H., 2020. The Status of Smokeless Tobacco Use and its Associated Factors among the Business Guilds

- Population of Chabahar. The Internet Journal of Allied Health Sciences and Practice. 18, 27-36.
8. Patel V.D., Jadhav K.B., Shah V.S., Gupta N.D., 2017. Comparative assessment of salivary cotinine level and psychological dependence among tobacco users. Dental Research Journal. 14, 125-130.
 9. Raja M., Garg A., Yadav P., Jha K., Handa S., 2016. Diagnostic Methods for Detection of Cotinine Level in Tobacco Users: A Review. Journal of Clinical Diagnostic Research. 10, 24-36.
 10. Jadgal M.S., Sayedrajabizadeh S., Sadeghi S., Nakhaei-Moghaddam T., 2020. Effectiveness of Nutrition Education for Elementary School Children Based on the Theory of Planned Behavior. Current Research in Nutrition and Food Science. 8, 308-317.
 11. Panahi R., Ramezankhani A., Dashtbani F., Javanmardi K., Javanmardi E., Anbari M., 2020. Effect of Health Belief Model based education on health literacy and smoking prevention among students. Journal of Health in the Field. 7, 47-58.
 12. Karimiankakolaki Z., Mazloomi Mahmoodabad S.S., Kazemi A., Fallahzadeh H., 2019. Designing an educational intervention on second-hand smoke in smoker men on the exposure of pregnant wives: a protocol for a randomized controlled trial. Reproductive Health. 16, 11-19.
 13. Bobrowska-Korzeniowska M., Jerzyńska J., Miśtał M., Podlecka D., Brzozowska A., Stelmach I., 2020. Effectiveness of ongoing face-to-face anti-tobacco intervention in children with asthma. Allergy and Asthma Proceeding. 41, 198-203.
 14. Hutchinson S.G., van Breukelen G., van Schayck C.P., Essers B., Hammond S.K., Muris J.W.M., Feron F.J.M., Dompeling E., 2017. Motivational interviewing and urine cotinine feedback to stop passive smoke exposure in children predisposed to asthma: a randomised controlled trial. Scientific Reports. 7, 15473.
 15. Ratschen E., Thorley R., Jones L., Opazo Breton M., Cook J., McNeill A., Britton J., Coleman T., Lewis S. A., 2018. A randomised controlled trial of a complex intervention to reduce children's exposure to secondhand smoke in the home. Tobacco Control. 27, 155-162.
 16. Ismail S., Abdul Rahman H., Abidin E.Z., Isha A.S., Abu Bakar S., Zulkifley N.A., Fuad A. F., 2016. The effect of faith-based smoking cessation intervention during Ramadan among Malay smokers. Qatar Medicine Journal. 2, 16-26.
 17. Fattahi E., Tavousi M., Niknami S.h., Zareban I, Hidarnia A., 2013. Effectiveness of an educational intervention for reducing Paan consumption among adolescents. Payesh. 12, 109-116.
 18. Rabiei M., Rahbar Taramsar M., Sadegh Kanjani M., Kazemnezhad Leyli E., Masoudi Rad H., Jafari Farshami M., 2014. Comparison of Salivary Cotinine Concentration in Cigarette Smokers, Water Pipe Smokers and Non-Smokers. Journal of Islamic Dental Association of Iran. 26, 39-45.
 19. Müssener U., Bendtsen M., Karlsson N., White I.R., McCambridge J., Bendtsen P., 2016. Effectiveness of Short Message Service Text-Based Smoking Cessation Intervention among University Students: A Randomized Clinical Trial. JAMA Internal Medicine. 176, 321-328.
 20. Lin Y., Wang L.X., Qiu L.X., Huang Q., Shu Q., Lin H.X., Meng X., Zeng X.L., Xiao L.X., Bam T.S., Chiang C.Y., 2015. A smoking cessation intervention among tuberculosis patients in rural China. Public Health Action. 5, 183-187.
 21. Whittaker R., McRobbie H., Bullen C., Rodgers A., Gu Y., 2016. Mobile phone-based interventions for smoking cessation. Cochrane Database System Review. 4, CD006611.
 22. Stead L.F., Lancaster T., 2012. Combined pharmacotherapy and behavioural interventions for smoking cessation. Cochrane Database System Review. 10, CD008286.
 23. Cahill K., Lancaster T., 2014. Workplace interventions for smoking cessation. Cochrane Database System Review. 2, CD003440.
 24. Cupertino A.P., Cartujano-Barrera F., Ramírez M., Rodríguez-Bolaños R., Thrasher J.F., Pérez-Rubio G., Falfán-Valencia R., Ellerbeck E.F., Reynales-Shigematsu L.M., 2019. A Mobile Smoking Cessation Intervention for Mexico: Single-Arm Pilot Study. JMIR Mhealth Uhealth. 7, e12482.

25. Liao Y., Wu Q., Kelly B.C., Zhang F., Tang Y.Y., Wang Q., Ren H., Hao Y., Yang M., Cohen J., Tang J., 2018. Effectiveness of a text-messaging-based smoking cessation intervention ("Happy Quit") for smoking cessation in China: A randomized controlled trial. *PLoS Med.* 15, e1002713.
26. Kim S.S., Sithisongkram S., Bernstein K., Fang H., Choi W.S., Ziedonis D., 2016. A randomized controlled trial of a videoconferencing smoking cessation intervention for Korean American women: preliminary findings. *International journal of women's health.* 8, 453-462.
27. Rigotti N.A., Tindle H.A., Regan S., Levy D.E., Chang Y., Carpenter K.M., Park E.R., Kelley J.H., Streck J.M., Reid Z.Z., Ylloja T., Reyen M., Singer D.E. 2016. A Post-Discharge Smoking-Cessation Intervention for Hospital Patients: Helping Hand Randomized Clinical Trial. *American Journal of Preventive Medicine.* 51, 597-608.
28. Li W.H.C., Ho K.Y., Wang M.P., Cheung D.Y.T., Lam K.K.W., Xia W., Cheung K.Y., Wong C.K.H., Chan S.S.C., Lam T.H., 2020. Effectiveness of a Brief Self-determination Theory-Based Smoking Cessation Intervention for Smokers at Emergency Departments in Hong Kong: A Randomized Clinical Trial. *JAMA Internal Medicine.* 180, 206-214.
29. Chen J., Ho E., Jiang Y., Whittaker R., Yang T., Bullen C. A., 2020. Mobile Social Network-Based Smoking Cessation Intervention for Chinese Male Smokers: Protocol for a Pilot Randomized Controlled Trial. *JMIR Research Protocols.* 9, e18071.
30. Huque R., Shah S., Mushtaq N., Siddiqi K., 2016. Determinants of Salivary Cotinine among Smokeless Tobacco Users: A Cross-Sectional Survey in Bangladesh. *PLOS One.* 11, e0160211.
31. Prabhakar M., Bottu K., Sivapathasundharam B., 2020. Estimation and comparison of serum cotinine level among individuals with smoking and tobacco chewing habit. *Indian Journal of Dental Research.* 9, 531-536.
32. Mushtaq N., Beebe L.A., Vesely S.K., 2012. Determinants of salivary cotinine concentrations among smokeless tobacco users. *Nicotine and Tobacco Research.* 14, 1229-1234.
33. Singh P.N., Natto Z., Saxena R., Banerjee H., Yel D., Khieng S., Job J.S., 2013. Cotinine levels among betel quid users and cigarette smokers in Cambodia. *Asia Pacific Journal of Public Health.* 25, 84S-91S.
34. Honarmand M., Nakhaee A., Moradi M., 2018. Comparison of Salivary Cotinine Concentrations in Male Smokers and Smokeless Tobacco Users. *Asian Pacific Journal of Cancer Prevention.* 19, 1363-1366.