Original Article

Comparison of Cytotoxic Effect of Cigarette and Waterpipe Smoking on Human Buccal Mucosa

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Abstract

Background: The evidences on cytotoxic effect of cigarette and waterpipe smoking are very rare and controversial. The aim was to compare the cytotoxic effect of cigarette and waterpipe smoking on human buccal mucosa cells. **Methods:** The study was case–control. Feulgen-stained samples of exfoliated buccal mucosa cells were evaluated. The cytology slides of 25 cigarette smoker, 25 waterpipe smoker, and 25 individuals in the never smoked were examined. The number of pyknosis, karyorrhexis, and karyolysis in 1000 cells/subject were counted. Exposing to cigarette and waterpipe smoke was considered by the number of pack × years. **Results:** There were significant differences among the groups in terms of karyolysis and pyknosis while there was no significant difference among the cigarette smokers group and waterpipe smokers group in terms of karyorrhexis ($P \leq 0.01$). The cytotoxicity effect of cigarette smoking was not significantly correlated to time exposure ($r = 0.099$, $P = 0.637$). The cytotoxicity effect of waterpipe smoking was significantly correlated to time exposure ($r = -370$, $P = 0.044$). **Conclusions:** The cytotoxic effect of cigarette and waterpipe smoking on buccal mucosa cells was significantly higher than nonsmokers. The effect of cigarette smoking on cellular death was higher than waterpipe. The cytotoxic effect of waterpipe smoking was dose dependent.

Keywords: *Buccal mucosa, cytotoxic agents, smoking*

Introduction

Cigarette and waterpipe smoking is a worldwide problem, especially in the Middle-East and Southeast Asia. The increasing tendency to waterpipe smoking expressly among youths and women basically originates from public believe about lesser harmful damage of waterpipe smoking on health status in comparing to cigarette.

It has been established that the genotoxic abnormalities of buccal mucosa in cigarette and waterpipe smokers is more than nonsmokers.[1,2] The genotoxic effects have been reported to be associated with nucleus abnormalities.[1-5] Spite recognized genotoxic effect[6,7] evidence on cytotoxic effect of cigarette and waterpipe smoking is very limited and controversial.

In a biologic process of cell death, all cellular functions terminate. The event completes by nuclear changes that are indicative of apoptosis.[8] Evaluating the frequency of pyknosis, karyorrhexis and karyolysis in a given cytotoxic exposure, cellular death could be assessed. Assessing the cytogenetic damage of buccal mucosa cells is a simple biomonitoring evaluation for demonstrating the biologic effects on tissues and estimation the risk of cancer.[9]

Study on the health-related effects of different forms of smoking is an important stride in appraising the users about harmful effects of their habits. The aim was to compare the cytotoxic effect of cigarette and waterpipe smoking on human buccal mucosa. This is the first study in comparison the cytotoxic effect of cigarette and waterpipe smoking on human buccal mucosa cells.

Methods

The study was case–control. The study was taken the approval number, IR.Shahed.Rec.1394.301 from the Ethical Committee on Biological Researches of Shahed University.

25 participants who were cigarette smokers, 25 waterpipe smokers, and 25 healthy controls (persons who never smoked waterpipe and cigarette) were entered the study.

Matching the case and control groups, all participants were selected from Iranian

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male between 25- and 50-years-old. The participants in waterpipe smokers group were selected from a local Water Pipe Café in Tehran, Iran. Omitting the hormonal impact on buccal mucosa, females have not been entered the study in both cases and control groups. The persons who have been exposed to radiography beam in recent 6 months, consumed drugs and suffered from systemic disease were excluded from all groups.

In waterpipe smokers group, participants have been selected from the smokers who had never smoked cigarettes or smoked utmost 100 cigarettes in whole their life.[2]

A signed inform consent were taken from all participates. The data were entered a coded registration form and participants were identified by received codes. Exfoliated buccal mucosa cells were obtained by scraping the mucosa. Applying gently pressure, buccal cells were removed by rubbing a wooden spatula over the inner part of cheek. Scarped buccal cells were spread on clean glass slides and fixed in Carnoy's fixative (methanol and glacial acetic acid in a ratio of 3:1) for 30–35 min. After drying at room temperature, Feulgen reaction used for staining.[1]

Feulgen staining completed using modified method of Thomas *et al*. as follows: dipping the slides in 1 N HCl at 60°C for 10 min, rinsing in distilled water for 3 min, immersing in Schiff's reagent for 90 min, immersing in normal saline for 10 min, immersing in 0.5% sodium metabisulfite solution for 3 times, rinsing with tap water, staining with 1% light green for 15 min, rinsing with tap water, and drying and finally mounting.[10]

The cells with nuclear phenomena of pyknosis, karyorrhexis, and karyolysis were encountered the study. Nuclear abnormalities were calculated in cells with distinctive cellular margin. The overlapped cells were not counted.

Based on Tolbert *et al*. the count of pyknosis, karyorrhexis, and karyolysis were recorded. The structures within cytoplasm with aggregated chromatin, nuclear disintegration, and nuclear dissolution were considered as pyknosis, karyorrhexis, and karyolysis, respectively [Figure 1]. The number of counted pyknosis, karyorrhexis, and karyolysis in 1000 cells/subject was determined.[11] The counts were completed with optic microscope (ZEISS, Germany) under × 1000 (×10 ocular and ×100 objective lenses) magnification in the form of double blind.

Exposure to cigarette and waterpipe smoking was considered by the number of pack × years (P × Y).[1]

Statistical analysis

The data were analyzed by a one-way ANOVA, and the comparison of means carried out with Duncan's multiple range test at the $P \leq 0.01$ probability level to determine the significant differences, using SPSS statistical software package (version 22; IBM, Chicago, IL, USA). The data in this research were presented as the mean value ± standard error of mean (SEM).

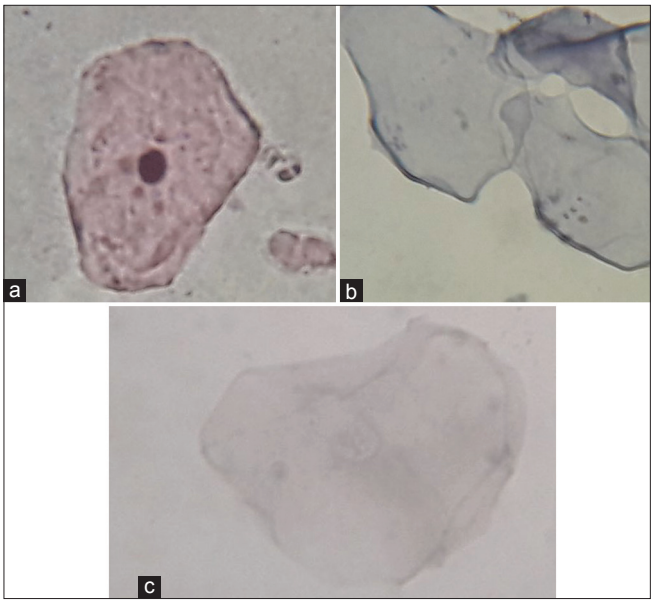

Figure 1: Nuclear abnormalities in human buccal mucosa cells: Pyknosis (a), karyorrhexis, (b) and karyolysis (c) (Feulgen staining, ×400)

Results

The average age of cigarette smokers, waterpipe smokers, and healthy controls were 44, 28, and 37 years, respectively. The average duration of cigarette and waterpipe smoking was 20 and 4 years, respectively. The mean ± SEM of P × Y in cigarette and waterpipe smokers were 5704.7 ± 730.9 and 292.6 ± 53.4, respectively.

The mean number of pyknosis in nonsmokers, cigarette, and waterpipe smokers were 0.17 ± 0.08, 3.64 ± 3.13, and 2.44 ± 1.51, respectively. The mean number of karyorrhexis in nonsmokers, cigarette, and waterpipe smokers was 0.96 ± 0.15, 5.08 ± 3.32, and 4.76 ± 2.83, respectively. The mean number of karyolysis in nonsmokers, cigarette, and waterpipe smokers was 1.21 ± 0.19, 9.24 ± 5.81, and 4.24 ± 2.24, respectively.

The ANOVA results revealed a high significant difference among the three groups in terms of karyolysis, karyorrhexis and pyknosis ($P \leq 0.01$). The comparison of means using Duncan's multiple range test indicated that there were significant differences among the groups in terms of karyolysis and pyknosis while there were no significant differences among the cigarette smokers group and waterpipe smokers group in terms of karyorrhexis [Figure 2]. The mean and SEM of groups based on P × Y were shown in Table 1.

The cytotoxicity effect of cigarette smoking was not significantly correlated to time exposure ($r = 0.099$, $P = 0.637$). The cytotoxicity effect of waterpipe smoking

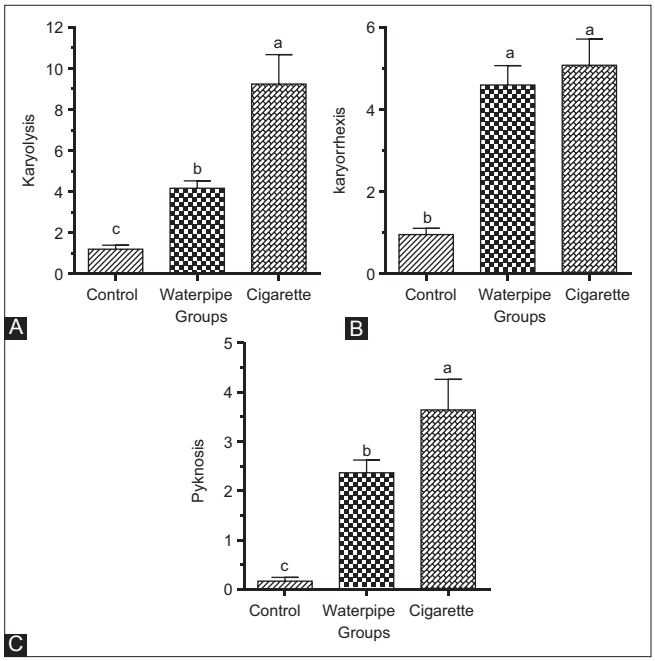

Figure 2: Comparison of the karyolysis (A), karyorrhexis, (B) and pyknosis (C) of the three studied groups using Duncan's multiple range test ($P \leq 0.01$). Different letters indicate significant difference between the values of pair of groups

Table 1: The mean and standard error of mean of studied characteristics in nonsmokers, cigarette smokers and waterpipe smokers based on pack per years

Groups	P × Y*	*n*	Mean±SEM		
			Karyolysis	Karyorrhexis	Pyknosis
Nonsmokers		25	1.21±0.19	0.96±0.15	0.17±0.08
Cigarette smokers	0-2000	3	8.67±2.60	5.00±1.53	2.67±1.45
	2001-4000	5	7.20±3.31	4.40±1.72	3.00±1.84
	4001-6000	9	10.67±3.18	5.00±1.19	3.67±1.07
	6001-8000	1	15.00±0.00	6.00±0.00	5.00±0.00
	8001-10,000	3	8.00±3.79	6.00±1.87	4.33±2.03
	>10,001	4	8.50±2.66	6.00±1.87	4.25±0.62
Waterpipe smokers	0-300	18	4.11±0.45	5.00±0.65	2.17±0.34
	>300	12	4.64±0.56	4.36±0.59	2.91±0.34

*Exposure to smoke based on P×Y. P×Y=Pack per years, SEM=Standard error of mean

was significantly correlated to time exposure ($r = -370$, $P = 0.044$).

Discussion

The cytotoxic effect of cigarette and waterpipe smoking on buccal mucosa cells was significantly higher than nonsmokers. The effect of cigarette smoking on cellular death was higher than waterpipe. The cytotoxicity effect of cigarette smoking was not correlated to time exposure. The cytotoxic effect of waterpipe smoking was dose dependent.

The findings of present study on cellular death of human buccal mucosa in cigarette and water pipe smokers are compatible with the previous studies on areca nut, chewed-tobacco, and snuff.[7,11]

The cytotoxic effect of cigarette smoke on alveolar and nasal epithelial cells has been shown in the previous studies.[12,13] These findings are compatible with the results of this study on buccal mucosa. No previous study was found on cytotoxic effect of waterpipe smoking.

The mutagenic agents stimulate cellular death to eliminating genotoxic damaged cells. Pyknosis, karyorrhexis, and karyolysis originate after cytotoxicity-induced cellular necrosis. Necrosis indicates the cytotoxicity of cells from cell proliferation to epithelial carcinoma.[11] Apoptosis is another form of cellular death that controls by natural genetic and physiologic process of tissues. Exposing to mutagenic agents stimulate the apoptosis. In this circumstances, apoptosis acts as a mechanism for removing the damaged cells. Pyknosis and karyorrhexis (without karyolysis) are cellular evidence of apoptosis.[14]

The present study showed that the frequency of pyknosis, karyorrhexis, and karyolysis in cigarette smokers, and waterpipe users were significantly higher than nonsmokers. This suggests the cytotoxic effect of both cigarette and waterpipe on epithelial cells. However, the average count of karyolysis in cigarette smokers was significantly higher than waterpipe smokers. This finding suggests the higher necrotic effect of cigarette than waterpipe. This finding is compatible with more incidence of cellular alternation toward cancer development.[8]

It has been shown that the individual responses to cytotoxicity of cigarette smoke are independent to age and smoking habits.[15] In the present study, the age range of participants was from 25 to 50 years. All selected participants were Iranian male. The sampling method decreased any possible biases in obtained results.

The study shows that the effect of cigarette smoking on cellular death was not dose dependent. Conversely, the correlation between cell death and time exposure to waterpipe smoke was dose dependent. The cytotoxic effect of cigarette smoke contributes to the presence of cytotoxic agents in the gas and particulate phases of cigarette smoke. The HCN and acrolein are specific cytotoxic agents in gas phase of cigarette smoke. The semi-volatile acidic and neutral fractions are cytotoxic agents in particulate phase of cigarette smoke.[16,17] Several chemicals including nicotine, tar, CO, phenanthrene, and fluoranthene produce after water pipe smoking.[18] It has been shown that CO exposure and carboxyhemoglobin production after waterpipe smoking are many times more than the cigarette smoke.[19,20]

Besides the chemicals, cigarette and waterpipe using methods and the amount of produced heat are different. Puffing characteristics comprising volume, duration, and frequency of each puff are different between cigarette and waterpipe smoking. Waterpipe smoking session is

longer than cigarette almost 1/2 h or more. One session of waterpipe smoking is equivalent to smoking of 10 cigarettes.[21] The waterpipe or hookah user inhales water-filtered smoke water decreasing the temperature of smoke. Heated tobacco in lower temperature reduces the cytotoxicity of smoke more than burning temperature. The reduction is higher in particulate phase than in gas phase.[22,23] The results of present study are in agreement to mentioned results.

Based on the findings of the present study, both cigarette and waterpipe have cytotoxic effect on epithelial cells. The cytotoxic effect of waterpipe smoking relates to exposing time. Increasing the number of waterpipe smoking can potentially increasing cytotoxic effect. The average usage of waterpipe is at most once in a day, however, daily usage of cigarettes are several numbers more. Based on results, waterpipe smoking is not safer than cigarette and increasing the number of waterpipe smoking can potentially increasing cytotoxic effect.

A general accepted protocol for studying the impact of dose and duration of waterpipe smoking on cell cytotoxicity is not create yet. This issue causes difficulties on comparing cigarette and waterpipe smoking with each other in obtained results from cytotoxic and genotoxic studies. Further researches need for obtaining additional results.

Conclusions

The cigarette and waterpipe smoking had cytotoxic effect on buccal mucosa cells. The effect of cigarette smoking on cellular death was higher than waterpipe. The cytotoxic effect of waterpipe smoking was dose-dependent. Increasing the number of waterpipe smoking can potentially increasing cytotoxic effect. The cytotoxicity effect of cigarette smoking was not correlated to exposing time and in any amount had cytotoxic effect.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Naderi NJ, Farhadi S, Sarshar S. Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian J Pathol Microbiol 2012;55:433-8.
2. El-Setouhy M, Loffredo CA, Radwan G, Abdel Rahman R, Mahfouz E, Israel E, *et al.* Genotoxic effects of waterpipe smoking on the buccal mucosa cells. Mutat Res 2008;655:36-40.
3. Haveric A, Haveric S, Ibrulj S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and non-smokers. Toxicol Mech Methods 2010;20:260-6.
4. Konopacka M. Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells. Neoplasma 2003;50:380-2.
5. Nersesyan A, Kundi M, Atefie K, Schulte-Hermann R, Knasmüller S. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. Cancer Epidemiol Biomarkers Prev 2006;15:1835-40.
6. Oliveira LU, Lima CF, Salgado MA, Balducci I, Almeida JD. Comparative study of oral mucosa micronuclei in smokers and alcoholic smokers. Anal Quant Cytol Histol 2012;34:9-14.
7. Joshi MS, Verma Y, Gautam AK, Parmar G, Lakkad BC, Kumar S, *et al.* Cytogenetic alterations in buccal mucosa cells of chewers of areca nut and tobacco. Arch Oral Biol 2011;56:63-7.
8. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: Methods development. Mutat Res 1992;271:69-77.
9. Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, *et al.* Buccal micronucleus cytome assay. Nat Protoc 2009;4:825-37.
10. Thomas P, Hecker J, Faunt J, Fenech M. Buccal micronucleus cytome biomarkers may be associated with Alzheimer's disease. Mutagenesis 2007;22:371-9.
11. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: A field test in snuff users. Am J Epidemiol 1991;134:840-50.
12. Hoshino Y, Mio T, Nagai S, Miki H, Ito I, Izumi T, *et al.* Cytotoxic effects of cigarette smoke extract on an alveolar type II cell-derived cell line. Am J Physiol Lung Cell Mol Physiol 2001;281:L509-16.
13. Comer DM, Elborn JS, Ennis M. Inflammatory and cytotoxic effects of acrolein, nicotine, acetylaldehyde and cigarette smoke extract on human nasal epithelial cells. BMC Pulm Med 2014;14:32.
14. Miller ML, Andringa A, Dixon K, Carty MP. Insights into UV-induced apoptosis: Ultrastructure, trichrome stain and spectral imaging. Micron 2002;33:157-66.
15. Hopkin JM, Steel CM. Variation in individual responses to the cytotoxicity of cigarette smoke. Thorax 1980;35:751-3.
16. Thayer PS, Kensler CJ. Cigarette smoke: Charcoal filters reduce components that inhibit growth of cultured human cells. Science 1964;146:642-4.
17. Matsukura N, Willey J, Miyashita M, Taffe B, Hoffmann D, Waldren C, *et al.* Detection of direct mutagenicity of cigarette smoke condensate in mammalian cells. Carcinogenesis 1991;12:685-9.
18. Shihadeh A, Saleh R. Polycyclic aromatic hydrocarbons, carbon monoxide, "tar", and nicotine in the mainstream smoke aerosol of the narghile water pipe. Food Chem Toxicol 2005;43:655-61.
19. Maziak W, Rastam S, Ibrahim I, Ward KD, Shihadeh A, Eissenberg T, *et al.* CO exposure, puff topography, and subjective effects in waterpipe tobacco smokers. Nicotine Tob Res 2009;11:806-11.
20. Eissenberg T, Shihadeh A. Waterpipe tobacco and cigarette smoking: Direct comparison of toxicant exposure. Am J Prev Med 2009;37:518-23.
21. Neergaard J, Singh P, Job J, Montgomery S. Waterpipe smoking

and nicotine exposure: A review of the current evidence. Nicotine Tob Res 2007;9:987-94.
22. Tewes FJ, Meisgen TJ, Veltel DJ, Roemer E, Patskan G. Toxicological evaluation of an electrically heated cigarette. Part 3: Genotoxicity and cytotoxicity of mainstream smoke. J Appl Toxicol 2003;23:341-8.
23. Patskan G, Reininghaus W. Toxicological evaluation of an electrically heated cigarette. Part 1: Overview of technical concepts and summary of findings. J Appl Toxicol 2003;23:323-8.