Ann Med Res

Current issue list available at AnnMedRes

Annals of Medical Research

journal page: www.annalsmedres.org

Micronucleus assay of buccal mucosa cells in cigarette and waterpipe smokers in Duhok, Iraq

Dian Jamel Salih[a,*], Sayran Kamal M. Salih[b], Rawa Muhammed Abdo[b], Chiman Saleem Rasool[c], Asaad A. Alasady[b]

[a] *University of Foggia, College of Medicine, Department of Clinical and Experimental Medicine, Italy*
[b] *University of Duhok, College of Medicine, Department of Anatomy, Duhok, Iraq*
[c] *University of Duhok, College of Nursing, Department of Basic Sciences, Duhok, Iraq*

ARTICLE INFO

Keywords:
Micronuclei
Hookah smoking
Cigarette
Buccal cells

Received: Oct 14, 2021
Accepted: Jan 18, 2022
Available Online: May XX, 2022

DOI:
10.5455/annalsmedres.2021.08.514

Abstract

Aim: Smoking and Hookah is widespread in Western countries and in the United States. The goal of this study was to compare the genotoxic/mutagenic effects of cigarettes and hookahs in oral mucosa cells using the micronucleus biomarker to see if there was a difference between the two types of fumes.

Materials and Methods: In the current study, 75 people were chosen at random from various parts of Duhok, Iraq. They were divided into three groups (25 participants for each group): a control group, a group of cigarette users only, and a group of narghile users only. The total number of micronuclei per 1,000 cells per subject was compared under light microscope.

Results: According to the findings, the groups with the highest frequency of micronucleus were those who only used hookah (10.2±9), followed by cigarette users (8.3±4) and non-smoker participant (2.5 ±7). Hookah use was found to be more genotoxic than tobacco consumption.

Conclusion: Cigarette and Hookah smoking had a significantly greater cytotoxic effect on buccal mucosa cells than nonsmokers. Increasing the duration of smoking could increase the frequency of micronucleus.

Introduction

Cigarette and Hookah smoking is a worldwide problem and widely used in Middle East countries and some parts of United states. It has gained popularity in recent years in European countries, particularly following the migration of youth from Middle Eastern countries to European countries. The reasons for its success are linked to a set of factors, including the captivating aromas that can be added to it, greater social acceptability, but also lax policies on its use and a general underestimation of its toxic effects [1]. "Use spring brackets instead of square brackets."

The spreading increase of Hookah smoking among the youth back to the public believe that smoking through a Hookah filter out the toxic components of tobacco and making it less harmful on health status than smoking cigarettes [2]. Many studies have well established that cigarette smokers are exposed to potentially harmful effects of dangerous chemicals in cigarettes. Another study confirms that prevalent smoking rates by Hookah smokers has become one of the most severe risks and unhealthy lifestyles, potentially increasing the risk of genotoxic responses and chronic various types of cancer [3, 4]. Cigarette smoking has been linked to a variety of cancers, such as mouth, throat, lung, stomach, urinary bladder, and kidney cancers. It is also suspected of causing a variety of other diseases, including acute myeloid leukemia and hepatocellular carcinoma. Hookah may increase the risk of head and neck, esophageal, and lung cancers, as well as possibly the risk of stomach and bladder cancer, according to the most recent high-quality studies [5, 6, 7]. The mutagenicity of substances present in tobacco, which number over 4,000 chemicals and 69 of which have been classified as carcinogens, determines the cytotoxic effects of cigarettes and hookah smoking [8]. The genotoxic abnormalities of exfoliated buccal mucosa in cigarette and Hookah smokers are more than nonsmokers. These genotoxic effects have been reported to be associated with nucleus abnormalities, Yet there is insufficient evidence on the cytotoxic effects of cigarette and hookah smoking [9, 10]. The mi-

*Corresponding author:
Email address: dian.salih@unifg.it (Dian Jamel Salih)

cronucleus assay for exfoliated cells is a popular tool for estimating DNA damage in humans and the genotoxic of smaller concentrations of carcinogens in human populations. This assay's results can be used to provide an advance indication of the possibility of developing long-term health problems [11]. Due to an increasing rate of cigarette and hookah smoking consumption among young people in the Duhok, Iraq, this study aimed to evaluate the harmful effects of these two types of tobacco smoking on cigarettes and hookah smokers and compare them with the control group via the micronucleus assay.

Materials and Methods

Ethical statement

The scientific research committee of the college of medicine/University of Duhok and the Ethics Committee of the Director of Health (No.68. 22/09/2020) approved the current study. All participants signed both oral and written consent forms.

Samples

The present study was performed according to the case-control study design. This study comprised 75 individuals in the age group ranged from 18 to 26 years, and they were selected randomly at different parts in the Duhok and Shexan Districts/ Kurdistan region of Iraq.

All participants were classified into three main groups: Group one consisted of 25 participants who were cigarette smokers. Group two consisted of 25 hookah smokers and 25 healthy controls who never smoked cigarettes and Waterpipes.

Each individual completed a questionnaire form that included information such as gender, age, medical history, daily smoking duration, and alcohol consumption habits. In the hookah smokers group, participants have been selected from the smokers who had never smoked cigarettes or smoked rarely.

Buccal micronucleus assay

Exfoliated buccal mucosa cells were softly collected from each participant's oral mucosa of cheeks using a soft toothbrush. Participants were asked to rinse their mouths completely with water before collecting buccal cells to remove any undesired material. Cell samples were taken from the inner walls of the cheeks on the right and left sides using a small-headed toothbrush.

The toothbrush was immersed in Phosphate buffer solution in a centrifuge tube. The cell suspension was made to produce slides after centrifugation. After drying, the slides were fixed in 80 percent ice-cold methanol, stained with Giemsa, and examined under a light microscope.

Statistical analysis

All statistics were done, and the average frequencies of MN cases and controls were evaluate by comparing with the Student's t-test. (P0.05) was considered to be statistically significant. To determine the frequency of various cell types, each participant had 1000 cells scored. Nuclear anomalies were scored using the criteria proposed by Tolbert et al. [11].

Table 1. Demographic characteristics of participants

Variable	Controls	Cigarette smokers	Hookah smokers
Participants	25	25	25
Age (Mean±St)	21.1±4	24.4±9	23.6±6
Duration of smoking (Years)	-	2.5-6	1-4.5
Alcohol consumption (Person)	-	-	-
No. of Cigarette/Hookah per day	-	16.3	0.8

Table 2. The mean numbers of micronuclei observed in the three groups

	Frequency of MN	Mean numbers of MN
Controls	0-4	2.5 ±7
Cigarette smokers	4-10	8.3±4
Hookah smokers	3-16	10.2±9

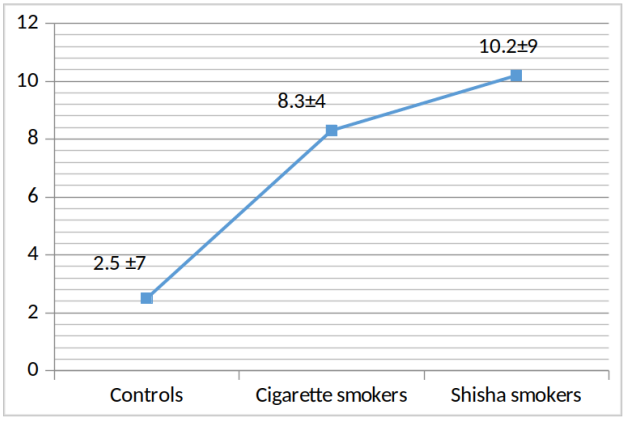


Figure 1. Mean number of micronuclei in three study groups

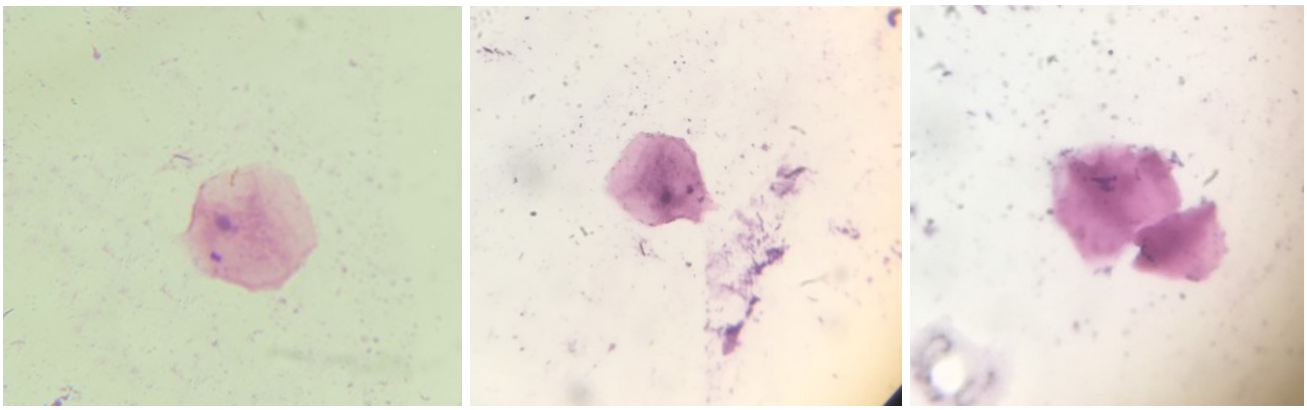
Results

A total of 75 male and female participants (25 healthy controls, 25 cigarette smokers, and 25 hookah smokers) were included in the current study. The age of the participants ranged from 18-26 years with mean "23.1(2.3)" years.

The duration of cigarette smoking ranged from (2.5-6) years with mean "4.5(8.1)" years while the duration of hookah smoking ranged from (1-4.5) years with mean "2.7(1.5)" respectively. The characteristics features of

Table 3. Mean number of Pyknosis, karyorrhexis and karyolysis in three study groups

	Pyknosis	Karyorrhexis	Karyolysis
Controls	0.1±0.8	0.9 ±0.3	0.2 ±0.1
Cigarette smokers	1.4 ±0.3	2.0 ± 1.1	2.16 ± 0.5
Hookah smokers	1.6 ± 0.5	1.3 ± 0.2	5.78 ± 2.7



(a) Micronucleus cell **(b)** Binucleated cells **(c)** Karyorrhexis

Figure 2. Cytosmears showing exfoliated buccal epithelial cells with Giemsa Stain x1000

the group exposed including age, sex, length of time of cigarette smoking, and alcohol consumption, were summarized in (Table 1).

Micronuclei frequency in exfoliated buccal cells varied from (0-4) in healthy controls, from (4-10) in smokers, and from (3-16) in hookah smokers. As shown in [Figure1] (Figure 1), the mean micronuclei frequency in healthy controls was (2.5±7), smokers (8.3±4), and hookah smokers (10.2±9) in 1,000 binucleated cells. Cigarette and Hookah smokers had a considerably higher frequency of micronuclei ($p \leq 0.05$). The mean numbers of micronuclei observed in the three groups are presented in (Table 2). The mean number of Pyknosis, karyorrhexis, and karyolysis of three study groups are summarized in (Table 3) and presented in (Figure 2).

Discussion

Smoking has already been identified as a significant health risk for a variety of health issues and different types of cancer, especially oral cancer. Cigarette smoking, hookah smoking, and tobacco consumption is one of the leading causes of oral cancer because they contain several carcinogens that activate in different tissues and cause DNA damages. [12, 13, 14].

In the present study, we evaluated the effects of cigarettes and hookah smoking on the formation of micronucleus in exfoliated oral cells using the micronucleus assay, which is a fast and reliable assay for evaluating mutagen and carcinogen exposure and detecting human cancer risks because most tumors are of epithelial origin.

This study tried to evaluate the contribution of cigarettes and hookah smoking in raising the micronucleus frequencies in exfoliated buccal mucosal cells of Kurdish people in Duhok city/ Kurdistan region of Iraq who smoked cigarettes for more than 5 years and hookah for more than 2 years routinely in comparison to control group who do not smoke cigarettes and Waterpipes.

The number of micronuclei in tobacco chewers and cigarette smokers was found to be greater than in the general population. According to reports, smokers have 1-2 times the number of micronuclei as non-smokers.The results showed that hookah smokers have nearly 1.5 times more micronuclei than people who have never smoked waterpipes, which is consistent with prior findings in cigarette smokers [4, 16].

The current research work showed a significantly higher frequency of micronuclei, as genotoxic indicators in the cigarettes and hookah smokers as compared to the control group (10.2±9, 8.3±4 and 2.5±7 respectively); The findings of the present study are compatible with the previous studies were done by Sarshar et al., Joshi et al. [15, 16].

Air pollution, agricultural pesticide exposure, and long-term Arsenic occupational exposure have all been associated with increased rates of micronuclei score in mucosal surface and peripheral blood lymphocytes [17].

Studies show that micronuclei develop as a result of continuous exposure and can disappear if genotoxic agents are not used any more. Based on this analysis, it is observed that this biomarker has occurred in all groups tested when examining results relating to the micronucleus [18, 19].

Our findings also show that hookah smoking has a significant impact on elevated micronucleus frequencies in youth ($p \leq 0.05$) when compared to cigarette smokers. The genotoxic effects of hookah smoking were found to be more prevalent in men and alcoholics. There was no considerable relationship between age and micronucleus occurrence in this study, possibly because all of the samples were almost same age.

The findings suggest that alcohol and cigarettes can provide suitable conditions for the development of micronuclei, as evidenced by the subjects who admitted to using these substances. However, they also show that alcohol and cigarettes do not provide the required conditions, as subjects who did not consume these substances had micronuclei as well.

Our findings show that smoking causes micronucleus incidence and other nuclear anomalies such as nuclear pyknosis, karyolysis, and karyorrhexis in human buccal cells, and that these results vary based on the extent of smoking and the number of cigarettes consumed per day. Previous studies on the cytogenetic effects of smoking on oral cells have found that these effects may be due to the nicotine levels of the cigarettes smoked by the participants.

Conclusion

The current study demonstrated that hookah and tobacco smoking cause cell instability, resulting in cellular changes such as the formation of micronuclei in the oral mucosa. However, other genotoxic agents can also cause it. Despite being an important indicator of instability, the presence of these changes does not guarantee that the subject will develop some disease as cancer.

Acknowledgments

The authors would like to thank the staff of the Department of Anatomy, College of Medicine, University of Duhok.

Authors' contributions

This work was carried out in collaboration between all authors equally. All authors read and approved the final version of the manuscript.

References

1. Khlifi R, Trabelsi-Ksibi F, Chakroun A, Rebai A, Hamza-Chaffai A. Cytogenetic Abnormality in Exfoliated Cells of Buccal Mucosa in Head and Neck Cancer Patients in the Tunisian Population: Impact of Different Exposure Sources. BioMed Research International. 2013;2:1-10.
2. Mohammad-Alizadeh-Charandabi S, Mirghafourvand M, Tavananezhad N, Karkhaneh, M. Prevalence of cigarette and Hookahsmoking and their predictors among Iranian adolescents. International Journal of Adolescent Medicine and Health. 2015;27(3):291-8.
3. Nersesyan A, Muradyan R, Kundi M. Knasmueller S. Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. Mutagenesis. 2010;26(2):295-301.
4. DehghanNezhad M, Jalayer Naderi N, Semyari H. Micronucleus Assay of Buccal Mucosa Cells in Hookah(Hookah) Smokers: A Cytologic Study. Iran J Pathol. 2020;15(2):75-80. doi:10.30699/ijp.2020.101701.2010
5. Haveric A, Haveric S, Ibrulj S. Micronuclei frequencies in peripheral blood and buccal exfoliated cells of young smokers and nonsmokers. Toxicology Mechanisms and Methods. 2010;20(5):260-66.
6. Jackson D, Aveyard P. Shisha smoking in students: Prevalence, risk factors, symptoms of addiction, and smoke intake. Evidence from one British university. BMC Public Health. 2008;8(1).
7. Chatterjee S, Dhar S, Sengupta B, Ghosh A, De M, Roy S, etal Cytogenetic monitoring in human oral cancers and other oral pathology: the micronucleus test in exfoliated buccal cells. Toxicol Mech Methods. 2009;19:427–33.
8. Hoffmann D, Hoffmann I, El-Bayoumy K. The Less Harmful Cigarette: A Controversial Issue. A Tribute to Ernst L. Wynder. Chemical Research in Toxicology. 2001;14(7):767-90.
9. Pasha M, Naderi N. Comparison of cytotoxic effect of cigarette and Shisha smoking on human buccal mucosa. International Journal of Preventive Medicine. 2017;8(1):98.
10. El-Setouhy M, Loffredo CA, Radwan G, Abdel Rahman R, Mahfouz E, Israel E, etal Genotoxic effects of Hookahsmoking on the buccal mucosa cells. Mutat Res. 2008;655:36–40.
11. Sellappa S, Sadhanandhan B, Francis A, Vasudevan S. Evaluation of Genotoxicity in Petrol Station Workers in South India Using Micronucleus Assay. Industrial Health. 2010;48(6):852-56.
12. Khabour OF, Alsatari ES, Azab M, Alzoubi KH, Sadiq MF. Assessment of genotoxicity of Hookahand cigarette smoking in lymphocytes using the sister-chromatid exchange assay: a comparative study. Environ Mol Mutagen. 2011;52:224–8
13. Özkul Y, Donmez H, Erenmemisoglu A, Demirtas H, Imamoglu N. Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis. 1997;12(4):285-87.
14. Salih D, Qader M, Ahmed M. The Effects of Benzene on Micronuclei Frequency of Workers in Petrol Stations in Duhok-Iraq. OTSBD. 2020; 5(2): 221-229.
15. Tolbert P, Shy C, Allen J. Micronuclei and Other Nuclear Anomalies in Buccal Smears: A Field Test in Snuff Users. American Journal of Epidemiology. 1991;134(8):840-50.
16. Sarshar S, Naderi N, Farhadi S. Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian Journal of Pathology and Microbiology. 2012;55(4):433.
17. Joshi M, Verma Y, Gautam A, Parmar G, Lakkad B, Kumar S. Cytogenetic alterations in buccal mucosa cells of chewers of areca nut and tobacco. Archives of Oral Biology. 2011;56(1):63-7.
18. Knishkowy B, Amitai Y. Water-pipe (narghile) smoking: an emerging health risk behavior. Pediatrics. 2005;116(1):e113–9.
19. Setúbal A. Micronucleus: an important intermediary biological marker in the prevention of oral cancer. Rev Odonto Ciência-Fac.Odonto.2005;20(28);1-4.