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Evaluation of genotoxic effect of different smoking habits by detecting micronucleus frequency in university students, A case control study

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Abstract

Background and objective: Cigarettes, shisha, and e-cigarettes are significant etiologic factors of oral cancers. Buccal mucosa is the first tissue that faces the pollutants and might reflect unhealthy processes in the body. Micronuclei are chromosomal fragments generated in interphasic cells. The present study was undertaken to investigate the association of cigarette, shisha, and e-cigarette use and micronucleus induction in the buccal mucosa.

Methods: The present study was carried out on 120 healthy volunteers (56 males and 64 females) aged 18–30 years old and divided into four groups: e-cigarette consumers, cigarette smokers, shisha smokers, and non-smokers. After filling out the questionnaire, participants were asked to rinse their mouths with water, and then oral mucosa samples were taken, processed, and stained for micronucleus detection.

Results: The frequency of micronucleus (mean ±SD) was 34.3±6.5 vs 16.76±4.27 (P <0.001), 37.1±8.31 vs 16.76±4.27 (P <0.001), 39.1±3.25 vs 16.76±4.27 (P <0.001) in cigarette vs control, shisha vs control, and e-cigarette vs control, respectively. Among the studied tobacco-related habits, and in respect to genotoxicity, e-cigarette revealed highest significant impact (P <0.001). In respect to the gender, no significant (P >0.05) differences were observed between male and female students who use tobacco related habits.

Conclusion: The rate of exfoliated buccal epithelial cells with micronuclei in the buccal mucosa of e-cigarette, shisha, and cigarette users was significantly higher compared with the non-smokers group, which might lead to undesired clinical consequences. However, further studies concerning the association of genetic aberrations on the nucleotide level and tobacco-related habits are recommended.

Keywords: Cigarette, E-cigarette, Genotoxicity, Micronucleus, Micronucleus assay, Shisha.

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Introduction

Cancer is one of the most common causes of mortality in the world (1). Oral cancer is among the ten types of highly prevalent malignant tumors worldwide. Tobacco smoking and betel quid chewing, either individually or in combination, are among the most common and established risk factors that contribute to the development of oral cancer (2).

Annually, over 8 million deaths are attributed to direct tobacco use. It remains a public health issue, with an estimated 186 million people using tobacco or tobacco-related products worldwide. In men, tobacco use was estimated to be associated with 92% of trachea, bronchus, and lung cancers, versus 62% among female users. In Iraq, about 264 new cases of lip and oral cancer were detected in 2020 (3). Tobacco smoke contains a mixture of over 5000 harmful chemicals, including 98 particularly dangerous substances (4). Smokers have a fivefold higher risk of developing oral cancer (5).

Two phases of cigarette smoke are well established: particulate and gas phases. The particulate phase contains nicotine, tar, and water, whereas the gas phase contains carbonyl compounds, including formaldehyde, acrolein, and tobacco-specific nitrosamines. Tar and nicotine are two essential harmful components of traditional tobacco. Moreover, previously published reports indicated that not only the particulate phase of tobacco but also its gas phase has various adverse effects on the body (6). Recent studies revealed that the tobacco smoke gas phase enhances the activation of nicotinamide adenine dinucleotide phosphate, which predisposes to cytotoxicity induction. Furthermore, the tobacco gas phase increases reactive oxygen species releases in human umbilical vein endothelial cells and inhibits thromboxane A2, which disturbs platelet aggregation (7).

In Asia and Africa, waterpipes have been used for tobacco smoking for many centuries. Nowadays, waterpipe smoking is a global problem. Published data reveals that waterpipe users are noticeably increasing among women and teenagers. Mucosal epithelial cells of the oral cavity and respiratory tract receive high levels of nicotine, which contain considerable levels of carbon monoxide and carcinogenic compounds, from waterpipe smoking (8). The risk of carcinogenesis induction is relatively intensified by waterpipe smoke (9). Furthermore, waterpipe smokers inhale greater doses of nicotine compared to cigarette smokers, and inhalation of chemical agents such as nicotine will significantly induce genetic damage (10, 11). Similarly, the cytotoxicity and genotoxicity of e-cigarette chemical constituents and aerosol condensate have also been documented in vitro (12, 13).

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Nowadays the number of young adults and teenagers who are users of e-cigarettes is noticeably increasing (14-16). The popularity of e-cigarettes came from a false perception that they were less harmful than traditional cigarettes, their lower price, the spread of the vaping trend, and the variety of flavors available (17). In Iraq the prevalence of smoking was about 31% and 4% among males and females, respectively, as reported by WHO in 2013 (18). Over the last ten years, the rate of tobacco use among Iraqi males has significantly increased to approximately 38%, accompanied by a notable rise in the number of schoolchildren who use tobacco (20% of males and 9% of females) aged 13 to 15 years old.

The oral cavity is a significant marker of an individual's health, since it is the first tissue that comes into contact with the invading pollutants (19). Many studies have shown that oral habits, such as chronic smoking and drinking alcohol, can induce carcinomatous changes in normal buccal mucosal epithelial cells that can be detected by micronucleus assay (2, 19). The micronucleus assay is a simple, reliable, cost-effective, and non-invasive method with a sensitivity, specificity, and diagnostic accuracy of 94%, 100%, and 95%, respectively, used for the detection of early genotoxic damage. Such relatively high diagnostic accuracy qualifies the micronucleus assay as a good prognostic indicator for oral squamous cell carcinoma (2). A significant increase in the frequency of micronuclei in buccal cells is an indicator of buccal mucosa cancer (19). Additionally, the comet assay is frequently used in combination with the micronucleus assay in human biomonitoring studies upon exposure to potentially genotoxic agents to detect DNA damage as an early biological effect of exposure (20). A micronucleus is a detached part of the nucleus formed during cellular division and generated from chromosomal fragments at interphase (21). These nuclei are cytoplasmic structures that measure between 1/5 and 1/3 the size of the cell nucleus with the same staining characters (11, 22).

In general populations, the frequency of cells with micronuclei ranges from 0.0% to 0.9%. Elevation of micronuclei frequency reflects a possible chromosomal alterations, and ats degree depends on the degree of carcinogenic effects (11).

To best of our knowledge so far, no studies concerning e-cigarette use and health behaviours among students in Erbil, have been conducted. The current study was designed to investigate the induction of micronucleation in the buccal cells of students using e-cigarette. This genotoxic marker was compared between e-cigarette users, regular smokers, shisha users and none

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smokers among students of International University of Erbil.

Methods

Study design, duration and setting: The present study is a case- control study carried out on 120 (56 males and 64 females) of apparently healthy volunteer students of International University of Erbil, their ages ranged from 18 years to 30 years old, during the period from October 1st 2024 to January 1st 2025. The volunteers comprised of four groups, e-cigarette consumers (n=30) who were using e-cigarette regularly and did not smoke cigarettes or shisha; current cigarettes smokers (n=30) who were smoking more than one cigarette per day and had never used an e-cigarette or shisha; shisha smoker group (n=30) who were smoking shisha and had never smoked cigarette and e-cigarette, and lastly non-smokers group (n=30), who had never smoked or used a regular cigarette, e-cigarette, and shisha.

A close endedquestioner, involved detailed data concerning the smoking habits, age, and health conditions, blood group, gender, diseases history and alcohol consumption habits, was filled through direct interview with the participant prior to collect the buccal epithelial samples.

Exclusion criteria: Participants who: (1) had oral lesions and wounds, (2) had teeth implants, (3) were taking periodontal treatment (4) were under regular medication, (5) were suffering from chronic diseases.

Sample collection and preparation: Participants were asked to rinse their mouth with water and then oral mucosa was scraped gently with a wooden spatula. Exfoliated buccal cells were scrapped and were placed on to glass slides. A volume of 100 to 200 µl of phosphate buffer solution (pH 7.2) was added. Ten replicated smears were done for each sample, left to dry at room temperature (22-25 ^{o} C), and fixed in 3:1 methanol/acetic-acid for 15 minutes. The dried fixed slides were stained with Giemsa stain (Atoms scientific Ltd, UK), and diluted with phosphate buffer saline (pH 7.2) for 5-10 minutes. The stained slides were viewed by compound microscope (Olympus, Japan) under oil immersion (1000X magnification power) to identify the micronuclei (Figure 1). The micronuclei frequency was calculated by the number of counted micronuclei per1000 cells per sample (11).

Ethical consideration:

The protocol was approved by the ethics committee in the College of Medicine, Hawler Medical University (Meeting code: 1, paper code: 53 on 22nd of September 2024). A verbal consent was taken from the participants, and we assured for them that their data will not be disclosed and will keep confidential.

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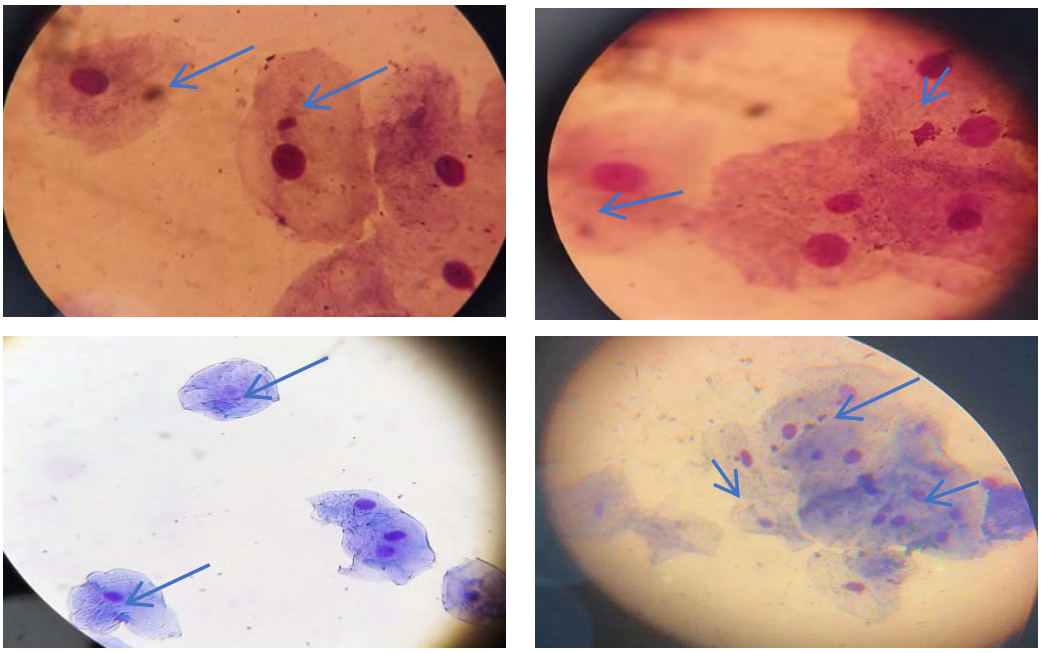

Figure 1. Giemsa- stained smears showing micronuclei in exfoliated buccal mucosal cells of smokers (1000X magnification)

Statistical analysis: The data was analysed using SPSS (version 24). Student's t-test was used for pairwise comparison of the micronucleus count in the studied groups, and one-way analysis of variance test for comparison among all groups. P value ≤0.05 was used to find statistically significant levels.

Result

The results revealed that the frequency of micronucleus (mean ±SD) was 34.3±6.5 vs 16.76±4.27 (P <0.001), 37.1±8.31 vs 16.76±4.27 (P <0.001), 39.1±3.25 vs 16.76±4.27 (P <0.001) in cigarette vs control, shisha vs control, and e-cigarette vs control, respectively. Statistical analysis revealed highly significant differences between each group experiencing tobacco- related habits in comparison with non-tobacco consumers, control group. From all three groups of tobacco-related habits, e-cigarette was more effective (P <0.001) with respect to genotoxicity in comparison with cigarette users and non- tobacco users, control groups. However, no significant differences were observed between e-cigarette vs shisha (P = 0.1138) and shisha vs cigarette (P = 0.0786) (Table 1).

From all three types of tobacco- related habits e-cigarette (39.1±3.25) was more effective with respect to genotoxicity, followed by the group who were using shisha (37.1±8.31). Despite, the less harmful type of smoking was cigarette

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(34.3±6.5), but it is significantly elevated the risk of genotoxicity.

In respect to the gender, the results revealed that the micronucleus frequency in buccal epithelial cells were 33.82±6.16 vs 35±7.15 (P=0.3200), 36.5±9.05 vs 37.78±7.66 (P = 0.3384), and 44.9±8.16 vs 45.31±6.12 (P = 0.4434) in male vs female participants who were cigarettes consumers, shisha users, and E-cigarette users, respectively. Statistical analysis revealed no significant differences between males and females in all groups of smoking habits (Table 1).

Table 1. The micronucleus frequency (mean ±SD) in cigarette, shisha, e-cigarette smokers and non-tobacco consumers (control groups)

Tobacco-related habits	**Male**	**Female**	**Total**	**P value ***	**P value****
Cigarettes (N 30)	33.82±6.16 (N 17)	35±7.15 (N 13)	34.33±6.51 (N 30)	P < 0.001	P < 0.001
	P=0.3200				
Shisha (N 30)	36.5±9.05 (N 16)	37.78±7.66 (N 14)	37.1±8.31 (N 30)	P < 0.001	
	P=0.3384				
E- cigarette (N 30)	44.9±8.16 (N 11)	45.31±6.12 (N 19)	39.1±3.25 (N 30)	P < 0.001	
	P=0.4434				
Control (N 30)	17.08±4.12 (N 12)	16.55±4.47 (N 18)	16.76±4.27 (N 30)		
Total (N 120)	33.17±11.67 (N 56)	33.48±12.91 (N 13)	33.34±12.29		
	P value = 0.3571				

*T test - each group versus its control; ** one-way analysis of variance between all groups; N- number of the participants

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Discussion

Statistical analysis revealed highly significant differences between each group experiencing tobacco- related habits compared with non-tobacco user control group. From all three groups of tobacco-related habits, e-cigarette was more effective (P <0.001) with respect to genotoxicity compared to cigarette users and non- tobacco users control groups.

This adverse impact of tobacco-related habits on the induction of genotoxicity might be due to high level of toxic substances in the tobacco smoke and of which 200 are well proved to be toxic to human being, as well as approximately 50 substances including carcinogenic polycyclic hydrocarbons and specific nitrosamines (23). Those released hydrocarbons will be enzymatically metabolized to produce powerful carcinogens such as aryl hydrocarbon hydroxylase which increases carcinogenic potential of tobacco smoke benzopyrene along with other products released during smoking that collectively induce further DNA damage (24). Furthermore, waterpipe smoke contains toxic chemicals including carbon-monoxide as well as heavy metals (14). The carbon-monoxide concentration in expired air of waterpipe and cigarette smoking were estimated to be 23.7 ppm and 2.7 ppm, respectively. The carboxy-haemoglobin level was found to be threefold higher after waterpipe smoking in compare with cigarette smoking (25). As it is clearly noticed, e-cigarette users are remarkably increasing among various age groups that might be due in part to the willingness to take risks to achieve different sensations and experiences, even though it will be ended by undesired risky consequences (26, 27).

Adolescents who trying cigarettes may be more likely to try e-cigarettes and vice versa, and falsely believing's, the majority of young adults look at e-cigarettes as safer than cigarettes. E-cigarettes contains a variable percentage of nicotine as well as elements such as propylene glycol and glycerol that combined with concentrated flavours (28). Quantitative and qualitative studies have distinguished a wide diversity of chemical elements in the cartridges, refill solutions, and aerosols of e-cigarettes (29). Kucharska *et. al.* (29), recognized 113 chemicals in 50 brands of liquids. Carcinogenesis, and respiratory and cardiac disease induction factors have been recognized in e-cigarette aerosols, cartridges, refill liquids, and environmental emissions.

Some of the identified tobacco-specific nitrosamines, aldehydes, metals, volatile organic compounds, phenolic compounds, polycyclic aromatic hydrocarbons, propylene glycol, and tobacco alkaloids are harmful or potentially toxicogenic harmful

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constituents (30).

In the current study, the statistical analysis revealed no significant differences between males and females in all groups of smoking habits. Similar figures were observed by previously published studies. Those studies observed that the spontaneous buccal cell micronucleus frequencies in males and females did not substantially differ, with a slight but not significant excess in males (31). In contrast, other studies observed significant association of micronucleus frequency and participant's gender (14, 26).

Previously published studies revealed a controversial finding concerning micronucleus induction in the buccal epithelial cells in response to smoking and smoking-related habits. However, those studies revealed that the frequency of micronucleus in the oral epithelial cells was much higher in smokers in compared with non-smokers. Such higher frequencies were significantly correlated with the quantity of the consumed cigarettes and exposure duration to tobacco smoking (31). On the other hand, Haveric *et al* (32) revealed that the frequency of apoptotic buccal cells was significantly higher in tobacco and tobacco-related users, and such apoptotic levels were found to be user's age and smoking duration dependent (33).

Conclusion

The rate of exfoliated buccal epithelial cells with micronuclei in the buccal mucosa of e-cigarette, shisha, and cigarette users was significantly higher compared with the non-smokers group, which might lead to undesired clinical consequences. However, further studies concerning the association of genetic aberrations on the nucleotide level and tobacco-related habits, as well as large-scale epidemiological studies to investigate the association of tobacco-related habits and incidence of oral cancer and lung pathology, in Erbil city are recommended.

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Competing interest

The author declares that she has no competing interests.

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