

## **Nuclear abnormalities and Oxidative Stress Induced by Hookah (Shisha) in Male Human in Erbil City**

**Natheer Jameel Yaseen<sup>1\*</sup>**

<sup>1\*</sup>Biology Department, College of Science, Salahaddin University, Erbil, Iraq

Email: [natheer.yaseen@su.edu.krd](mailto:natheer.yaseen@su.edu.krd)

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### **ABSTRACT**

Nowadays hookah smoking has dramatically increased especially in the Middle East. Because Hookah tobacco contains many harmful substances, it has dangerous impacts on human beings. The goal of the current study is to examine the oxidative stress in blood and clastogenic effects of hookah smoking on exfoliated buccal cells. One hundred and twenty five healthy adult males were involved in this study. The participants were distributed into five groups: control, passive smokers, and three hookah smoker groups. Samples of blood from all groups were examined for Malondialdehyde (MDA) level and exfoliated buccal cells were examined thorough micronucleus (MN) test for frequencies of nuclear abnormalities including Micronucleus (MN), Binucleates (BN) and Karyolysis (KL), were evaluated as well. Frequencies of all nuclear abnormalities Micronucleus (MN), Binucleates (BN) and Karyolysis significantly increased in all exposed groups compared to the control group and the effect was exposure duration dependent. Malondialdehyde level also significantly increased in all exposed groups compared to the control group and the effect was exposure duration dependent.

**Keywords:** Hookah Smoking, Clastogenic effects, Micronucleus test, and Malondialdehyde.

### **التغيرات النووية والاكسدة الخلوية المسببة عن طريق تدخين النركيلة في الذكور في مدينة اربيل**

نذير جميل ياسين<sup>1\*</sup>

<sup>1\*</sup>قسم علوم الاحياء, كلية العلوم, جامعه صلاح الدين, اربيل- العراق

#### **الخلاصة**

حديثاً، انتشر تدخين النركيلة بشكل كبير وخاصة في الشرق الاوسط. ولان تبغ النركيلة يحتوي عل مواد مضرّة للصحة، فهي تسبب مشاكل صحية خطيرة للبشر المدخنين والغير المدخنين. تناول هذا البحث دراسة تاثيرات الجينية في خلايا الداخلية للفم والاكسدة الخلوية في الدم لتدخين النركيلة عل الذكور في مدينة اربيل. شمل البحث مئة وخمسة وعشرون شخصاً سليماً وتم تقسيمهم الى خمسة مجاميع بالتساوي وشملت: مجموعة السيطرة، مجموعة المدخنين السالبيين، وثلاث مجاميع من مدخنين النركيلة حسب فترة التدخين. تخم اخذ عينات من الدم لتحليل الاكسدة الخلوية (Malondialdehyde) وتم اخذ عينات من الفم لفحص التاثيرات النووية ( nuclear abnormalities) عن طريق فحص (Micronucleus (MN) test). النتائج: نسبة التشوهات النووية والتي تشمل كلا من (Micronucleus (MN), Binucleates (BN) and Karyolysis (KL)) زادت معنوياً مقارنة مع مجموعة السيطرة وتأثير

الزيادة كان معتمدا على طول فترة التدخين. و نسبة (Malondialdehyde) في الدم زاد معنويا عند المدخنين في كل المجاميع مقارنة مع مجموعة السيطرة والتاثير كان اكبر كلما زادت فترة التدخين.

**الكلمات المفتاحية:** تدخين النركيلة, التاثيرات الجينية, فحص ال Micronucleus, المالوندايديهايد (Malondialdehyde).

## **Introduction**

During the past decade, hookah (also known as shisha or waterpipe in some locations) use has elevated, which has motivated more researchers to assess its relation with tissue and organ damage. The novel studies show that hookah use is as or even more deleterious than cigarettes. Constituents of hookah tobacco lead to oxidative stress and inflammation (1). Carbon monoxide (2), nicotine (3), volatile organic chemicals (4), acrolein, and heavy metals (5) are main deleterious constituents of hookah. One inhalation in hookah pipe contains smoke equal to 100 or more of cigarette smoke inhalation (6).

Micronucleus assay (MN-assay) is a bioassay used to evaluate the harmful influence of environmental factors, genetic and lifestyle on genomic firmness in humans. MN-assay is favored due to its simplicity in use and low cost in relation to the accuracy of visualizing a larger number of cells (7). Micronuclei (MN) are nuclei like in appearance but smaller, and they are mainly pieces or complete chromosomes stayed in the anaphase stage of nuclear division. Existence of these nuclear anomalies in cells reflects presence of mutations in the chromosomes originated from nuclear division (8).

Lipid peroxidation in cells causes elevation in free radicals' level and then increases the production of Malondialdehyde (MDA). One of the main markers for detection of oxidative stress and antioxidant content of the cell is level of Malondialdehyde, which is highly linked with development of dangerous diseases (9). The aim of this research is to investigate the clastogenic effects in exfoliated buccal cells and the oxidative stress of hookah on male humans.

## **Materials and methods**

### **Participants:**

One hundred and twenty five hookah smokers located in the center of Erbil city were included in our study. The participants were divided into the following groups: control group (G1 group, n=25), group of passive smokers (G2 group (workers in cafes), n=25), and three groups of healthy males divided according to duration of exposure into 3 groups (each with n=25). The exposed groups were: third group (G3 group, duration of exposure was zero to 2 years), fourth group (G4 group, duration of exposure was 3 to 5 years), and the fifth group (G5, duration of exposure was 5 to 10 years). Ages of participants were between (20 to 35 years) and they were informed about the study and asked a group of questions about style of living and special factors to know important information for data collection.

### **Assessment of nuclear anomalies by Micronucleus test:**

Samples from buccal cavity were collected from the inner mucosa of the mouth by clean and sterilized cotton with plastic stick. The samples were smeared on slides and left at room temperature for drying, then fixed using methanol for five minutes. After that, samples were stained by the Geimsa stain. For each participant, two slides were prepared and 500 cells were assessed per slide to estimate the number of nuclear anomalies by using compound microscope, Olympus at 1000x. Anomalies of nucleus (binucleates, micronucleus and karyolysis) were classified depending on Tolbert *et al.*, (10) and the practical work was done in the genetics laboratory at the Biology Department in the College of Science, Salahaddin University, Erbil, Iraq.

### **Blood samples collection:**

Blood samples were collected by vein puncture from a vein of upper arm. Then venous blood transmitted by clean sterilized syringe to fresh white tubes (without anticoagulants) to allow the blood to clot and obtain serum, centrifuged at 3000 rpm for 5 minutes, then the serum (supernatant) was isolated

to another plain tube and used for Malondialdehyde estimation. Malondialdehyde in serum was calculated as conjugate with TBA (Thiobarbituric acid) by spectrophotometer at 534 nm.

**Data analysis:**

Results are shown as the mean  $\pm$  standard error. Normality of data and statistical analysis was done for the results using student's t-tests for independent samples. P-values  $\leq 0.05$  were significantly different. Analyses of all results were done using the program GraphPad Prism 8 on PC.

**Results**

Table 1 and Figures 1, 2, 3, 4 and 5 show the mean, frequency, and slides of clastogenic effects of hookah in exfoliated buccal cells by micronucleus test. According to this table, a clear statistical difference between all of the treated groups in comparison to the control group was noticed in total normal cells parameter, and the highest effect was in (G5) group (lowest number of normal cells,  $422.2 \pm 7.959$ ), then in passive group ( $439.1 \pm 4.918$ ). While the lowest effect was in (G4) group ( $450.7 \pm 1.796$ ) compared to the control group (also shown in Figure 1). According to Karyolysis parameter, there was a statistical difference between all of the treated groups compared to the control group. The effect was highest at the (G5) ( $59.6 \pm 7.788$ ), then in passive group (G2,  $51.9 \pm 3.746$ ), while the lowest effect was in (G3) group ( $36.4 \pm 6.446$ ) compared to the control group (also seen in Figure 2). While in Binucleate parameter, there was a clear difference between all of the treated groups except (G3) compared to the control group, and the effect was highest in G5 group ( $13.6 \pm 1.708$ ), then in (G4) group ( $9.1 \pm 1.433$ ), while the lowest effect was in (G3) group ( $3.5 \pm 0.473$ ). There was also no significant difference between the control group and (G3) group (as presented in Figure 3). But in Micronucleus parameter it can be seen that there is no statistical difference between the exposed groups (G2, and G3) in comparison with the control group, but (G4, and G5) showed a clear difference compared to the control group. The highest effect was reported in G5 group ( $4.6 \pm 0.796$ ) and the lowest effect was in G3 group ( $0.9 \pm 0.233$ ) in comparison to the control group (as shown in Figure 4).

**Table 1: Frequency of nuclear anomalies of control and exposed groups**

Groups	Micronucleus test Parameters			
	Total normal cells	Karyolysis	Binucleate	Micronucleus
G1 ( Control)	$486.4 \pm 1.296^a$	$11.4 \pm 1.343^a$	$1.7 \pm 0.395^a$	$0.5 \pm 0.233^a$
G2 ( passive smokers)	$439.1 \pm 4.918^{be}$	$51.9 \pm 3.746^{bcd}$	$7.2 \pm 1.597^{be}$	$1.8 \pm 0.326^{abc}$
G3	$459.2 \pm 6.420^{cd}$	$36.4 \pm 6.446^{bc}$	$3.5 \pm 0.473^{ae}$	$0.9 \pm 0.233^{ab}$
G4	$450.7 \pm 1.796^d$	$37.6 \pm 2.177^c$	$9.1 \pm 1.433^{ce}$	$2.6 \pm 0.452^{bc}$
G5	$422.2 \pm 7.959^e$	$59.6 \pm 7.788^d$	$13.6 \pm 1.708^d$	$4.6 \pm 0.796^c$

**Note: In each column, same symbols means there is no significant difference but columns with different symbols indicates to a significant difference between them.**

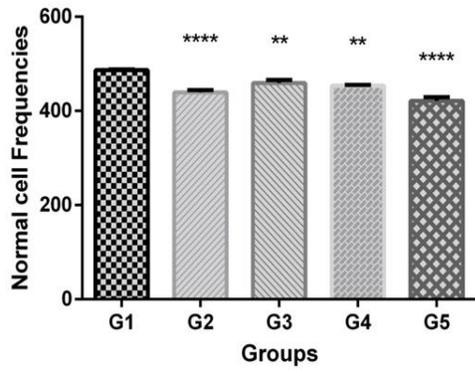


Figure 1: Frequency of distribution of total normal cells in control and treated groups.

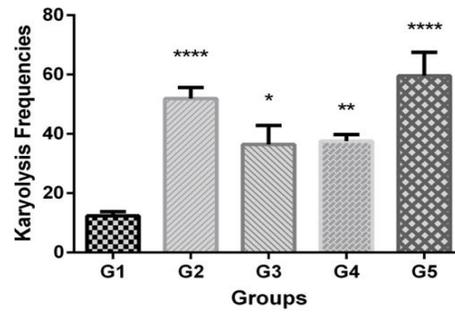


Figure 2: Rate of Karyolysis in control and treated groups.

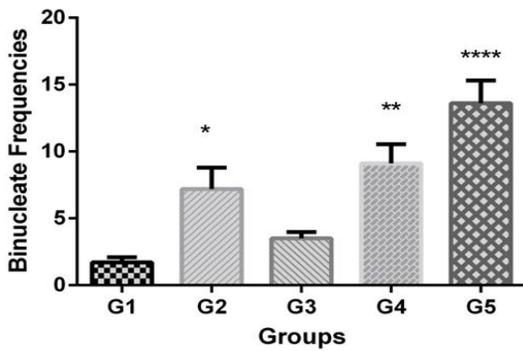


Figure 3: Rate of Binucleate in control and treated groups.

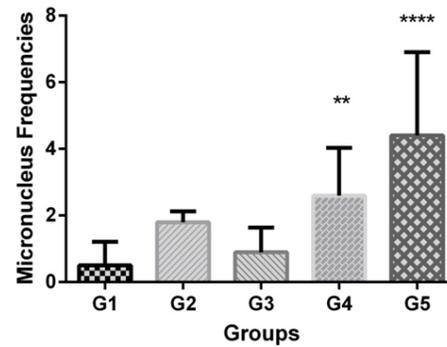


Figure 4: Rate of Micronucleus in control and treated groups.

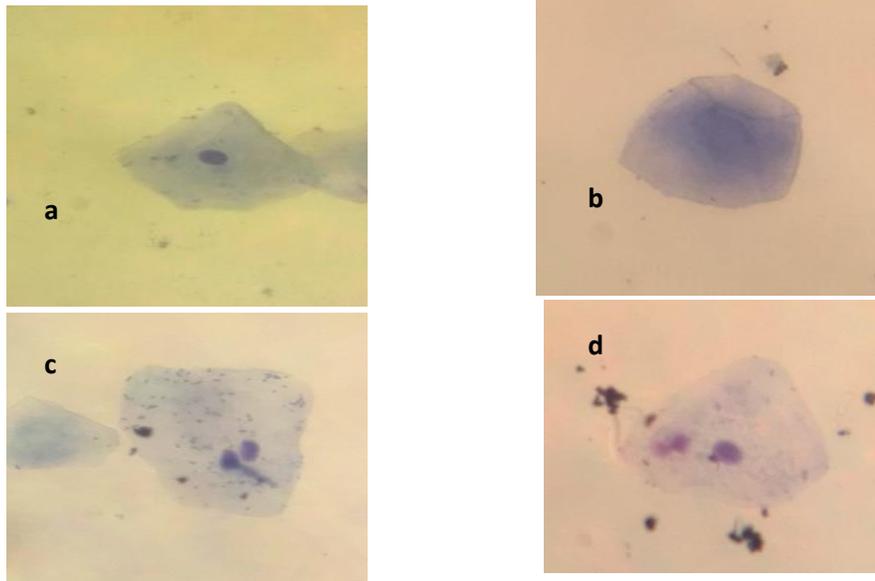


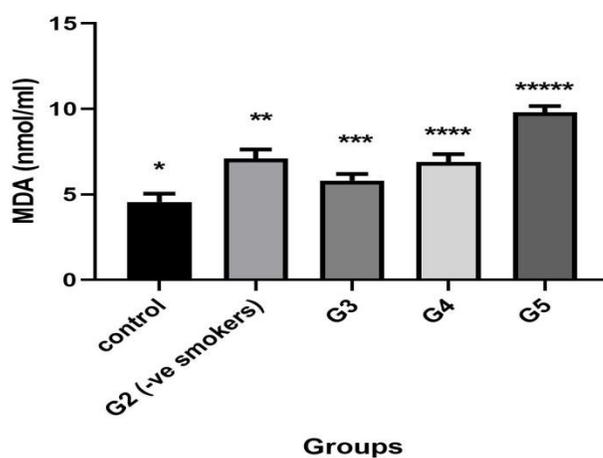
Figure 5: (a) Cells with normal nucleus, (b) Karyolysis, (c) Binucleated cells, and (d) Micronucleated cell (Giemsa stain, 1000x).

Table 2 and Figure 6, shows the oxidative stress effect of hookah smoking on male human measured by malondialdehyde estimation in serum of male human. As shown in the table, there is clear statistical difference between control and all of the treated groups. The effect is dependent on duration of exposure. The highest effect was in the last group (G5), followed by negative smokers group, while the lowest effect was shown by the (G3) group.

**Table 2: Comparison of mean Malondialdehyde (nmol/ml) between control and exposed groups.**

Groups	MDA
G1 ( Control)	4.30±0.487 <sup>a</sup>
G2 ( passive smokers)	7.10±0.523 <sup>b</sup>
G3	5.80±0.378 <sup>c</sup>
G4	6.90±0.457 <sup>d</sup>
G5	9.80±0.371 <sup>f</sup>

**Note: In each column, same symbols mean there is no significant difference but columns with different symbols indicate to significant difference between them.**



**Figure 6: Frequency of distribution of mean Malondialdehyde (nmol/ml) in control and exposed groups.**

**Discussion**

Daily, about 100 million people use hookah (11). The current study tested the link between hookah smoking with anomalies of nucleus in mucosal cells of inner mouth by using micronucleus test and oxidative stress by measuring serum malondialdehyde. According to the findings (Table 1 and Figures 1, 2, 3, 4 and 5) hookah smoking causes a considerable elevation in nuclear abnormalities of mucosal cells, and the effect was higher with longer exposure duration to hookah smoke subjects. Because the smoke contains many toxic chemicals, it can cause damage to DNA (genetic material) in many mechanisms such as DNA methylation and DNA nicking and thereby changing the genes or their expression (12). Acrolein and formaldehyde are among the toxic chemicals that are abundantly present in hookah smoke that can adduct DNA and proteins and promote reactive oxygen species (ROS) production (13). Findings of the current study are in agreement with the following research results. Derici-Eker and his colleagues (14) revealed that people who smoke hookah showed clear significant statistical differences compared to others who do not smoke in terms of micronucleus, binucleates, fragments and gap parameters, supposing that smoking hookah may cause genotoxic effects. Nersesyanyan and Muradyan (15)

found that frequency of nuclear anomalies in smokers who use the strongest cigarettes (high nicotine content cigarettes) were significantly elevated (3-fold) compared to control group, while the other indicators of nuclear anomalies (broken eggs (BE) and binucleates (BN)) increased in the MF (medium nicotine content cigarettes) group in comparison with control group. Also, Nersesyan and his colleagues (16) found that anomalies of nucleus were elevated in both pre- and post-menopausal women smoker groups. The anomalies were more obvious in the postmenopausal group compared to other group. No remarkable damage effects were recorded by tar and nicotine on chromosomes but obvious relation with longer accumulated exposure was indicated. However, Lu and Morimoto (17) said that genotoxicity of cigarette smoking in male Japanese smokers is related sensitively to level of exposure to cigarette tar or nicotine (mg/day).

Generally, inhalation of smoke causes oxidative stress and can be estimated by elevation of plasma malondialdehyde (MDA) (18). Thus, the current study evaluated the effect of hookah smoke use on oxidative stress by estimation of serum Malondialdehyde levels. As shown in (Table 2 and Figure 6), hookah smoking causes significant increase in serum MDA in groups of hookah smokers especially in long exposure. This result is due to many harmful components in hookah including carbon monoxide (19) and nicotine (20) which cause an increase in reactive oxygen species (ROS) as hydroxyl anion, super oxide anion and hydrogen peroxide, which in turn increase lipid peroxidation and free radical production (21). Results of the current work are compatible with results of other studies such as Safyudin and Subandrate (22), and Ahmed *et al*, (23). Those authors reported that the elevated plasma levels of Malondialdehyde mark to an elevation in the rate of free radicals production and thereby causing oxidative stress.

In conclusion, this study concluded that hookah smoking; even passive smoking, could cause DNA damage by increasing nuclear anomalies and causing oxidative stress through increasing Malondialdehyde levels in the body. Also the passive smokers are negatively affected and have showed an increase in MDA and nuclear anomalies in comparison to the control group.

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