MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 4490-4494
DOI: 10.12659/MSM.898593

Received: 2016.03.18
Accepted: 2016.04.21
Published: 2016.11.21

# Determination of Genotoxic Effects of Hookah Smoking by Micronucleus and Chromosome Aberration Methods

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Data Interpretation D
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**Source of support:** This study was supported by the Scientific Research Projects Unit, Mersin University (BAP-ECZ F MBB (EDE) 2009-6)

**Background:** Use of a hookah (a type of water pipe) is a traditional way of smoking tobacco, particularly in the Middle East. In Turkey, its popularity has been growing in recent years, especially among young people. It is known that cigarette smoking has genotoxic effects and causes mutations, but no comprehensive study has been done on the genotoxic effects of hookah usage, particularly in Turkey.

**Material/Methods:** We collected peripheral blood/buccal smear samples from 30 subjects who did not smoke cigarettes but who regularly smoke a hookah an average of 2 times per week, and from 30 control subjects who had never smoked cigarettes or a hookah. Chromosome analyses were performed on the samples obtained from peripheral blood of each individual, 25 metaphase plaques were counted for each, and chromosome/chromatid breakage/gap parameters were evaluated. Micronucleus analysis was done on buccal smear samples and micronucleus/binucleus parameters were investigated by counting 2000 cells of each individual.

**Results:** Chromosome breakage ratios were found to be 0.64±0.86 and 0.46±0.71 in the study and control groups, respectively, while chromatid breakage ratios were 0.53±0.83 and 0.53±0.71; fragment ratios were 0.82±1.24 and 0.21±0.49 ($p<0.05$); and gap ratios were 0.57±0.83 and 0.18±0.53 ($p<0.05$), respectively. Micronucleus ratio was 6.03±2.06 and 4.43±2.27 ($p<0.05$) in the study and control groups, respectively, and binucleus ratios were 8.53±3.23 and 12.15±5.18, respectively ($p<0.05$).

**Conclusions:** Results of our study reveal significant statistical differences between the individuals who smoked hookah and those who did not in terms of fragment, gap, micronucleus, and binucleus parameters, suggesting that smoking a hookah may cause genotoxic effects.

**MeSH Keywords:** Chromosome Aberrations • Micronucleus Tests • Mutagenicity Tests • Smoking

**Full-text PDF:** http://www.medscimonit.com/abstract/index/idArt/898593

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## Background

According to World Health Organization (WHO) data, 5 million people die each year due to tobacco use. Tobacco has been the leading cause of avoidable death in the world, especially in the United States, and accounts for almost half a million deaths yearly [1,2].

Use of hookahs, also known a water pipe, narghile, shisha, goza, and hubble-bubble, is gradually becoming more widespread in cafes in Turkey. A hookah passes charcoal-heated air over flavored or unrefined tobacco to become smoke [3]. The smoke is bubbled through a cup of water and inhaled through a nozzle linked by a pipe to the upper part of the cup [3–6].

More young people have begun smoking hookahs in recent years and a single hookah-smoking session is the same as smoking 1–50 cigarettes, depending on the calculated toxin. Therefore, hookah smokers have similar health risks as cigarette smokers [6–9].

Scientific studies have shown that smoking a hookah decreases pulmonary functions by at least 30% and causes particularly respiratory tract, esophageal, oral, and gingival cancers. It also causes respiratory tract problems and heart diseases. Nicotine addiction is also among the associated risks. Sharing the same mouthpiece, which is common in many societies, may lead the transmission of tuberculosis, herpes, and hepatitis infections [9–11].

The micronucleus test is a genotoxicity test used to detect damage to genes of individuals who smoke hookahs [12]. Genotoxicity can be defined as the capacity of physical, chemical, or biologic agents to damage genetic material. A genotoxic substance may cause many different diseases; however, the most frequent is cancer. It has been shown by studies that many of the carcinogenic substances in humans are also genotoxic. Thus, the link between the carcinogenesis and genotoxicity is clear. Genotoxicity is regarded as the event that initiates the cancer mechanism [12,13].

Micronucleus (MN) is the formation which arises during the mitotic division of the cell; it is not included in the main nucleus and originates from the full or acentric chromosome fragments [13]. Increased MN number is regarded as an indirect indicator of quantitative and structural chromosomal disorders of cells due to various agents. The MN test became a widespread technique in cytogenetic damage detection due to its advantages, which include easy use compared to chromosome analysis, ability to count more cells, and having more significant statistical results [13–15].

The aim of this study was to determine the effects of hookah smoking on chromosome aberrations and micronucleus ratios in individuals who have used a hookah for at least 1 year.

## Material and Methods

The study group consisted of 30 individuals who did not smoke cigarettes but who regularly smoked a hookah an average of 2 times per week. The control group consisted of 30 individuals who had never smoked cigarettes or hookahs. The average age of both groups ranged from 18 to 25 years. The sex distribution of both groups were similar. All individuals gave written consent. Under aseptic laboratory conditions, we obtained 3-ml samples of heparinized blood, as well as buccal smears, from all subjects.

To investigate the chromosome aberrations, 5 ml medium (RPMI-1640 Biological Industries, USA) was put into the test tubes first, then 35 µl L-Glutamine (Biological Industries, USA) and 35 µl phytohemagglutinin (Biochrome, USA) were added. Six drops of blood were added to this mixture and incubated at 37°C for 72 h. After 70 h of incubation 25 µl colchicine (Biological Industries, USA) was added. At 72 h, samples were removed from the incubator and centrifuged at 1200 rpm for 10 min. Cells were swollen and burst by hypotonic solution. Cells were fixed with Carnoy fixative, spread on microscope slides, dyed with Giemsa, and evaluated with a binocular light microscope under a 100× objective (Nikon Eclipse E200, JAPAN). We evaluated 25 metaphase plaques for each individual during all these investigations [13–15].

For the micronucleus test, buccal smear samples from each individual were spread on microscope slides and dried at room temperature. Then, they were fixed with methanol for 12 h. They were put into 1N HCl at room temperature for 10 min and then put into 1N HCl at 60°C for another 10 min. They were dyed by putting them into Feulgen solution for 90 min [14]. All preparations were evaluated by use of a binocular light microscope under 40× objectives (Nikon Eclipse E200, JAPAN). We evaluated 2000 epithelial cells for each individual and the number of micronuclei within the cells was recorded to determine the total micronucleus frequency (MN%).

All experiments were repeated 3 times and the average was taken. The independent-samples *t* test was used to determine whether there was a statistically significant difference between the study and control groups in terms of chromosome aberration and micronucleus. SPSS for Windows v16 program was used for the analyses.





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**Table 1.** Evaluation of the aberrations counted on the 25 metaphase plaques of the study and control groups.

| | Control group (n=30) | Study group (n=30) |
|---|---|---|
| Chromosome breakage | 0.46±0.71 | 0.64±0.86 |
| Chromatid breakage | 0.53±0.71 | 0.53±0.83 |
| Fragment | 0.21±0.49 | 0.82±1.24* |
| Gap | 0.18±0.53 | 0.57±0.83* |

* Statistically higher when compared to the control group (p<0.05). Values were given as mean ± standard deviation.

**Table 2.** Number of micronucleus and binucleus in 2000 cells counted in the study and control groups.

| | Control group (n=30) | Study group (n=30) |
|---|---|---|
| Micronucleus | 4.43±2.27 | 6.03±2.06* |
| Binucleus | 12.15±5.18 | 8.53±3.23** |

* Statistically higher when compared to the control group (p<0.05); ** Statistically lower when compared to the control group (p<0.05). Values were given as mean±standard deviation.

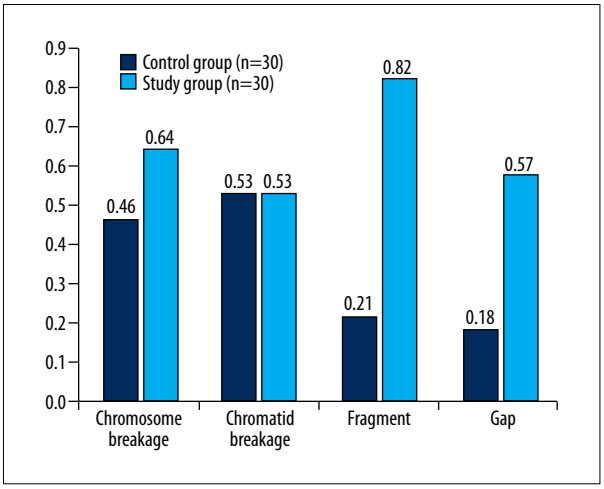


**Figure 1.** Graphical evaluation of the aberrations.

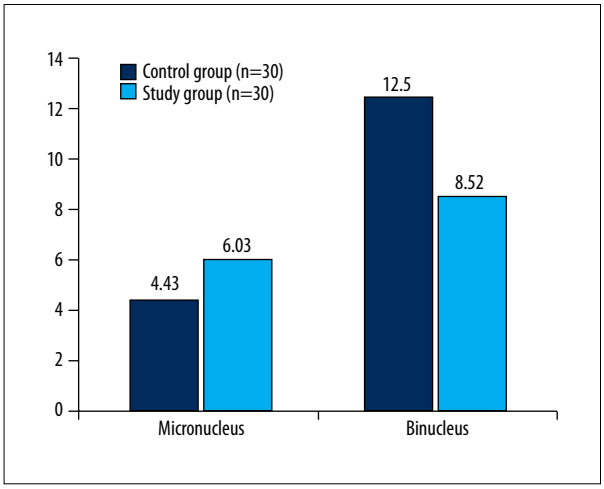


**Figure 2.** Graphical evaluation of micronucleus and binucleus.

## Results

The aim of this study was to determine whether smoking a hookah has toxic effects. Thus, chromosome breakage was investigated by chromosome analysis (Table 1), and then the possible toxic effects of hookah smoking were investigated by micronucleus method (Table 2).

In the chromosome analysis; the number of chromosome breakages per 25 metaphase plaques was 0.46±0.71in the control group and 0.64±0.86 in the study group. Although the number of chromosome breakages in the study group was higher, this difference was not statistically significant (p=0.399). The number of chromatid breakages per 25 metaphase plaques was 0.53±0.71 in the control group and 0.53±0.83 in the study group (Figure 1). There was no statistically significant difference between the study and control groups in terms of chromatid breakage (p=0.982).

The number of fragment per 25 metaphase plaques was 0.21±0.49 in the control group and 0.82±1.24 in the study group. The number of fragments in the study group was significantly higher than in the control group (p=0.022).

In the chromosome analysis, the number of gaps per 25 metaphase plaques was 0.18±0.53 in the control group and 0.57±0.83 in the study group. The number of gaps in the study group was significantly higher than in the control group (p=0.043).

In the micronucleus tests, the number of micronuclei per 2000 epithelial cells in the control group was 4.43±2.27 and





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6.03±2.06 in the study group. The number of micronuclei in the study group was significantly higher than in the control group (p=0.006).

The number of binuclei per 2000 epithelial cells investigated by the micronucleus test was 12.15±5.18 in the control group and 8.53±3.23 in the study group (Figure 2). The number of binuclei in the study group was significantly lower than in the control group (p=0.002).

## Discussion

In Turkey, tobacco smoking using a hookah has been increasing in recent years, particularly among young people. Hookah smoking causes tobacco addiction and has negative effects on health [16,17]. It has thousands of chemical substances, which cause pulmonary cancer, cardiovascular blockade, and many other diseases. An individual who smokes a hookah for 1 h inhales 100–200 times more smoke than an individual who smokes cigarettes for 1 h. The water within the hookah catches some of the nicotine, so one needs more time and more smoke to get the same amount of nicotine produced by cigarette smoking [17]. Thus, hookah smokers inhale more carbon monoxide and other carcinogenic substances than a person who smokes cigarettes.

Tobacco smoke inhaled through use of a hookah mainly contains nicotine, tar, and heavy metals (e.g., arsenic, chrome, and lead). According to a study by Shihadeh, a standard hookah protocol of 100 inhalations of 3 s each with 30-s intervals between inhalations delivers 2.25 mg nicotine, 242 mg tar, and more amounts of arsenic, chrome, and lead than smoking a single cigarette. Increasing the inhalation frequency also increases the amount of tar but has minimal effect on the amount of nicotine. Removing water from the hookah does not change the amount of tar but increases the amount of nicotine [18–22].

Carbon monoxide (CO) is a main contributory mediator in cardiovascular disease among smokers. It is important to note that the CO-to-nicotine ratio of hookah smoke is around 50:1 compared to 16:1 for cigarettes. Therefore, if hookah smokers titrate for nicotine as do some cigarette smokers, they can be exposed to considerably greater CO while seeking equivalent nicotine delivery [22].

Monzer et al. emphasized that toxins generated by the charcoal do not mean that the charcoal poses the only important risks to smokers; toxins transferred from the tobacco (e.g., nicotine, tar, and carcinogenic nitrosamines) may pose equivalent or worse health hazards [23].

The buccal mucosa micronucleus test is used as a biomarker to show environmental pollution (e.g., pesticide, arsenic, and formaldehyde) and genetic damages due to medical procedures (e.g., radiotherapy and/or chemotherapy) or factors of natural life (e.g., malnutrition, or alcohol and tobacco consumption) and it is also used in the detection of congenital genetic defects of DNA repair [23,24].

Maraş powder (*Nicotiana rustica* L.) is a powerful type of tobacco which is used instead of cigarettes in southeastern Turkey. A study recruited and compared 3 groups – Maraş powder smokers, cigarette smokers, and non-smokers – and MN amounts were calculated in the buccal mucosa cells to evaluate genotoxicity. MN amounts were found to be much higher in Maraş powder smokers compared to cigarette smokers and non-smokers. Maraş powder was also reported to have genotoxic effects [25,26].

In another study, as a most common reason of oral cancer, effects of cigarette smoking on the MN frequency were investigated. The MN frequency detected in the palatal and buccal mucosa epithelial cells of individuals who smoke cigarette was significantly higher than that of the control group subjects, while the increase in MN frequency in the lingual epithelial cells was insignificant [27].

Carboxyhemoglobin concentrations were calculated at 10–40 min after hookah smoking in a study with 1832 volunteers in Saudi Arabia. Average carboxyhemoglobin concentration of hookah smokers was 10.1%, while it was 6.5% in cigarette smokers and 1.6% in non-smokers [28].

Cigarette and hookah smoking is perceived as a means of socialization, particularly among university students, and the usage rate is gradually rising as the cafes that offer hookahs become widespread. A study found that the frequency of hookah usage among students is higher than in other social groups. In another study, most of the participants believed that the water in a hookah filters many of the toxins out, and that there is virtually no tar or nicotine in hookah tobacco smoke. Most (77.5%) of the participants mentioned that hookah smoking can increase the risk of cardiovascular and respiratory diseases. This reflects that among university students, the addictive effect of hookah smoking is not adequately understood by users and shows the urgent need for education about the harmful effects of hookah smoking [29,30].

## Conclusions

Today, people suffer the mutagenic and carcinogenic effects of genotoxic agents in their daily life or work environment. Certain addictions like cigarette smoking and hookah use also





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cause genotoxic effects. Thus, investigation of the effects of mutagenic and carcinogenic substances becomes crucial. The micronucleus test is a simple, fast, and sensitive method to detect the toxicity due to environmental agents, and today it is being considered for use as a biologic dosimeter. Its area of use is gradually increasing due to easy sampling and application. In the present study, the micronucleus frequency in the hookah smokers was found to be higher than in non-smokers. This shows that hookah usage, which is becoming more common, particularly among young people, damages the genetic structure.

In this study, the MN test was applied to the buccal epithelial cells as a biological marker, and the extent of cytotoxicity due to hookah usage was determined. Overall, MN numbers were found to be 36% higher than those of the non-smokers. This study is one of the first to determine the relationship between hookah usage and MN. Further studies with larger subject populations and applications which combine other methods will provide more specific results.

### Conflict of interests

The authors declare no conflict of interests.

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