RESEARCH ARTICLE

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Micronucleus Assay of Buccal Mucosal Cells in Hairdressers: The Importance of Occupational Exposure

Sareh Farhadi[1], Maryam Jolehar[1]*, Fatemeh Safapour[2]

Abstract

Background and objective: Today, the chemical materials available in hair dyes are considered risk factors for many cancers, particularly oral cancer. This study was performed to study the effect of occupational exposure on micronucleus (MN) frequency of buccal mucosa cells in hairdressers. **Materials and methods:** This historical cohort study was performed on 28 hairdressers and 28 control samples. To eliminate the gender variable, all the samples were women and they were matched by age. Buccal mucosa cells were removed using a wet spatula and after fixation, Papanicolaou staining method was applied. The percentage of the cells containing MN was registered. T-test was used to compare the results between the two groups. **Results:** The mean percentages of MN in buccal mucosa cells of hairdresser's and control sample were 16.61±4.95 and 8.84±4.74, respectively, with a significant difference (P<0.001). In addition, higher MN mean percentage was reported in subjects working more than 60 hours weekly compared with those working 60 hours and less; however, the difference was not statistically significant (P=0.14). **Conclusion:** In the present study, hairdressers demonstrate significantly higher average of MN in buccal mucosa cells. Also, it seems increment in their working time can increase MN frequency in these studied samples.

Keywords: Micronucleus- nuclear change- hairdresser- buccal mucosa

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Introduction

One of the hairdressers' concerns is long-term exposure to chemicals available at work, consisting of potential carcinogenic substances in hair dyes (Babish et al., 1991). WHO considers cosmetology as "probably carcinogenic"; for example, there is a higher risk of bladder cancer in hairdressers (Harling et al., 2010). There are more than 5000 different chemical substances in hair dye products, some of which have been reported to be "carcinogenic" in animals (Bolt and Golka, 2007). Considering the wide use of hair dye products, even a small increase in carcinogenic risk may significantly affect the public health (De Sanjosé et al., 2006).

Individuals in this profession are exposed to thousands of chemical substances found in hair dyes, bleachers, shampoos and hair conditioners. Moreover, hairdressers might be exposed to solutions, stimulant substances, aerosols of hair sprays as well as formaldehyde, methacrylate and nitrosamines available in most hair care products. Some of these substances, particularly those available in hair dyes, are potentially carcinogenic and some of these materials have been found in hairdressers' urine in some research studies (Silverman et al., 1992). Furthermore, for the first time in late 1970s some studies showed the relationship between use of hair dye and breast cancer; therefore, the effects of hair dyes in carcinogenicity were taken into consideration (Shafer, 1976).

On the other hand, as there is no primary clinical diagnostic marker, cancer is usually diagnosed in advanced phases (Kamboj and Mahajan, 2007). However, nuclear changes strongly happen in the initial phases of cancer. These changes in the buccal cells were introduced for the first time by Stich et al., (1983). Today, they are used as a biomarker in genetic damages (Palaskar and Jindal, 2010; Farhadi et al., 2017), paving the way for the survey of nuclear changes in the cells as carcinogenicity before clinical symptoms of cancer appear (Stich et al., 1984). The examination of nuclear changes in cytology of samples collected from patients' buccal mucosa is simple and non-invasive and somewhat painless as well (Saeed and Younis, 2012).

Exposure to poison results in the formation of nuclear objects called nucleoli (MN) in interphase during nuclear fission of cell cycle. MNs are separate small nuclei added to main nucleus, containing some parts of chromosomes or full chromosomes. MNs are one of the biomarkers for genotoxicity, formed in human erythrocytes, lymphocyte, reticulocytes and buccal mucosa cells (Marez et al., 1993). Micronucleus assay is one of the most sensitive DNA damage markers. This marker is used for investigation of genotoxicity of different chemical substances (Fenech,

*[1]Department of Oral and Maxillofacial Pathology, Dental Branch of Tehran, Islamic Azad University, [2]Dentist, Tehran, Iran. *For Correspondence: joleharm@gmail.com*

1993). This criterion is used for investigation of genetic damages caused by vocational and environmental parameters in human epidemiologic surveys and animal experiments as well (Ishikawa et al., 2000). Moreover, Francielli de Oliveira reported that MN assay is a good parameter for identifying subjects with high risk of cancer and determining biologic situations of oral lesions (Francielli de Oliveira et al., 2011).

According to our knowledge of available resources, there are limit studies on the effect of occupational exposures on buccal mucosa nuclear changes in hairdressers, with some reporting contradictory results (Rickes et al., 2010; Carlin et al., 2013). Therefore, this study was performed to study the effect of occupational exposure on micronucleus (MN) frequency of buccal mucosa cells in hairdressers.

Materials and Methods

This historical cohort study was performed using purposive sampling. The subjects consisted of a group of hairdressers and a control group. The subjects with history of smoking, alcohol drinking, radiotherapy, present systemic disease, consuming any drugs and occupation in relation to chemical agents were excluded from the study.

In next process, 28 samples with at least a year of working experience (at least 40 hours per week) in crowded beauty salons were considered as the cases and 28 persons with no work experience in beauty salons as the control group (Carlin 2013). All the case subjects were female to eliminate the effect of sex variable. Meanwhile, the case and control groups were matched in terms of age mean.

After ethical approving from the "Dental Branch, Islamic Azad University ethical committee", the subjects were requested to wash their mouth carefully before collecting buccal mucosa cells. Buccal mucosa cells were scraped by a wet spatula and distributed on small clean glass slides. After fixation by Pathofix spray and drying at room temperature, Papanicolaou staining method was used for evaluation of the cells containing micronuclei.

Cell examination was performed under an optical microscope (Nikon Ys-100) at ×400 magnification and the criteria applied by Tolbert et al were used for the evaluation of micronucleus (Naderi et al., 2012). A total of 500 cells were counted for each sample and the presence of cells with micronuclei was reported in percentages. Figure 1 demonstrates buccal mucosa cells of hairdressers containing MN using Papanicolaou staining and Figure 2 present buccal mucosa cells of case group without containing MN.

Finally, t-test was used for statistical analysis of the related data.

Results

Table 1 presents the means of micronuclei in the buccal mucosa cells of hairdressers and control subjects. The difference was significant (P<0.001), with hairdressers exhibiting significantly higher MN frequencies than the control group.

Table 2 presents comparison between mean MN percentages in hairdressers working 60 hours and less in a week and those working more than 60 hours. Although MN frequency was higher in those with more working time, the difference was not significant (P=0.14). Despite the absence of significant differences, effect coefficient (r = 0.26) in this study appears to indicate that

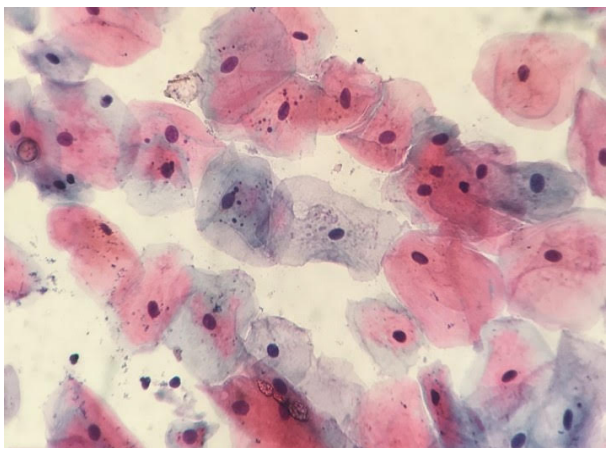

Figure 1. Buccal Mucosa Cells of Hairdressers Containing MN Using Papanicolaou Staining at ×400 Magnification

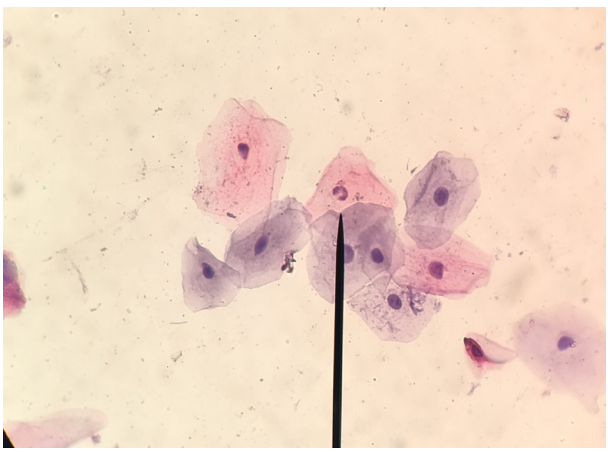

Figure 2. Buccal Mucosa Cells of Hairdressers without Containing MN Using Papanicolaou Staining at ×400 Magnification

Table 1. Comparison between Mean MN Percentages in Buccal Mucosa Cells of Hairdressers and Controls

	Mean MN percentage ± SD	Minimum	Maximum	P-value
Hairdressers	16.61±4.95	3.9	34.5	P<0.001
Control	8.84±4.74	2.1	21.2	

Table 2. Comparison between Mean MN Percentages in Buccal Mucosa Cells of Hairdressers and Controls in Terms of Duration of Work in a Week

Working time during a week	average of MN percentage ± SD	
More than 60 hours	18.61 ± 6.74	P=0.14
60 hours and less	15.66 ± 3.71	

more working time has moderate effects on MN frequency according to Cohen interpretation system.

Discussion

In this study, hairdressers exhibited a significant increase in mean MN in their buccal mucosa. Furthermore, an increase in working time resulted in an increase in micronucleus mean in the buccal mucosa.

Aslantürk and Çelik (2017) and Rickes et al., (2010) reported a significant increase in nuclear anomaly, such as micronuclei, in exfoliated cells of hairdressers' buccal mucosa. Carlin et al., (2013) also reported similar significant increase in the above-mentioned nuclear anomaly in mucous cells exfoliated from the lateral border of hairdressers' tongue. The results of studies above are consistent with those of the present study in relation to nuclear anomalies. On the other hand, the effect of working time has not been considered in any of the above studies in contrast to the present report. Furthermore, Vlastos and Ntinopoulos (2011) evaluated micronuclei in lymphocytes of peripheral blood of hairdressers and Espinoza et al., (2008) evaluated the cells lining hairdressers' urinary tract and reported a significant increase. Micronucleus survey of buccal mucosa cells might be less invasive than its evaluation in peripheral blood and urology cell. So, it is highly recommended in this field.

Generally there is limited information on the effects of chemical substances used in beauty salons on health and available studies have shown contradictory results. Rotenberg et al., (1969) found chronic poisonous effects in rats by these substances and Tyl reported it in rabbits and rats.

Correa et al., (2000) reported a relationship between use of hair dyes and leukemia; however, the kind of product, duration of application and its long time were not determined. A study in Japan by Nagata et al., (1999) demonstrated a relationship between myelodisplastic syndrome and use of hair color cream. There are some limit studies regarding oral diseases particularly increasing the risk of dysplastic lesions and malignancy.

The products used by hairdressers contain poisonous substances such as (TGA) thioglycolic acid, which can be absorbed by skin and damage animal organs and systems. Comedolytic use of TGA results in some disorders in rats' reproduction cycle and increases micronuclei frequency in marrow cells. Considering the results, it is advised that some studies be performed on humans to evaluate reproduction and mutagenic problems. (Gan et al., 2003) Another researchers conducted a study on 65 female hairdressers aged 20–40 years, with a mean work experience of 6–7 years, who were constantly exposed to hair straightening material containing 12% TGA (purity 99%), 8% ammonia solution, about 2 grams of sodium carbonate and 80% ionizied water. They found some anomalies in menstrual cycle and an increase in cells containing micronuclei in these hairdressers. They concluded that the function of reproduction in female hairdressers can be influenced after long-term exposure. Some studies have demonstrated that a combination of risk factors such as employment effect in combination with population/social factors like age, habits such as smoking and use of alcohol, nutritional status and chronic and infectious diseases might have important effects on the formation and increase in MNC (Bolukbas et al., 2006).

According to these results, it can be concluded that hairdressers might exhibit an increase in the cells containing micronuclei due to the genotoxic side effects of the products, to which they are exposed chronically. However, further studies are recommended on nuclear changes other than micronuclei for more concrete evidence.

In conclusion, this study showed that hairdressers exhibit significantly higher mean of MN in buccal mucosa cells. In addition, it appears an increase in working time can increase the frequency of MN in such subjects.

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