

## Detection of the Micronucleus Frequency in Buccal Mucosa Epithelial Cells Among Sudanese Hairdressers in Shendi City

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### Abstract

Hairdressing is one of the fastest-growing occupations in today's society. Hairdressers help with styling, cutting, coloring, perming, curling, straightening hair, and various treatments for customers. Hairdressers are regularly exposed to chemicals that can harm the human genome, including formaldehyde, hydrogen peroxide, thioglycolic acid, and aromatic amines found in hair products. The micronucleus is one of the most effective biomarkers for processes associated with the induction of DNA damage. The study aimed to determine the micronucleus frequencies in buccal mucosa epithelial cells of hairdressers exposed to chemicals in hair products. This descriptive comparative cross-sectional study included one hundred smears collected from a hairdresser gently using a toothbrush and divided into two groups: the non-exposed (control) group and the exposed (case) group. After sterilization under strict precaution, the smears were smeared on a cytology glass forested slide, fixed in 95% ethanol, rehydrated through a descending grade of alcohols, then water, and smears stained with papanicolaou stain. A significant difference in micronucleus frequency between 2 groups was reported in 40 (53.3%) in the exposed (case) group compared to 9 (36.0%) in the non-exposed (control) group. The *P* value was 0.133; the age was categorized into three groups, group A (16-30 years), B (31-45 years), and C (more than 45 years); the micronucleus positive was 27 (67.5%), 11 (27.5%), and 2 (5.0%), respectively; the micronucleus negative was 22 (62.9%), 12 (34.3%), and the *p*-value was 0.757. The duration was categorized into three groups: group A (less than one year), group B (one year–15 years), and group C (16 years–45 years). The micronucleus positive was 2 (5.0%), 34 (85.0%), and 7 (14%), respectively, and the micronucleus negative was 1 (2.9%), 27 (77.1%), and 7 (20.0%), respectively, and the *P* value was 0.443. The study concluded that the chemical substances in hair products increase the micronucleus frequency of the epithelial cells in the buccal mucosa of hairdressers. Additionally, the most positive rate was observed among males within the age group (16–30) years. This may direct the establishment of Personal Protective Equipment (PPE) protocols for protecting workers' health and the development of guidelines and policies. Protective protocols (face masks, gloves, etc.) in the salon should be followed and carried out by most hairdressers and researchers to look for more information about detecting the micronucleus among the hairdressers.

**Keywords:** Micronucleus, Nuclear Change, Hairdresser, Buccal Mucosa

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## 1. Introduction

One of the hairdressers' concerns is long-term exposure to chemicals available at work, including potentially carcinogenic substances in hair dyes [1]. WHO considers cosmetology "probably carcinogenic"; for example, there is a higher risk of bladder cancer in hairdressers [2]. There are more than 5000 chemical substances in hair dye products, some of which have been reported to be "carcinogenic" in animals [3]. Considering the wide use of hair dye products, even a tiny increase in carcinogenic risk may significantly affect public health [4]. Millions of workers are exposed to carcinogenic substances, of which only a limited number have been evaluated by International Agencies. An estimated 3–6% of all cancers worldwide are caused by exposure to carcinogens in the workplace. To reduce the number of workers affected by occupational cancer, it is necessary to reduce exposure to carcinogens (primary prevention), and where this is not possible, monitor workers exposed to carcinogens [5]. Hairdressing is one of the fastest-growing occupations in today's society. Hairdressers help with styling, cutting, coloring, perming, curling, straightening, and various treatments for customers. The human genome may be harmed by the chemicals that hairdressers are continuously exposed to, including formaldehyde, hydrogen peroxide, thioglycolic acid, and aromatic amines found in hair products. The micronucleus is one of the effective biomarkers for processes associated with the induction of DNA damage [6].

However, further research is needed to fully understand the health risks faced by hairdressers due to chemical exposure. The oral epithelial cells represent a target site for earlier genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. Buccal mucosa cells are the first barrier to metabolizing carcinogens into reactive products. It is known that chronic exposure of the oral mucosa to toxic substances leads to keratinization through the synthesis of keratin bodies. In a recent study, the excess risk for cancer of the upper aerodigestive tract, lungs, colon, cervical, and pancreatic was identified in hairdressers [7]. A study done by Rickes L, Alvarado M, Souza T, et; studied the increased micronucleus frequency in exfoliated cells of the buccal mucosa in hairdressers; their study included mucosa of 50 hairdressers and 50 controls; an assessment was carried out on the incidence of MNC, the data were analyzed with SPSS, the mean number of anomalies in hairdressers was  $2.02 \pm 3.60$ ; in controls, it was  $0.36 \pm 1.06$ . We concluded that exposure to substances used by hairdressers is genotoxic for men [8]. According to our knowledge of available resources, there are limited studies on the effect of occupational exposures on buccal mucosa nuclear changes in hairdressers, with some reporting contradictory results [8,9]. Therefore, this study was performed to study the effect of occupational exposure on the micronucleus (MN) frequency of buccal mucosa cells in hairdressers among Sudanese Hairdressers in Shendi City.

## 2. Materials and Methods

### 2.1. Study Design

This descriptive comparative cross-sectional study aimed at detecting micronucleus frequency among hairdressers in the

Shendi locality.

### 2.2. Study Area

This study was conducted in River Nile state at Shendi market hairdresser salons and shops from Feb 2023–February 2024, Shendi locality—River Nile state-Sudan. Shendi is a town in northern Sudan on the east bank of the Nile, 150 km northeast of Khartoum (16 41 N, 33 25 E). The Gaaleen Tribe inhabits the area.

### 2.3. Study Population

The study population represents all men who work in the hairdressing profession in Shendi City and its suburbs from 2023 to 2024.

### 2.4 Inclusion Criteria

Hairdressers, including all age groups, and exposure to chemicals in hair products.

### 2.5. Exclusion Criteria

Hairdressers who locate site mean areas, habits such as alcohol consumption, systemic diseases, and cases with no adequate data were also excluded.

### 2.6. Study Samples

Include One hundred 95% ethanol-fixed buccal smears.

### 2.7. Sample Processing

One hundred smears were collected gently from the hairdresser using a toothbrush after sterilization under strict precaution and smeared on a cytology glass-forested slide, fixed in 95% ethanol, then rehydrated through a descending grade of alcohols, and then water. Smears were stained by Papanicolaou stain.

### 2.8. Tools of Data Collection

Participants' information from questionnaire sheets included age, duration, dye use, and behavior.

### 2.9. The Method Used For The Detection Of Micronucleus

The stain uses both essential and acidic dyes, such that the basic dye stains the acidic components while the acidic dyes stain the integral component of the cells. This is based on the ionic charges of the element; the cell follows the principle of attraction and repulsion of the ions and the dye. Five dyes are used in three solutions, and the primary reagent used in the stain is hematoxylin, a neutral dye that stains the cell. In nuclear blue, the dye attaches to the sulfate groups of DNA because it has a high affinity for atomic chromatin. The most common hematoxylin dyes are Harries Hematoxylin; OG6 is an acidic counterstain that stains the cytoplasm of mature keratinized cells. The component of the target stain is orange in varying intensities of the dye. EA; its second counterstain combination of eosin y, light green SF, and eosin stain the cytoplasm of mature squamous cells, nucleoli, red blood cells, and cilia pink, the eosin dyes commonly used area Area 31 and EA 50, while EA 65 is a light green stain the cytoplasm of active cells such as columnar cells, parabasal squamous cells, and intermediate squamous cells are blue.

### 2.10. Result Interpretations

A stained slide was observed by microscope with lenses 10x\0.25 and 40x\0.65. Minor, round, to oval cytoplasmic chromatin segments next to the nucleus, indicate that the micronucleus is positive.

### 2.11. Quality Control

Quality control was performed at each step and during each procedure during this study to ensure reliable performance and correct reporting of results.

### 2.12. Data Analysis

Data was analyzed using the SPSS-28 computer program; frequencies and person chi-square tests were calculated.

### 3. Results

One hundred smears were examined cytopathologically and assessed for micronucleus frequency. The cytopathological examination revealed that the demonstration of micronucleus by PAP stain was positive in 40 (53.3%), negative in 35 (46.7%) in the case group, and positive in 9 (36.0%), cases and negative in

16 (64.0%) in the control group (Table 1). Our study illustrated that the frequency of the micronucleus among the study group was 40(53.3%), and the control group was 9 (36.0%) (Table 2). Our study revealed that the comparison between the case and control group in the detection of micronucleus, micronucleus was reported in 40 (53.3%) in the study group compared to 9 (36.0%) in the control group, the P. value was 0.133 (Table 3).

The correlation between micronucleus and age groups within the study subject, the age was categorized into three groups: group one (16\_30 years), (31-45yeas), and (more than 45 years), micronucleus positive was 27(67.5%), 11(27.5%) and 2(5.0%) respectively. Micronucleus negative was 22(62.9%), 12(34.3%) and 1(2.9%) respectively, and the p-value was 0.757 (Table 4). Our study illustrated the correlation between micronucleus and duration within study subject, the duration was categorized into three groups, group one (less than one year), (one year – 15 years ),( 16 years – 45 years) the micronucleus positive was 2(5.0%), 34(85.0%), 7 (14%)and 4(10.0%) respectively, and micronucleus negative was 1(2.9%), 27(77.1%) and 7(20.0%) respectively, and the P. value was 0.443 (Table 5).

Micronucleus	Positive	Negative	Total
Case group	40	35	75
Control group	9	16	25

**Table 1: Show Micronucleus By Pap Staining Technique Among Case And Control Groups**

Micronucleus	Frequency	Percentage %
Case group	40	53.3%
Control group	9	36.0%

**Table 2: Show The Frequency Of The Micronucleus Among Case And Control Groups**

Micronucleus	Study population			P value
	Case	control	Total	
Positive	40(53.3%)	9(36.0%)	49(49.0%)	0.113
Negative	35(46.7%)	16(64.0)	51(51.0%)	
Total	75(100%)	25(100%)	100(100%)	

**Table 3: Show A Comparison Between Case And Control Group In The Detection Of Micronucleus**

Micronucleus	Age groups				P value
	16-30	31-45	More than 45	Total	
Positive	27(67.5%)	11(27.5%)	2(5.0%)	40(100%)	0.757
Negative	22(62.9%)	12(34.3%)	1(2.9%)	35(100%)	
Total	49(65.3%)	23(30.7%)	3(4.0%)	75(100%)	

**Table 4: Show The Correlation Between Micronucleus And Age Groups Within The Study Subject**

Micronucleus	Duration				P value
	Less than one year	1-15year	16- 45year	Total	
Positive	2(5.0%)	34(85.0%)	4(10.0)	40(100%)	0.443
Negative	1(2.9%)	27(34.3%)	7(20.0%)	35(100%)	
Total	3(4.0%)	61(81.3%)	11(14.7%)	75(100%)	

**Table 5: Show The Correlation Between Micronucleus And Duration Within The Study Group**

#### 4. Discussion

In this study, the mean MN in the buccal mucosa of hairdressers increased significantly. Moreover, the micronucleus means in the buccal mucosa increased with increased working time. Our Finding shows that the frequency of micronucleus within the exposed group (cases) compared to the non-exposed group (control), micronucleus was reported in 40 (53.3%) in the exposed group compared to 9 (36.0%) in the non-expose group, the P. value was 0.133, these findings were in agreement with the study done by Sareh Farhadi, Maryam Other, and FatemahSafapour in 2018, which studied the micronucleus of buccal mucosal cells in hairdressers and the importance of occupational exposure, They reveal that the micronucleus in buccal mucosa cells of the hairdresser's and control sample were (16.61±4.95) and (8.84±4.74), respectively, with a significant difference (P<0.001) [7], and also in agreement with the study done by KohHui Yee et al. (2015), which studied the micronucleus frequency in exfoliated buccal cells from a hairdresser who was exposed to hair products, they concluded that the mean of micronucleus frequency of non-exposed and exposed group was higher than the micronucleus frequency in the exposed group [6, 7]. This indicated that exposure to chemical substances in hair products could increase the frequency of micronuclei in oral buccal epithelial cells. Also, our study illustrated that a higher positive rate of micronuclei was found in ages between 16 and 30 years old, representing (67.5%) overall age groups, which supports that MN has significantly increased with a longer duration. This may be explained by the fact that young people use dyes more than older people. Our Findings reveal a correlation between micronucleus and duration within the exposed group. The result showed a significant increase in the frequency of micronuclei with the increase in the duration of exposure. These findings were in agreement with the study done by KohHui Yee et al. in 2015, which studied the micronucleus frequency in exfoliated buccal cells from a hairdresser who was exposed to hair products; they revealed that result shows there was an increase in the frequency of micronuclei in subjects who work as a hairdresser with a longer duration [6]. The increased length of time exposed to harmful substances will increase the risk for adverse health effects. Our findings prove that the chemical substances in hair products increase the micronucleus frequency of the epithelial cells in the buccal mucosa of hairdressers.

#### 5. Conclusions

This study found that the frequency of micronuclei among Sudanese hairdressers in Shendi City was 53.3%. The highest rate was observed in men within the age group of 16–30. We also noted an increase in micronuclei frequency among hairdressers with

more prolonged exposure durations, indicating a direct correlation between the two. Importantly, our study identified that chemical substances in hair products significantly affect the micronucleus frequency of the epithelial cells in the buccal mucosa of hairdressers. Furthermore, the study suggests that increased working time can lead to a higher frequency of micronuclei in such subjects.

#### Recommendations

1. Personal Protective Equipment (PPE) protocols to protect worker's health should be established.
2. There is a need to develop guidelines for hairdressers and many occupational activities.
3. Policy and protective protocol (face masks, gloves) in the Salon should be followed and carried out by most of the hairdressers.
4. Further study, including significant sample size, should be carried out to provide more information about the micronucleus among hairdressers.

#### Ethical Consideration

The study was approved by the Department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, and it was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area. This study's aims and benefits were explained with the assurance of confidentiality. All protocols in this study were meticulously followed, per the Declaration of Helsinki (1964), ensuring the highest ethical standards.

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#### Conflict Of Interest

The authors have declared that no competing interests exist.

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