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Research Report

Micronucleus frequency in exfoliated buccal cells from hairdresser who expose to hair products

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ABSTRACT

Background: *Hairdresser is one of the fastest growing occupations in today's society. Hairdresser help styling, cutting, colouring, perming, curling, straightening hair and various treatment to customer. Somehow, hairdresser are constantly exposed to chemical substances such as aromatic amines, hydrogen peroxide, thioglycolic acid, formaldehyde in hair products which can cause damage to human's genome. Micronucleus is one of the effective biomarker for processes associated with the induction of DNA damage.* ***Purpose:*** *The aim of this study was to determine the micronucleus frequencies in buccal mucosa epithelial cells of hairdresser who were exposed to chemical of hair products.* ***Method:*** *This study was conducted on twenty female subjects, who were divided into 2 groups: exposed and non-exposed (control) group. All subjects recruited were working in the same beauty salon. Buccal cells were obtained from each individual by using cytobrush. The cells were stained with modified Feulgen-Ronssenback method and counting of micronucleus per 1000 cell was done under light microscope. The data were analyzed using independent t-test and one-way Anova ($p<0.05$).* ***Result:*** *The result showed a significant difference in micronucleus frequency between 2 groups. There were a significantly increase of micronucleus frequency in hairdressers and increase of micronucleus frequency with the longer duration of exposure.* ***Conclusion:*** *It concluded that the chemical substances of hair products had affected the micronucleus frequency of the epithelial cells in buccal mucosa of hairdressers.*

Keywords: *Hairdresser; micronuclei; genome*

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INTRODUCTION

Hairdressers or hairstylist represent a large and fast growing group of professionals. This is due to people of today's society always want to look good and feel proud to be considered as fashionable or fashion and style conscious. Hairdressers and hairstylists help provide hair care services for those who want to improve their appearance. They offer a wide array of services such as styling, cutting, straightening, curling, and coloring hair. The growing popularity of specialized hairstyling services have contributed to job growth in this occupation.

Hairdressers are exposed daily to a great variety of chemical substances through inhalation, absorption or accidentally ingested.[1] These professionals are exposed to several thousands of chemicals contained in colourants, bleaches, shampoos and hair conditioners. They may also exposed to volatile solvents, propellants and aerosols from hairsprays such as formaldehyde, methacrylates and nitrosamines.[2] Hairdressers and their clients are chronically exposed to variety of chemical and mechanical hair treatment that contain a high number of potentially harmful chemicals, including acetone, benzaldehyde, valeraldehyde,[3] lead acetate, p-phenylenediamine, hydrogen peroxide or bisthmuth citrate.[4] Toxic substances such as thioglycolic acid (TGA), which can absorb through the skin and cause damage to organs and animal systems. TGA caused a disorder in the reproductive cycle of rats and

increased the frequency of micronuclei in bone marrow cells.[5]

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 which are capable of metabolizing carcinogens to reactive products. It is known that chronic exposure of oral mucosa to toxic substances leads to keratinisation with synthesis of keratine bodies.[6] In recent study, excess risk for cancer of the upper aerodigestive tract, lungs, colon, cervical and pancreatic were identified in hairdressers.[5]

There are several biomarkers for studying individuals exposed to known or potential genetic agent. Chromosome alterations, such as chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronuclei, which are directly visible as changes in chromosome structure. Among these biomarkers, micronucleus appears to be the most suitable for epidemiological studies because it is simple and can reflect changes due to early-stage carcinogenesis.[7] Micronuclei originate from chromosomal region lagging or regularly migrating at anaphase. They can derive from acentric chromatid or chromosome fragments produced by chromosome breakage, or from entire chromatids lagging on account of spindle disturbances. In the course of telophase these chromosome regions are included in the daughter cells where they can fuse with the main nucleus or can form one or more smaller secondary nuclei.[8] It has been suggested that micronucleus plays an important role in the occurrence of genetic instability and cancer.[9] The increased micronucleus frequency in exfoliated cells of the buccal mucosa in hairdressers due to daily exposure to harmful chemical substances.[1]

Besides exposure of chemical substances in hair products, genomic damage is produced by lifestyle such as alcohol consumption, smoking habits, tobacco chewing, intake of drugs and stress. Others risk factors are medical procedure for instance radiation and chemicals, micronutrient deficiency, and genetic factors .[10] Ultraviolet light is an environment factor that causes skin cancer. Ultraviolet irradiation may modify DNA base pairs, and produces a reactive oxygen species, which directs cells toward carcinogenesis.[11] The climate of Yogyakarta is generally tropical and receive a great amount of sunlight and ultraviolet irradiation since Yogyakarta is located at the latitudinal and longitudinal of 7 47 S and 110 22 E.[12] Hormones that can affect micronucleus are estrogen and progesterone. Both of these hormones can affect the metabolism and the biochemical system of cells and may induce chromosomal damage.[13]Micronucleus frequency was higher in women than men because of the influence of sex hormones.

Evaluation of micronuclei in epithelial cells of the buccal cavity of the mouth is a method of detection of DNA damage that is non-invasive in humans. Cells observed were cells exfoliated using a cytobrush, carried by staining with Feulgen-Rosssenbeck modified method and observation under a microscope by counting the frequency of micronuclei in epithelial cells.[10] The aim of this study was to determine the effect of exposure to harmful chemical substances in hair product to the micronucleus frequency of buccal mucosa epithelial cells of hairdressers. This study can be used as a pre-research for further investigating research that will be used as a tool for early detection of diseases of the mouth, particularly on the risk of oral cancer due to exposure to harmful chemical substances in hair products.

MATERIALS AND METHODS

A total of 10 female hairdressers were included in this study. A control group composed of 10 female not occupationally exposed as hairdresser was selected. This study case stated after received a letter of ethical clearance issued by the Ethics and Advocacy Unit of the Faculty of Dentistry, Universitas Gadjah Mada (No. 510/KKEP/FKG-UGM/EC/2013) as well as permission letter from Faculty to conduct study case in Salon and Laboratory of Histology. Criteria for subject requited were female hairdressers who exposed to chemical substances in hair products for minimal 2 years, age 18-30, and habits such as alcohol consumption, oral contraceptive and systemic disease were completely omitted. Subject who fulfilled the inclusion criteria and did not have any of the exclusion criteria, were given informed consent forms to sign under ethical clearance as an agreement to take part in this study. Before they signed, explanation about the procedures of the study, their role as a participant and the content of the informed consent form, was given to them. Informed consent form was given to each subject who satisfied the criteria of this study.

Subjects were asked to rinse to avoid contamination of debris in the oral cavity and to remove exfoliated dead cells. Buccal cells were obtained from each individual by gentle brushing of the buccal mucosa with a cytobrush damped with 0.09% NaCl. Epithelial swabbing was done by rotating cytobrush at least 360° on the buccal mucosa. Every subject was swab with the same type of cytobrush with the similar brushing force to attain exfoliated buccal cells. After swabbing the buccal mucosa, cells that had been attached to the cytobrush smeared on glass object by rotating oppose to the direction of rotation on the buccal mucosa. The slides then fixed in a fixation solution (3:1, methanol-acetic) which has been prepared earlier. Staining was performed with modified Feulgen-Rossenbeckmethod.[14] Specimens were immersed in a solution of 5M HCl at room temperature for 15 minutes, then washed with distilled water for 10-15 minutes. The first staining using Schiff's reagent for 90 minutes and followed by staining with fast green counterstain 1% for one minute. Identification is then performed with a light microscope magnification of 200 times and a computer monitor with a magnification

of 100times. A total of at least individual 1000 cells were screened per subject.

Buccal cells were collected and analyzed to a standard protocol described by Titenko-Holland *et al.*[15] Only cells that were not smeared clumped or overlapping and that contained intact nuclei were included in the analysis. Micronucleus was identified according to certain characteristics. The micronucleus should be less than 1/3 diameter of the main nucleus. Micronucleus is smooth oval or round shape and has the same colour, texture and refraction as the main nucleus. The micronucleus is clearly separated from the main nucleus.[15] Cells were observed at 200x magnification with a light microscope to determine the presence of micronucleus cells. Micronucleus performed in dark green with modified Feulgen-Rossenbeck staining method. Number of identified micronucleus was recorded using handy counter. Micronucleus frequencies in units per 1000 cells were recorded. The data obtained were analyzed by using one-way Anova, followed by Post Hoc LSD test to compare duration of exposure among exposure group and also analyzed using independent sample t-test for exposed group and control group.

RESULTS

Micronucleus is round or oval in shape. They are not linked or connected to the main nuclei. The scoring is done according to the criteria which were based on the morphological features of micronucleus cells. In this study, it was found that the diameter of micronuclei varies between 1/16 and 1/3 of the mean diameter of the nuclei and located within cellular cytoplasm (Figure 1). Surprisingly, it was also found that a cell can contain more than one micronucleus as shown in Figure 2.

Micronucleus frequency of exposed group and the control group were expressed as the number of cell that contain micronucleus per 1000 cells. The mean of micronucleus frequency of non-exposed and

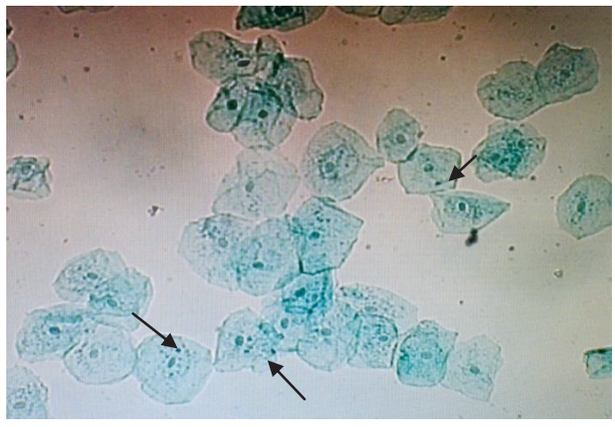

Figure 1. Cells that contain micronucleus (arrow) under magnification of 200.

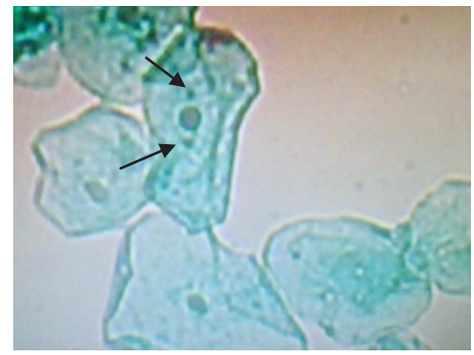

Figure 2. A cell with two micronuclei (arrow) under magnification of 400.

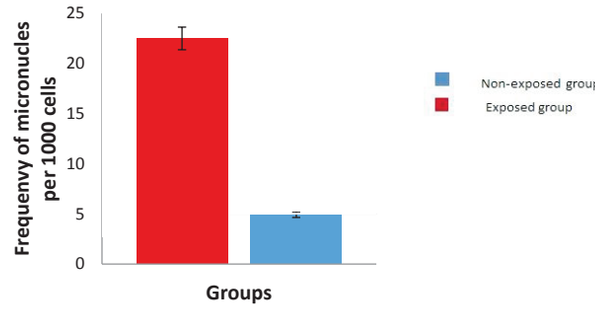

Figure 3. The micronuclei frequency of buccal epithelial cells base on group.

exposed group were showed in Figure 3. There was an increase of micronucleus frequency in the exposed group (hairdressers).

Shapiro-Wilk normality test showed significance values exposed group and control group, respectively for 0.528 and 0.393. This showed that both groups had normal distribution of data. Levene's test showed a p value of 0.01 which indicated that the data did not show homogeneity of variances (alternative hypothesis accepted). The data were further analyzed and the significance of the mean difference between the two groups was determined by using independent samplet-test test. Table 1 shows the summary of the results of independent samplet-test.

Table 1 showed significant differences between the mean micronucleus frequency of exposed group compared to the control group. This indicated that exposure to chemical substances in hair product can increase the frequency of micronuclei in oral buccal epithelial cell.

The data of exposed group then analyzed further to investigate if the duration of exposure will cause an effect in the increase of micronucleus frequency. The summary result of one-way Anova test among exposure group was showed in Table 2. The results of Table 2 showed significant differences in the value of micronucleus frequency exposure duration between groups. This indicated that the increase of micronucleus frequency is directly proportional to the

Table 1. Result of independent sample t-test (p<0.05)

Group	Micronucleus frequencies					
	t	df	Sig.	Mean Diff	Std. Error	CI(95%)
Exposed Non-exposed	10.06	18	0.02**	17.60	1.74	13.924-21.434

Tabel 2. Summary results of one-way Anova test among exposure duration subgroup (p <0.05)

Duration of exposure	Mean and standard deviation of micronucleus	F	Sig.
< 5 years	16.00±2.0	36.347	0.00**
6-10 years	21.50±2.1		
>10 years	26.80±1.5		

Tabel 3. Summary results of Post Hoc LSD test duration of exposure between exposed subgroup (p<0.05)

	5 years	6-10 years	>10 years
<5 years		0.011**	0.000**
6-10 years	0.011**		0.008**
>10 years	0.000**	0.008**	

duration of exposure.

This result shows there was an increase in the frequency of micronuclei in subjects who work as hairdresser with a longer duration. Furthermore, LSD Post Hoc test was used to determine the significance of differences between groups micronucleus frequency duration of exposure and the results was summarized in Table 3. The result revealed a significant increase in the frequency of micronuclei with the increase of duration of exposure.

DISCUSSION

Hairdressers and their clients are chronically exposed to chemical and mechanical hair treatments that contain a high number of potentially harmful chemicals. Many of these chemicals are either known or suspected allergens, carcinogenic or mutagens.[16] The micronucleus test in exfoliated cells of buccal mucosa is a potentially excellent biomarker to detect genome damage.[10]

Hairdressers have a significant increase in micronucleus frequency compared to control group. This result in line with the study of Galiotte *et al.*[16] that stated the increase in genetic damage could be associated in part with the occupational conditions to which hairdressers are constantly exposed to potentially hazardous chemical products.

There was an increase of micronucleus frequency with the duration of exposure. This result was supported by the study of Jeffrey that stated the DNA damage, mutagenic and genotoxic effect increase with the duration of exposure to chemical composition in hair product.[17] The increase length of time exposed to harmful substances will increase the risk for adverse health effects.

Chemical substances found in hair product include amines, resorcinol, hydrogen peroxide, ammonium, formaldehyde. Hair dyes contain a variety of chemical agents, some which are considered proven, probable or possible human carcinogens.[18] Many permanent and semi-permanent dyes used by hairdressers were shown to contain aromatic amine derivates such as 2-amino-4 nitrophenol, 2-amino-5 nitrophenol and 1,4-diamino-2-nitrobenzene, many of which are mutagenic.[3] Aromatic amines used in oxidative hair dyes were identified as mutagenic or carcinogenic in rodents.[14] Ammonium releaser such as ammonium chloride or ammonium phosphate are used in the process of hair dye in order to improve the hair penetration so that hair strand could open up and absorb color. Formaldehyde is substance used in hair straightening solutions and have been found carcinogeic by the Internatioal Agency for Research on Cancer.[3]

Protective equipments are also used to avoid or reduce irritation due to exposure of hazardous substances in working environment.[22] In this study, we found that the micronucleus frequency were increase, this phenomena could caused by the indiscipline behavior of the hairdresser. Some of the subjects in this study admitted their improper usage of protective equipment. They do not adjust their face masks to fully cover up their nose and mouth during hair dying or straightening process. Besides that, a few of the subjects do not change their gloves during hair dying process when the gloves were torn. Some of the regular customers of the Salon told that the hairdresser actually did not even put on any gloves or face mask. Many of the hairdressers also told that protective equipments are inconvenient and uncomfortable to put on. Personal protective equipment (PPE) protocols is one of the most crucial elements to protect worker's health in any workplace. Policy and protective protocol in the Salon were not followed and carried out by most of the hairdresser. Improper usage of protective equipment will increase risk of toxic exposure.

The oral cavity is the first barrier of the inhalation or ingestion of carcinogens and may play a role in metabolism to a reactive substance.[23] Buccal mucosa is very sensitive to toxic exposure and readily to form micronucleus.[24]

Inhalation and dermal absorption are considered the most frequent routes of exposure to chemicals agent among hairdressers.[21] Personal protective equipment (PPE) may be the most practical and effective way of minimizing genotoxic risk. Examples of personal protective equipment in hair salons are gloves, aprons, protective masks and eyes protection.

Micronucleus is an effective biomarker of disease and process is associated with the induction of DNA or genome damage. The scoring is done based on the number of cell that contain micronucleus but not the number of micronucleus in each cell. This scoring was done in this study for cell that has more than 1 micronucleus as well as the cell that contains 2 micronuclei as shown in figure 5. This study in line with the study of Pawitan, in which stated that the diameters of micronuclei are almost 1/3 of the mean diameter of the nuclei and must be located within cellular cytoplasm. A cell can contain 1-3 micronuclei. There can be more than one micronucleus forming when more genetic damage has happened due to more chromosomal breakage in anaphase.[25]

Micronucleus is genotoxicity biomarkers in human erythrocytes, lymphocytes, reticulocytes, and buccal mucosa cells.[26] According to Takkouche, hairdresser has a higher risk of cancer than the general population. The risk increase is substantial for lung, larynx, bladder cancer and multiple myeloma. An increased risk was observed for cancer of pancreas, aerodigestive tract, cervix, in situ of the skin and colorectal adenocarcinoma in hairdresser. [18]

In this study, micronucleus also found in non exposed group. This is because a normal healthy person also contain about 1-9 micronucleus per 1000 cells of buccal mucosa epithelial cell.[27] Micronucleus frequency was higher in women than men because of the influence of sex hormones.[1] Hormones that can affect micronucleus are estrogen and progesterone. Both of these hormones can affect the metabolism and the biochemical system of cells and may induce chromosomal damage.[13] Hence, only female were chosen as the inclusive criteria of the subject. The increase in micronucleus frequency in females can be accounted for by the greater tendency of the X Chromosome to be lost as an micronucleus relative to other chromosome and females have two copies of chromosome compared to only one in male. Thus subject selected were all female and age range between 18-35.[28,29]

Other than exposure of harmful chemical substance, smoking, alcohol consumption, stress, pharmacotherapy and intake of drugs are risk factor of micronucleus.[10] In this study, subjects selected were those who without smoking habit and alcohol consumption. Cigarette contains several carcinogens. These materials activate in different tissues, which cause the DNA adducts products. The time influence on DNA adducts has controversial results.[30] It has been shown that the synergic effect of cigarette smoking and alcohol consumption is more than 5.5 fold.[31] None of the subjects were alcohol consumers, so the role of confounding factors especially consumption of alcohol has omitted completely. Hairdresser should be aware of the increase of micronucleus frequency since forming of micronuclei in associated with the induction of DNA or genome damage.[26]

In conclusion, chemical substances of hair products had affected the micronucleus frequency of the epithelial cells in buccal mucosa of hairdressers.

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