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Increased micronucleus frequency in exfoliated cells of the buccal mucosa in hairdressers

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ABSTRACT. Hairdressers are exposed daily to chemical substances, such as dyes, chemical straighteners and curling chemicals, which can be absorbed, inhaled or possibly ingested. We analyzed the frequency of micronuclei (MNC) in exfoliated cells of the buccal mucosa of 50 hairdressers and 50 controls in Pelotas, RS, Brazil. An assessment was carried out on the incidence of MNC, binucleated cells (BNC), broken egg cells (BEC), budding cells (BC), and the sum of anomalies (SA), in 2000 cells per individual. The data were analyzed with SPSS, using the Mann-Whitney U-test, $\alpha = 0.05$. The mean number of anomalies in hairdressers was 2.02 ± 3.60 MNC; 8.50 ± 5.07 BNC; 9.06 ± 3.83 BEC; 0.32 ± 0.62 BC, and 19.90 ± 9.61 SA; in controls it was 0.36 ± 1.06 MNC; 5.20 ± 4.73 BNC; 5.92 ± 2.67 BEC; 0.10 ± 0.36 BC, and 11.58 ± 6.67 SA; the differences for all parameters were significant. The non-occupational factors did not significantly influence the alterations. A significant increase of BEC ($P = 0.003$) was observed in the hairdressers and SA ($P = 0.033$) in females. The lowest income level influenced MNC ($P = 0.044$), and the habit of not smoking influenced SA ($P = 0.020$). We concluded that exposure to substances used by hairdressers is genotoxic for men.

Key words: Hairdressers; Micronucleus tests; Exfoliated cells; Micronucleus

INTRODUCTION

Damage to the genome is probably the most important and fundamental cause of the development of anomalies and degenerative diseases. It has been established that genomic damage is produced by exposure to genotoxic substances, medical procedures (radiation and chemicals), micronutrient deficiency (folic acid), life style (alcohol, smoking, drugs, stress), and genetic factors, such as defects in metabolism and/or in the repair of DNA. Hence, it is essential to perform biomonitoring with minimally invasive markers. The micronucleus trial in exfoliated cells of the buccal mucosa is a potentially excellent biomarker candidate for monitoring studies (Holland et al., 2008).

The micronucleus test in buccal mucosa cells is one of the less invasive methods to measure DNA damage in humans. This test was proposed in 1983 and continues to gain in popularity as a biomarker of genetic damage (Stich and Rosin, 1983). And its information can be used as an early warning of potential risk of developing long-term health problems (Au, 1991).

Hairdressers are exposed daily to a great variety of chemical substances, which can be absorbed, inhaled or possibly ingested. Hair treatment includes dying, discoloring and straightening reagents. The coloring used can contain oxidants and dying substances, like p-phenylenediamine or 2-nitro-p-phenylenediamine and even hydrogen peroxide, whereas the progressive colorings contain metallic salts, such as lead acetate or bismuth citrate (Cho et al., 2003). Thioglycolic acid is used in the straightening or curling process, which can potentially damage the health of users and the hairdresser (Gan et al., 2003).

Few studies have been performed on hair coloring. However, there is a relationship between the increase in chromosome aberrations and the use of substances involved in the coloring and straightening of hair (Taioli, 1999). Cho et al. (2003) performed a study with 20 women who used dyes, the composition of which was p-phenylenediamine, m-aminophenol, 2,4-dichlorodiamino-phenoxyethanol, N-phenyl-p-phenylenediamine sulfate and hydroxypropyl-bis (N-hydroxyethyl-p-phenyldiamine). This study was performed on volunteer women who dyed their hair regularly and who had continued to do so over a number of years. In this study, the comet assay of peripheral blood was performed on these volunteer women and significant differences of damage to DNA were found after the use of coloring.

This study had the purpose of investigating the frequency of micronucleus while a biological marker for the cellular exposure to carcinogens, in exfoliated cells of the oral mucosa of hairdressers, seeking to demonstrate the priority and urgency in taking measures that regulate the use and exposure to harmful substances.

MATERIAL AND METHODS

All volunteers answered a questionnaire, in accordance with the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens (Carrano and Natarajan, 1988). They provided information on occupational and non-occupational exposure, habits and diet, with free and clarified consent. The project was approved by the Ethics Committee of the Universidade Católica de Pelotas.

To obtain cells of buccal mucosa, an oral scraping (right and left sides) was performed on every individual, with the help of a wooden tongue depressor, previously washed with filtered water. After finishing the scraping, each tongue depressor was transferred to a centrifuge tube containing phosphate, pH 6.8. Subsequently, they were centrifuged for 10 min and then

fixed with methanol:acetic acid (3:1), hydrolysis was done with 1 N HCl at 60°C for 10 min and the coloring of the slides was performed with Schiff-fast-green, according to the methodology described by Roth et al. (2008).

The analysis of the cells was done under a common optical microscope, binocular, with an objective of 100X and oculars of 10X. Two thousand cells per individual (exposed group and non-exposed group) were observed and the results are reported as number of cells with micronucleus (MNC), broken egg cells (BEC), binucleated cells (BNC), and cells with buds (BC) (Figure 1), registered in specified files. Only non-fragmented, non-accumulated, non-overlaid cells, as well as those containing an intact nucleus were considered. Criteria used to identify a micronucleus were established by Picker and Fox (1986).

The data obtained were codified, tabulated, and stored in the database of the SPSS 10.0 for Windows program, using the Mann-Whitney U-test, Kruskal-Wallis for analysis and Spearman's rho correlation, with a probability of 0.05 or less.

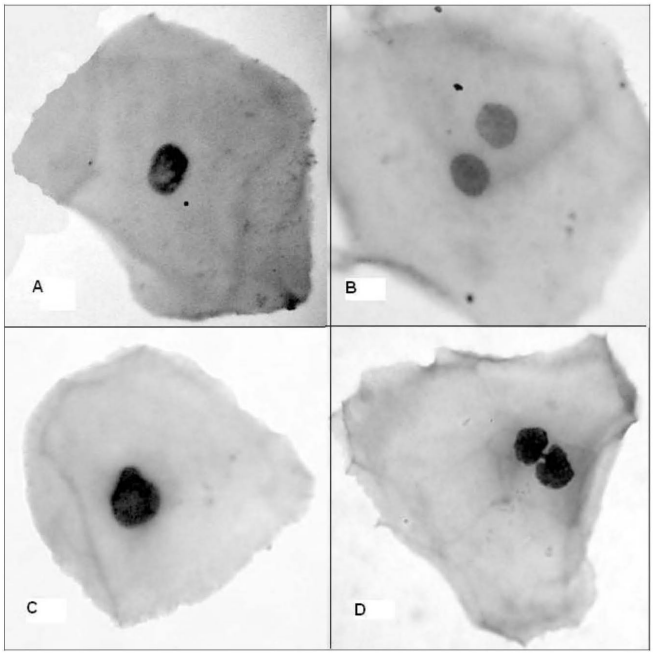

Figure 1. Nuclear anomalies. **A.** Cell with micronucleus. **B.** Binucleated cells. **C.** Cell with bud. **D.** Broken egg cells. Source: Genetics Laboratory of the Universidade Católica de Pelotas.

RESULTS

The main socio-demographic characteristics of the exposed group (cases) and the non-exposed group (controls) are described in Table 1. Both groups were characterized for gender, age, working time, family income, smoking and alcohol consumption, dermatological (dermatitis), ophthalmological (irritation) and respiratory (coughing, sinusitis, bronchitis) diseases, and central nervous system symptoms (cephalalgia, dizziness).

Table 1. Socio-demographic profile of hairdressers and controls in the city of Pelotas, RS.

Variable	Case (N = 50)	Control (N = 50)
Gender		
Female	25 (50%)	25 (50%)
Male	25 (50%)	25 (50%)
Age (years)		
Minimum	15	18
Maximum	66	65
Mean ± SD	37.40 ± 12.09	37.02 ± 12.00
Working time		
Minimum	0.1 month	0.7 month
Maximum	42 years	30 years
Mean ± SD	10.13 ± 9.17	7.25 ± 6.90
Family income (reais, R$)		
Minimum	500	0
Maximum	6000	7600
Mean ± SD	1.799 ± 1.262	2.289 ± 1.627
Smoking habits		
Yes	19 (38%)	11 (22%)
No	31 (62%)	38 (76%)
Drinking habits		
Yes	36 (72%)	34 (68%)
No	14 (28%)	16 (32%)
Dermatological disease		
Yes	19 (38%)	16 (32%)
No	30 (60%)	33 (66%)
Ophthalmalogical disease		
Yes	18 (36%)	20 (40%)
No	30 (60%)	30 (60%)
Respiratory disease		
Yes	26 (52%)	19 (38%)
No	23 (46%)	31 (62%)
Symptoms of CNS		
Yes	30 (60%)	20 (40%)
No	18 (36%)	29 (58%)

SD = standard deviation; CNS = central nervous system (cephalalgia, dizziness).

In Table 2, the average and standard deviations are shown for the number of MNC, BNC, BEC, BC, and SA of both groups investigated. The evaluation of the frequency of the micronuclei in exfoliated cells of oral mucosa revealed a significant increase (P = 0.0001) in the individuals exposed to chemical products in relation to the non-exposed group (control). The same was observed in relation to BNC (P = 0.0001), BEC (P = 0.0001), BC (P = 0.029), and SA (P = 0.0001).

Table 2. Average and standard deviation of the number of cells with micronuclei, binucleated cells, broken egg cells, cells with buds, and sum of anomalies observed in 2000 cells of hairdressers and controls in the city of Pelotas, RS.

Anomalies	Hairdressers (N = 50)	Controls (N = 50)
MNC		
Mean ± SD	2.02 ± 3.60	0.36 ± 1.06
P	0.0001	
BNC		
Mean ± SD	8.50 ± 5.07	5.20 ± 4.73
P	0.0001	
BEC		
Mean ± SD	9.06 ± 3.83	5.92 ± 2.67
P value	0.0001	
BC		
Mean ± SD	0.32 ± 0.62	0.10 ± 0.36
P	0.029	
SA		
Mean ± SD	19.90 ± 9.61	11.58 ± 6.67
P	0.0001	

SD = standard deviation; MNC = cells with micronuclei; BNC = binucleated cells; BEC = broken egg cells; BC = cells with buds; SA = sum of anomalies.

Table 3 shows that some socio-demographic variables, habits and diseases influenced on the incidence of micronuclei. Also classified were age, working time and family income in hairdressers and controls. The results demonstrated that female hairdressers presented a higher frequency of anomalies than males. However, only the differences in relation to BEC and SA were significant (P = 0.003 and 0.033, respectively). In relation to age, the eldest age group presented a higher number of anomalies in general. The same fact repeated itself in relation to working time.

Lower income brackets also presented a higher number of alterations than higher income brackets, presenting a negative correlation (P = 0.05). Those who did not smoke, nor drink alcoholic beverages presented more anomalies than the others in control and case groups.

The hairdressers presented complaints of dizziness and cephalalgia that were designated by symptoms of the CNS. This fact was important, because a higher incidence of alterations was observed in this group, although there was only a significant increase in BC (P = 0.013) in controls.

DISCUSSION

The group of hairdressers evaluated presented five times the number of cells with micronuclei than the control group, indicating that the hairdressers are exposed to substances that are harmful to their genetic material. These substances are contained in dyes, straighteners and curlers used by these professionals in the processes of dying, straightening and curling hair.

There is little data in the literature evaluating the effects of these substances on health. The study carried out thus far shows conflicting results. In rats, Rotenberg et al. (1969) found an acute toxicological effect. In rabbits and in rats, Tyl et al. (2003) found toxic effects through chronic inhalation. However, in the Ames test the result was negative.

According to Correa et al. (2000), there is a correlation between people who use hair dye and leukemia. However, the type of product, the frequency of use and the duration of the dye were not ascertained. Another study done in Japan by Nagata et al. (1999) also suggests that there is a relationship between myelodisplastic syndromes and users of hair dye.

Kersemaekers et al. (1995) investigated possible reproductive alterations in Dutch hairdressers, evaluating 9000 hairdressers and comparing them to 9000 controls. They discovered that the female hairdressers presented longer times of pregnancy, a higher rate of abortions, and children with lower weights. They also verified a larger frequency of malformations in the children of these female hairdressers. This finding was corroborated by Keshava and Ong (1999), who state that workers exposed to genotoxic agents can also present genetic alterations in germinative cells, resulting in greater reproductive problems, with an estimated 50% greater chance of the loss of fetus, 30% of mentally retardation, 20% of congenital malformations, and 2% of infertility in men.

The products used by hairdressers contain toxic substances like thioglycolic acid (TGA), which can be absorbed through the skin and cause damage to organs and animal systems. The percutaneous administration of TGA caused a disorder in the reproductive cycle of rats and increased the frequency of micronuclei in bone marrow cells. Taking into account these results, Gan et al. (2003) recommended studies on humans that would evaluate reproductive and mutagenic problems. In a subsequent case study and control, Gan et al. (2003) evaluated

Table 3. Average number of cells with micronuclei, binucleated cells, broken egg cells, cells with buds, and sum of anomalies in relation to the socio-demographic variables analyzed in hairdressers and controls in the city of Pelotas, RS.

Variables	MNC		BNC		BEC		BC		SA	
P*	CA	CO	CA	CO	CA	CO	CA	CO	CA	CO
Gender										
Female	2.44 ± 4.46	0.32 ± 1.22	9.20 ± 5.34	5.96 ± 5.98	10.76 ± 3.76	6.04 ± 3.12	0.44 ± 0.71	0.12 ± 0.33	22.84 ± 10.97	12.44 ± 8.28
Male	1.60 ± 2.48	0.40 ± 0.91	7.80 ± 7.80	4.44 ± 2.93	7.36 ± 3.13	5.80 ± 2.20	0.20 ± 0.50	8.00 ± 0.40	16.96 ± 7.09	10.72 ± 4.56
P*	0.548	0.776	0.306	0.518	0.003	0.206	0.176	0.970	0.033	0.799
Age (years)										
15 to 25	1.58 ± 2.15	0.23 ± 0.83	8.42 ± 5.43	4.15 ± 2.41	8.75 ± 4.03	6.38 ± 2.36	0.41± 0.66	0	19.16 ± 7.83	10.76 ± 3.39
26 to 50	2.07 ± 4.17	0.37 ± 1.16	8.57 ± 5.22	5.03 ± 4.23	8.90 ± 4.01	5.07 ± 1.93	0.20 ± 0.48	0.16 ± 0.46	19.73 ± 10.82	10.63 ± 5.38
51 to 66	2.50 ± 3.30	0.67 ± 1.21	8.38 ± 4.53	8.83 ± 8.95	10.13 ± 3.00	9.00 ± 4.29	0.62 ± 0.91	0.00 ± 0.00	21.62 ± 7.78	18.50 ± 13.24
P (2x3)**	0.605	0.401	0.996	0.463	0.520	0.017	0.271	0.259	0.718	0.142
Working time (years)										
0.1 to 8	1.54 ± 1.90	0.37 ± 1.16	8.73 ± 5.05	4.80 ± 3.24	9.23 ± 4.56	5.17 ± 2.04	0.26 ± 0.60	0.13 ± 0.43	19.76 ± 9.08	10.46 ± 4.71
9 to 19	2.20 ± 3.12	0.00 ± 0.00	8.33 ± 4.62	6.17 ± 6.37	9.20 ± 2.88	5.00 ± 0.63	0.40 ± 0.73	0.16 ± 0.40	20.13 ± 6.54	11.33 ± 6.31
20 to 42	3.50 ± 7.37	0.75 ± 1.50	8.50 ± 6.65	11.00 ± 10.68	8.88 ± 2.80	9.50 ± 5.57	0.37 ± 0.51	0	21.25 ± 15.72	21.25 ± 16.13
P**	0.726	0.473	0.776	0.533	0.944	0.185	0.662	0.710	0.586	0.341
Family income (reais - R$)										
0 to 1700	2.71 ± 4.40	0.58 ± 1.50	9.18 ± 5.38	5.74 ± 5.01	9.32 ± 3.74	5.32 ± 2.00	0.39 ± 0.68	5.26 ± 0.20	21.60 ± 10.46	11.68 ± 6,54
1750 to 3840	0.83 ± 2.29	0.20 ± 0.70	6.58 ± 3.80	5.25 ± 5.38	7.67 ± 2.71	6.70 ± 3.33	8.33 ± 0.28	0.20 ± 0.52	15.16 ± 5.60	12.35 ± 8.00
3850 to 8000	0.67 ± 0.58	0.14 ± 0.38	8.33 ± 4.93	3.71 ± 2.14	11.33 ± 7.51	5.43 ± 2.07	0.33 ± 0.57	0	20.66 ± 13.20	9.28 ± 3.81
P**	0.044	0.614	0.256	0.748	0.352	0.561	0.339	0.376	0.770	0.599
Smoking habits										
Yes	0.74 ± 0.81	0.36 ± 0.92	6.58 ± 2.67	3.36 ± 3.14	8.00 ± 2.21	6.64 ± 2.01	0.26 ± 0.56	9.09 ± 0.30	15.57 ± 3.70	10.45 ± 5.42
No	2.81 ± 4.37	0.37 ± 1.13	9.68 ± 5.83	5.76 ± 5.05	9.71 ± 4.46	5.74 ± 2.85	0.35 ± 0.66	0.10 ± 0.38	22.54 ± 11.12	11.97 ± 7.09
P*	0.129	0.852	0.124	0.036	0.093	0.104	0.668	0.928	0.020	0.405
Drinking habits										
Yes	2.36 ± 4.07	0.29 ± 0.80	8.72 ± 5.10	5.21 ± 4.48	8.58 ± 3.71	6.15 ± 3.00	0.33 ± 0.67	5.88 ± 0.34	20.00 ± 10.07	11.70 ± 6.88
No	1.14 ± 1.75	0.50 ± 1.51	7.93 ± 5.14	5.19 ± 5.36	10.29 ± 3.99	5.44 ± 1.79	0.28 ± 0.46	0.18 ± 0.40	19.64 ± 8.66	11.31 ± 6.43
P*	0.383	0.732	0.508	0.489	0.203	0.776	0.817	0.066	0.871	0.883
Symptoms of SNC										
Yes	2.60 ± 4.21	0.55 ± 1.47	9.27 ± 5.76	6.70 ± 6.66	9.40 ± 4.33	6.30 ± 3.60	0.33 ± 0.60	0.25 ± 0.55	21.60 ± 10.91	13.80 ± 9.46
No	1.22 ± 2.37	0.24 ± 0.69	7.50 ± 3.82	4.14 ± 2.49	8.67 ± 2.47	5.79 ± 1.74	0.27 ± 0.66	0	17.66 ± 6.54	10.17 ± 3.36
P*	0.069	0.537	0.507	0.320	0.562	0.585	0.523	0.013	0.141	0.424

Data are reported as average ± standard deviations. CA = cases (hairdressers); CO = controls; MNC = cells with micronuclei; BNC = binucleated cells; BEC = broken egg cells; BC = cells with buds; SA = sum of anomalies. *Mann-Whitney U-test; **Kruskal-Wallis test. $P < 0.05$ compared to the variable groups.

hairdressers in China, using a sample of 65 women between the ages of 20 and 40 years, with an average working time of 6 to 7 years and exposed constantly to straighteners, the composition of which was 12% TGA (99% purity), 8% ammonia solution, approximately 2 g sodium carbonate and 80% deionized water. In this study, Gan et al. (2003) found anomalies in the menstrual cycle and an increase in cells with micronuclei in female hairdressers. They concluded that the reproductive function of the female hairdressers can be affected after long periods of exposure.

Some studies report an important role that a combination of risk factors can have in the formation and elevation of MNC, such as the effect of the occupational factors combined with socio-demographic factors (age), habits (smoking, alcohol), nutritional state, and chronic and infectious diseases (Bolukbas et al., 2006).

The difference in the frequency of MNC between genders has been well documented in biomonitoring studies, being considerably higher in women. This fact has been justified by various factors, including the loss of the chromosome-X. Fenech (1998) reported 1.2 to 1.6 more micronuclei in women than in men. Our data point to a ratio of 1.5 MNC for women over men. However, this figure has been quite controversial in other studies.

In relation to age, an increase in micronuclei may be due to the increase in the age of the individuals, in both case and control groups, as was expected, based on other study. The same occurred in relation to the working time, although this increase was not statistically significant for the two variables.

Family income was classified in three levels, and it was verified that those with a lower income presented a higher number of MNC than those with a higher income. Family income is related to purchasing power and consequently to the intake of food, that is, with improved access to proper nutrition for those with a higher income. There is strong evidence that vitamin deficiency is associated with chromosome damage and incidence of MNC. According to Battershill et al. (2008), the function of micronutrients like folic acid and vitamin B12 is associated with the synthesis and repair of DNA. Hence the results found in this study in relation to income can be related to the nutritional habits of the individuals, which give rise to a better or worse quality of life.

There is a clear relationship between cancer and exposure to carcinogen substances present in tobacco, although when the association and frequency of MNC is analyzed, it is not so clear. There are studies that show a significant increase in MNC in smokers (Fenech, 1993). However, other studies have not found significant differences or even a decrease in MNC in smokers (Battershill et al., 2008), similar to the data found in this study.

The use of alcoholic beverages can be associated with some kinds of head and neck cancer, and can lead to an increase in the formation of micronuclei. The results found in the literature are controversial, and in this study no relevant result was found for this factor.

Some studies report an increase of allergic and respiratory problems in hairdressers (Gan et al., 2003); however, in the current study this fact was not verified, although a significantly higher number was detected in people with symptoms of the CNS (cephalalgia, dizziness) in hairdressers than in controls. Nevertheless, only the number of BC was significantly higher in controls.

Based on these results, it can be concluded that hairdressers present an increase in cells with micronuclei and other anomalies, probably due to the genotoxic action of the substances to which they are exposed and that age, working time, and alcohol use did not influence the frequency of nuclear alteration in the workers exposed and evaluated in this study.

Only family income presented a negative correlation to MNC, which could have a connotation with quality of life.

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