

**EFFECT OF LONG TERM USE OF HAIR DYES ON THE DNA DAMAGE IN
HEALTHY FEMALE SUBJECTS**
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ABSTRACT :To date, the toxic effects of hair dyes on DNA have been investigated by several in vivo and in vitro tests and various conflicting results have been reported. To clarify this toxicity, we used "comet assay" which is a new and more sensitive method and it was used first time for the determination the effect of long term use of hair dyes on the DNA damage. Thirty-nine healthy female subjects were selected who have been using hair dyes for 4 to 12 years, and 24 healthy females who had never used hair dyes before were studied as a control group. The rate of damaged (limited and extensive migrated) cells in the study group were significantly higher than that of the control group ($p < 0.05$) indicating a detectable DNA damaging effect of hair dyeing on human lymphocytes. In conclusion, we suggest that the caution should be recommended in subjects whose family history of cancer is positive. Although our conclusion, however, can not be extended to the developing fetus in utero, women had better not use hair dye especially before and during pregnancy.

[Key words: Hair dyes, teratogenicity, DNA damage, comet assay]

INTRODUCTION

Hair dyes are widely used as cosmetic agents to change the color of hair, and/or to color the gray hair around the World in recent years. It is more common in women. There are some side effects of hair dyes such as allergic and irritant contact dermatitis (most common), leukoderma, photosensitivity, purpuric eruptions, angioedema, urticaria and rhinitis, asthma, syncope (1-5). The most important side effect is possibly the toxicity on DNA. This possible toxic effect is more important in women considering the possibility of pregnancy; if such toxicity is effective, it is clear that they are at greater risk of adverse pregnancy outcomes, including complications which may affect the fetus. Congenital

malformations have received the most attention. They may have caused a greater risk of developing congenital malformations and malignancy than are those in the general population. Although the first trimester of pregnancy, in particular week 2 to 8 after fertilization, is the most critical period, some malformations may occur throughout the pregnancy. The decision to continue or terminate hair dyeing during pregnancy for fetal safety should be made and its toxicity on DNA should also be considered. Several investigations were conducted to determine this toxicity, but conflicting results were reported.

The comet assay, also called single-cell gel electrophoresis, is a simple, yet powerful tool for demonstrating the damaging

effects of different compounds or physical treatments on DNA at the individual cell level. It permits quantitative evaluation of DNA-damaging or apoptosis inducing mechanism and agents in various target cell population. Electrophoresis of target cells suspended in agarose, causes the migration of unwound or fragmented DNA out of the cells resulting a comet-like appearance that can be imaged microscopically by appropriate fluorescent staining. Advances in DNA damage detection have allowed scientists to determine the impact of certain environmental factors or medical treatments on DNA integrity. It is used both in vitro and in vivo tests. The introduction of alkaline conditions makes the relationship with single-strand breakage more obvious. (6-8). Although other methodologies exist for detecting damaged DNA, as the comet assay have some advantages (9-11), it is rapidly increasing in popularity.

The purpose of this study was to determine the toxic effects of hair dyes on DNA using the comet assay in the peripheral blood lymphocytes of the women who have been using hair dyes for a long time, because this assay is a new and more sensitive method and has not been used on this subject until now.

MATERIALS AND METHODS

Thirty-nine healthy females who have used hair dyes for at least four years, and a control group consisted of 24 healthy females were selected from the neighborhood where the study group lived, and they had never used hair dyes. In both groups, ages were between 25 and 43, and all the subjects were not smoking, not using any contraceptive or any other long lasting drugs, and having normal menstrual cycles. To our knowledge, neither the study group nor the control subjects were exposed to other mutagenic agents (e.g., radiation, chemicals, lifestyle) for years, and none of them presented any chronic or neoplastic diseases. Age, frequency of dyeing, and usage period were also recorded. All subjects were

healthy. The blood samples were taken at 20th and 27th days following from the beginning of their menstrual bleeding.

Five ml of blood was carefully layered over 8 ml Lymphocyte Separation Medium and centrifuged at 2000 x g for 15 min. After the plasma layer was removed and kept, the buffy coat was carefully removed and the cells were washed with TC-199 medium and then collected by 10 min centrifugation at 1000 x g. Lymphocytes were resuspended using approximately 10^7 / ml in TC-199 medium with 20% v/v plasma and 10% v/v plasma and v/v DMSO. Lymphocytes were transferred to microfuge tubes and stored at -20°C. The comet assay was performed as described previously (12). Comets from as broken ends of the negatively charged DNA molecule becomes free to migrate in the electric field towards the anode. The assay provides direct determination of the extend of DNA damage in individual cells and the extent of DNA damage can be assessed from the length of DNA migration which is derived by subtracting the diameter of the nucleus from the total length of the image. The degree of damage was determined by grading the cells as; normal (undamaged - no migration), limited migration (at low damage levels, stretching of attached strands of DNA, rather than migration of individual pieces is likely to occur), and extensive migration (with increasing numbers of breaks, DNA pieces migrate freely into the tail forming comet images). A minimum of 100 cells were analyzed for each subject. Slides were scored blindly by two independent investigators. Student's t-test analysis was used in the statistical evaluations of the data. The effects of age, frequency and usage period in the study group were also investigated by regression analysis.

An appropriate institutional review board approved the project and informed consent was obtained from the subjects after the nature of the procedures had been explained fully.

RESULTS

The mean age of the study subjects was 34.9 ± 4.9 years and that of the control group was 32.1 ± 6.0 years. The duration of hair dyeing until the study ranged from 4 to 12 years, with a mean of 8.4 ± 2.4 years. The mean frequency of hair dyeing was once per 1.6 ± 0.8 months (ranged from 1 to 3 months). Twenty-four healthy females of similar age and socioeconomic background were studied simultaneously as controls. The statistical comparison of the ages in two groups showed no significant difference. However, the rate of damaged (limited and extensive migrated) cells in hair dyeing females were higher than that of the controls ($p < 0.05$ and $p < 0.01$). There were no significant correlation noted between comet scores and age, frequency, and usage period.

DISCUSSION

Potential carcinogenic and teratogenic effects of hair dyes have been evaluated by various tests, but the results were not consistent. In an investigation to evaluate the toxicity of two chemicals in the semi-permanent hair dyes, it has been found that one of them produce statistically significant elevation of micronuclei in female mice (13). In another investigation, it has been speculated that two hair dye components generate active oxygen species causing DNA damage which leads to the carcinogenesis (14). Positive associations between hair dye use and the development of the cancer in urinary tract also have been reported (15,16). In a case-control study carried out to examine the relationship of personal hair dye use and environmental factors to myelodysplastic syndromes, it has been found that there were statistically significant trends in risk with increasing duration and number of hair dye use and occupational exposure was also marginally associated with this risk (17). In a similar study, their results were modestly supportive of the hypothesis that exposure to hair dyes, particularly dark hair dyes, is a risk factor for

myeloid leukemia and refractory anemia with excess of blasts (18). In a multicenter case-control study on risk factors for acute leukemia and preleukemia, a moderate leukemogenic effect of hair dyes use was suggested (19).

Contrary to these results above, analysis of data failed to show any significant effects of hair dyeing in an epidemiological survey of bladder cancer (20). No association was observed between hair dye use and cutaneous malignant melanoma, either (21). In a case-control study, it has been found that neither duration nor average frequency of hair dyeing was related to breast cancer, and risk was also unaffected by darkness of color used (22).

When hair dyes are used frequently, especially during pregnancies, their toxic effect (teratogenicity) should be considered. In a pair-matched case-control study on the gestational risk for Wilms' tumor, the use of hair-coloring products was strongly associated with cases in which Wilms' tumor was diagnosed before 2 years of age (23). A relationship between hair dye use and the risk of another childhood tumor, neuroblastoma was also reported (24). On the other hand, in a large national collaborative clinical trial which examined the nonoccupational risk factors for Wilms' tumor, no association was found with maternal exposure to hair coloring products during pregnancy (25).

Several studies have been published about the toxic effect of hair dyes on DNA, but none of them is based on the comet assay analysis. In comparison with other methods currently used in human biomonitoring, the comet assay seems to be able to identify low level exposures with greater sensitivity (26-28). In the present study, this toxicity of hair dyes was investigated by alkaline comet assay for the first time. The comet scores between the hair dyeing group and control group were significantly different ($p < 0.05$). There was a statistically significant increase at the damaged cells in the peripheral lymphocytes of hair dyeing women. Moreover, the frequency of extensive migrated cells was also significantly

higher in the study group compared to that of the controls ($p < 0.01$). We could not find any significant relation between age, frequency and usage period. The factors that may have influence on the comet scores (age, sex, race, nutrition, environment etc.) were similar in both groups. Physiological factors that may have effects on DNA are reproductive hormones; evaluation of SCE frequencies during a normal menstrual cycle demonstrated a higher rate of ovulation, and in the luteal phase as compared to the early follicular phase (29). In our study, all the subjects (patients and the control group) were at the same phase of the menstrual cycle (within 20th and 27th days following the beginning of menstrual

bleeding) at the time of sampling. All the factors that may have influences on the comet scores were similar in both groups. Therefore, we think that the difference in the comet scores was induced by the hair dye application for a long-time.

In conclusion, our results support that long-term use of hair dye in adult female healthy subjects appear to have some toxic effects on DNA. We suggest that caution should be recommended in subjects whose family history of cancer is positive. Although our conclusion, however, can not be extended to the developing fetus in utero, women had better not use hair dye especially before and during pregnancy.

Table : Statistical results.

	CONTROL		HAIR DYE		p
	X ± SD	N	X ± SD	N	
Age	32.1±6.0	24	34.9±2.4	39	0.1
Undamaged	95.0±2.7	24	87.2±2.98	39	0.3
Limited damaged	3.3±1.8	24	7.7±1.27	39	0.03
Extensive damaged	1.75±1.7	24	5.1±2.75	39	0.009

X: mean, SD: Standard deviation

REFERENCES

1. Belton AL, Chira T: Fatal anaphylactic reaction to hair dye. *Am J Forensic Med Pathol*, 18: 290-292, 1997
2. Garcia Ortiz JC, Terron M, et al: Contact allergy to henna. *Int Arch Allergy Immunol*, 114: 298-299, 1997
3. Sahoo B, Handa S, Penchallaiah K, et al: Contact anaphylaxis due to hair dye. *Contact Dermatitis*, 43: 244., 2000
4. Santucci B, Cristaudo A, Cannistraci C, et al: Hypertrophic allergic contact dermatitis from hair dye. *Contact Dermatitis*, 31: 169-171, 1994
5. Wang GH, Jiang XF, Luo WC: Report of 2 cases of extrinsic allergic alveolitis and review of relevant literature. *Zhonghua Jie He He Hu Xi Za Zhi*, 17: 367-368, 1994
6. Singh NP, McCoy MT, Tice RR, et al: A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, 175: 184-191, 1988
7. McKelvey-Martin VJ, Green MH, Schmezer P, et al: The single cell gel electrophoresis assay (comet assay): a European review. *Mutat Res*, 288: 47-63, 1993

8. Fairbairn DW, Olive PL, O'Neill KL: The comet assay: a comprehensive review. *Mutat Res*, 339: 37-59, 1995
9. Tafazoli M, Kirsch-Volders M: In vitro mutagenicity and genotoxicity study of 1,2-dichloroethylene, 1,1,2-trichloroethane, 1,3-dichloropropane, 1,2,3-trichloropropane and 1,1,3-trichloropropene, using the micronucleus test and the alkaline single cell gel electrophoresis technique (comet assay) in human lymphocytes. *Mutat Res*, 371: 185-202, 1996
10. Frenzilli G, Betti C, Davini T, et al: Evaluation of DNA damage in leukocytes of ex-smokers by single cell gel electrophoresis. *Mutat Res*, 375: 117-123, 1997
11. Hartmann A, Speit G: The contribution of cytotoxicity to DNA-effects in the single cell gel test (comet assay). *Toxicol Lett*, 90: 183-188, 1997
12. Baltaci V, Aygun N, Akyol D, ve ark. : Chromosomal aberrations and alkaline comet assay in families with habitual abortion. *Mutat Res*, 417: 47-55, 1998
13. Parton JW, Beyers JE, Garriott ML: Mutagenicity evaluation of HC Blue No. 1 and HC Blue No. 2, I. Effect on the induction of micronuclei in mouse bone marrow cells. *Mutat Res*, 241: 139-144, 1990
14. Chen F, Oikawa S, Hiraku Y, et al: Metal-mediated oxidative DNA damage induced by nitro-2-aminophenols. *Cancer Lett*, 126: 67-74, 1998
15. Ohno Y, Aoki K, Obata K, et al: Case-control study of urinary bladder cancer in metropolitan Nagoya. *Natl Cancer Inst Monogr*, 69: 229-234, 1985
16. Nomura A, Kolonel LN, Yoshizawa CN: Smoking, alcohol, occupation, and hair dye use in cancer of the lower urinary tract. *Am J Epidemiol*, 130: 1159-1163, 1989
17. Nagata C, Shimizu H, Hirashima K, et al: Hair dye use and occupational exposure to organic solvents as risk factors for myelodysplastic syndrome. *Leuk Res*, 23: 57-62, 1999
18. Mele A, Szklo M, Visani G, et al: Hair dye use and other risk factors for leukemia and pre-leukemia: a case-control study. *Am J Epidemiol*, 139: 609-619, 1994
19. Mele A, Stazi MA, Pulsoni A, et al: Epidemiology of acute promyelocytic leukemia. *Haematologica*, 80: 405-408, 1995
20. Sullivan JW: Epidemiologic survey of bladder cancer in greater New Orleans. *J Urol*, 128: 281-283, 1982
21. Osterlind A: Malignant melanoma in Denmark. Occurrence and risk factors. *Acta Oncol*, 29: 833-854, 1990
22. Koenig KL, Pasternack BS, Shore RE, et al: Hair dye use and breast cancer: a case-control study among screening participants. *Am J Epidemiol*, 133: 985-995, 1991
23. Bunin GR, Kramer S, Marrero O, et al: Gestational risk factors for Wilms' tumor: results of a case-control study. *Cancer Res*, 47: 2972-2977, 1987
24. Kramer S, Ward E, Meadow AT, et al: Medical and drug risk factors associated with neuroblastoma: a case-control study. *J Natl Cancer Inst*, 78: 797-804, 1987
25. Olshan AF, Breslow NE, Falletta JM, et al: Risk factors for Wilms tumor. Report from the National Wilms Tumor Study. *Cancer*, 72: 938-944, 1993
26. Vodicka P, Bastlova T, Vodickova L, et al: Biomarkers of styrene exposure in lamination workers; levels of 06-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. *Carcinogenesis*, 16: 1473-1481, 1995
27. Binkova B, Lewtas J, Miskova I, et al: Biomarkers studies in the Northern Bohemia. *Environ. Health Perspect*, 104: 591-597, 1996

28. Andreoli C, Leopardi P, Crebelli R: Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. *Mutat Res*, 377: 95-104, 1997
29. D'Souza D, Thomas IM, Das BC: Variation in spontaneous chromosomal damage as a function of biologic rhythms in women. *Hum Genet*, 79: 83-85, 1988

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