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The Oxidative Stress by Hair Dyeing Changes the Antioxidant Defense Systems and Strongly Relates to the Plasma Vitamin E Concentration*

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Reactive oxygen species can be generated in the skin by hair dyeing. The aim of this study was to find out the effects of the oxidative-type hair dye application in young women on the antioxidant systems. We investigated the lipid peroxide levels, glutathione (GSH) levels, and the antioxidant enzyme activities including superoxide dismutase (SOD), glutathione peroxidase (GSHPx) in plasma and erythrocytes and catalase (CAT) in erythrocytes, and DNA damages in lymphocytes. Also, plasma concentrations of antioxidant vitamins, vitamin A and E, were measured and the correlations between various antioxidant parameters and oxidative damages were evaluated. The antioxidant enzyme activities in plasma (GSHPx) and in erythrocytes (SOD and CAT) were decreased significantly after hair dyeing. The lipid peroxide and GSH levels were not affected in both plasma and erythrocytes. No significant difference was found in the concentrations of both vitamin A and E between before and after hair dyeing. However, DNA damages expressed as the tail extent moment (TEM) and tail length (TL) were significantly ($p<0.001$) increased. The plasma vitamin E concentration was correlated with DNA damages (TEM: $r=-0.590$, $p<0.01$ and TL: $r=-0.533$. $p<0.01$) and RBC SOD activity ($r=0.570$, $p<0.05$). In turn, RBC SOD activity was significantly correlated with both plasma MDA levels ($r=-0.412$, $p<0.05$) and DNA damages (TM: $r=-0.546$, $p<0.01$, TL: $r=-0.493$, $p<0.01$). Our results demonstrated that the exposure to hair dyeing produced lymphocyte DNA damage and modification of the antioxidant enzyme activities. Also, there were very strong associations between plasma vitamin E concentration, RBC SOD activity and DNA damage induced by hair dyeing. It suggests that the antioxidant status of a subject is likely to be related to the extent of the harmful effects caused by hair dyeing.

Key words: Oxidative stress, Hair dyeing, Antioxidant enzymes, Plasma vitamin E, Lymphocyte DNA damage

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INTRODUCTION

The endogenous antioxidant system is able to deal with reactive oxygen species (ROS) under normal conditions, however, when this system cannot handle ROS, oxidative stress arises. Oxidative stress induces a variety of cellular insults including aging, DNA mutations leading to cancer and immunosuppression and pathogenesis of other diseases.[1)] The endogenous antioxidant system contains catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase as enzymatic antioxidants as well as nonenzymatic antioxidants including glutathione, uric acid, alpha-lipoic acid and antioxidant vitamins, such as vitamin A and E.

Hair dyes are widely used around the world. It has been estimated that over one-third of women above the age of 18 in Europe, North America and Japan[2)] and over 89% of women above the age of 20 years in Korea use some type of hair dye. Among the types of hair dye, the oxidative-type dyes, in which hydrogen peroxide is used to oxidize aromatic amines, account for about 75% of the annual hair-dye market.[3)] P-phenylenediamine is the main aromatic amine in hair dye and a frequent cause of contact sensitization in an exposed person,[4)] which is likely to be related to the generation of oxygen radicals.[5)] It has been reported that most of commercial oxidative-type hair-dye formulations are mutagenic *in vitro*,[6)] and carcinogenic in experimental animals.[7)] Also, small amounts of these potentially carcinogenic substances are

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absorbed percutaneously during normal use.[8,9] Recent studies showed that acute exposure to hair dyes could cause oxidative DNA damage in lymphocytes.[10] However, topically pre-treated antioxidants, such as alpha-tocopherol and superoxide dismutase, effectively protected the skin cells from oxidative damage induced by ultraviolet light.[11]

In order to find out the effects of the oxidative-type hair dye application in young women on oxidative damages, we investigated the antioxidant parameters (TBARS levels, glutathione levels, ROS scavenging enzyme activities in plasma and erythrocytes, and DNA damages in lymphocytes). Also, we measured the plasma concentrations of antioxidant vitamins, vitamin A and E, and evaluated the correlations between various antioxidant parameters and oxidative damages induced by hair dye to suggest some appropriate way to attenuate the harmful effects of hair dye on the tissues exposed.

MATERIALS AND METHODS

1. Subjects

Thirty young women of early twenties were recruited and provided black hair dyeing according to the manufacture's instructions. The ingredients of hair dye were p-phenylenediamine (1.8%), p-aminophenol (0.3%), m-aminophenol (0.1%), resorcinol (1.0%) and others. All of the subjects were informed and consented about participation in this study.

2. Sample Preparations

Blood samples were collected by venipuncture from each subject before and 6 h after hair dyeing, and centrifuged at 3000 rpm for 20 min and separated to plasma and blood cells. For studying DNA damage in the Comet assay, lymphocytes were separated using Histopaque 1077 from heparinized whole blood. The separated lymphocytes were washed, resuspended with PBS, and used for the Comet assay immediately.

3. Antioxidant Enzyme Activities and Glutathione (GSH) Levels

Glutathione peroxidase (GSHPx) activity in plasma and erythrocyte was measured spectrophotometrically (Hitachi U-3010, Japan) at 37 ℃ and 340 nm according to the method of Paglia *et al.*.[12] The GSHPx values were expressed as umole NADPH oxidized/min/L and umole NADPH oxidized/min/g Hb, respectively.

The superoxide dismutase (SOD) activity in plasma and erythrocyte was assayed by the method described by Flohe *et al.*.[13] This method is based on the reduction of superoxide, which is produced by xanthine/xanthine oxidase system, by cytochrome C. One unit of SOD was determined as the amount that reduces cytochrome C's reduction by 50%. Results are expressed as U/L and U/g Hb, respectively.

The catalase (CAT) activity in erythrocyte was measured spectrophotometrically by the method of Aebi *et al.*[14] and expressed as k/g Hb.

The reduced glutathione (GSH) content of the plasma and erythrocyte was measured using fluorometric method (Hitachi F-4500, Japan) of Hissin *et al.*[15] The GSH levels were expressed as mmole/L and umole/g Hb, respectively.

4. Lipid Peroxidation

The end-product of polyunsaturated fatty acid peroxidation, thiobarbituric acid reacting substances (TBARS), was determined by the method of Yagi *et al.*.[16] The values of TBARS were expressed in terms of MDA umol/L for plasma and umole/g Hb for RBC.

5. Plasma Vitamin A and E Concentrations

Plasma concentrations of vitamin A and E were assayed by the HPLC method of Bieri *et al.*[17] using reverse phase C18 column, and expressed as ug/mL plasma.

6. Comet Assay

The comet assay was performed under alkaline conditions essentially following the procedure of Singh *et al.*[18] Fully frosted slides were covered with 0.65% of normal agarose as the first layer, a mixture of cell suspension and 0.65% of low melting agarose (LMA) as the second layer, and finally with 0.65% of LMA as the third layer. The slides were allowed to solidify at 4 ℃ in the dark for 5 min, and placed in cold lysis buffer and kept at 4 ℃ for 1 hr, and placed in a horizontal electrophoresis tank. The slides were left in the electrophoresis solution for 20 min to allow DNA unwinding and expression of alkali labile damage before electrophoresis. Electrophoresis was conducted at 4 ℃ for 20 min using 25 V and 300 mA. After electrophoresis, the slides were neutralized in neutralization buffer, stained with ethidium bromide, and examined using a fluorescence microscope (200x, Leica). Images of 50 randomly selected cells from each slide and two slides per each sample were analyzed with an image analysis system (Version 5.0, Kinetic Imaging, UK). The results were expressed in terms of DNA Tail length in micrometers and Tail extent moment (the fraction

of DNA in the tail multiplied by the tail length).

7. Statistics

Statistical analysis was performed with SPSS version 11. The results were given as mean±SD. Differences before and after hair dyeing were tested with paired-t test. The correlations between variables were evaluated with Pearson's correlation coefficient.

RESULTS AND DISCUSSION

The effects of hair dyeing on the antioxidant enzyme activities, GSH levels and lipid peroxidation in plasma and erythrocytes are shown in Table 1. The antioxidant enzyme activities of SOD, GSHPx and CAT were determined before and 6 h after hair dyeing. The decrease of SOD activities was observed both in plasma (24%) and in erythrocytes (18%), but the changes in plasma were not significant. The GSHPx activity in plasma (24%, $p<0.001$) and CAT activity (15%, $p<0.05$) in erythrocytes were also significantly decreased after hair dyeing. The GSH levels in both plasma and erythrocytes remained constant despite of changes in antioxidant enzyme activities 6 h after hair dyeing. Also, the amount of MDA production was not affected by hair dyeing in both plasma and erythrocytes.

Oxidative stress can be defined as any derangement between pro-oxidants and antioxidants in which the former prevail.[1] Picardo *et al.*[19] reported that treatment with hair dye induced an oxidative stress due to the generation of free radicals during spontaneous oxidation of its components. Also, they observed a decrease in antioxidant enzyme activities following the exposure to the hair dye. Our findings were consistent with the results of Picardo *et al.*. They treated normal human keratinocytes with p-phenylenediamine, an aromatic amine and a contact allergen in hair dye, to investigate the cause of contact sensitization and found that the antioxidant systems, including SOD, CAT and GSH, were decreased and membrane lipid peroxidation was increased depending on pPD concentrations and the period of exposure. Similarly in this study, the involvement of antioxidants as a response to the oxyradicals formed after hair dye treatment was shown by the modifications of SOD, GSHPx and CAT activities in plasma and erythrocytes. The lower enzyme activities observed after hair dyeing were most likely due to the enzyme inactivating activity of ROS induced by the ingredients of hair dye. But hair dye application had different effects on the activities of antioxidant enzymes in plasma and erythrocytes. There were only negligible changes in GSH and MDA levels in erythrocytes and plasma after hair dye application.

The concentrations of the plasma antioxidant vitamins, vitamin A and E, were measured before and 6 h after hair dyeing. Table 2 shows that no significant difference was found in the concentrations of both vitamin A and E between before and after hair dyeing.

Several studies have indicated that topical alpha-tocopherol applications have photoprotective effects on the epidermis.[20-22] Alpha-tocopherol is the major lipid-soluble chain-breaking antioxidant in the blood plasma and membranes,[23] and acts as a scavenger of free radicals or singlet oxygen.[21] The protective effects of plasma antioxidant vitamins on the skin alterations by hair dyeing can be expected but has not been demonstrated yet. As we observed in antioxidant enzyme activities, it could be expected that the plasma antioxidant vitamin levels were lowered due to the oxidative stress induced by hair dyeing. But the concentrations of the vitamins have not changed. It could be assumed that 6 hrs after hair dyeing was not enough time to affect the plasma concentrations of these antioxidant vitamin levels.

The induction of DNA damage in human lymphocytes has been compared after the treatment of hair dyeing in the Comet assay (Table 3). Six hours after hair dyeing,

Table 1. Effects of hair dyeing on plasma and erythrocyte antioxidant enzyme activities, glutathione and TBARS levels.

	Before	After
Plasma		
SOD (U/L)	6770.6±2196.9	6139.2±2594.7^{ns}
GSHPx (umole/min/L)	105.8±11.0	78.1±12.6***
GSH (mmole/L)	1.04±0.13	1.06±0.11^{ns}
TBARS (umole/L)	10.33±2.79	11.74±6.55^{ns}
Erythrocyte		
SOD (U/g Hb)	2697.9±733.0	2222.5±741.6**
GSHPx (umole/g Hb)	13.20±2.53	12.61±2.26^{ns}
CAT (k/g Hb)	371.2±62.3	328.7±108.3*
GSH (mmole/g Hb)	6.56±0.85	6.49±1.24^{ns}
TBARS (umole/g Hb)	55.94±23.94	55.47±10.44^{ns}

Each value represent the mean±SD of 30 subjects.
The value with an asterisk is significantly different from before dyeing value by paired t-test (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).
Ns: not significant.
Abbreviations: SOD, superoxide dismutase: GSHPx, glutathione peroxidase: CAT, catalase: GSH, glutathione: TBARS, thiobarbituric acid reactive substances

Table 2. Effects of hair dyeing on plasma vitamin A and E concentrations in subjects. (ug/ml)

	Before	After
Vitamin A	0.323±0.061	0.344±0.142^{ns}
Vitamin E	3.73±0.68	4.03±0.92^{ns}

Each value represent the mean±SD of 30 subjects.
Ns: not significant.

Table 3. Effects of hair dyeing on lymphocyte DNA damage in subjects.

	Before	After
Tail Extent Moment (au)	0.25±0.05	0.58±0.44*
Tail Length (um)	5.88±0.62	8.07±2.27*

Each value represent the mean SD of 30 subjects.
The value with an asterisk is significantly different from the value before dyeing by paired t-test (*: $p<0.001$).

the tail extent moment (TEM) and tail length (TL) were significantly ($p<0.001$) higher than before the hair dyeing. TEM was increased from 0.25±0.05 to 0.58±0.44, and TL from 5.88±0.62 to 8.07±2.27, before and 6 h after hair dyeing, respectively.

Biological markers, associated with genotoxic changes, may be useful for improving the prediction of risk to human health posed by environmental exposure.[24] The Comet assay, the alkaline version in particular, has become a very popular method for the analysis of DNA damage caused by various chemical and physical agents because of its simplicity and rapidity.[25] Cavallo *et al.* investigated the possible correlation between hair dye exposure and direct-oxidative DNA damage, production of tumor necrosis factor alpha (TNFa) and allergic inflammatory diseases in 19 hairdressers. He suggested that occupational exposure to hair dye could induce DNA damage, increase the TNFa levels and induce allergic sensitization.[6] A recent study demonstrated that acute exposure of hair dyes caused DNA damage in lymphocyte.[26] In this study, we could observe the DNA damage induced by hair dyeing. When comparing the parameters of DNA damage in Comet assay, tail length and tail moment were increased by 137% and 232%, respectively, after hair dyeing compared to those before dyeing. Watanabe *et al.* has reported that pPD, a key ingredient of oxidative hair dyes, may become mutagenic in the presence of hydrogen peroxide.[27] But other studies has reported that the activity was disappeared or dramatically decreased in the presence of coupler substances such as resorcinol in hair dyeing formulation. Since commercial oxidative hair dyes do not contain dye bases such as pPD alone but always include couplers, the activity of pPD plus peroxide alone is not relevant for a human health risk.[28] A number of genotoxicity studies in humans were published in the scientific literatures. For example, two studies in female volunteers exposed to up to 13 cumulative hair dye applications found no increased incidence in sister chromatid exchanges (SCEs) or chromosome breaks/aberrations in peripheral lymphocytes.[29,30] Moreover, no evidence of increased incidence of DNA strand breaks or SCEs in peripheral lymphocytes or Salmonella-positive mutagenic activity in the urine was found in 15 heavily exposed hairdressers.[31] *In vitro* genotoxicity tests on hair dye ingredients frequently had positive results, although their correlation with *in vivo* carcinogenicity for the oxidative hair dye ingredients (aromatic amines) is uncertain. Therefore, it is noteworthy that even positive *in vivo* genotoxic activity of a hair dye may not always suggest a risk to human health, and more human study is required to investigate the effects of hair dye on human health.

In order to see whether the plasma antioxidant vitamins are correlated with the different antioxidant parameters and DNA damage, the correlation coefficients were calculated between variables 6 h after hair dyeing (Table 4). The correlation coefficient (r) between plasma vitamin E concentration and DNA damages expressed as TEM and TL was -0.590 ($p<0.01$) and -0.533 ($p<0.01$), respectively.

Table 4. Correlation coefficients (r) between variables after hair dyeing in subjects.

		Plasma				Erythrocyte					Plasma vitamins		DNA damage	
		SOD	GSHPx	GSH	TBARS	SOD	GSHPx	CAT	GSH	TBARS	Vt. A	Vt. E	TEM	TL
Plasma	SOD	1												
	GSHPx	-0.001	1											
	GSH	-0.214	-0.275	1										
	TBARS	0.228	-0.037	-0.088	1									
Erythrocyte	SOD	-0.107	0.203	0.006	**-0.412***	1								
	GSHPx	-0.107	-0.178	0.145	-0.270	0.085	1							
	CAT	-0.009	-0.023	-0.231	0.181	-0.322	-0.266	1						
	GSH	-0.061	0.090	-0.316	-0.049	-0.011	-0.290	**0.442***	1					
	TBARS	-0.094	-0.086	-0.157	-0.257	0.251	-0.068	-0.261	0.105	1				
Plasma	Vt. A	-0.014	-0.039	-0.117	-0.237	-0.161	0.113	0.336	0.280	-0.169	1			
vitamins	Vt. E	-0.035	0.043	0.167	-0.110	**0.570***	0.164	-0.040	-0.177	-0.286	0.014	1		
DNA	TEM	0.041	0.109	-0.055	0.109	**-0.546****	-0.074	0.125	0.117	0.096	0.314	**-0.590****	1	
damage	TL	0.072	0.210	0.052	0.030	**-0.493****	-0.030	0.017	0.064	0.104	0.235	**-0.533****	**0.941*****	1

The value with an asterisk is significantly correlated. (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)

The plasma vitamin E concentration was also significantly correlated with RBC SOD activity ($r=0.570$, $p<0.05$). In turn, RBC SOD activity was inversely correlated with both plasma TBARS levels ($r=-0.412$, $p<0.05$), and DNA damages as TEM ($r=-0.546$, $p<0.01$), TL ($r=-0.493$, $p<0.01$). We also analyzed the correlations between variables before hair dyeing, and between before and after hair dyeing (data not shown). Before hair dyeing, there were no correlations between plasma vitamin E and RBC SOD ($r=0.194$), and RBC SOD and TEM ($r=-0.057$). But the strong correlation between plasma vitamin E level before hair dyeing and RBC SOD activity after hair dyeing ($r=0.614$, $p<0.001$) was observed. The results revealed a very strong association between plasma vitamin E concentration, RBC SOD activity and DNA damage induced by hair dyeing. It suggests that the plasma vitamin E, which scavenges reactive oxygen species produced by hair dyeing as well as protects the cell membranes exposed to hair dye from oxidative damage, reduces the lipid peroxidation and SOD inactivation by ROS, therefore protects cells from DNA damage.

Vitamin E is the major lipid-soluble chain-breaking antioxidant in the plasma and membranes.[23] Hairless mice were fed diets containing different levels of vitamin E or received topical applications of vitamin E for three weeks before a single exposure of ultraviolet light. The results suggested that both dietary and topical vitamin E were effective in protecting the epidermis against some of the early damages induced by UV radiation as found in the decreased lipid peroxidation and DNA damage in skin epidermis.[32] Hamanaka *et al.* reported that topical SOD protected skin from photo-oxidative damage.[33] However, there is no report about the skin protecting efficacy of dietary or topical vitamin E and SOD from the oxidative damage induced by hair dyeing. Therefore, further study is required to investigate if the dietary or topical use of antioxidant vitamins, especially vitamin E, protects the skin from oxidative stress induced by hair dyeing. Also, the use of antioxidant substances can be suggested as an alternative way to reduce the possible health risk caused by hair dyeing.

Hair dyes and their ingredients have moderate to low acute toxicity. The use of hair dyes has dramatically increased in industrialized countries during the last decades, and the prevalence of sensitization to hair dyes in the general and professional populations has increased. Our results demonstrated that exposure to hair dyeing produced lymphocyte DNA damage and modification of the antioxidant enzyme activities. Also, there were very strong associations between plasma vitamin E concentration, RBC SOD activity and DNA damage. It suggests that the antioxidant status of subjects is likely to be related to the extent of harmful effects of hair dyeing. Therefore, further research is required to investigate the relations between the antioxidant status and the toxicity of hair dyeing in human.

Literature Cited

1) Halliwell B, Gutteridge JMC. Protection against lipid peroxidation. In: free Radicals in Biology and Medicine. 2nd ed pp.92-98, Oxford, Clarendon Press, 1989
2) La Vecchia C, Tavani A. Epidemiological evidence on hair dyes and the risk of cancer in humans. *Eur J Cancer Prev* 4:31-43, 1995
3) Voluntary filing of cosmetic product ingredient composition statements (21 CFR 720). Food and Drug Administration. FDA, 2002
4) Picardo M, Cannistraci C, Cristaudo A. Study on cross-reactivity to the para group. *Dermatologica* 181:104-108, 1990
5) Picardo M, Zompetta C, Marchese C. Paraphenylene diamine, a contact allergen, induces oxidative stress and ICAM-1 expression in human keratinocytes. *Br J Dermatol* 126:450-455, 1992
6) Ames BN, Mccann J, Yamasaki E. Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. *Proc Nat acad Sci* 72:2423-2427, 1975
7) Sontag JM. Carcinogenicity of substituted-benzenediamines (phenylenediamines) in rats and mice. *J Natl Cancer Inst* 66:591-602,1981
8) Maibach HI, Leaffer MA, Skinner WA. Percutaneous penetration following use of hair dyes. *Arch Dermatol* 111:1444-1445, 1975
9) Marshall S, Palmer WS. Dark urine after hair colouring. *JAMA* 226:1010, 1973
10) Cavallo D, Ursini CL, Setini A, Chianese C, Cristaudo A, Iavicoli S. DNA damage and TNFalpha cytokine production in hairdressers with contact dermatitis. *Contact Dermatitis* 53(3):125-129, 2005
11) Hamanaka H, Miyachi Y, Imamura S. Photoprotective effect of topically applied superoxide dismutase on sunburn reaction in comparison with sunscreen. *J Dermatol* 17(10):595-598, 1990
12) Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70(1):158-169, 1967
13) Flohe L, Otting F. Superoxide dismutase assays. *Methods in Enzymology* 105:93-104, 1984
14) Aebi H. Catalase in vitro. *Methods in Enzymology* 105: 121-126,1984
15) Hissin PJ, Hilf R. A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Anal Biochem* 74:214-226, 1976

16) Yagi K. Assay for blood plasma or serum. *Methods in Enzymology* 105:328-331, 1984
17) Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by HPLC. *Am J Clin Nutr* 32:2143-2149, 1979
18) Singh NP. DNA damage and repair with age in individual human lymphocytes. *Mut Res* 237:123-130, 1990
19) Picardo M, Zompetta C, Grandinetti M, Ameglio F, Santucci B, Faggioni A, Passi S. Paraphenylene diamine, a contact allergen, induces oxidative stress in normal human keratinocytes in culture. *Br J Dermatology* 134:681-685, 1996
20) Quevedo WC Jr, Holstein TJ, Dyckman J, McDonald CJ, Isaacson EL. Inhibition of UVR-induced tanning and immunosuppression by topical applications of vitamins C and E to the skin of hairless mice. *Pigment Cell Res* 13:89-98, 2000
21) McVean M, Liebler DC. Inhibition of UVB induced DNA photodamage in mouse epidermis by topically applied alpha-tocopherol. *Carcinogenesis* 18:1617-1622, 1997
22) Eberlein-Konig B, Placzek M, Przybilla B. Protective effect against sunburn of combined systemic ascorbic acid and d-alpha-tocopherol. *J Am Acad Dermatol* 38:45-48, 1998
23) Kagan VE, Packer L. Light-induced generation of vitamin E radicals: Assessing vitamin E regeneration. *Methods Enzymol* 234:316-320, 1994
24) Mercier MJ, Robinson AE. Use of biologic markers for toxic end-points in assessment of risks from exposure to chemicals. *Int Arch Occup Environ Health* 65(1):7-10, 1993
25) Cebulska-Wasilewska A, Nowak D, Niedzwiedz W, Anderson D. Correlations between DNA and cytogenetic damage induced after chemical treatment and radiation. *Mut Res* 421:83-91, 1998
26) Cho JA, Oh E, Lee E, Sul D. Effects of hair dyeing on DNA damage in human lymphocytes. *J Occup Health* 45:376-381, 2003
27) Watanabe T, Kawata A, Koyanagi T. Mutagenicity of hair dyes. *J Cosmetic Chem Japan* 41(1):20-25, 1980
28) Bracher M, Faller C, Grotsch W, Marshall R, Spengler J. Studies on the potential mutagenicity of p-phenylenediamine in oxidative hair dye mixtures. *Mut Res* 241:313-323, 1990
29) Hofer H, Bornatowicz N, Reindl E. Analysis of human chromosomes after repeated hair dyeing. *Food and Chemical Toxicology* 21(6):785-789, 1983
30) Turanitz K, Kovac R, Tuschi H, Pavlicek E. Investigations on the effects of repeated hair dyeing on sister chromatid exchanges. *Food and Chemical Toxicology* 21(6):791-793, 1983
31) Sardas S, Augun N, Karakaya AE. Genotoxicity studies on professional hair colorists exposed to oxidation hair dyes. *Mut Res* 394:153-161, 1997
32) Record IR, Dreosti IE, Konstantinopoulos M, Buckley RA. The influence of topical and systemic vitamin E on ultraviolet light-induced skin damage in hairless mice. *Nutr Cancer* 16(3-4):219-225, 1991
33) Hamanaka H, Miyachi Y, Imamura S. Photoprotective effect of topically applied superoxide dismutase on sunburn reaction in comparison with sunscreen. *J Dermatol* 17(10):595-598, 1990