SCE Frequency Analysis in the Lymphocytes of the Healthy Turkish Women who are Regular Users of Hair Dye

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Abstract

Although hair dyes are widely used cosmetic agents, their toxic effect on DNA has not been entirely demonstrated. These are of primary importance in women, particularly those who are pregnant or lactating, since any potential toxic effect on DNA becomes more significant. On the other hand, compositions of hair dyes vary in different countries because of differences in regulations. Therefore, toxicity studies should be taken under consideration for every countries. In the present study, the potential genotoxic effects of long term use of hair dyes (minimum 5 years) were investigated by means of incidence of sister chromatid exchange (SCE) using the peripheral blood lymphocytes of healthy Turkish women at 25-43 age range. The mean frequency of SCE in women using hair dyes (n=39, mean SCEs/cell ± SD: 14.26 ± 3.05) was significantly higher (P < 0.0001) than that of the control group (n=24, mean SCEs/cell ± SD: 8.5 ± 1.59). Regarding age, hair color, usage period and the frequency of hair dyeing there was no significant difference in the SCE scores. In conclusion, when used long period of time, hair dyes sold in Turkish markets have toxic effects on DNA, and we suggest that these preliminary data should also be confirmed by other toxicology detection methods.

Keywords: *DNA damage, genotoxicity, hair dye, SCE*

Düzenli Saç Boyası Kullanıcısı olan Sağlıklı Türk Kadınlarında SCE Sıklık Analizi

Özet

Saç boyası sık kullanılan bir kozmetik ajan olmasına rağmen, DNA üzerine toksik etkisi yeterince açık değildir. Kadınlarca daha sık kullanıldığından, özellikle hamile-

lik ve süt verme durumunda böyle bir toksik etki daha da önemli hale gelir. Öte yandan, saç boyalarının yapısı çeşitli ülkelerdeki kurallara göre değişiklik göstermektedir.Bu nedenle, bu olası toksik etki her ülkede araştırılmalıdır. Bu çalışmada, yaşları 25-43 arasında değişen sağlıklı Türk kadınlarının periferik lenfositlerinde sister chromatide exchange (SCE) yöntemi kullanılarak uzun süre (en az 5 yıl) saç boyası kullanımının genotoksik etkisinin olup olmadığı araştırıldı. Kontrol grubu ile (n=24, ortalama SCE sıklığı/hücre ± SD: 8.5 ± 1.59) karşılaştırıldığında ortalama SCE sıklığının saç boyası kullanan kadınlarda (n=39, ortalama SCE sıklığı/hücre ± SD: 14.26 ± 3.05) önemli derecede arttığı saptandı (P < 0.0001). Saç boyası kullanan kadınların yaşı, kullanım süresi, boyanın rengi ya da kullanım sıklığı dikkate alındığında SCE sonuçlarında önemli bir farklılık gözlenmedi. Sonuç olarak Türkiye'de satılmakta olan saç boyaları uzun süre kullanıldığında DNA üzerinde toksik etki oluşmakta olup bu öncül verilerin diğer toksikoloji testleriyle de doğrulanmasının faydalı olacağını düşünmekteyiz.

***Anahtar kelimeler**: DNA hasarı, genotoksisite, saç boyası, SCE*

Introduction

Hair dyes are cosmetic agents used worldwide to change the color of hair and/or to color the gray hair. There are three main groups of hair dyes; 1) Botanical, 2) Metallic, 3) Synthetic. Each group contains different chemical substances and their actions change related to those compounds. Moreover, their compositions vary according to the regulations accepted by the legal authorities in the country where they are manufactured and/or sold. Therefore, the important side effects should be investigated specifically for each country. Some common side effects such as allergic and most commonly irritant contact dermatitis, leukoderma, photosensitivity, purpuric eruptions, angioedema, urticaria and rhinitis, asthma, syncope were reported in various countries (1-5). Hair dying is a common issue among women. Particularly, those at productive ages are subjects to teratogenesis if the compounds used in manufacturing of dying agents have toxic effect on the DNA. They are at 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 through out the pregnancy. It is a common idea that toxicity of hair dyes on DNA should be considered and the decision to continue or terminate hair dyeing before or during pregnancy for fetal safety should also be made.

DNA damage is known as one of the major mechanisms responsible for teratogenesis and carcinogenesis. Most chemical and physical agents causing DNA damage, such as various chemotherapeutic, antineuplastic drugs and ultraviolet have an influence on sister chromatid exchange (SCE) frequency (6), which is known to result from reciprocal DNA interchange in homologous loci of sister chromatids in the replication process and it occurs spontaneously at certain rates in all cells (7). Therefore, SCE analysis has come into use as a sensitive means of monitoring DNA damage.

The aim of this study is to evaluate the association of exposure to hair dyes with DNA damage by SCE analysis in the peripheral blood lymphocytes of the healthy Turkish women who have been using hair dyes for a long period of time.

Materials and Methods

39 healthy females who have been using hair dyes for at least five years except the one used for 2 years and the one for 4 years have been selected for the study. A control group consisted of 24 healthy females not using hair dyes and matched to the patient group according to their age were selected randomly from the neighborhoods where the study group lived. Both the study and the control groups were between the ages 25 and 43, not smoking, not using any contraceptive or any other long lasting drugs, and all of them had 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), and none of them presented chronic or neoplastic diseases by the time the study was conducted. Age, frequency of dyeing, usage period and hair color were also recorded to evaluate. The study was approved by the hospital's research ethics board and informed consent was provided from all patients. The blood samples were taken from the control and patient groups within 20th and 27th days following the beginning of their menstrual bleeding. All subjects were healthy at the time of sampling.

Peripheral venous blood was drawn aseptically into heparinized tubes from each subject. 200 Ìl of the whole blood was added within the same day of sampling to 5 ml medium TC 199 (Gibco) supplemented with 10 % fetal calf serum (Gibco), 2 % phytohemagglutinin (Sigma), 5 Ìg/ml 5-bromodeoxyuridine (Sigma), 150 U/ml penicillin and 150 Ìg/ml steptomycin. Cultures were incubated in the dark for 68 h at 37_C. After the treatment with demecoline (Colcemide, Gibco, 0.1 Ìg/ml) for 3 hours, microscope slides were prepared by a conventional method and stained by fluorescence plus the Giemsa technique of Perry and Wolff (8). The mean SCE frequency was calculated as SCE per cell from 20 selected cells per individual.

Statistical evaluations were performed using SPSS 10.0 (SPSS Inc, Chicago, Il, USA) statistical package program. Comparisons between the study and control groups were based on Student's t-test. The effects of age, hair-dye color, frequency and usage period in the study group were assessed using Pearson correlation, Kruskal-Wallis one way variance analysis and Mann-Whitney U test.

Results

Clinical data and the mean frequencies of SCE per cell in hair dyeing and control women are presented in Table 1. The difference between the mean ages of two groups (study group: 34 ± 5.5 yr, control: 32 ± 6 yr) was insignificant ($P > 0.05$).

The mean frequency of SCE in women using hair dyes (n=39, mean SCEs/cell ± SD: 14.26 ± 3.05) was significantly higher ($P < 0.0001$) than that of the control group (n=24, mean SCEs/cell ± SD: 8.5 ± 1.59) (Student's t-test).

In order to assess if there is correlation between usage period, monthly frequency of hair dyeing and SCE frequency, a calculation has been made giving the term "exposure": Exposure = Usage year x frequency per month x 12. Using this equality, between SCE frequency and

exposure in hair dyeing women, no correlation has been found (Pearson, r = 0.093, P = 0.572).

When three colors (dark, red, light) have been taken, assuming they have different effects on the DNA, no difference in SCEs has been detected (Kruskal-Wallis one way variance analysis, P = 0.515). Giving the colors as two groups: dark (red and dark) and light (light), again no difference has been found in SCE frequencies (Mann-Whitney U test, P = 0.253).

Age differences within the study group were also not correlated with the SCE frequencies (Pearson, r = 0.015, P = 0.928).

Discussion

Potential carcinogenic and teratogenic effects of hair dyes have been evaluated using various tests, but the results have not always been 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 (9). In another investigation, it has been speculated that two hair dye components generate active oxygen species causing DNA damage which leads to the carcinogenesis (10). Positive associations between hair dye use and the development of the cancer in urinary tract also have been reported (11,12). 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 relationship with increasing duration and number of hair dye use (13). 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 (14). In a multicenter case-control study on risk factors for acute leukemia and preleukemia, a moderate leukemogenic effect of hair dyes use was suggested (15).

Contrary to these results above, analysis of data failed to show any significant effects of hair dyeing in an epidemiological survey of bladder cancer (16). No association was observed between hair dye use and cutaneous malignant melanoma, either (17). 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 (18).

Genotoxicity should also be taken under consideration for children as women often continue to use hair dyes during pregnancy and lactation. In a pair-matched case-control study on the gestational risk for Wilms' tumor, it has been reported that use of hair-coloring products was strongly associated with cases in which Wilms' tumor was diagnosed before 2 years of age (19). A positive relationship between hair dye use and the risk of another childhood tumor, neuroblastoma has also been reported(20). On the other hand, in a large national collaborative clinical trial which examined the non-occupational risk factors for Wilms' tumor, no association was found with maternal exposure to hair coloring products during pregnancy (21).

SCE is a reciprocal exchange of DNA segments between sister chromatids at identical loci (22). This phenomenon occurs during DNA synthesis. The detailed mechanism underlying SCE formation is not clear; however, it has been related to the processes of replication and repair (23). During the past years, quanti-

tative analysis of SCEs has been used as a sensitive method of detecting damage to the DNA, thereby evaluating the mutagenic and carcinogenic potential of various agents (24,25). According to various reports, a variation in SCE frequency exist among healthy individuals (26). Variation is associated with different experimental conditions in different laboratories. However, it still exists when conditions are kept constant. Age, sex, different physiologic parameters, as well as different genomes may affect the frequency of SCEs (27). Increased SCE frequency may occur following exposure to chemicals, irradiation and drugs (28,29). Physiological factors that may affect SCE frequency are reproductive hormones; evaluation of SCE frequencies during a normal menstrual cycle demonstrated a higher rate around ovulation and in the luteal phase as compared to the early follicular phase (30). 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. Moreover, all the subjects were living in the same region, and in similar conditions. The factors that may have influence on the SCE frequencies (e.g. age, sex, race, nutrition, environ etc.) were similar in both groups. Therefore, we suggest that the difference in the SCE frequencies was induced by the use of hair dyes produced and/or sold in Turkey. Hair dyes are usually compounded of 7 to 12 aromatic substances. These constituents including certain phenylenediamines, nitrophenylenediamines, diaminotoluenes and diaminoanisoles should be investigated separately by in vitro tests, and if responsible chemical substance can be found, it would be enough to remove it from the hair dyes composition.

Dark hair colors, frequent dyeing (more than five times a year), and long usage period (about ten, to fifteen years) have been found to have significant oncogenic effects (31,32). Contrary to these results, in our previous study, we did not detect any influence of usage period in hair-dyeing women by the comet assay. This finding consistent with our recent study suggests that this DNA damaging effect may begin before the 5th year of hair dyeing. In previous comet study, we did not find any significant effect of age, frequency and hair color on the SCE scores, either.

In conclusion, our results support the idea that long-term use of hair dye in adult Turkish healthy women appear to have some toxic effects on DNA, which may be associated with carcinogenesis, and even teratogenesis. We suggest that caution should be recommended to persons with positive family history in cancer development and to those who seek to use the hair dyes for a long period of time. We also suggest that, until proved otherwise, the potential toxic effect of hair dyes should be taken under consideration by women who are pregnant or lactating.

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Table 1. SCE frequencies from the hair dye using women and the controls.

Subject	*Age(year)*	*Usage Period (year)*	*Frequency (month)*	*Dye color*	*SCE frequencies of women using hair diye (percell)*	*AGE (control)*	*SCE frequencies of control women (per cell)*
1	30	11	1	Red	20	27	10
2	28	7	1	Light	18	30	8
3	42	10	2	Dark	16	35	9
4	35	12	2	Light	11	29	6
5	27	5	1	Red	12	39	8
6	39	13	1	Red	10	35	10
7	38	15	1	Red	17	25	9
8	40	10	3	Dark	15	40	11
9	30	8	1	Light	18	36	7
10	38	18	2	Dark	13	28	7
11	32	8	2	Light	18	21	8
12	43	15	1	Dark	18	23	8
13	26	4	1	Light	16	32	10
14	33	10	1	Light	13	38	12
15	35	9	1	Light	12	30	11
16	41	6	3	Red	15	38	9
17	23	2	1	Light	16	26	7
18	30	10	1	Light	14	34	8
19	37	10	2	Red	12	25	9
20	25	5	1	Light	13	33	8
21	31	7	1	Light	11	43	7
22	36	9	1	Dark	13	40	7
23	41	15	3	Dark	15	34	6
24	39	10	1	Dark	10	29	9
25	39	13	2	Dark	12		
26	27	6	1	Red	11		
27	33	9	1	Light	20		
28	37	12	1	Light	18		
29	40	10	3	Light	17		
30	43	8	2	Red	13		
31	30	8	1	Light	12		
32	28	5	1	Light	9		
33	34	7	2	Light	17		
34	36	9	1	Red	14		
35	37	6	1	Dark	14		
36	38	8	2	Light	17		
37	30	7	1	Dark	16		
38	37	10	1	Dark	8		
39	28	7	1	Light	12		
Mean	34.256	9.076			14.256*	32.083	8.5
± SD	5.452	3.343			3.05	5.956	1.588

* : $P < 0.0001$ (Student's t-test)