TOPICAL APPLICATION OF HAIR DYE CONTAINING PARAPHENYLENE DIAMINE – EVALUATION OF ANTIOXIDANT STATUS AND RENAL PARAMETERS IN DIABETIC AND NON-DIABETIC USERS

R. Sangeetha*, N. Karthi

Department of Biochemistry, School of Life Sciences, Vels University, Chennai, Tamilnadu, India

Author For Correspondence:
Dr. R. Sangeetha,
Assistant Professor,
Department of Biochemistry, School of Life Sciences,
Chennai – 600 117. Tamilnadu, India.
Tel: 044-22662500
E-mail: sara_dna@yahoo.co.in

Summary

Hair dyes, especially that containing p-phenylene diamine are toxic when consumed, administered or topically applied. p-phenylene diamine exerts oxidative stress as the chemical can penetrate the scalp and skin. This study aimed at investigating the oxidative stress and other biochemical alterations induced by the topical application of p-phenylene diamine in diabetic hair dye users when compared to non-diabetic controls. Alterations in the levels of enzymic and non-enzymic antioxidants, glucose, cholesterol and renal parameters were studied. A statistically ($p<0.05$) significant increase in the levels of all these parameters was found in the test subjects when compared to control. Lipid peroxidation was also increased ($p<0.01$) in the diabetic hair dye users. Zymogram analysis indicated increased activity of superoxide dismutase in the hair dye users when compared to non-user controls.

Key words: antioxidants, diabetes, p-phenylene diamine, renal parameters

Introduction

Personal grooming has become an art; coloring and dyeing the hair has become inevitable to fulfill the desire to improve appearance. Hair dyeing is the most highlighted component of anti-ageing tools and the use of hair dyes has increased considerably in the last few decades. Different kinds of hair dyes which have been categorized as temporary, semi-permanent and permanent are available commercially. Hair dyes are also classified as reactive hair dyes and adhering hair dyes depending on their mode of action to color hair. The former has found the utmost usage as the best choice for covering grey hair and has high toxicity than the latter (1).

The permanent hair dyes are marketed as hair dye components and coupler in one bottle and an oxidizer or developer which is usually hydrogen peroxide in the other bottle. The components of both bottles are mixed before use. The chemicals commonly used as dye are p-phenylene diamine (PPD), p-toluene diamine, p-aminophenol. p-phenylene diamine shows the highest toxicity and there are reports on poisoning following its oral consumption (2, 3) and undesirable side effects following its topical application (4). Human skin is permeable to topically applied substances and hair dyes penetrate percutaneously through scalp during hair dyeing or coloring (5). Brown and McGeown, 1987 (6) have reported that chronic renal failure is associated with topical application of PPD. In this study we have investigated the susceptibility of diabetics to the toxicity of topical application of PPD.

Diabetes mellitus is a disorder and the global population affected by this disorder is increasing at alarming rates. Diabetes is associated with many metabolic and physiological complications. Diabetes is also characterized by alterations in lipid peroxidation and status of antioxidants. Free radicals have been implicated in the development of complications associated with this disorder (7). The complications associated with diabetes precipitate with habits like smoking and alcoholism (8). Hence we proposed to study the impact of the practice of dyeing the hair in diabetic subjects and the aim of the present study was to analyze the influence of topical application of hair dyes containing PPD on the levels of enzymic and nonenzymic antioxidants in diabetics and nondiabetics. The difference in the extent of lipid peroxidation was also analysed. Zymogram analysis of SOD was performed as a biomarker of oxidative stress in both diabetic and nondiabetic hair dye users. The alterations in the renal parameters and activity of choline esterase were also observed.

Materials and methods

Subjects

All the subjects were male volunteers chosen on visits to 2 salons located in Chennai, India. Informed consent was obtained from all the volunteers. The age of the subjects was between 40-50 years. The hair dye users dyed their hair once in 45 days and were in the practice of dyeing hair for atleast 5 years. Samples were collected on the day subsequent to the day of hair dyeing. The diabetic subjects chosen for both user and non-user groups had a diabetic history of minimum 5 yrs and a maximum of 7yrs (Table 1). The volunteers were grouped as non-diabetic controls (Group 1) and diabetic controls (Group 2) who did not practice hair dyeing; subjects who were hair dye users were grouped as non-diabetic tests (Group 3) and diabetic tests (Group 4).

Table 1. Demographic details of subjects

Particulars	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=20)
Age (yrs)	45 ± 4	45 ± 5	44 ± 5	45 ± 6
Weight (kg)	59 ± 5	62 ± 4	57 ± 6	62 ± 5
Duration of diabetes (yrs)		6.2 ± 1		6.3 ± 1
Duration of hair dyeing(yrs)			5.8 ± 0.8	5.7 ± 0.4

All the values are expressed as mean ± SEM

None of the subjects were under antioxidant supplementation and lipid lowering drugs. None of the subjects had any history of infection, inflammation, disorder or any disease for the past 6 months. The blood samples were collected, transferred to a fresh sterile tubes and stored in ice-box (-4 ºC) for transportation to the laboratory for analysis.

Biochemical analysis

Glucose was estimated using o-toluidine method (9). Cholesterol was estimated by Zak's method (10). Creatinine was measured by Jaffe's method (11). Urea was measured using diacetyl monoxime method and uric acid by the method described by Jung and Parekh (12). Ascorbic acid was measured using phosphotungstic acid as colouring reagent (13). Superoxide dismutase (SOD) was measured by pyrogallol oxidation inhibition assay of Marklund (14). Activity of Glutathioine peroxidase (GPx) was determined by the method of Rotruck et al. (15). Lipid peroxidation was determined by assaying thiobarbituric acid reactive substances (16). Choline esterase activity was determined by the method of Ellman, et al. (17). The zymogram study of SOD was performed by the method of Beauchamp and Fridovich (18).

Statistical analysis

All values are reported as mean ± SEM. Statistical significance was assessed using Student's t-test.

Results

The random glucose and cholesterol levels were significantly ($p<0.05$) high in the test subjects when compared to controls while no significant difference in glucose levels were observed between diabetic and non-diabetic tests. The renal parameters urea, uric acid and creatinine were significantly ($p<0.05$) elevated in the diabetic hair dye users when compared to non-diabetic hair dye users and diabetic control subjects. The activity of choline esterase was significantly elevated ($p<0.05$) in the diabetic users. The levels of vitamin C and the activity of GPx in both diabetic and non-diabetic test subjects were high when compared to controls. The SOD activity in the non-diabetic hair dye users was very high when compared to non-diabetic controls though both the test subjects exhibited significantly ($p<0.05$) high activity of SOD when compared to the respective controls. The lipid peroxidation was significantly high ($p<0.01$) in the diabetic hair dye users (Table 2).

Table 2. Antioxidant status and levels of renal and biochemical parameters in diabetic and nondiabetic subjects

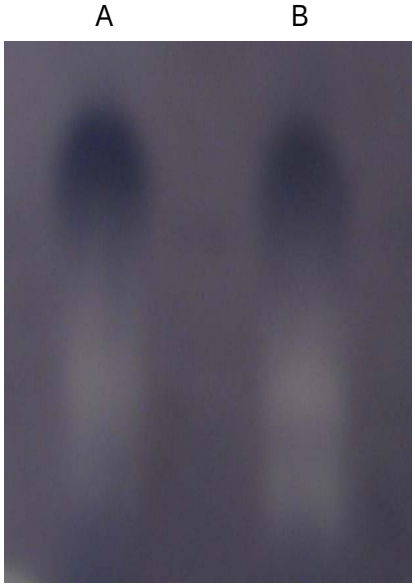
Particulars	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	Group 4 (n=20)
Glucose(mg/dl)	85.3 ± 4.2	173.4 ± 7.4	91.6 ± 3.8	198.1 ± 5.6
Total cholesterol (mg/dl)	151 ± 2.4	164.6 ± 3.8	168.2 ± 3.9	182.9 ± 3.4
SOD (U/ml)	5.6 ± 0.42	3.2 ± 0.45	6.8 ± 0.7	5.4 ± 0.7
GPx (U/ml)	13.0 ± 0.22	11.8 ± 0.15	14.52 ± 0.38	13.4 ± 1.2
Vit C (mg/dl)	1.7 ± 0.25	1.42 ± 0.15	1.5 ± 0.07	1.53 ± 0.09

MDA (nmol/ml)	0.89 ± 0.24	1.88 ± 0.32	1.52 ± 0.37	2.59 ± 0.52
Choline esterase (mmole/min)	7.4 ± 0.2	7.9 ± 0.4	9.02 ± 0.31	15.4 ± 0.42
Urea (mg/dl)	20 ± 1.4	20.3 ± 0.9	26.2 ± 1.8	29.6 ± 2.64
Creatinine (mg/dl)	0.8 ± 0.04	0.9 ± 0.08	1.2 ± 0.4	1.5 ± 0.64
Uric acid (mg/dl)	4.3 ± 0.41	4.6 ± 0.36	5.8 ± 0.95	6.8 ± 0.85

All the values are expressed as mean ± SEM

The zymogram study further performed to analyse SOD activity showed remarkable activity of SOD as a large achromatic zone against a blue background (Figure 1). Visual observation revealed high SOD activity in the diabetic test sample when compared to the diabetic control sample.

Figure 1. Zymogram analysis for the activity of SOD



Lane A – diabetic control and Lane B- diabetic test

Discussion

Topical application of PPD containing hair dyes can exert oxidative stress and there are reports on acute and chronic renal failure associated with topical application of PPD. Oxidative stress serves as a primary component in the development of diabetic complications (19). Hyperglycemia was observed with the diabetic and non-diabetic hair dye users. The increased levels of glucose in these subjects can be attributed to the impairment in pancreatic production of insulin required to control blood glucose. Free radicals produced as a result of oxidative stress can impair and damage the pancreatic islet cells and thus decrease insulin production (20).

The levels of antioxidants were analysed and the results showed increased levels of vitamin C, SOD and GPx in the hair dye users and lipid peroxidation was also found to be increased in the test subjects. This indicates the increased generation of free radicals as a result of exposure to PPD containing hair dyes. Lipid peroxidation and free radical formation have previously shown to be associated with exposure to PPD in human keratocytes and in guinea pig liver (21, 22). The oxidative stress following the application of PPD results in the formation of reactive oxygen species like superoxide and hydrogen peroxide. These free radicals are scavenged by enzymic and non-enzymic antioxidant defenses and hence the levels of SOD, GPx and Vitamin C were found to be increased. Decrease in insulin and the associated increase in β-oxidation of fatty acids causes increased production of H_2O_2 also enhances activity of GPx (23).

The activity of choline esterase was found to be significantly elevated ($p<0.05$) in the diabetic dye users when compared to non-diabetic tests and diabetic controls and indicated the neurotoxicity induced by the PPD containing hair dye which can permeate the scalp during the dyeing process (5). Also increased values of choline esterase can be seen in sickle cell anemia type – II, Diabetes mellitus, hyperthyroidism and nephritic syndrome. Increase in choline esterase activity is related to severity of diabetes and the choline esterase activity increased with increase in blood sugar levels (24).

The statistically significant elevation in urea, uric acid and creatinine levels in the diabetic hair dye users indicates impaired renal functioning and there are reports on acute and chronic renal failure caused by the topical application of PPD (25, 6). Elevated levels of creatinine are merely associated with abnormal renal function, more specifically with reduced glomerular filteration rate. Urea constitutes nearly half the non-protein nitrogenous substance in the blood; it is synthesized in the liver and mainly excreted through the kidney. Therefore, blood urea is traditionally used to monitor renal function. Rats and chicken exposed to PPD had renal cell necrosis following exposure to PPD (26, 27). Chronic application of dye to the skin can lead to glomerular injury (2).

Thus the practice of dyeing the hair causes renal impairment and exerts oxidative stress and these negative effects were found to high in the diabetic hair dye users and thus can precipitate the complications associated with diabetes. With increase in importance given by people of all ages to appear young and attractive, the usage of dyes to colour hair and to mask grey hair has dramatically increased. Hence measures to prevent percutaneous absorption of hair dyes should be evaluated. A temptation exists not to thoroughly wash the hair as washing may reduce the hair colour (4) and this promotes higher rates of percutaneous absorption of dyes. Goetz et al, 1988 (28) have reported a five to ten fold decrease in the penetration of PPD by protecting the scalp with clay prior to dye application.

The results of the present study suggest that prolonged use of hair dyes poses detrimental effects on health particularly in diabetic patients and thus precipitates the major physiological and metabolic complications associated with diabetes mellitus.

References

1. Kumar R, Singh B, Sharma SR, Singh N, Singh J. Acute renal failure due to paraphenylene diamine intoxication (hair colouring dye): Report of a case and discussion of Management guidelines based on a review of literature. Medico-legal update 2006; 6:33-35.
2. Suliman SM, Fadlalla M, Nasr MM et al. Poisoning with Hair-Dye Containing Paraphenylene Diamine: Ten Years Experience. Saudi J kidney Dis Transpl 1995; 6:286-289.
3. Filali A, Semlali I, Ottaviano V et al. A restrospective study of acute systemic poisoning of paraphenylenediamine (occidental takawt) in Morocco. Afr J Trad CAM 2006; 3(1):142-149.
4. Nott H.W. Systemic Poisining by Hair dye. Br Med J 1924; 421.

5. Wolfram L.J, Maibach H.I. Percutaneous penetration of Hair dyes. Arch Dermatol Res 1985; 277:235-241.
6. Brown JH, McGeown MG. Chronic renal failure associated with topical application of paraphenylene diamine. Br Med J 1987; 294:155.
7. Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular disease. Bratisl Lek Listy 2000; 101(10):541-551.
8. Dutt D, Roy G, Chatterjee P. Risk Factor Assessment for Type II Diabetes Mellitus in a Tertiary Hospital in Kolkata. Ind J Community Med 2004; 29:4.
9. Sasaki T, Masty S, Sonnae A. Effect of acetic acid concentration on the color reaction in the o-toluidine-boric acid method for blood glucose estimation. Rinsho Kagaku 1972; 1:346–353.
10. Zak B, Boyel AJ, Zlatkis A. A method for the determination of serum cholesterol. J Clin Med 1953; 41:486-492.
11. Husdan H, Rapport A. Estimation of creatinine by Jaffe's reaction. Clin Chem 1968; 4:222-238.
12. Jung DH, Parekh AC. An improved reagent system for measurement of serum uric acid. Clin Chem 1970; 16:247-250.
13. Kyaw A. A simple colorimetric method for ascorbic acid determination in blood. Clin Chim Acta 1978; 86:153-157.
14. MarkLund. Extra cellular superoxide dismutase and superoxide dismutase isoenzyme in tissue. Biochem 1984; 222:649-655.
15. Rotruck JT, Pope AC, Ganther HE et al. Selenium biochemical role as component glutathione peroxidase purification and assay. Science 1973; 179:558-590.
16. Draper HH, Hadley M. Malondialdehyde determination as an index of lipid peroxidation. Methods Enzymol 1990; 186:421–31.
17. Ellman GL, Countney KD, Anders VJ and Featherstone RM. A new rapid colorimetric determination of acetyl choline esterase, activity. Biochem Pharmacol 1961; 7:88-95.
18. Beauchamp CO, Fridovich I. Superoxide dismutase improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44:276-287.
19. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40:405–412.
20. Strain JJ. Disturbances of micronutrient and antioxidant status in diabetes. Proc Nutr Soc 1991; 50:591-604.
21. Picatdo M, Zompella C, Marhese C et al. Paraphenylenediamine, in control allergen, induced oxidative stress and ICMA- expression in human keratocytes, Br J Dermato 1992; 126:450-455.
22. Mathur AK, Gupta B N, Singh S. Biochemical and histopathological changes following dermal exposure to paraphenylenediamine in Guinea pigs. J App Toxicol 1990; 10:383-386.
23. Gupta MM, Chari S. Lipid peroxidation and antioxidant status in patients with diabetic retinopathy. Indian J Physiol Pharmacol 2005; 49(2):187–192.
24. Fokina AA. Serum cholinesterase activity of rats with experimental diabetes. Bull Exp Biol Med 1964; 58(4):1173-1175.
25. D'Arcy PF. Fatalities with the use of henna dye. Pharm International 1982; 3:217-218.
26. Wadaan MAM. Blood chemistry of Domestic Rabbits exposed to a oxidative hair dye. Int J Pharm 2006; 2(4):431-434.
27. Saad HA, Mousa HM, Ali BH. Some toxicological observations on paraphenylene diamine (Hair dye) in rats and chickens, Pakistan J Biol Sci 2000; 3(6):953-956.
28. Goetz N, Lasserre P, Bore P, Kalopissis G. Percutaneous absorption of p-phenylene diamine during an actulal hair dyeing procedure. Int J Cosmetic Sci 1988; 10(2):63-73.