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Full Length Research Paper

Histopathological effects of maternal hair dye use on the cornea

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The aim of this study is to investigate and compare the histopathological effects of hair dye additives, 2-amino-5-nitrophenol (2A5NP) and 2-nitro-p-phenylendiamin (2NPPD) on cornea of neonates from pregnant rats that have been administered these additives subcutaneously. The study included 90 neonates of 26 nulligravida wistar-albino rats among which ten were given 100 mg/kg/day 2A5NP (Group I), ten rats received 150 mg/kg/day 2NPPD (Group II) and control rats received saline (Group III) injections subcutaneously between 7th and 15th gestational days. No sign of toxicity was observed during the treatment and there was no gross abnormality in both the study and control groups. Histopathological changes of cornea were seen in 22 of 30 newborn rats in Group I (73.4%), in 23 of 30 rats in Group II (76.7%) and only 5 of 30 rats in the control saline injected Group III (16.7%). Histopathological effect of the two additives were statistically significant when compared to the control group (Chi-square:27.63, p = 0.0001), but there was no difference between the effects of 2A5NP and 2NPPD additives on cornea (Chi-square:0.089, p = 0.766). The present experimental study on rats confirmed the histopathological effect of 2A5NP and 2NPPD on cornea beyond doubt. In the light of which, we can speculate that maternal exposure of hair dyes during pregnancy has some teratogenic effects on newborn rat cornea.

Key words: 2-Amino-5-nitrophenol, 2-nitro-p-phenylendiamin, cornea, neonatal rats.

INTRODUCTION

The use of hair dyes can be traced back to at least 4000 years. Today, millions of consumers use hair dyes. P-phenylenediamine or p-diaminobenzene or ursol-D($C_6H_8N_2$) is a commonly used product for cosmetic purposes in this modern age of beauty consciousness. Paraphenylenediamine is an aniline dye, naturally available in white to slightly red crystals. It is readily soluble in water and darkens on exposure to air. By virtue of this property, it is being used as hair dye in humans (Jain et al., 1981).

Hair dyes are separated into four groups; oxidative hair dyes, direct dyes, metal salts and natural dyes. Oxidative dyes (80%) are the most common dyes used in the US and EU. Oxidation of primary intermediates (para-phenylenediamine, para-toluenamine, substituted para-diamines, orto or para-amino phenols) and coupling with modifiers like 2-amino-5-nitrophenol (2A5NP) and 2-nitro-p-phenylendiamin (2NPPD) result in coloured reaction products (Nohynek et al., 2004). The genetic and ocular teratogenic effects of hair dyes are not known exactly (Nohynek et al., 2004;Sosted and Menne, 2005;Grant; 1962

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Abbreviations: 2A5NP, 2-Amino-5-nitrophenol; **2NPPD,** 2-nitro-p-phenylendiamin.

Czene et al., 2003). Periocular dermatitis, conjunctivitis, corneal ulcers, cyclitis, secondary glaucoma, gangrene of lids, optic neuritis and proptosis are known toxic effects of hair dyes which occur upon contact to eyebrow and eyelashes or ingestion (Sosted and Menne, 2005; Grant, 1962; Czene et al., 2003).

This study is aimed at investigating and comparing the histopathological effects of hair dye additives, 2A5NP and 2NPPD, on corneas of neonates of pregnant rats. This is the first study on this subject in the literature, to the best of our knowledge.

MATERIALS AND METHODS

The local ethics committee of Gaziantep University reviewed the study, and all the experiments conformed to the Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences' Committee on Educational on Animal Research (Committee on Educational Programmes in Laboratory Animal Science, 1991).

The study was done on 26 female young, adult, nulligravid wistar-albino rats. The rats were kept in six metal cages containing 3 or 5 rats each, with 20 - 22°C room temperatures and 12 h light period. There was one male rat in each cage. After the determination of copulation by the presence of vaginal sperm, each group consisting of five rats were put in different cages on day zero of gestation. Between 7th and 15th days of gestation, ten rats were chosen randomly and 2NPPD was administred 150 mg/kg /day subcutaneously. To another group of ten randomly selected pregnant rats, 2A5NP was administered 100 mg/kg/day subcutaneously. Sterile saline was injected to the control group at the same time period.

The study included 90 neonates of rats which were given 2A5NP (Group I), 2NPPD (Group II) and saline (Group III). The rats were sacrificed with intra-abdominal injection of Pentothal on the 20th day of gestation and the litters were delivered by caesarian-sectio. The fetuses were inspected for any gross abnormality after drying. Thirty fetuses from each three groups of rats were sent to pathology laboratory in 10% formaldehyde buffer. The groups were coded randomly. Litters were decapitated on postnatal day 1 in the pathology department after which the eyes were enucleated and examined for histopathological characteristics with the pathologist blinded for the study groups. Only one randomly selected eye (right eyes) was used.

The globes were separately numbered, fixed in a solution of 10% formaldehyde, and prepared for the histological examination. The tissues were embedded in paraffin wax; sections with 4 - 6 micrometers thickness were obtained, mounted on slides and stained with hematoxyline and eosine for routine light microscopy. The slides were histologically investigated by the same pathologist, masked as to the treatment.Chi square test was used to compare differences between the groups.

RESULTS

Histopathological examinations of the corneas by light microscope in normal group disclosed normal findings such as regular stromal collagen fibers, descement membrane and a monolayer, regular corneal endothelium (Figures 1 and 2). Pathological findings showed four

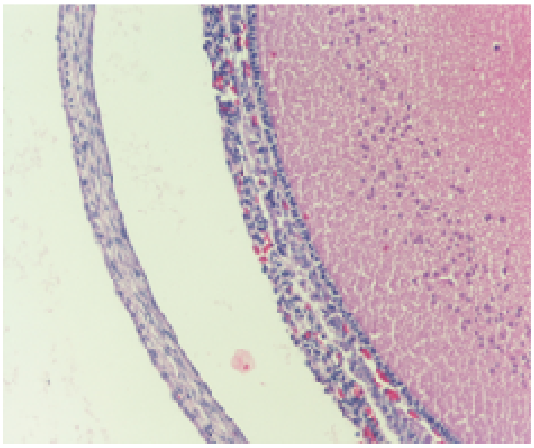

Figure 1. Normal eye (original magnification × 200, Hematoxyline and Eosine staining).

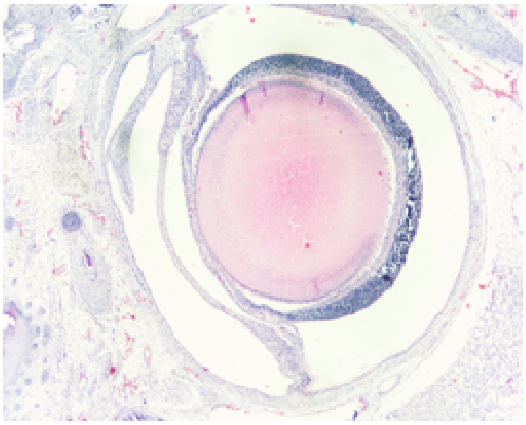

Figure 2. Normal eye (original magnification ×40, Hematoxyline and Eosine staining).

histopathological changes. These were teratogenic corneal agenesis, corneal epithelial, endothelial and stromal proliferations (Figures 3 - 7).

Corneal epithelial proliferation included hyperchromosia, polymorphism, and mitosis. Endothelial changes were multilayered cellular proliferations with hyperchromosia, polymorphism and mitosis. There were irregular and widely separated destructive fibers and increased mitosis in stromal proliferation. Teratogenic corneal agenesis was total agenesis of corneas in immature fetal eyes. Corneal histopathological changes were seen in 73.4% in Group I (22 of 30 newborn rats), in 76.6% in Group II (23 of 30 rats), and only 5 of 30 rats in the control saline injected group. Furthermore, the difference of corneal stromal proliferation rate was statistically significant when compared to the control group ($p = 0.04$).

Eight normal eyes were seen in 2A5NP (26.6%), 7 eyes (23.3%) in 2NPPD and 25 eyes (83.3%) in the control group

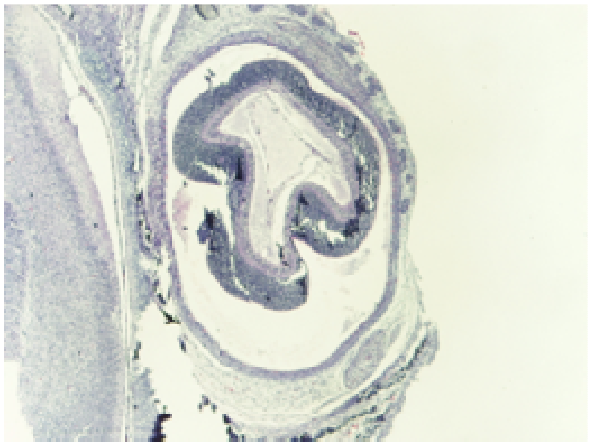

Figure 3. Anomalism of eye (original magnification × 40, Hematoxyline and Eosine staining).

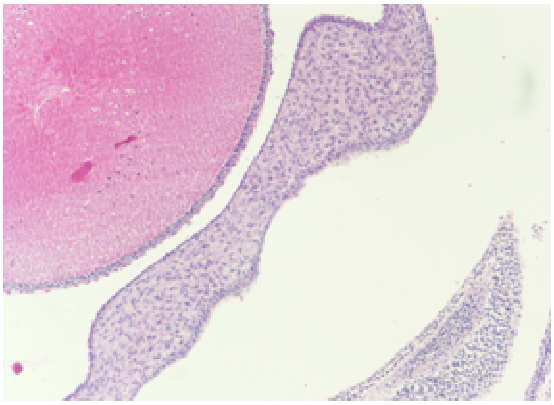

Figure 4. Corneal epithelial, endothelial and stromal proliferations (original magnification × 40, Hematoxyline and Eosine staining).

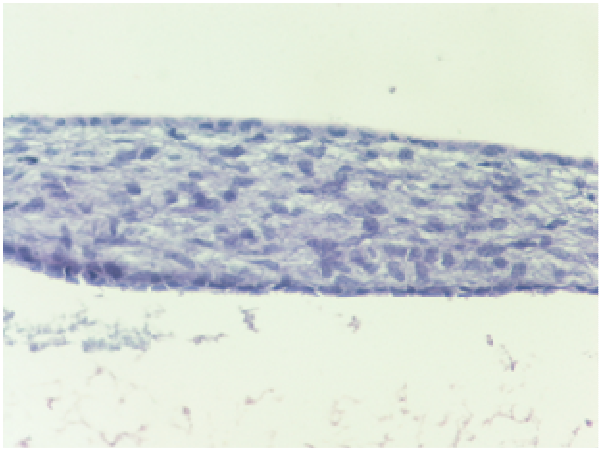

Figure 5. Corneal epithelial, endothelial and stromal proliferations (original magnification × 400, Hematoxyline and Eosine staining).

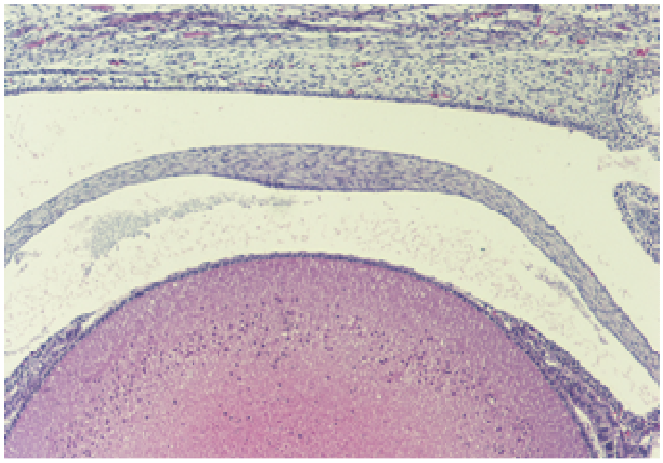

Figure 6. Corneal epithelial, endothelial and stromal proliferations (original magnification × 40, Hematoxyline and Eosine staining).

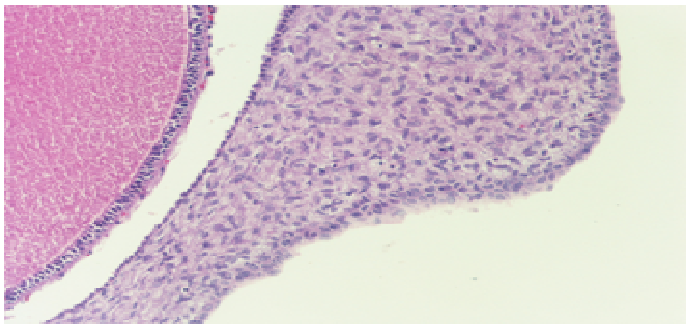

Figure 7. Corneal epithelial, endothelial and stromal proliferations (original magnification × 400, Hematoxyline and Eosine staining).

(p = 0.0001). Corneal epithelial proliferation was inspected in 2A5NP [2 eyes (6.7%)] and 2NPPD [5 eyes (16.7%)]. There was epithelial proliferation only in 1 eye in the control group (3.3%) (p=0,168). While there were endothelial proliferation in 5 eyes (16.7%) of group I (2A5NP) and in 3 eyes (10%) of group II (2NPPD), none was observed in the eyes of the control group (p = 0.073). Stromal proliferation of corneal were seen in 5 eyes (16.7%) of Group I, in 6 eyes (20%) of Group II and none in the control group (P = 0.04). Teratogenic corneal agenesis was observed in 10 eyes (33.3%) of 2A5NP group, in 9 eyes (30%) of 2NPPD group and in 4 eyes (13.3%) of control group (p = 0.163). Histopathological effect of the two additives were statistically significant when compared to the control group (Chi-square:27.63, p = 0.0001), but there was no difference between the effects of 2A5NP and 2NPPD additives on cornea (Chi-square:0.089, p = 0.766). The results for all groups and histopathological changes are given on Table 1.

Table 1.Histopathological changes of cornea.

Histopathological changes of cornea	Group I (100 mg/kg 2A5NP)		Group II (150 mg/kg 2NPPD)		Group III (Control saline)		P value
	N	%	N	%	N	%	
Epithelial proliferation	2	6.7	5	16.7	1	3.3	0,168
Endothelial proliferation	5	16.7	3	10	0	-	0,073
Stromal proliferation	5	16.7	6	20	0	-	0,04
Teratogenic agenesis	10	33.3	9	30	4	13.3	0,163
Normal eyes	8	26.6	7	23.3	21	70	0
Total	30	100	30	100	30	100	

DISCUSSION

Two types of reactions have been described with paraphenylenediamine. First of all, acute toxic reactions of this dye include conjunctivitis, corneal ulcers, lid gangrene, proptosis and cyclitis. Other reactions are allergic in nature and include dermatitis, angioneurotic edema, allergic conjunctivitis and retrobulbar neuritis. Both reactions are of acute onset and so easily noticeable. Some of these allergic reactions have been reported by the hair dyes (Zapolanski and Jacob, 2008; Fautz el al., 2002). Hair dyes and their ingredients have moderate to low acute toxicity. Human poisoning accidents are rare and have only been reported following oral ingestion. Contact sensitization to hair dyes has been a safety issue, mainly as a consequence of unprotected professional exposure. *In vitro* genotoxicity tests on hair dye ingredients frequently had positive results, although their correlation with *in vivo* carcinogenicity for the chemical class of oxidative hair dye ingredients (aromatic amines) is uncertain (Bolt and Golka 2007). Some studies suggested that evaluation of fetal external, visceral, and skeletal anomalies revealed no statistically significant differences between dye treated and control groups (Dinardo et al., 1985; Marks et al 1981). Although 2NPPD administration led to a significant increase in the average percentage of malformed fetuses at 160 mg/kg/day and above, these effects occurred only at dosages which produced significant maternal toxicity (Marks et al., 1981).

The present study is the first investigating maternal hair dye exposure during pregnancy which demonstrated serious deleterious effect on embryo-fetal cornea development in neonatal rats. In our study, hair dye additives, 2A5NP and 2NPPD, were given subcutaneously during the organogenesis period (9 - 20 days). Corneal histopathological changes were seen in 80% in Group I (24 0f 30 newborn rats), in 83.3% in Group II (25 of 30 rats), and only 5 of 30 rats in the control saline injected. Corneal stromal proliferations were statistically significant when compared to the control group. Furthermore, impaired corneal maturation with striking histopa-thological changes was observed. On the other hand, macroscopic changes were not observed in any of the corneas.

There are studies demonstrating the absorption of such hair dye additives from the skin in which the metabolite of this dye (P-Toliendiamine) had been shown in the human urine (Wernick and Lanman, 1975). Marks et al. (1981) showed increased number of malformed newborn mice born to mother albino rats which were administered 2NPPD 150 mg/kg/day subcutaneously between 7th and 15th days of gestation in their experimental study. Moreover maternal exposure during the 7th and 15th days of gestation of such known teratogenic agents like caffeine has been shown to cause teratogenic effects in newborn rat cornea (Evereklioglu et al., 2004). The most evident alterations were multilayered endothelial cellular proliferation with hyperchromosia and polymorphism and increased corneal stromal mitotic activity.

Erbagci et al. (2006) speculated that maternal exposure of hair dyes during pregnancy is potentially a risk factor to congenital cataract. Posterior subcapsular cataract results from the migration of the lens epithelial cells over the posterior lens capsule behind the equator. Jain et al. (1979) showed the cataractogenous effects of paraphenylenediamine in their experimental study. Keratitis and corneal opacities were observed by local instillation, lenticular opacities were observed by dyeing skin.

In conclusion, our study demonstrated that use of hair dyes in pregnant rats induced histopathological cornea changes without doubt. These results may suggest that pregnant women should refrain from using hair dye in excess, especially during the 1st trimester. The ocular effects are needed to be assessed by further studies both on newborn and surviving litters.

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