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DNA Damage and Repair Studies in Individuals Working with Photocopying Machines

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ABSTRACT Photocopiers are one of the most common machines in the market today. They are a source of employment for a large number of people. The personnel working with photocopiers are commonly exposed to toners and their byproducts during reloading and running of the machines. The main components of these toners are carbon black, styrene, resins, formaldehyde and polycyclic aromatic hydrocarbons (PAHs) and they are known to be genotoxic. The present investigation was undertaken to screen 29 individuals working with photocopying machines, for possible genotoxic effects, using comet assay, on their peripheral blood leukocytes. For comparison 26 controls were included. DNA repair studies were also undertaken on 10 exposed and 10 control subjects. 50 cells per treatment were scored for comet tail length, which is an estimate of DNA damage. A significant increase in basal DNA damage and a decrease in the repair efficiency were observed in the exposed group compared to the controls.

INTRODUCTION

The risk of human exposure to hazardous substances in environment has been known to potentiate genotoxic effects. A person spends, on an average, one third of his life at his work place and therefore the environment in which he works can be a major factor in determining his health. Exposure to toxic substances at the work-places like in shoe-making, tobacco and rubber industries or exposure to petrochemicals can pose a serious health hazard (Pitarque et al. 1999; Zhu et al. 1999; Somorovska et al. 1999b; Lebailly et al. 1998). Apart from the occupational hazard, workers may also be exposed to additional toxic substances due to their social habits, such as smoking, tobacco chewing and alcohol consumption. An assessment of health hazards arising from occupational exposure to chemicals is based on the levels of toxic chemicals in the work environment. It has therefore become extremely important to have some knowledge of the effects of chemicals, which can be analyzed and assessed for their adverse effects.

Personnel working on photocopying machines are exposed to toners. The photocopying toners have in their composition, carbon black, polycyclic aromatic hydrocarbons (PAHs) (Venier et al. 1987), styrene, resins, magnetite and nitropyrenes (Rosenkranz et al. 1980). The toners were found to be mutagenic in *Salmonella* tester strains TA98 in the absence of rat liver metabolic activation, suggesting that the compounds present in the toners were mutagenic and could cause frameshift mutations (Lofroth et al. 1980, Rosenkranz et al. 1980). To our knowledge, information on the genotoxic effects of toners on the mammalian systems is not known. Hence, the present study was undertaken to assess the genotoxic effects, among the personnel working on photocopying machines, using comet (alkaline single cell gel electrophoresis, SCGE) assay (Singh et al. 1988). This assay is found to be a sensitive and reliable technique and allows the detection of intercellular differences in DNA damage and repair at individual cell level, in virtually any eukaryotic system. The technique can be used to detect a wide variety of DNA damage including single-strand breaks, double-strand breaks, alkali labile sites, cross-links and base damage. It requires only small cell samples (~10,000 cells) and results can be obtained in a relatively short time (6-8 hours).

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MATERIALS AND METHODS

The chemicals used in this experiment were purchased from the following suppliers: RPMI 1640, normal melting point agarose (NMA), low melting point agarose (LMA), Triton-X 100, sodium N-lauryl sarcosinate, and silver nitrate from Sigma chemicals, St. Louis, USA; tungstosilicic acid from Koch-Light Laboratories, Colnbrook-Bucks, England; potassium chloride, potassium dihydrogen phosphate, sodium phosphate dibasic from Glaxo, Bombay, India; sodium chloride, sodium hydroxide, ethylene diamine tetra acetic acid (EDTA), Tris, dimethyl sulphoxide, acetic acid, glycerol, formaldehyde, zinc sulphate and ammonium nitrate from Fischer Inorganics and Aromatics Ltd, Madras, India.

Subjects and Sampling: A well designed proforma was prepared in order to collect detailed information from the individuals on: age, duration of work, health history, family history, diet, habits, etc. After taking an informed consent from the subjects, peripheral blood samples were collected in sterile, heparinized capillary tubes by the finger prick method. All the samples were collected in the morning before the workers started their work. The samples were brought to the laboratory in ice and processed within one hour of collection.

Two sets of experiments were carried out: In the first set of experiments, basal DNA damage was studied in the peripheral blood leukocytes of 29 exposed males (age range of 17-40 years), working 8-10 hours per day with photocopying machines, for more than a year. From each of the subjects 40µl of peripheral blood was drawn by finger prick in a heparinized capillary tube. The blood samples were analyzed for basal DNA damage by comet assay. As controls, 26 age-matched individuals working in different professions (like clerks, attenders and students) and with same socio-economic status were included.

In second set of experiments, repair efficiency among the individuals working with photocopying machines was studied. The study was carried out on 10 of the exposed and 10 of the control subjects. From each of these subjects, 0.5ml of peripheral blood was drawn in a heparinized tube. This was divided into 11 aliquotes of 40µl each. Each of these aliquotes was mixed with 300µl of RPMI 1640 medium, supplemented with 20% fetal calf serum and incubated for the time intervals of 0, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 minutes at 37°C. After incubation, the samples were processed for comet assay to assess the repair efficiency among the subjects working with photocopying machines.

Comet Assay or Single Cell Gel Electrophoresis Assay (after Singh et al. 1988)

Slides were prepared in duplicate per sample, for the two experiments as mentioned above. Dust free, plain slides were covered with a layer of 140µl of 0.67% NMA (prepared in PBS) and allowed to dry for 10 min in hot air oven. This layer served as an anchor for additional layers to prevent slippage. About 110µl of NMA was layered as first layer and was immediately covered with a cover-slip and the kept at 4°C for 10 min to allow the agarose to gel. 20µl of blood sample was mixed with 110µl of warm 0.5% LMA (prepared in PBS) and this mixture was layered as second additional layer and gelled. A third additional layer of 110µl of LMA was added on top and gelled again. The slides were treated overnight in freshly prepared, chilled lysis buffer solution (25mM sodium chloride, 100mM sodium EDTA, 10mM Tris, 1% sodium lauryl sarcosinate; 1% Triton X -100 and 10 % DMSO added before use and pH adjusted to 10) at 4 °C.

Electrophoresis: The slides were removed from the lysing solution, incubated in alkaline electrophoresis buffer (10N NaCl, 20mM EDTA; pH adjusted to 13) for 20 min followed by electrophoresis (0.67 V/cm, 300mA) for 30 min, in the same buffer. The slides were then neutralized with Tris buffer (0.4 M Tris, pH adjusted to 7.5) and rinsed with distilled water. All of these steps were conducted under dim yellow light to prevent the occurrence of additional DNA damage from exposure to light. The slides were left to air-dry.

Fixation: The slides were left in the fixing solution (15% w/v trichloracetic acid, 5% w/v zinc sulphate and 5% w/v glycerol) for 10 min, washed several times with water and drained thoroughly.

Silver staining (after Ahuja and Saran 1997): The slides were immersed in a freshly prepared mixture of staining solutions containing 32ml of solution A (5% w/v Na_2CO_3) and 68ml of solution B (0.02% w/v NH_4NO_3, 0.02% w/v $AgNO_3$, 0.1% w/v tungstosilicic acid and 0.05% v/v formaldehyde). The slides were stained for about 10 min, and this step was repeated a couple of times with fresh staining solutions until a grayish brown color developed on the slide. Then the slides were dipped in 1% acetic acid for 5 min to stop the reaction. Finally the slides were rinsed in distilled water and air-dried. These slides were stored in dust free boxes till screened.

Scoring: The slides stained with silver nitrate were screened under a bright field transmission light microscope. Comet tail length was measured for 50 cells per treatment using an ocular micrometer fitted in the eyepiece. Mean±SD of DNA damage was calculated for each sample.

Statistical Analysis: For assessing the basal DNA damage in both the groups, the data were analyzed using Student's t-test and significance was adjudged at 5% level unless stated otherwise. Linear regression analysis was used to study if there was any confounding effect of age, years and hours of exposure of photocopying personnel on DNA damage.

RESULTS

The results on the basal DNA damage obtained on individuals working with photocopying machines are shown in table 1. The data clearly show that there was a significant difference in basal DNA damage in the exposed group compared to the control subjects ($p<0.01$). However, there was no significant difference in basal DNA damage between smokers and nonsmokers in both control and exposed subjects. Regression analysis did not show any effect of age, years of service and hours of exposure/day on the basal DNA damage in the exposed group.

Table 1: Mean ±SD (μ) comet tail length of the exposed and controls groups. Sample size is given within parenthesis

Subjects	*Exposed (mean±SD)*	*Control (mean±SD)*
Smokers+Nonsmokers	15.86±5.06 (29)	4.82±0.74 (26)
Smokers	16.45±5.84 (14)	4.43±0.90 (11)
Nonsmokers	5.56±4.52 (15)	3.37±0.52 (15)

The results on repair studies are presented in table 2 and figure 1. Our results show that in the exposed group there was a significant increase in mean tail length at 15 and 30 minutes of incubation when compared to the basal level (0 min). There was a gradual decrease of mean tail length from 45 to 150 min of incubation. In the control group there was no such increase in the tail length at 15 and 30 minutes of incubation, but a gradual decrease right from basal level (0 min) upto 60 min and then it stabilized at that level upto 150 min.

Table 2: Mean ± SD (μ) comet tail length of the exposed and control groups at different repair timings

S.No.	*Time (minutes)*	*Exposed*	*Control*
1.	0	17.32±3.67	5.57±0.37
2.	15	19.90±2.80	4.73±0.16
3.	30	21.11±4.11	3.76±0.16
4.	45	16.13±3.56	2.82±0.16
5.	60	13.11±2.83	2.72±0.11
6.	75	8.48±0.76	2.72±0.11
7.	90	6.97±0.44	2.72±0.11
8.	105	4.65±0.23	2.72±0.11
9.	120	3.84±0.31	2.71±0.13
10.	135	2.88±0.13	2.71±0.13
11.	150	2.88±0.13	2.71±0.13

DISCUSSION

Main components of toners are carbon black, styrene, resins, formaldehyde and nitrated PAHs. It was also reported that the photocopiers emit ozone, nitrogen dioxide and volatile organic compounds like 1,1,biphenyl, p-dichlorobenzene, styrene, propylebenzene, tetrachloroehthylene aldehydes and nitrated PAHs (Beland 1991; Selway et al. 1980; Brown 1999; Tuomi et al. 2000; Stefanik et al. 2000). Earlier studies revealed that occupational exposure to the above mentioned compounds induces mutagenic/carcinogenic effects, which are listed in the table 3.

There was a significant increase in the basal DNA damage in the exposed group when compared to the control group (Table 1). This increase could be explained mainly due to the presence of styrene, formaldehyde, ozone, PAHs and other chemical compounds present in toners. Relationship between the total duration, hours of work per day and age did not correlate with DNA

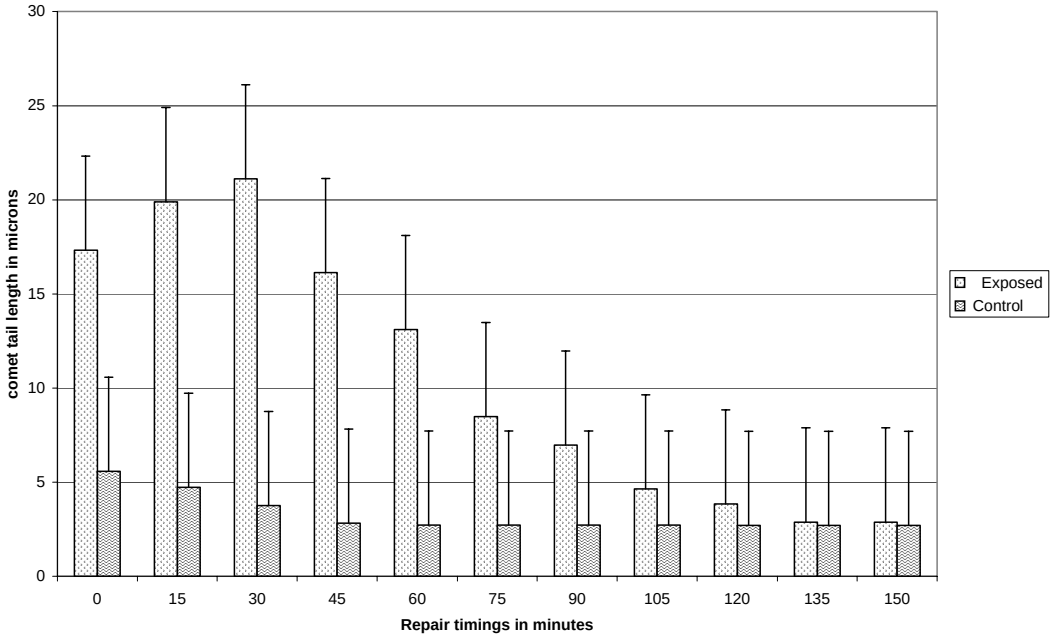

Fig. 1. Repair studiesin exposed and control groups

Table 3: Components of toners with their mutagenic/carcinogenic/ health effects

Chemical	*Mutagenic/Carcinogenic effects*	*Reference*
Styrene	DNA damage, sister chromatid exchanges and chromosomal aberrations in Humans lymphocytes.	Vaghef and Hellman (1998) Somrovska et al. (1999a)
Benzene and its metabolites	Single strand breaks or alkali labile sites in human lymphocytes.	Andreoli et al. (1997)
Chlorobenzene	DNA damage in peripheral blood lymphocytes in mice.	Vaghef and Hellman (1995)
PAHs	DNA single strand breaks by oxidative damage.	Popp et al. (1997)
Ethyl benzene, propyl benzene and other aliphatic hydrocarbons	Headaches, sleep disorders, increased irritability and tendency of anxiety in human.	Indulski et al. (1996)
Ozone	DNA damage in human in nasal epithelial cells.	Garciduenas et al. (1997)

damage. Smoking had a small but insignificant effect on the basal DNA damage in the exposed as well as the control group (Table1). As far as confounding factors mentioned above, there are some workers who have seen the effects whereas others have not (see review by Moller et al. 2000).

The leukocytes of the control subjects, when allowed to go through repair there was a gradual decrease at 15, 30 and 60 min duration as compared to the start (Table 2). However, in the exposed group there was a significant increase in DNA damage after incubation of sample for 15 and 30 min duration after the start (Table 2). This increase in the DNA damage could be due to some lesions, which were converted into single strand breaks while the cell was going through repair process. Mendiola-Cruz and Morales-Ramirez (1999) also saw a similar increase in DNA damage during repair process when they treated murine leukocytes to gamma rays.

CONCLUSION

Our preliminary results indicate that personnel working with photocopying machines have an increased basal DNA damage probably due to exposure to components present in the toners and their byproducts when the machine is in operation. Moreover, DNA repair process also showed an initial increase followed by a decline, indicating an interaction of this complex mixture of chemicals with DNA. These are indicators of health hazards. In order to confirm our results we have planned to study a larger sample size.

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