DNA Damage in workers occupationally exposed to photocopying machines in Coimbatore south India, using comet assay

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Abstract

The frequencies of DNA damage in peripheral blood lymphocytes of 74 workers occupationally implicated in photocopying industry were studied. Toxic components of the photocopiers are from their emissions, toners and extremely low frequency electromagnetic fields. DNA frequency of the workers increased with length of the chemical exposure period. So the present study used comet assay to determine DNA damage of photocopiers and control subjects. A significant increase in the incidence of DNA damage was observed in the experimental subjects when compared to their respective controls. However significant association was found between years of exposure, smoking, age, gender, alcohol consumption and high level of DNA damage ($P > 0.05$). Our findings indicate that occupational exposure to toxic components from photocopy machines could cause genomic damage in somatic cells of the workers with various etiological factors.

INTRODUCTION

Photocopying has become one of the common modes of employment in India. Toner is used in the photocopiers to produce an image on paper or transparency. Two essential components of dry toners are colorants (most common being carbon black) and binder resins. According to Rosenkranz et al.[1] the apparatus of toners comprise polycyclic aromatic hydrocarbons and styrene. The operators are exposed to the toners (while reloading and unloading the machines) and to toxic gases like ozone, nitrogen dioxide, volatile organic compounds and extremely low frequency electromagnetic fields during their operation [1, 2, 3, 4, 5]. Iravathy Goud [6] has reported parallel genotoxic effect in the workers occupationally exposed to print copying machine.

In the past 5 years the comet or single cell gel electrophoresis (SCGE) assay gained on popularity as a method for evaluation of DNA damage and repair in various in-vitro and in-vivo studies and it has been found to be a very sensitive method for measuring DNA damage [7, 8, 9].

Therefore the DNA damage in peripheral blood lymphocytes (PBL) it can be used as a biomarker of health outcome, measuring genetic damage due to exposure those results from non-repaired primary lesions in chemical exposure.

In this view, the focal aim of present investigation is whether individuals working with photocopying machine more DNA damage with the increase in the exposure period, alcohol and smoking habituate. Although the photocopy machine exposure has been extensively investigated, to the greatest of our knowledge, this is the first study to carry out a genetic analysis in this region.

MATERIALS AND METHODS

DEMOGRAPHIC PROFILE

Coimbatore, the Manchester of South India is located in the western region of Tamilnadu, bordered by the panaromic Western Ghats, the pristine nilgiris hills. The total population of the Coimbatore district is 42.25 lakhs (21, 56,280 males and 20, 67,817 females). Due to the existence and predominance of industries the silent toll of human lives due to numerous diseases is on the rise.

STUDY POPULATION

The study involved 148 subjects divided into two categories. The first categories consist of 74 workers employed in photocopy machine. The average duration of their employment was 7.04 years (range 3–13 years). Another category consists of 74 control subjects and positive and negative controls were selected from various professionals in

same city.

The selection criteria of study subjects were based on the survey. At the time of survey, workers signed a term of informed consent and replied to a questionnaire elaborated to determine the profile and habits of the both control and experimental subjects. All the subjects sub-divided based on the gender, years of exposure, age, smoking and alcohol consumption. All the subjects had been taking no medicines nor had been exposed to any kind of radiation (diagnostic and therapeutic) for 12 months before the blood sampling. Demographic characteristics of all subjects are shown in Table 1.The research procedures used in the present study were approved by the local Ethical Committee.

Figure 1

Table.1. Demographic distinctiveness of control and experimental subjects

Parameter		Control subjects (n = 74)	experimental subjects (n = 74)
Age (years) (mean±S.D.)		32.7±5.1	34.1±4.2
Years of exposure (mean±S.D.)		-	7.04±2.3
Gender (n) (%)	Female	27(36.49)	22(29.72)
	Male	47(63.51)	52(70.28)
Smoking (n) (%)	Yes	34(45.9)	36(48.64)
	No	40(54.1)	38(51.36)
Alcohol (n) (%)	Yes	27(36.49)	25(33.78)
	No	47(63.51)	49(66.22)

PHOTOCOPYING MACHINES

The photocopying machines used by the subjects are of Canon make and other machines are Sharp and Modi with matching brands of toners in powder form.

SAMPLE COLLECTION

5 ml of Fresh or thawed peripheral blood was collected from experimental and control subjects, under sterile conditions by venipuncture into heparinized tubes. The time of blood sampling were identical to that both subjects.

SINGLE CELL GEL ELECTROPHORESIS (SCGE) ASSAY

This assay was performed as described by Singh [7], with minor modifications. Briefly, after alkaline lysis (2.5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10), 10% of DMSO and 1% of triton X-100 at 4ºC for 1 h, slides were placed on a horizontal electrophoresis unit. The DNA was allowed to unwind for 20 minutes in electrophoresis running buffer solution (300 mM NaOH, and 1mM EDTA, pH 13). Electrophoresis was conducted for 20 minutes at 25 V and 300 mA. All technical steps were conducted using very dim indirect light. After electrophoresis, the slides were gently removed and alkaline (pH neutralized with 0.4 M Tris, pH7.5). Ethidium bromide (75 ml of a 20mg/ml solution) was added to each slide and a cover glass was placed on the gel, DNA migration was analyzed on a Nikon Optiphot-2 microscope with fluorescence equipment (filter G-2A), and measured with a scaled ocular. For the evaluation of DNA migration (total image length), 50 cells were scored for each individual. The length of the DNA migrated in the comet tail, which is an estimate of DNA damage was measured using an ocular micrometer. Quantification of the DNA damage for each cell was calculated as: comet tail length (μm) =/ (maximum total length)–(head diameter). DNA damage was further quantified by visual classification of cells into categories of ‘comets’ corresponding to the amount of DNA in the tail according to Anderson et al [10]. Images of 100 randomly selected cells (50 cells from each of two replicate gels) were analyzed from each individual. Comet tail lengths (nuclear region + tail) were measured in arbitrary units. One unit was approximately 5 m at 200X magnification. The fluorescence microscope was equipped with a BP546/12-nm excitation filter and a 590-nm barrier filter.

STATISTICAL ANALYSIS

All calculations were performed using MINITAB RELEASE II Software package for windows. Mean values and standard deviations (S.D.) were computed for the scores and the statistical significance (PB/0.05) of effects was determined using analysis of variance (ANOVA). Simple linear regression analyses were performed to assess the association between endpoints and independent variables.

RESULTS

The single cell gel (comet) assay was used to measure DNA damage. DNA strand break was represented by the mean tail moment and tail intensity. Table 2 and 3 represents DNA damage with age, smoking, gender, alcohol and exposure period of experimental and control subjects. Among these experimental subjects male (T % 4.63 and TL 4.78 m) workers showed more number of DNA damage than females (T % 4.08 and TL 4.61m) as well as male experimental subjects shows more (1.61: 0.52) DNA damage than (1.63:

1.76) females. Statistically significant difference ($p<0.05$) between experimental and control subject was observed. To determine the effect of exposure period on DNA damage showed no statistically significant results found with increasing the duration of exposure. Low number of DNA damage was observed in experimental subjects when compared to their respective controls. Alcoholic users of experimentals subjects showed significant ($p<0.05$) amount of DNA damage than alcoholic users of control subjects and non alcoholic users in experimental subjects, this date showed no statistically significant. An age related increase of DNA damage was observed in ≥ 31 years than ≤ 30 years but age factors did not showed any difference between experimental and control subjects. An increased level of DNA damage was observed in the workers with smoking habits when compared with smoking controls and nonsmoking workers. A clear and statistically significant ($p < 0.05$) increase in DNA damage was observed in experimental group (table 3) when compared to control groups (table 2). As analyzed smoking, alcohol, and gender differences shows that exposed subjects carry more number of DNA damage than control subjects.

Figure 2

Table 2. Shows the comet tail percentage and tail length of control subjects.

Parameter		n=74	T (%) (percentage DNA in tail)	TL µm (Tail length)
Gender	Female	27	1.61 [a] (0.35–.21)[b]	0.52 (0.32–4.64)
	Male	47	1.63 (1.91–3.09)	1.76 (0.59–3.89)
Smoking (*n*) (%)	Yes	34	2.18 (1.47–4.75)	2.43 (1.02–4.17)
	No	40	0.60 (0.90–1.23)*	0.72 (0.32–2.79)*
Age (years)	≤30	27	3.96 (0.20–4.31)	3.28 (0.29–3.76)
	≥31	47	3.86 (0.59–3.12)	3.27 (0.89–4.31)
Alcohol (*n*) (%)	Yes	27	1.52 (0.56–3.11)*	1.43 (0.93– 3.39)*
	No	47	1.42 (1.00–3.26)*	1.52 (0.32–3.79)*

Abbreviation:

[a] Median ; [b] Range; *p < 0.05; n: number of subjects studied

Figure 3

Table 3. Shows the comet tail percentage and tail length of experimental subjects

Parameter		n=74	T (%) (percentage DNA in tail)	TL µm (Tail length)
Gender	Female	22	4.08 [a] (2.15–9.05)[b]*	4.61 (3.21–11.73)*
	Male	52	4.63 (3.91–7.11) *	4.78 (3.75–12.78)*
Years of exposure	≤8	35	4.82 (3.02–11.21)	4.38 (2.65–13.96)
	≥9	39	5.62 (3.21-11.81)	4.91 (3.21–14.23)
Smoking (*n*) (%)	Yes	36	5.27 (4.56–7.86)*	5.32 (4.91–12.28)*
	No	38	4.60 (3.81–10.11)	4.61 (4.21–13.68)
Age (years)	≤30	37	3.96 (3.31–7.32)	3.17 (3.10–7.89)
	≥31	37	3.97 (3.71–8.01)	3.16 (3.91–7.42)
Alcohol (*n*) (%)	Yes	25	4.63 (3.71–7.01)	4.32 (4.91–12.28)
	No	49	4.01 (3.82–6.38)*	4.61 (4.21–13.68)*

Abbreviation:
[a] Median ; [b] Range; *$p < 0.05$; n: number of subjects studied

DISCUSSION

Mutagenesis involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to the development of pernicious illnesses, many times through mechanisms that involve genetical changes. In order to evaluate the possible impact of environmental and occupational exposition on health, it is essential to identify the effects of exposure. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposition and to monitor populations that are excessively exposed [11, 12]. In this regard a constant exposure of humans to toxic compounds in the workplace may lead to mutagenic/carcinogenic effects. In the present study, individuals working with photocopying machines could be exposed either by nasal or oral inhalation of the volatile organic compounds (VOCs) emanating during running and reloading of the machines, as well as the electro magnetic fields generated, or by physical contact with toners during reloading of the machines. Mullin et al. [13] and Iravathy Goud et al [6] have reported that similar symptoms of workers occupationally exposed to print copying machine. The present study was designed to assess the DNA damage among photocopy machine workers who are occupationally exposed to toners chemical. The comet assay is an easy, quick and accurate test that has been widely used in molecular epidemiology studies where DNA damage evaluated by comet assay is used as a biomarker of exposure[14]. Comet assay was used in various in vitro and in vivo studies to monitor exposure to mutagens and

carcinogens that induce DNA damage[15]. In the present study a notable DNA damage was observed among the healthy controls. Occupational exposures epithelial cells are in direct route of airborne pollutants and they can metabolize proximate carcinogens [16]. Previous epidemiological studies, genetic changes in these cells and PBL are of particular interest because these cells are frequently targets to toxic agents.

Of the various confounding factors studied, duration of exposure showed significant effects and it has also shows a positive effect on DNA. Similarly for example, workers of phosphate fertilizer factory, paint manufacture industry, out-door painters, cement workers, aluminium sulphate worker, those exposed to sulphur dioxide and environmental factors and low dose ionizing radiation showed increased frequency of genetic damage with increase in duration of work suggesting a cumulative genotoxic effect [17, 18, 19, 20, 21, 22, 23, 24, 25]. Photocopy machine workers shows exposure period also shows significant effects on DNA damage when compared to nonexposures. Alcohol consumption has been associated with different pathologies such as cirrhosis, acute and chronic pancreatitis, cardiovascular disease, psychological and neurological dysfunction [26, 27] and immune system damage [28, 29, 30]. The effects of ethanol intake on peripheral lymphocytes chromosome and DNA have also been well described in human alcoholics [31, 32, 33, 34]. A similar result was found the present study and which is statistically significant effect on the DNA damage with alcohol users. Recently, age and gender factors have been found to be strongly associated with the frequency of DNA and it's reported the relationship between aging and structural genetic damage[35], whereas others have found no association with age and DNA damage at all [36]. Furthermore DNA damages in aged person maybe some additional factors such as apoptosis, physical activity, the individual's nutritional state and environmental pollution [37, 38]. Similarly present study also shows no significant differences between DNA damage with age. Nevertheless, it is in contrast with data proposed by Singh et al. [9], who found DNA damage to be five times more frequent in samples from the most elderly persons in comparison with younger subjects. Nonetheless, the author points out that this results are influenced by characteristics of the studied sample. On the other hand in our study demonstrate the importance of gender (male sex) on the presence of DNA damage, in that the elderly males in our population having more number of DNA damage than females. This becomes relevant if we associate with the study of Collins et al. [39, 40] in which a strong association between DNA damage and gender. This could explain to a certain extent the greater longevity in females, as reported worldwide by the United Nations [41]. Similarly, present study it was confirmed that male sex factor constitutes a risk factor for more DNA damage.

The photocopy machine workers with smoking habits also shows more amount of DNA damage was observed in smokers which shows cigarette smoking has synergistic effect on inducing DNA damage. Some previous findings reported similar results on bidi, smokeless tobacco users [22, 23, 28]. Furthermore the present study correlates with smoking and photocopiers. Smoking-related DNA adducts have been detected by a variety of analytical methods in the respiratory tract, urinary bladder, cervix and other tissues. On terms of biological activity, cigarette smokers and its conductors have been shown to form adducts with DNA protein and to induce chromosome aberrations.

Nakayama et al. [43] reported DNA strand break in smokers due to the effect of electrophilic substances in tobacco. The "slower" response of peripheral lymphocytes of smokers compared to non-smokers found may be an expression of influence of chemical exposure with cigarette smoke components on cellular immune response in human. Although cigarette smokers were more likely than those who had never smoked to have a smokeless tobacco lesion, our previous finding shows little evidence of independent effect of cigarette smoking and alcohol consumption on selected population [25, 44]. Jayakumar and Sasikala [45] reported cigarette smoking habitual has a synergistic effect on inducing DNA damage among the jewellery workers are occupationally exposed to nitric oxide. On the other hand experimental conditions used our comet assay study reveal an effect of cigarette smoking on the more amount of DNA damage than other factors. This finding is in agreement with previous study [46] and the majority of findings reviewed human biomonitoring studies, which also positive approach to shows an effect of smoking on DNA migration in the comet assay [47, 48]. Some of these components, either directly or through their metabolites, may have formed DNA adducts. These adducts during faulty repair process could probably open up and form single strand breaks which after replication could result in double strand breaks/micronuclei. Similar results were reported in workers occupationally exposed to chemical mutagen [49]. Occupational exposure to these xenobiotics may result in covalent binding of their molecules to DNA, which leads to chromosome alterations

and may be an initial event in the process of chemical carcinogenesis [50, 51, 52]. The present findings highlight the importance of using comet assay to detect DNA damage and genotoxic effect of various confounding factors, since this information provides an increased degree of identification for the positive response.

In conclusion biomonitoring studies of workers exposed to photocopy machine are rather specific because each population has a different life style factors but same occupation in different areas under different climatic and environmental conditions and are exposed to indistinguishable of mutagen. This could explain why some studies find an increase of genetic damage in general populations. Another explanation for the genotoxic damage observed can be the lack of protective measures taken by the workers. Therefore, there is a need to educate those who work with photocopy machine about the potential hazard of occupational exposure and the importance of using protective measures. Since DNA damage is an important step in events leading from carcinogen exposure to cancer disease, our study represents an important contribution to the correct evaluation of the potential health risk associated with exposure. To the preeminent of our acquaintance this is the first study in our region, which has been conceded out on a large sample size in subjects occupationally using photocopying machines, employing sensitive biomarkers in order to evaluate the genetic effects of combined exposure to chemicals.

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