ORIGINAL ARTICLE

Cytogenetic damage in the buccal cells of photocopying workers in Lahore, Pakistan

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Abstract

Objective: To assess the genotoxic effects associated with workers in relation to the emissions at photocopying centres.

Methods: This case-control study was conducted at the Lahore College for Women University, Lahore, Pakistan, from August to November 2015, and comprised photocopying operators and controls. Micronuclei and other nuclear abnormalities in exfoliated buccal cells were analysed. A structured questionnaire was designed and interviews were conducted face-to-face. Statistical analysis was conducted to evaluate the significance of differences.

Results: Of the 200 participants, there were 100(50%) in each group. There were 24(24%) smokers and 76(76%) non-smokers among the controls compared to 29(29%) smokers and 71(71%) non-smokers among the cases. The mean age was 31.08±10.97 years and 28.19±7.58 years in the controls and the cases, respectively. The frequency of deoxyribonucleic acid damage and cell death parameters was high in photocopying operators compared to the controls ($p<0.05$). Positive correlation was observed between work span and the frequency of deoxyribonucleic acid damage ($r=0.226$) and cell death parameters ($r=0.115$). Smoking was positively associated with the frequencies of deoxyribonucleic acid damage ($r=0.65$) and cell death parameters ($r=0.37$). The differences were statistically significant ($p<0.001$).

Conclusion: A highly significant difference was observed in deoxyribonucleic acid damage and cell death of workers associated with photocopy profession.

Keywords: Photocopying operators, Genotoxic, DNA damage, Cell death, Toner. (JPMA 67: 275; 2017)

Introduction

Printing devices and photocopying machines emit harmful pollutants including engineered nanoparticles, metals, semi-volatile organics and volatile organic compounds which create toxicological issues associated with long-term exposures to the emitted pollutants.[1] Deoxyribonucleic acid (DNA) damage has been found to be influenced by confounding factors such as age, alcohol usage, smoking and duration of exposure. There is a lack of information available concerning the consequence of smoking and other agents in combination.[2]

The nuclear abnormalities including binucleated cell (BN), pyknotic nuclei (PN), nuclear buds (NBUDs), condensed chromatic (CC), karyolysis (KL) and Karyorrhexis (KR) have found to be associated with the exposure to tobacco smoke, drugs as well as alcohol.[3]

The biomonitoring studies involving human subjects use cytogenetic biomarkers. In order to evaluate the influence of medical, occupational as well as environmental factors on the stability of genome, these biomarkers are substantially used.[4] The application of exfoliated buccal cells for micronuclei (MN) assay determines the genotoxic potential on the targeted tissues in response to the long-term exposure to cytotoxic as well as genotoxic chemicals.[5]

The most widely accepted biomarkers include the manifestation of MN as well as nuclear abnormalities (NA). The effective monitoring of individuals as well as population upon exposure to genotoxic, mutagenic and teratogenic chemicals can be done by the identification of MN and NA, predominantly the micronucleogenic cells in epithelial tissues.[6]

The buccal cavity is a reflector that contemplates the alterations in the disease. Besides, most of the pollutants including tobacco smoke or alcohol make contact with the oral cavity. It is a representative of systemic state as well as undesirable effects owing to radiotherapy and chemotherapy.[7]

In Pakistan, an elevated ratio of diseases and injuries associated with occupation has been found. Unfortunately, there is an inadequate availability of data regarding these issues due to deficit of reporting.[8] In Pakistan, millions of workers get exposed to similar

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chemicals on a regular basis. Lack of proper reporting is responsible for the availability of adequate data on such issues.[9]

The photocopiers are associated with the release of ozone, particulate matter, semi-volatile organic compounds and volatile organic compounds.[10]

The present study was planned to assemble the information on the genotoxic effects of this occupation to conduct an appropriate assessment of the risk to the health that can be beneficial for instigating the functional approach associated with health care.

Subjects and Methods

This case-control study was conducted at the Lahore College for Women University, Lahore, Pakistan, from August to November 2015, and comprised photocopying operators and controls. Approval was obtained from the institutional ethics committee.

The exposed group consisted of photocopying operators as well as toner fillers working at different photocopying units working indifferent areas of the city. It was ensured that the cases and controls did not markedly differ from each other except for the occupational exposure. Like the exposed group, the healthy controls belonged to similar socio-economic and educational background. Healthy subjects aged between 17 and 65 years were included. Subjects with known chronic illnesses like cardiovascular diseases, diabetes mellitus, chronic respiratory problems, epilepsy, cancer, etc., were excluded. The selected subjects were not on consistent medications. The buccal cell samples were collected from photocopying operators, toner fillers and healthy control individuals without photocopying experience currently or formerly. Participants were further branched as smokers and non-smokers. On the basis of age, the two categories were<30 years and >30 years. On the basis of job duration, the exposed subjects were categorized as <10 years and >10 years. All the subjects filled in the structured questionnaire covering customary demographic information as well as occupational information. Body mass index (BMI) was calculated from the body weight divided by the square of body height of an individual. Before collecting the buccal cell samples, all participants were told about the purpose of the study and informed written consent was taken with the assurance of anonymity.

The buccal cells sampling was done according the criteria set up by Tolbert et al.[11] After rinsing the mouth to wipe off surplus debris, the buccal cell samples were attained using non-pointed wooden tooth picks.[12] The tooth picks were immersed in the Eppendorf tubes twice or thrice gently containing 0.5 ml of phosphate-buffered saline (PBS) of pH 7.4 to ensure preservation.

The Eppendorf tubes were centrifuged at 1,500 revolutions per minute(rpm) for 10 minutes. The supernatant was drained out and freshly prepared PBS was added. The centrifugation was done thrice in order to eliminate bacteria as well as to immobilise endogenous and deoxyribonuclease (DNase) to avoid the interference in scoring.[4] The resultant pellet was gently smeared on clean 4-6 microscopic slides per sample. After air drying at room temperature, the mixture of cold methanol and glacial acetic acid (3:1) was used as a fixative. The staining was carried out using giemsa stain. In several studies, giemsa stain has been widely used at various concentrations.[13] The slides were rinsed using double distilled water and air dried. After coding with specific numbers to avoid misinterpretation, the slides were observed under a light microscope under a magnification of 100x, and 400x.

The defined specifications for the cell scoring[11] include: unimpaired cytoplasm, smooth position of the cell, little or no detritus, smooth and normal nucleus, and little or no overlapping with the vicinal cells. It has been commended to review from 3,000 to 4,000 cells.[14] The abnormalities in the nucleus are considered to be expressive of cell death as well as damage to DNA. The scoring of exfoliated buccal cells was done as per the criteria established by Thomas et al.[15] with minor modifications. The present study aimed at scoring 3,000 cells per subject to evaluate the presence of normal cells, micronucleated cells, binucleated cells, karyolitic cells, as well as karyorrhetic cells.

For the assessment of data related to DNA damage and cell death, mean and standard deviation (SD) were calculated for each biomarker. The statistical significance of the effects (exposure, smoking and age) was determined using one-way analysis of variance (ANOVA). The significance of the differences between control and experimental groups' means were analysed using Student's t-test where $p<0.001$ was considered significant. Pearson's correlation was computed in order to determine the r-values.

Results

Of the 200 participants, there were 100(50%) in each control and experimental group. There were 24(24%) smokers and 76(76%) non-smokers among controls

Table-1: Frequency of nuclear abnormalities with respect to smoking in control and photocopying operator.

Characteristics	Sample Size	Age (Years)	BMI (kg/m^2)	Duration of Exposure (Years)	No of Cigarettes / day	DNA Damage MN (M+SD)	DNA Damage BN(M+SD)	Cell Death KL(M+SD)	Cell Death KR(M+SD)
Control (Total)	100	31.08+10.97	23.46+4.1	-	-	2.17+2.16	2.76+2.42	8.45+6.4	8.95+5.29
Control (Smokers)	24	31.38+8.56	24.61+4.16	-	9.75+6.54	5.71+0.86	6.63+0.49	19.21+3.11	16.83+3.95
Control (Non Smokers)	76	30.99+11.68	23.09+4.04	-	-	1.05+0.81	1.54+1.17	5.05+1.51	6.46+2.45
Experimental (Total)	100	28.19+7.58	23.57+4.26	6.5+6.54	-	6.94+1.96	8.69+2.54	26.22+1.31	19.16+1.23
Experimental (Smokers)	29	29.86+7.94	22.31+3.38	8.84+7.28	10.93+6.89	9.07+1.67	12.03+1.72	27.21+1.61	19.66+1.78
Experimental (Non Smokers)	71	27.51+7.38	24.09+4.49	5.54+6.01	-	6.07+1.29	7.32+1.20	25.82+0.92	18.96+0.85

BMI: Body mass index
DNA: Deoxyribonucleic acid
MN: Micronuclei
BN: Binuclei
KL: Karyolysis
KR: Karyorrhexis
M: Mean
SD: Standard deviation.

Table2: Frequency of DNA damage and cell death with respect to duration of exposure and age in control and photocopying operator.

Characteristics	Control (n=100) Sample Size	Control MN Frequency (M±SD)	Control BN Frequency (M±SD)	Control KL Frequency (M±SD)	Control KR Frequency (M±SD)	Experimental (n=100) Sample Size	Experimental MN Frequency (M±SD)	Experimental BN Frequency (M±SD)	Experimental KL Frequency (M±SD)	Experimental KR Frequency (M±SD)
Age <30 years	54	1.66±1.93	2.25±2.19	7.29±5.77	8.17±4.44	65	6.86±2.04	8.70±2.69	26.26±1.31	19.2±1.21
Age >30 years	46	2.76±2.28	3.34±2.56	9.80±6.87	9.87±6.06	35	7.08±1.80	9.03±2.17	27.05±1.41	20.08±1.27
Exposure <10 years	-	-	-	-	-	76	6.78±1.88	8.55±2.58	26.21±1.32	19.01±1.22
Exposure >10 years	-	-	-	-	-	24	7.46±2.15	9.13±2.43	26.25±1.33	19.63±1.13

DNA: Deoxyribonucleic acid
MN: Micronuclei
BN:Binuclei
KL: Karyolysis
KR: Karyorrhexis
M: Mean. SD: Standard deviation.

compared to 29(29%) smokers and 71(71%) non-smokers among the experimental group. The mean age was 31.08+10.97years and 28.19+7.58years in the control and the experimental groups, respectively, whereas BMI was 23.46±4.1 and 23.57±4.26 kg/m^2. DNA damage and cell death in terms of average frequency of nuclear abnormalities, including MN, Binuclei (BN), KL and KR, was 2.17±2.16, 2.76±2.42, 8.45±6.4 and 8.95±5.29, respectively, among the controls, and 6.94±1.96, 8.69±2.54, 26.22±1.31 and 19.16±1.23 among the cases. The difference was significant (Table-1).

The frequency of MN, BN, KL, KR per 3000 cells studied in the photocopying experimental smokers group was greater than the frequency in control subjects. A strong correlation existed between the smoking habit and DNA damage parameters (r=0.65) and cell death parameters (r=0.37) in photocopying operators whereas in case of control subjects with the smoking habit revealed the same results with the DNA damage parameters (r=0.77) and cell death parameters (r=0.73). The results from both the groups demonstrated statistically significant difference ($p<0.001$) regarding smoking habit and nuclear abnormalities.

In terms of age 54(54%) controls were aged <30 years and 46(46%) >30 years. In the experimental group, the corresponding values were 65(65%) and 35(35%). Mean MN, BN, KL and KR frequencieswere1.66±1.93, 2.25±2.19, 7.29±5.77 and 8.17±4.44, respectively, among controls aged <30 years compared to 2.76±2.28, 3.34±2.56, 9.80±6.87 and 9.87±6.06 among those aged >30 years.

As for the effect of exposure period on genetic damage, the experimental group was divided into two

categories; exposure <10 years, and >10 years. The data revealed less MN, BN, KL, KR in the workers with exposure <10 years compared to the workers with exposure >10 years. The duration of work showed statistically significant results with the nuclear abnormalities ($p<0.001$) (Table-2).

Discussion

It has been found that photocopying operators can get exposed to volatile organic compounds via inhalation during operational phase, or toners while reloading operation via physical contact. Therefore, the symptoms may lead to short-term as well as long-term consequences. Reported short-term effects include nausea, eye irritation, breathing issues, coughing, sneezing, physical discomfort, fatigue and headache.[16]

A relatively vigorous correlation has been found between age and MN frequency and the chromosomal loss has been considered the ascertaining factor in this elevation. Data clearly depicts that with the increase in age the frequency of damage increases significantly.[17]

In the present study, nuclear abnormalities were observed in the buccal epithelial cells since the sensitivity of the buccal cells to the manifestation of cytogenetic damage due to toxic agents is higher as compared to the lymphocytes.[18] The present study reported an elevation in the frequency of nuclear abnormalities including MN, BN, KL and KR in photocopying operators. A study conducted in India related to the MN detection in buccal epithelial cells reported the similar increase in the frequency of MN reflecting the mutagenesis in the epithelial cells owing to the constituents of toner with the mutagenic potential.[16]

Predominantly, the working span as well as smoking habits revealed significant outcome in the present study. The elevation in the frequencies of micronuclei in exfoliated buccal epithelial cells has been reported in individuals attributable to the exposure to solvents organic in nature.[19] A study revealed considerable increase in micronucleates associated with high smoking in comparison to non-smokers.[20] A remarkable elevation in the cytogenetic parameters has been demonstrated in case of hookah and bidi smokers.[21]

Conclusion

A highly significant difference was observed in DNA damage and cell death of workers associated with photocopy profession. Millions of workers get exposed to assorted types of chemicals in their daily routine. Workers are unaware of the toxic chemical agents as well as their adverse levels. There is a paucity of reporting and the situation is becoming worse. Most of the workers at their workplaces avoid wearing personal protective equipment which is beneficial for their health and safety. There is a need to focus on such issues to curtail the adverse effects.

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