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Micronucleus Frequencies and Nuclear Anomalies in Exfoliated Buccal Epithelial Cells of Firefighters

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ABSTRACT To determine the genotoxic effects of combustion fumes on the target tissues, micronucleus (MN) assay was carried out in exfoliated buccal epithelial cells of 47 firefighters. Compared to a mean value of 1.25 MN per 1000 cells in 40 matched controls, the firefighters had 3.91 MN per 1000 epithelial cells in their oral cavity, thereby showing 3-fold rise over control. Besides MN, the firefighters exhibited higher prevalence for several other nuclear anomalies like 'broken egg' nuclei (5.69 vs. 1.73/1000 cells), pyknotic and condensed nuclei (2.86 vs. 0.62/1000 cells), karyorrhexis (24.12 vs. 6.45/1000 cells), and karyolysis (152.6 vs. 21.5/1000 cells). All these changes were statistically significant ($p<0.05$). The nuclear changes were more prevalent in firefighters with relatively longer duration of service. MN and other nuclear anomalies reflect genetic changes, events associated with carcinogenesis. Therefore the results unveil a high risk of developing cancer among firefighters.

INTRODUCTION

Firefighters are entrusted with the important task of fire extinguishing that saves materials and precious lives. In accomplishing their duties, they are potentially exposed to a vast array of toxic combustion and pyrolysis products such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, acrolein, nitrogen dioxide, and volatile organic compounds (VOCs) (Moen and Ovrebo 1997; Austin et al. 2001; Caux et al. 2002). Many of these PAH and VOCs, such as benzo(a)pyrene, benzene, 1,3-butadiene and benzene are potential carcinogens for humans. Accordingly, PAHs and VOCs have been implicated as sources of long-term health risks like cancer to firefighters (Austin 2001). Despite these reports, no study has so far been carried out on the genotoxic effects of combustion fumes in firefighters of the country. Assessment of genotoxic changes in these subjects is important for identification of persons with high risk of developing cancer. This information may also serve as the basis of any therapeutic intervention.

Genotoxicity of combustion product mixture can be studied directly in target cells of the buccal epithelium by the micronucleus assay. The technique involves examination of epithelial smears to determine the prevalence of cells containing micronuclei. The micronucleus (MN) is defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus (Schmid 1975). Micronuclei originate from aberrant mitoses and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated into the daughter nuclei during mitosis. The MN test is the most frequent technique used to detect chromosome breakage or mitotic interference, events thought to be associated with increased risk for cancer (Stich et al. 1982; Tolbert et al. 1991). Considering these, we have analyzed in this study the MN frequency in exfoliated buccal epithelial cells of the firefighters who are occupationally exposed to high level of combustion fumes.

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MATERIALS AND METHODS

Subjects: A total number of 87 adult male individuals were included in this study. Of these, 47 (mean age 43 yr) were fire service personnel of the twin city of Kolkata and Howrah engaged in firefighting for the last ten years or more. The rest 40 individuals were age-matched (mean age 44 yr) controls consisting of either administrative staff of West Bengal fire service not engaged in

fire fighting and government employees of other departments with office jobs. To eliminate different confounding factors that are known to influence MN count, the firefighters and their controls were chosen in a way so that they were comparable in age distribution, ethnicity, food habit, lifestyle like smoking and alcohol consumption, nutritional status and the extent of indoor air pollution at their homes (Table 1).

Sample Collection and MN Assay: Exfoliated epithelial cells from buccal mucosa were collected by scraping the middle part of the inner cheeks with wooden spatula after moistening the mouth with water. The cells were smeared on slide, dried in air and fixed with cold solution of 1% glutaraldehyde in 0.1M phosphate buffer (pH 7.5) for 20 min. Then the slides were stained by Feulgen reaction essentially by the modified procedure of Belien et al. (1995): hydrolysis in 5N HCl for 30 min at 27^0C, washing in distilled water for 5 min, staining with fresh Schiff reagent (Sigma Chem, USA) for 45 min, and washing in tap water for 15 min. Slides were then counterstained with 0.1% naphthol-yellow for 20 sec. At least 2000 cells of each person were analyzed under light microscope (Leitz, Germany) with 40x dry-lens objective. The slides were illuminated with a halogen light source and the fields of vision were filtered with a monochromatic green filter (λ= 550 nm) for which Feulgen stain shows maximum absorption. An object was considered as MN if it fulfils the established criteria of MN having eight characteristic features (Belien et al. 1995): i. consists of nuclear material, ii. fully separated from the parent nucleus, iii. area is <1/5th of parent nucleus, iv. has lighter staining intensity, v. not fragmented, vi. either round or oval, vii. located within 4-fold the shortest axis of parent nucleus, and viii. not more than two MN in a cell. Besides MN, other nuclear anomalies like 'broken egg' (nuclei that appeared cinched), pyknosis (shrunken nuclei), karyorrhexis (nuclear disintegration), and karyolysis (dissolution of nucleus) were evaluated following the criteria of Tolbert et al. (1991).

Statistical Analysis: The results were statistically analyzed by Students't' test, and $p<0.05$ was considered significant.

Table 1: Characteristics of the study population

Variable	*Firefighter (n=47)*	*Control (n=40)*
Mean age (range) in year	43.2 (27-56)	44.0 (25-55)
Current smoker (%)	0	0
Ex-smoker (%)	4.2	2.5
Tobacco, betel quid chewer (%)	12.8	15
Alcohol consumption (%)	4.2	5
Use of mosquito repellant at home (%)	95.8	100
LPG as cooking fuel at home (%)	89.4	90
Food habit: veg/non-veg mixed (%)	97.9	97.5

RESULTS

Descriptive characteristics of the study populations consisting of 47 firefighters and 40 controls (all males) are compared in Table 1. It is evident that they were similar with respect to age, lifestyle, and indoor air pollution at home etc. This was done in order to minimize the effects of these confounding factors on MN frequency, so that only the effect of combustion products are taken into account.

MN frequency in exfoliated buccal epithelial cells of firefighters was found 3-times higher than the matched controls (3.91 vs. 1.25 per 1000 cells), and the change was highly significant ($p<0.01$, Table 2). Like MN, other nuclear anomalies were more prevalent in firefighters compared with that of controls. For example, 'broken egg' type of nuclear change was recorded in 3.3-times higher frequency in firefighting personnel. The increase in the prevalence of karyorrhexix and pyknosis was even more, 3.7-times and 4.6-times over control values respectively. However, greatest difference between the firefighters and control subjects was recorded in the frequency of karyolysis: 152.6 per 1000 cells in the former group against 21.5/1000 cells in controls, thereby showing a rise of 7-fold over control (Table 2).

In an attempt to examine the impact of the duration of exposure to combustion fumes on

Table 2: Micronucleus frequency and nuclear anomalies in firefighters

Outcome	*Control (n=40)*	*Firefighter (n=47)*
MN per 1000 cells (‰)	1.25 ± 0.12	3.91 ± 0.19*
'Broken egg' (‰)	1.73 ± 0.22	5.69 ± 0.49*
Karyorrhexis (‰)	6.45 ± 0.76	24.12 ± 1.48*
Karyolysis (‰)	21.5 ± 2.2	152.6 ±10.19*
Pyknosis (‰)	0.62 ± 0.13	2.86 ± 0.47*

Results are mean ± SE; *, $p<0.05$ compared with control

MN and other nuclear changes, a comparison was made between persons with relatively shorter (10-19 yr) and longer (20-32 yr) service as firefighter. The results are depicted in Table 3. It is evident that the MN and other nuclear changes were more prevalent in persons having longer service periods. Except for pyknotic nucleus, the differences between these two groups were statistically significant ($p<0.05$, Table 3).

Table 3: Micronucleus frequency and nuclear anomalies in firefighters in relation to length of service

Outcome	*Less than 20 years of service (n=20)*	*≥ 20 years of service (n=27)*
MN per 1000 cells (‰)	3.21 ± 0.24	4.43 ± 0.32*
'Broken egg' (‰)	4.42 ± 0.53	6.63 ± 0.47*
Karyorrhexis (‰)	18.2 ± 1.2	28.5 ± 1.7*
Karyolysis (‰)	105.4 ± 8.7	187.5 ± 11.3*
Pyknosis (‰)	2.62 ± 0.42	3.05 ± 0.51

Results are mean ± SE; *, $p<0.05$ compared with firefighters with < 20 years of service

DISCUSSION

Micronuclei are regarded as markers of abnormal mitoses involving chromosomal breakage and missegregated chromatin (Tolbert et al. 1992). Different laboratories have reported variable normal background MN frequency in human oral epithelial cells: 0.04% (Karahalil et al. 1999) , 0.16% (Tolbert et al. 1991), 0.1-0.3% (Fenech et al. 1999) and 0.33% (Burgaz et al. 2002). In the control group of this study we found 0.125% MN, which is in general agreement with the published reports. Increase in the MN frequency in exfoliated cells of firefighters as observed in the present study can be due to the presence of mutagens like PAH and VOCs in combustion products. Several lines of evidence support this hypothesis. First, PAH including mutagenic benzo(a) pyrene are produced by incomplete combustion of organic matter, and they increase MN frequency in oral epithelium (Karahalil et al. 1999). Second, urinary 1-hydroxypyrene, a measure of PAH exposure, has been found significantly elevated in firefighters (Moen and Ovrebo, 1997; Caux et al. 2001). Third, very high concentrations of VOCs have been recorded in municipal structural fires (Austin et al. 2001), and the concentration of urinary trans,trans-muconic acid, a measure of benzene exposure, has been found greatly elevated in firefighters (Caux et al. 2001).

Besides elevated MN frequency, the firefighters exhibited raised prevalence of several other nuclear anomalies like 'broken egg', pyknosis, karyorrhexis and karyolysis. 'Broken egg' nuclei have no relationship with MN, and its significance is not known (Roberts 1997). Pyknosis and condensed chromatin, on the other hand, are normal features of epithelial cell differentiation and maturation. But they occur at elevated levels in response to cellular injury. While karyolysis is associated with cytotoxicity, pyknosis and karyorrhexix accompany apoptosis (Tolbert et al. 1991), a process under genetic control. Increase frequency of these nuclear abnormalities in buccal epithelial cells of firefighters indicates adverse cellular reaction and/or a surveillance mechanism to eliminate cells with genetic damage.

Micronuclei in exfoliated cells reflect genotoxic events that occurred in the dividing basal cell layer one to three weeks earlier (Tolbert et al. 1991). The MN assay is regarded as an important biomarker to predict the relative risk of cancer in upper aero-digestive tract (Bloching et al. 2000). Thus, greater prevalence of MN in firefighters implies that they are highly exposed to mutagenic insults and therefore are at a greater risk of developing cancer in the upper airways and oropharynx.

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