Evaluation of DNA Damage and Oxidative Stress in Road Pavement Workers Occupationally Exposed to Polycyclic Aromatic Hydrocarbons

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***Abstract*—**Occupational exposures to bitumen and their emissions during road paving are possibly carcinogenic to humans. Road pavement workers are exposed to numerous known carcinogens in their complex occupational environment. The aim of this study was to evaluate the oxidative stress and DNA damages in road pavement workers, who occupationally exposed to polycyclic aromatic hydrocarbons (PAHs) from bitumen fumes. Micronucleus test was studied in both exposed and matching unexposed subjects. Frequency of micronucleus was statistically significant between the two studied groups. Levels of serum glutathione and malondialdehyde were measured spectrophotometrically. The glutathione concentration in exposed group was notably lower than in controls but the concentration of malondialdehyde was considerably elevated indicating oxidative stress. Results imply that PAHs from working environment may be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

***Keywords*—** DNA damage, Oxidative stress, Occupational exposure, Micronucleus test.

I. Introduction

BITUMENS are used mainly in road paving, roofing and flooring, more than 80% of bitumens are used in road construction and maintenance. Road construction work is usually performed by unskilled labourers; moreover they execute diverse tasks, such as bitumen asphalt preparation and road paving, which chronically expose workers to polycyclic aromatic hydrocarbons and heterocyclic compounds. Many of the organic compounds found in asphalt fumes have been shown to be mutagenic or carcinogenic [1].

Epidemiological studies of occupational exposure to bitumens showed the incidence of respiratory diseases and probable association between lung cancer risks in road paving workers [2], [3]. An increase in genetic damage was reported [4] in lymphocytes of road paving workers, whereas few studies contrast with this. At present there is deficient evidence to establish a causal relationship between occupational asphalt exposure and cancer risk [5].

The exposure to various toxic substances increase the production of reactive oxygen species (ROS) with the consequent disturbance of the oxidative balance in the cell disturbs the metabolism and causes oxidative stress [6]. The oxidative stress causes the damage to membrane lipids which manifests as the increase of the malondialdehyde (MDA) concentration. MDA is one of the better-known secondary products of lipid peroxidation. Products of lipid peroxidation are released into plasma as result of membrane damage, and MDA can be used as an indicator of cellular injury [7].

Oxidative stress induces a cellular redox imbalance which may be related to oncogenic stimulation [8]. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been noted in various tumors, strongly implicating such damage in the etiology of cancer [9]. Currently, several cytogenetic techniques served as helpful tools in assessing genotoxicity of physical and chemical agents and the most sensitive among those are micronucleus test [10].

The aim of the present study was the assessment of genetic damage and oxidative stress in PAH exposed road pavement workers. We used, exfoliated buccal epithelial cell micronucleus assay for the detection of genetic damage. In addition, serum GSH and MDA levels were evaluated as oxidative damage markers.

II. Procedure

A. Subjects

The study population composed of 34 male road pavers and 30 unexposed controls. The exposed group included 20 smokers and 14 non-smokers, from different road pavement sites located in Coimbatore City, South India. The control groups were matched for age, sex, smoking habits and had no occupational exposition to toxic agents. All subjects were

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selected based on questionnaire which included items about age, occupational exposure, smoking habit, use of drugs/alcohol, virus illnesses, recent vaccinations, and radiological exams. All the individuals who agreed to participate in the study were healthy, and they answered a detailed questionnaire according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens. For the exposed group, a further questionnaire was completed to evaluate the use of protective measure. These workers were engaged in work for more than 8 hrs per day with a minimum 5 yrs of exposure duration. None of these study groups showed significant differences with regard to lifestyle and personal factors. At the time of sample collection the subjects signed a term of informed consent. The study procedures used in the present study were approved by the Institutional ethics committee.

B. Sampling

Venous blood was drawn from workers and age and sex matched control volunteers and immediately transferred to laboratory in an ice box. Each sample was centrifuged at 4000 rpm and the plasma was separated and stored at -20 °C until analysis. Buccal cells (BCs) were collected from consented volunteers at the end of the work shift according to the criteria established by Tolbert and his co-workers [11]. Prior to BC collection the mouth was rinsed thoroughly with water to remove any unwanted debris. Buccal cell samples were obtained by rubbing the inside of both cheeks using a wooden spatula. The cells were collected in tubes containing 3ml sterile saline.

C. Biochemical assays

The measurement of amounts of protein was based on the method described by Bradford [12]. The GSH level was estimated by the method of Moron et al [13]. The TBARS levels were estimated as per the spectrophotometric method described by Ohkawa et al [14].

D. Micronucleus assay

Buccal cells were smeared on slide, dried in air and fixed with cold methanol: acetic acid (3:1) solution 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 and co-workers [15].

E. Statistical analysis

All data were expressed as mean ± standard deviations (SD). The comparison between two samples was performed by Student's t-test and the *p* values of <0.05 were considered as significant. All calculations were performed using Windows statistical package, version 11.5 (IL, USA).

III. Results

The characteristics of the subjects used in the study are shown in Table I. The individuals were classified according to their age, length of occupational exposure, smoking, and alcohol drinking habits. The two groups studied had similar demographic characteristics.

TABLE I
CHARACTERISTICS OF EXPOSED GROUPS AND CONTROLS

Characteristics	Exposed group	Control group
Number	34	30
Age (years ± S.D)	42.19±7.5	39.65 ±6.3
Exposure (years ± S.D)	10.35±8.3	-
Smoking (N) Y/N	19 / 15	15 / 15
Alcohol (N) Y/N	18 /16	14 / 16
Alcohol (N) Y/N	34	30

The concentrations of GSH and MDA were also measured (Table II). Concentration of GSH was lower in exposed group than the controls which was significant ($p<0.05$) whereas, mean value of MDA in the exposed group was significantly increased than control group ($p<0.05$).

Results on micronuclei frequency is given in Table II. Assessment of MN frequencies in exfoliated buccal cells revealed a significant difference between exposed workers and controls ($p<0.05$). Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei in comparison to non smokers and non alcoholics. Workers who are smokers showed a highly significant increase ($p<0.05$) in MN frequency when compared to all other groups and subgroups.

TABLE II
PLASMA LEVELS OF GLUTATHIONE (GSH) AND MALONDIALDEHYDE (MDA) IN EXPOSED GROUPS AND CONTROLS

Groups		N	GSH (mg/dl)	MDA (nmol/l)	MN (mean ± SD)
Controls (n=30)	Smokers	15	2.82±0.04	1.33±0.08	6.08±0.62
	Non-smokers	15	4.33±0.05	0.33±0.05	3.12±0.54
	Alcoholics	14	2.50±0.13	0.50±0.02	6.11±1.22
	Non-alcoholics	16	4.73±0.33	0.75±0.33	2.33±0.57
Exposed groups (n=34)	Smokers	19	2.04±0.10*	2.33±0.03*	7.80±0.68*
	Non-smokers	15	2.85±0.12*	1.50±0.05*	4.95±1.02*
	Alcoholics	18	1.65±0.17*	1.00±0.07*	8.61±0.93*
	Non-alcoholics	16	2.01±0.08*	2.00±0.08*	4.63±0.55*

All data are shown as mean ± SD; *p<0.05 significant vs. control subjects.

IV. Discussion

PAHs have been identified as cancer-inducing chemicals for animals and humans [16]. Also, there is sufficient evidence that exposures in the occupational settings are carcinogenic or probably carcinogenic to human. High occupational exposure to toxic substances such as PAHs and other petroleum products are the main toxicants for the exposed subjects [17]. It is generally accepted that PAHs may cause direct/indirect cytotoxic and genotoxic effects, thus genotoxicity biomarkers have received a considerable interest as tools for detecting human genotoxic exposure and effects. Searching of association between biomarkers will help to select most advantageous biomarkers for further competent monitoring of various human exposures [18].

Many studies have linked excess generation of reactive oxygen species (ROS) with cellular damage. Malondialdehyde is a decomposition product of autooxidation of polyunsaturated fatty acids is used as an index of oxidative

damage [19]. The present study observed a significantly high level of MDA in road pavement workers compared to the normal control group ($P<0.05$), which is in agreement with the previous studies.

GSH is the most abundant intracellular thiol-based antioxidant and plays a significant role in the cellular protection cascade against oxidative injury [20]. This study revealed a significant correlation between PAH exposure and GSH level. The findings are in well agreement with the previous investigations. The significantly decreased levels of GSH in this study could be to increased oxidative stress. A decreased GSH level is significantly correlated with altered DNA repair efficiency. Altered DNA repair efficiency is an additional risk factor for the development of cancer.

Buccal cells are the primary barrier for the inhalation and are capable of metabolizing proximate carcinogens to reactive products [21], [22]. Approximately 90% of human cancers originate from epithelial cells [23]. The oral epithelial cells represent a target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. Exfoliated buccal epithelial cells were used to evaluate the genotoxic effects and are an efficient tool for biomonitoring studies [24], [25]. The MN assay in buccal cells was also used as a biomarker in several genotoxicological studies [26]-[28].

Influence of lifestyle factors such as alcohol consumption and smoking habits by measuring the MN frequency greatly influenced the cytogenetic damage, which is inline with different occupational studies [29], [30]. Cigarette smoking is one of the daily life related public health threads that may influence the rate of cytogenetic damage [31]. In addition smoking may quantitatively influence the MN frequency in buccal cells [32], [33]. Our findings indicate that cigarette smoking significantly increases the frequencies of MN in both exposed and non exposed workers, these are consistent with recent reports suggesting an association between smoking and occupational exposure [34]. Since cigarette smoke contains about 50 potent carcinogens, including poly-aromatic hydrogen carbons (PAHs) and other organic chemicals, which may interfere with the production of ROS and the activity of free radicals during oxidative metabolism. Hence, the increase in MN by cigarette smoking is biologically believable. Serum malondialdehyde as an index of lipid peroxidation, and the serum enzyme GSH provides valuable information on biomonitoring test [35].

The results confirm that MN is a sensitive indicator for use in monitoring occupational exposure. Hence it is recognized that oxidative and genetic damage may be a sign of possible health risks. Employees, who may be exposed directly or indirectly to PAHs, need to be made aware of the genotoxic effects and ensure safe and healthy working atmosphere to alleviate the health hazards that they may encounter.

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