

Evaluation of Genotoxicity in Automobile Mechanics Occupationally Exposed to Polycyclic Aromatic Hydrocarbons Using Micronuclei and Other Nuclear Abnormalities

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Abstract

Background: Occupational and environmental exposures mostly represent mixtures of genotoxic agents, whereas the specificity of biomarker measurements varies widely. Exploration of correlations among biomarkers contributes to the further progress of molecular cancer epidemiology and to the selection of the optimal biomarkers for the investigation of human exposure to carcinogens. The aim of this study was to assess the potential cytogenetic damage associated with occupational exposure to Polycyclic Aromatic Hydrocarbons (PAHs) among automobile mechanics by using Micronuclei (MN) and other Nuclear Abnormalities (NA) as a biomarker.

Methods: The study population composed of 110 occupationally exposed automobile mechanics and 100 unexposed controls. All the study participants were males. Both the exposed and control individuals were selected from automobile garages located in the urban area of Coimbatore City, South India. Exfoliated buccal cells were collected from 110 automobile mechanics and 100 age and sex matched controls. Further, cells were examined for MN frequency and Nuclear Abnormalities (NA) other than micronuclei, such as binucleates, broken eggs and karyolysis.

Results: Results showed a statistically significant difference between occupationally exposed automobile mechanics and control groups. MN and NA frequencies in automobile mechanics were significantly higher than those in control groups ($p < 0.05$) and also significantly related to smoking habit ($p < 0.05$). In addition, a higher degree of NA was observed among the exposed subjects with smoking, drinking, tobacco chewing, which is an indicative of cytogenetic damage in these individuals.

Conclusion: MN and other NA reflect genetic changes, events associated with carcinogenesis. Therefore, the results of this study indicate that automobile mechanics exposed to PAHs are under risk of significant cytogenetic damage. Therefore, it is important to provide and offer better awareness of occupational hazards among these workers to promote occupational safety.

Keywords: DNA damage; Micronucleus test; Polycyclic Aromatic Hydrocarbon; Occupational exposure

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Introduction

Occupational health deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards. The health

of the workers has several determinants, including risk factors at the workplace leading to cancers. Humans are diverse in their responses to exogenous exposures because of variability, the rate of

metabolism, DNA repair processes and other factors [1]. Millions of workers in a variety of occupational settings have the potential to be exposed to hazardous substances. They can be present in the occupational environment in the form of gases, vapors, fumes and particles [2].

Occupational exposures to hazardous chemicals are common in industries using solvent based materials; furthermore, people are exposed to volatile organic compounds from various sources [3], among the most common contaminants Polycyclic Aromatic Hydrocarbons (PAHs) are of particular concern as they are comprised of a group of highly lipophilic, non-polar and persistent substances with remarkable mutagenic and carcinogenic properties. Exposure of PAHs can come from both occupational and environmental sources arise from use of coal tar, exposure to automotive exhaust, gases, tobacco smoke and many other sources [4, 5].

Automobile mechanics, because of their occupation, are exposed to complex mixture of PAHs, which have been implicated in vast number of toxicological manifestations in human [6]. PAHs are a class of compounds composed of two or more aromatic rings. Over a hundred PAHs have been identified and these are usually found as complex mixtures [7]. PAHs are a major class of environmentally hazardous organic compounds due to their known or suspected carcinogenicity [8, 9] and are known to be toxic [10]. Automobile mechanics exposed to PAHs are at an increased risk of lung, urinary tract and skin cancer [11]. The work environment plays a critical role in the potential exposures among mechanics and they frequently work with dirty and greasy automotive parts. The multiple work tasks performed results in a heterogeneous mixture of potential exposures among mechanics.

Some of PAHs are tumorigenic due to their metabolites and their ability to generate DNA adducts and oxidative DNA damage through the production of reactive oxygen species during metabolism [12]. While the PAHs in their initial form may not exhibit any carcinogenic properties, the metabolites of the parent compound often do show some carcinogenic tendencies. When humans interact with chemicals in their workplaces or natural environments, it may result in either detoxification or activation processes, which ultimately leads to an interaction with hemoglobin, proteins, DNA, or normal cells and formation of biological end products that may result in genotoxic effects.

Micronucleus (MN) assay for exfoliated cells have been used to evaluate the genotoxic effects

produced by low doses of carcinogenic substances or carcinogenic mixtures, to which human populations are exposed [13].

In South India, automobile garages are located on streets and workers at the garages, have a higher opportunity for exposure. Automobile mechanics are engaged in adjusting, repairing and overhauling of automotive vehicles, there are a number of sub-specialties, most of the mechanics do not wear personal protective equipments, and personal hygiene varies in their work place. Therefore, the occupational exposure to PAHs and other derivatives may possess genotoxic risk. Occupational exposure to carcinogens is of great public health concern. To add further knowledge to the genetic risk related to PAHs exposure, we applied the MN and other NA as a biomarker in occupationally exposed automobile mechanics.

Materials and Methods

The study population composed of 110 automobile mechanics and 100 controls. All of the study subjects were men aged 27 to 50 years. The workers had varying durations of exposure (7-15 years). The exposed group included 30 smokers and 30 non-smokers, 32 smokers with alcohol drinking and 18 smokers with tobacco chewing selected from 23 automobile garages located in the urban area of Coimbatore City, South India. The respective control groups were matched for age and sex (31 smokers, 28 non-smokers, 30 smokers with alcohol drinking, and 11 smokers with tobacco chewing) and had no occupational exposition to toxic agents. At the time of the sample collection, the subjects signed a term of informed consent. All subjects were selected based on a questionnaire which included items about age, occupational exposure, smoking habit, use of drugs, such as alcohol, virus illnesses, recent vaccinations, and radiological exams. All the individuals who agreed to participate in the study were healthy, and answered a detailed questionnaire according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens [14]. For the exposed group, a further questionnaire was completed to evaluate the use of protective measure. None of these study groups showed significant differences with regard to lifestyle and personal factors. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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 [15]. Prior to BC collection, the subjects' 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.

The 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 [16]. In short, hydrolysis in 5N HCl for 10 min at room temperature, washing in distilled water for 5 min, stained with fresh Schiff reagent (Sigma Chem, USA) for 90 min, and washing in tap water for 15min. The cells were counter stained in Coplin jars containing 1% Fast Green reagent for 2-5 min and rinsed with distilled water. Slides were analyzed under light microscope (Leitz, Germany) with 1000X magnification. A total of minimum 2000 cells per individual were scored for analysis of micronuclei. The slides were randomized and scored by a single observer.

Micronuclei were scored in normal cells. In addition, the frequencies of NA, namely Binucleates (BN), Broken Eggs (BE), and Karyolysis (KL) were recorded. MN and other NA were classified according to Tolbert and co-workers [16]. MN satisfied the following conditions: a) consisting of nuclear material; b) be completely separated from the parent nucleus; c) be less than 1/3 of the diameter of associated nuclei; d) be smooth, oval- or round shaped; e) be on the same plane of focus ; f) be of the same color, texture and refraction as the main nucleus. Cells with two nuclei were considered to be binucleate. Besides MN, other NA, such as BN, BE, and KL were recorded separately. The role of nonparametric factors was analyzed by the Mann-Whitney test, while the Students t-test was used for age and time comparisons. All calculations were performed using Windows statistical package, SPSS, version 11.5 (IL, USA). Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking and age) was determined.

Results

The characteristics of the study subjects are demonstrated in Table 1. The individuals were classified according to their age, length of occupation, smoking, tobacco chewing and alcohol drinking habits. The two studied groups had similar demographic characteristics.

The frequency of MN and NAs was studied in 110 automobile mechanics and in 100 controls. Workers

revealed a significant induction of MN when compared with controls ($p < 0.05$). Assessment of MN frequencies in exfoliated buccal cells revealed a significant difference ($p < 0.05$) between exposed workers with smoking (7.90 ± 1.91) and controls with smoking habit (2.88 ± 1.20). Smoking had a marked effect on MN frequency among controls (2.88 versus 1.93 between smokers and non-smokers, respectively). The average MN frequency in the exposed non-smokers was 6.94 ± 1.76 , and in the control non-smokers was 1.93 ± 0.88 ($p < 0.05$). The average MN frequencies were 7.66 ± 2.15 and 2.66 ± 1.12 between exposed smokers with tobacco chewing and unexposed smokers with tobacco chewing, respectively (Table 2).

The average MN frequencies in the exposed smokers with drinking were 7.59 ± 2.18 , and the unexposed control smokers with drinking were 2.26 ± 1.07 , respectively. A significant correlation ($p < 0.05$) was observed between MN induction and duration of exposure in workers. Like MN, other NA was more prevalent in PAHs exposed automobile mechanics compared with that of controls. These results show that individuals with smoking, drinking, tobacco chewing and exposure to PAHs have significantly higher frequencies of MN induction, indicative of cytogenetic damage in these individuals.

Discussion

Genotoxicity biomarkers have received a considerable interest as tools for detecting human genotoxic exposure and effects, especially in health surveillance programs dealing with chemical carcinogens. Exploration of correlations between biomarkers will contribute to the development of human biomonitoring to genotoxic exposures and will help to select optimal biomarkers for more efficient monitoring of various human exposures [17].

Micronucleus test has been receiving increased attention as a simple and sensitive short-term assay for detection of environmental genotoxicants [18]. Analysis of exfoliated buccal mucosa cells provides evidence of other nuclear abnormalities such as binucleates, karyorrhexis and karyolysis [19]. Our earlier study provides an evidence of MN and other nuclear abnormalities in the buccal mucosa cells of tobacco chewers [20].

Many PAHs have been identified as cancer-inducing chemicals for animals and humans [21]. In addition, there is sufficient evidence that exposures in the occupational settings are carcinogenic or probably carcinogenic to humans. Automobile mechanics are known to have a high occupational

Table 1. General characteristics of groups studied

Study group	N	Age (years) mean \pm SD	Average no of cigarettes/day	Alcohol intake in last 1 yr (g alcohol drinking/day) mean \pm SD	Duration of employment (years) mean \pm SD
Control (n=100)	Smokers	31	35.60 \pm 2.28	20	-
	Non-smokers	28	33.28 \pm 1.18	-	-
	Smoking with Drinking	30	36.08 \pm 3.01	18	88.91 \pm 33.53
	Smoking with tobacco chewing	11	32.31 \pm 1.23	16	-
Workers (n=110)	Smokers	30	32.33 \pm 2.20	21	-
	Non-smokers	30	31.76 \pm 1.73	-	-
	Smoking with Drinking	32	30.33 \pm 2.04	17	92.12 \pm 31.05
	Smoking with tobacco chewing	18	29.21 \pm 2.18	18	-

Table 2. The frequencies of micronuclei and other nuclear abnormalities in exfoliated buccal epithelial cells of control and PAHs exposed automobile mechanics

Study group	N	MN (mean \pm SD)	BN (mean \pm SD)	BE (mean \pm SD)	KL (mean \pm SD)	
Control (n=100)	Smokers	31	2.88 \pm 1.20	4.08 \pm 1.28	6.77 \pm 1.30	3.52 \pm 2.25
	Non-smokers	28	1.93 \pm 0.88	3.01 \pm 1.31	3.52 \pm 2.25	2.80 \pm 1.47
	Smoking with Drinking	30	2.26 \pm 1.07	3.76 \pm 1.36	5.91 \pm 1.82	3.38 \pm 2.31
	Smoking with tobacco chewing	11	2.66 \pm 1.12	3.87 \pm 1.23	5.01 \pm 1.16	3.45 \pm 2.35
Workers (n=110)	Smokers	30	7.90 \pm 1.91*	8.95 \pm 1.36*	14.91 \pm 5.21*	8.50 \pm 1.19*
	Non-smokers	30	6.94 \pm 1.76*	7.31 \pm 1.12*	11.17 \pm 4.06*	6.77 \pm 1.30*
	Smoking with Drinking	32	7.59 \pm 2.18*	8.80 \pm 1.20*	13.94 \pm 6.32*	8.13 \pm 1.32*
	Smoking with tobacco chewing	18	7.66 \pm 2.15*	8.85 \pm 1.51*	13.57 \pm 7.63*	8.19 \pm 1.53*

MN=cells with Micronuclei; BN=Binucleated cells; BE= Broken Egg cells; KL= Karyolytic cells *significantly different with their respective controls, p<0.05.

exposure to toxic substances, which include PAHs, which are found in petroleum products and are the main source of contamination for the exposed subjects [22].

An increase in the frequency of MN was noticed in the blood lymphocytes of PAHs exposed garage workers [23]. Similarly, PAHs exposed Coke oven workers showed significantly higher frequency of MN and other NAs [24]. In the present study, we report an increase in MN and other NAs of PAHs exposed

automobile mechanics than non-exposed control group.

Normally tobacco smoke results in exposure to several carcinogenic PAHs [25]. Burgaz and co-workers [26] found a significant increase of micronucleated cells (p<0.001) in smokers, as compared to non-smokers. Smoking is an important route of direct exposure to PAHs and a well-known source of many other chemicals [27]. Although the link between smoking and cancer is strong and

exposure to genotoxic carcinogens present in tobacco smoke has been convincingly demonstrated [28], and the same convincing association is apparent when assessing biomonitoring studies of genotoxicity. In our previous study, we found an elevated frequency of buccal cell MN in smoking group of petrol station attendants, metal arch welders, tannery workers and road paving workers [29-31].

The present investigation suggests that automobile mechanics under their particular conditions of exposure (cigarette smoke, alcohol and tobacco) revealed clear evidence of genotoxicity in exfoliated buccal cell when evaluated by MN test. Besides, elevated MN frequency, PAHs exposed automobile mechanics exhibited raised prevalence of other NAs like BN, BE and KL. These abnormalities occur at an elevated level in response to cellular injury. Increased frequency of these NA in buccal epithelial cells of PAHs exposed automobile mechanics indicates adverse cellular reaction and/or a surveillance mechanism to eliminate cells with genetic damage. Our analysis showed that smoking status affected genetic damage in the both studied groups, but a significant association emerged only among exposed workers. In our study, smoking, tobacco chewing, and alcohol drinking is associated with a significant induction of MN among PAHs exposed automobile mechanics. This shows synergistic effect between habitual usage and occupational exposure. Thus, the results of the present study make it clear that the PAHs exposed automobile mechanics had a significant increase in cytogenetic damage. These workers may not be aware that they have been exposed to genotoxic agents nor do they know the type and amount of agent to which they have been exposed. Therefore, there is a need to educate those who work with PAHs, about the potential occupational hazards and the importance of using protective measures.

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Conflict of Interest

There is no conflict of interest for this study.

Authors' Contribution

Sellappa Sudha designed the study. Mohammed Rafiq Khan contributed to the study design, literature

review, analyzed the data and wrote the first draft of the manuscript. Both the authors read and approved the final manuscript.

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