

**ASSESSMENT OF GENOTOXICITY IN EXFOLIATED BUCCAL EPITHELIAL CELLS OF FOUNDRY WORKERS OCCUPATIONALLY EXPOSED TO POLYCYCLIC AROMATIC HYDROCARBONS**SARANYA RAMALINGAM SINGARAVELU<sup>1</sup> AND SUDHA SELLAPPA<sup>1\*</sup>

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Received: 4 April 2013, Revised and Accepted: 23 April 2013

**ABSTRACT**

**Objectives:** Foundry workers are exposed to a mixture of chemicals and polycyclic aromatic hydrocarbons (PAHs), which is suspected to cause genetic damage. The aim of this study was to assess PAHs exposure by urinary excretion of 1-hydroxypyrene (1-OHP), a biological exposure marker. Moreover, we aimed to assess the level of genetic damage in south Indian foundry workers exposed to PAHs. **Methods:** Forty eight foundry workers and a standardised control group were examined for frequencies of micronucleus (MN) in buccal epithelial cells. Current exposure to PAHs was assessed by measurement of 1-OHP in urine samples. **Results:** An elevated concentration of 1-OHP was found in the exposed group ( $2.15 \pm 0.87 \mu\text{g/g}$  creatinine) relation to the control group ( $0.79 \pm 0.58 \mu\text{g/g}$  creatinine) ( $p < 0.05$ ). The genetic damage observed in the buccal cells of foundry workers was significantly higher than that in controls. Cigarette smoking was also related to genetic damage since the MN observed in PAHs exposed groups with smoking habits was significantly higher than non-smoking workers. **Conclusions:** Occupational exposure of PAHs from foundry workplaces has been associated with the increased genetic damage and smoking habit represents an additional risk factor. Exposure to PAHs may be aetiologically related to increased risk of cancer in foundry workers.

**Keywords:****INTRODUCTION**

Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) has been reported in a number of occupational settings. Several carcinogenic agents have been identified in contaminated foundry air; PAHs are among the most prevalent air pollutants. Foundry processes produce many hazardous chemicals (1) such as heat, metal dusts, fumes, silica, polycyclic aromatic hydrocarbons and metals that are released into the workplace and may have damaging effects on the health of the workers (2). PAHs are produced from the incomplete combustion of organic materials and have been considered as a possible cause of lung cancer among foundry workers (3).

Previous studies have demonstrated that increased concentration of PAHs in the workplace environment could induce the formation of DNA damage of exposed workers at road paving, bitumen and fire-proof plants (4). In addition, an increased risk for oxidative damage was found in PAH exposed coke-oven workers. Another study showed a surplus frequency of symptoms of chronic bronchitis in PAH exposed foundry workers (5), and an epidemiological study was also found an increased risk of cancers in foundry workers (6).

Human genotoxic exposures are encountered environmentally, occupationally, medicinally or through lifestyle choices. Biomarkers can be employed as end-points for the assessment of human-genotoxic interactions from exposure to effects and individual host susceptibility (7). Two widely used biomarkers of carcinogen exposure are urinary metabolites that indicate the internal exposure dose and genetic biomarkers like micronucleus (MN) assay that reflect the biologically effective dose. Mutations and cytogenetic changes are generally considered to be biomarker of effects (8, 9).

The purpose of the present investigation was to assess PAHs exposure by urinary excretion of 1-hydroxypyrene (1-OHP), a biological exposure marker. To include additional data to the genetic risk associated to foundry exposure, we applied the micronucleus (MN) assay to assess the biomarkers of early biological effects such as genotoxicity in foundry workers.

**SUBJECTS AND METHODS****Study Population**

The study population consisted of 95 male subjects (48 foundry workers and 47 controls). Foundry workers were employed in melting, machine moulding, casting, or sand preparation in and

around Coimbatore city, South India. The foundry workers had varying durations of exposure (5-20 years) and they were in the age group of 25-60 years. The experimental group was further branched as smokers (20) and non-smokers (28). The control group was selected from the general population with no history of occupational exposure to toxic fumes or any known physical or chemical agents in the workplace, but belonged to the same age group and socio-economic status as the foundry workers. The selection criteria were based on a questionnaire. The questionnaire covered standard demographic questions (sex, age, marital status, medical history, life style) and occupational questions (per day and years of exposure). We ensured that the workers and the controls did not markedly differ from each other except occupational exposure. Tobaccos, cigars, smoked were translated to cigarettes when calculating pack-years. In all cases, individuals who smoked more than five cigarettes per day for at least one year and those who consumed 120 gm of alcohol/day were considered as smokers and alcohol consumers. The study was conducted in accordance with the principles for human experience as defined by the Helsinki declaration. The research protocol was accepted by the institutional ethics committee.

All workers under study did not wear any safety materials as protective equipment. The exposure assessment was carried out in different foundry sites in and around Coimbatore city from January to December 2012. Biological monitoring of the exposure and evaluation of effects in foundry workers were performed on samples of urine and buccal cells collected simultaneously in the same working week.

**Buccal cell collection**

The buccal cells were collected from each subject after the work shift. Workers were asked to rinse the mouth with distilled water. The buccal cells were collected using a toothbrush by scrapping the inside cheek of the mouth. The toothbrush was agitated in 30 ml cold PBS buffer and the suspension was centrifuged at 2500 rpm at 4°C for 10 min. The cell pellet was resuspended in 100  $\mu\text{l}$  PBS buffer.

**Micronucleus test**

Cell suspension of 10  $\mu\text{l}$  was smeared on a microscopic slide, fixed and stained with Papanicolaou (PAP) stain. Whole of the smear was screened for counting of micronuclei. A total of minimum 2000 cells

per individuals were scored for analysis of MN. The criterion which was developed by Tolbert et al was used for counting the micronuclei, parameters for identifying micronucleus are rounded smooth perimeter, less than a 1/3 the diameter of associated nucleus, staining intensity similar to nucleus, texture similar to main nucleus, same focal plane as main nucleus and absence of overlap with or bridge to nucleus (10). Dead or degenerating cells (karyolysis, Karyorrhexis, nuclear fragmentation) were excluded from evaluation. Nuclear blebbings were also not considered.

#### Urinary 1-Hydroxypyrene analysis

Urine samples were collected in polyethylene bottles (which had been washed with 0.2% HNO<sub>3</sub>) at the end of the work shift and they were kept cold ( $\pm 4^{\circ}\text{C}$ ) during collection and with 20% glycerol (volume for volume) to minimize cell loss due to lysis after freezing ( $-20^{\circ}\text{C}$ ). Urine samples were analyzed for 1-hydroxypyrene according to the method developed by Jongeneelen *et al* 1987 (11). In brief, the urine was acidified and the glucuronic acid and sulfate were enzymatically removed. Creatinine levels were used to estimate urinary dilution using a colometric test, based on the Jaffe reaction between creatinine and sodium picrate (12). Urinary 1-hydroxypyrene was expressed as micromoles per mole of creatinine.

#### STATISTICAL ANALYSIS

Data were analyzed using the statistical package for social sciences (SPSS) version 11.5 for Windows. The following statistical methods were employed: Student's t-test was used for age and time comparisons, Mean values and standard deviations were computed for the scores and the statistical significance ( $P < 0.05$ ) of effects (exposure, smoking, alcohol consumption and age) was determined using analysis of variance (ANOVA).

**TABLE 1: Showing demographic features of the study population and mean urinary 1-OHP concentrations among foundry workers and controls**

Study group	(n=95)	Age (years) <sup>1</sup>	Cigarettes daily <sup>1</sup>	Frequency <sup>2</sup>	Duration <sup>3</sup>	Urinary1-OHP <sup>4</sup>
<b>Controls (n=47)</b>						
Smoking Yes	24	43.00 $\pm$ 11.28	9.91 $\pm$ 2.48	-	-	0.79 $\pm$ 0.58
No	23	43.17 $\pm$ 10.64	-	-	-	0.39 $\pm$ 0.49
Alcohol Yes	25	40.96 $\pm$ 10.84	-	450.01 $\pm$ 239.02	-	0.68 $\pm$ 0.47
No	22	40.18 $\pm$ 11.35	-	-	-	0.59 $\pm$ 0.50
<b>Workers (n=48)</b>						
Smoking Yes	20	41.45 $\pm$ 8.83	11.05 $\pm$ 1.93	-	12.45 $\pm$ 4.72	2.15 $\pm$ 0.87*
No	28	41.21 $\pm$ 10.11	-	-	13.03 $\pm$ 4.29	1.75 $\pm$ 0.75*
Alcohol Yes	27	40.70 $\pm$ 9.93	-	517.03 $\pm$ 234.59	12.62 $\pm$ 4.27	1.70 $\pm$ 0.72*
No	21	41.90 $\pm$ 10.05	-	-	13.57 $\pm$ 3.91	1.47 $\pm$ 0.51*

<sup>1</sup>(Mean $\pm$ SD); <sup>2</sup>(EtOHgm/day); <sup>3</sup>(yrs); <sup>4</sup> $\mu\text{mol/mol}$  creatinine; \* $P < 0.05$

**TABLE 2: Showing frequency of Micronuclei in association with smoking habit and alcohol consumption in controls and workers**

Study group			N = 95	MN (Mean $\pm$ SD)
Controls (n=47)	Smoking status	Yes	24	4.08 $\pm$ 1.24
		No	23	3.34 $\pm$ 1.19
	Alcohol status	Yes	25	3.72 $\pm$ 1.20
		No	22	3.04 $\pm$ 1.17
Workers (n=48)	Smoking status	Yes	20	5.8 $\pm$ 1.36*
		No	28	4.5 $\pm$ 1.64*
	Alcohol status	Yes	27	5.14 $\pm$ 1.09*
		No	21	4.38 $\pm$ 1.11*

#### DISCUSSION

Occupational exposures to hazardous chemicals are common in industries using solvent based materials; furthermore, people are exposed to volatile organic compounds from various sources (13), among the most common contaminants Polycyclic Aromatic Hydrocarbons (PAHs) have been identified as cancer inducing chemicals for animals and humans (14).

Occupational exposure to PAHs has been reported for its association with several cancers (15-19). High levels of PAHs exposure in foundry workers have been reported earlier (20-22) and special PAHs, such as anthracene (Ant), fluorene (Flu), naphthalene (Nap) and phenanthrene (PA), are reported in foundries.

#### RESULTS

The demographic characteristics of the study subjects are presented in Table 1. The age, alcohol consumption and smoking status distributions were similar among exposed workers and controls. Among the smokers and alcoholics, the years of smoking/ alcohol consumption were more or less similar in the two groups.

The mean urinary level of 1-OHP concentration of foundry workers ( $2.15 \pm 0.87 \mu\text{mol mol}^{-1}$  of creatinine) were significantly higher ( $P < 0.05$ ) than the mean ( $0.61 \pm 0.51$ ) displayed by the controls (Table 1). A higher level of mean urinary level of 1-OHP was observed in the workers with smoking habits in contrast to controls with smoking habit. Similarly alcoholics also revealed an increase in mean urinary 1-OHP levels in workers when compared to their respective controls. A clear and statistically significant ( $P < 0.05$ ) increase in mean urinary 1-OHP levels was observed in experimental group when compared to control groups. As analyzed smoking and alcohol differences shows that exposed subjects carry more urinary exposure than control subjects.

The results of MN assay are shown in Table 2. The frequency of MN was studied in 48 male foundry workers and in 47 controls. Workers showed a significant induction of MN when compared with controls ( $P < 0.05$ ). Those of the exposed as well as control groups with smoking and alcohol habits displayed an increased frequency of micronuclei when compared to the non-smokers and non-alcoholics. A significant increase ( $P < 0.05$ ) in MN frequency was observed in smokers and alcoholics when compared to non smokers and non alcoholic groups. A slightly significant correlation was observed between MN induction and period of exposure in workers (Table 2).

To foresee total internal exposure to PAHs we used urinary level of 1-OHP as internal biomarker of occupational exposure. In the present study, an elevated concentration of urinary level of 1-OHP was observed in the urine samples of PAHs exposed foundry workers. Similarly, 1-hydroxypyrene in urine has been used as a biomarker in studying occupational exposure to PAHs in paving workers (23), coke oven workers (24), and foundry workers (25).

The urinary level of 1-OHP assay proved to be able to detect differences in PAH exposure not only at high occupational exposures but also in cigarette smokers (26). The present study validated that the level of 1-hydroxypyrene in smokers sub-group and found an increased level of 1-OHP for combined exposure to PAHs.

Micronucleus test has been receiving increased attention as a simple and sensitive short-term assay for detection of environmental genotoxicants (27). MN could arise from the two basic phenomena in mitotic cells, which are chromosome breakages and the dysfunction of the mitotic apparatus (28). MN assay can be regarded as an important biomarker to predict the relative risk of occurrence of cancer (29). Concerning the effect of smoking on MN frequency, the data reported in biomonitoring studies are contradictory (30-36). In this study, we found a significant increase of MN among smokers when compared to non-smokers in exposed and non-exposed groups. Although the findings of our study are in accordance with the previous studies where, we found an elevated frequency of buccal cell MN in smoking group of petrol station attendants, metal arch welders, tannery workers, road paving workers, building construction workers, automobile mechanics and textile printing workers (37-39;40,41).

Alcohol use can enhance the number of micronuclei (42). Similarly, in the present study a significant increase in MN frequency was observed in workers than controls with drinking habits, which may signify the existence of an influence of alcohol use on the MN formation. Agreeably our previous work on peripheral blood lymphocytes of welders revealed significant increase of MN among workers with alcohol habits (43).

Our study reported an elevated level of MN frequency among PAHs exposed foundry workers. The existing analysis implies that foundry workers under the particular conditions of exposure (tobacco smoke and alcohol) revealed a clear evidence of genotoxicity in buccal epithelial cells as evaluated by MN test. The possible confounding factors that proved variation among the sub-groups which we measured could influence the results.

#### CONCLUSION

Our findings suggest that not only occupational exposures but also smoking plays a vital role in the elevation of 1-hydroxy-pyrene in urine. The present study showed that exposure to PAHs induces genotoxic effect in buccal epithelial cells, indicating a potential health risk for foundry workers. Therefore, to ensure maximum occupational safety, biomonitoring is of great value for assessing the risk for foundry workers.

#### ACKNOWLEDGMENTS

The authors are grateful to the authorities of Karpagam University, Coimbatore, and Tamilnadu, India for providing facilities and for their encouragement. The authors also grateful to Dr. B. Ravichandran, Scientist B, Regional Occupational Health Centre, Bangalore for his valuable suggestions. We are thankful to the industrial workers and control group who had volunteered and came forward to donate their samples, without whose cooperation the study would have been incomplete

#### DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

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