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FREQUENCY OF MICRONUCLEI IN EXFOLIATED BUCCAL EPITHELIAL CELLS OF SAND MOLDING FOUNDRY WORKERS IN TAMILNADU, SOUTH INDIA

SARANYA. R. S., SUDHA, S [*].

[*]Molecular diagnosis and drug discovery laboratory, Department of Biotechnology, School of life sciences, Karpagam University, Coimbatore, Tamilnadu, India

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***Correspondence for Author:**

*** Dr Sellappa Sudha**,
Associate Professor
Molecular diagnosis and drug discovery laboratory,
Department of Biotechnology
School of life science
Karpagam University,
Coimbatore, Tamilnadu, India
sudhasellappa@yahoo.co.in,

ABSTRACT

Occupational exposure to carcinogenic substances and metals has been reported in foundries. In the present investigation, totally 74 foundry workers involved in sand molding process and 52 control subjects with similar mean ages, smoking prevalence and alcohol consumption were enrolled for the analysis of micronucleus (MN) as a measure of genotoxicity in exfoliated cells of buccal mucosa. For each individual, 2,000 exfoliated buccal cells were analyzed. There was a significantly higher frequency of micronucleated cells in the exposed workers (4.96±0.72) to silica than in the unexposed control population (1.89±0.37). Smoking and drinking (alcohol) habits, age and length of occupation represent significant factors in terms of increased MN frequency in the exposed population. Further research is needed with large sample size to confirm the relationship between the exposure and MN frequency.

Key words: Cytogenetic damage, Biomarker, Silica, Occupational exposure.

INTRODUCTION

In the last few decades, genotoxicity biomarkers have received wide attention as tools for identifying human genotoxic exposure and effects, particularly in health observation programs dealing with occupational exposure. Populations of industrial areas are extremely exposed to chemicals that can cause mutations, cancer and other defects [1].

Occupational agents can induce several types of cancer, such as lung, urinary tract, skin, larynx and pancreatic cancers and leukaemia [2]. Occupational exposure to silica dust is found to be the main cause of lung cancer [3]. One mode to study the effects on an exposed population is to conduct bio monitoring studies using appropriate biomarker assay for human genotoxic exposure and effect. The obtained information can be used as an early warning about the potential health risk associated in the long run [4].

The foundry industry is very diverse in materials and processes, resulting in occupational exposures to a wide variety of substances like toluene, phenol, sulphur oxides, carbon monoxide, etc. [4]. Substantial exposures to silica dust continue to occur in many foundries.

It is estimated that nearly two million workers are exposed to respirable silica in foundry sand molding process, over 1, 00,000 of them being in high-risk [5]. The principal molding material used in foundry is silica sand, where respirable siliceous dust is produced as a product of furnaces, molding sand, and shakeout of castings. These silica forms are an occupational carcinogen. Although the mechanisms of their carcinogenesis are not clear yet, it is generally believed that generation of reactive oxygen species (ROS) and abnormal regulation of apoptosis play a critical role in neoplastic development [6]. Micronucleus (MN) assay designed for exfoliated cells have been used to evaluate the genotoxic effects formed by low doses of carcinogenic substances or carcinogenic mixtures, to which human populations are exposed [7-9]. Micronuclei are produced from the intact chromosomes which are stimulated by genotoxic stress such as clastogen or aneugen. Clastogenic and aneugenic agents are well-known to disrupt the spindle checkpoint, where chromatin is detached from the newly forming nucleus and forms an individual nucleus-like structure, the micronucleus [10-13].

The working environment of sand molding in foundries is extremely hazardous and characterized by multiple simultaneous chemical and physical hazards like silica dust, metal dust, various chemicals, noise, heat and radiations, etc. [14, 15]. The workers in their working environment are less aware of the carcinogenic exposure and having minimal level of protective measure. Taking into account that occupational exposure to such hazards may possess genotoxic risk, we aimed to investigate the cytogenetic damage in exfoliated buccal cells obtained from foundry sand molding workers and control subjects, using the micronucleus test. Frequencies of micronuclei were compared in smokers and alcoholics in order to examine the possible effects of smoking and drinking.

MATERIALS AND METHODS

Samples

A total of 126 individuals (74 sand molding workers and 52 controls) were analyzed in this study. The workers in the age group 37-58 years with varying exposure duration (5-31 years) were included in the study. The experimental group was further divided as smokers, non-smokers, alcoholics and non-alcoholics. The participation of each subject was voluntary and the subjects could withdraw at any time during the study. Subjects with both smoking and alcohol consumption were excluded from the study. The control group was selected from the general population with no history of exposure to any kind of toxic chemicals, any serious medical problem and intake of drugs or other therapeutic medicines (at least from the past one year from the day of sampling). Controls were matched by age and sex to the exposed workers.

Micronucleus test

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 [17]. Prior to BC collection, the mouth was rinsed thoroughly with water to remove any unwanted debris. BC samples were obtained by scrapping both cheeks using a wooden spatula. The cells were collected in tubes containing 3ml sterile saline. Exfoliated cells were stained by the Feulgen reaction and counter stained with Fast green as previously described by Stich et al. [18] with some minor modifications. The cytoplasm was stained a pale blue-green and the nuclei and micronuclei purple red. A total of minimum 2000 cells per individuals were scored for analysis of micronuclei. The slides were randomized and scored by a single observer.

The following criteria for MN analysis were used in oral epithelial cells. An MN must be less than one third the diameter of the main nucleus; must be on the same focal plane; must have the same colour, texture and refraction as the main nucleus; must have a smooth oval or round shape; and must be clearly separated from the main nucleus.

Statistical analysis

All calculations were performed using Windows statistical package, SPSS, version 11.5 (IL, USA). 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).

RESULTS AND DISCUSSION

The effect of occupational exposure to silica dust on the levels of genetic damage in foundry sand molding workers and control subjects was assessed by the MN assay. Table 1 represents the distribution of subjects with respect to age, years of exposure, smoking habits, and alcohol consumption. 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 and non smokers/non alcohol consumption were similar in the two groups. The mean age of the workers was 39.65±6.60 and that of controls were 38.32±6.60 ranging from 37 to 58 years.

The results of MN assay are shown in Table 2. The frequency of MN was studied in 74 foundry sand molding workers and in 52 controls. Molders revealed a significant induction of MN when compared with 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 (5.78 and 5.25) Vs. (1.67 and 2.14) when compared to the non-smokers and non-alcoholics (4.08 and 4.74) Vs. (2.28 and 1.5). A highly significant increase ($p<0.05$) in MN frequency was observed in smokers and alcoholics when compared to all other groups and subgroups.

Foundry workers are exposed to a unique collection of environmental challenges including noise, heat, vibration, organic and inorganic chemical dusts, residue, aerosols, gases, acids and other pollutants, many of which have the potential to cause cancer [19].

Although many changes have occurred in foundry technology and materials, the basic process and these potential hazards remain more or less the same. In fact, sand molding is still a labour-intensive and complex process demanding a great amount of repetitive manipulation and stressful physical and chemical loads, which are associated with work safety hazards including chronic diseases [20].

The use of biological markers to determine the extent of prior exposures to a specific chemical and to predict future disease outcome holds great promise. Exploration of correlations between biomarkers will contribute to the development of human bio monitoring to genotoxic exposures and will help to select optimal biomarkers for more efficient monitoring of various human exposures [21].

The present study was conducted to determine the level of genotoxicity in foundry sand molding workers exposed to crystalline silica-rich dust, by applying the MN assay.

Buccal cells are the first barrier for the inhalation and are capable of metabolizing proximate carcinogens to reactive products [22, 23]. Thus, it could be dispute that oral epithelial cells be an ideal target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion. The MN assay test has been increasingly accepted as a reliable biomarker of genotoxicity in occupationally exposed groups [24]. The MN assay has been considered to be an effective biomarker of disease and processes associated with induction of DNA damage [25].

The results indicated that, overall, MN frequency was higher in exposed workers than in controls. The detection of an elevated frequency of micronuclei in smokers indicates increased risk of cancer [26]. Similarly in the present study, a significant increase in the MN frequency was observed among the exposed group with smoking. Although the findings of the present study are in line with the previous studies where, we found an elevated frequency of buccal cell MN in smoking group of petrol station attendants, metal arch welders, road paving workers, building construction workers, automobile mechanics, and textile printing workers [26-31]. Smoking had a significant effect on micronucleated cell rates in all studies [32]. Cigarette smoking is one of the factors that may influence the rate of cytogenetic damage [33-35]. The results of present cytogenetic analysis clearly showed that the combined exposure to cigarette smoke and silica dust enhances the frequency of micronuclei and an increase in DNA damage in buccal epithelial cells of foundry sand molding workers.

Alcohol use can increase the number of micronuclei [36]. Likewise in the present study a minor increase in MN frequency was observed between foundry sand molding workers and controls with drinking habit that may indicate the existence of an influence of alcohol use on the micronuclei formation.

Table 1: General characteristics of groups studied

Study group		N	Age (yr)	Average no of cigarettes/ d	Alcohol intake in last 1 yr (g alcohol drinking /d)	Duration of employment (yr)
Controls (n=52)	Smokers	31	39.87 ± 7.59	9.64±4.8	-	-
	Non-smokers	21	35.42 ± 3.96	-	-	-
	Alcoholics	28	39.21± 7.69	-	393.21±202.75	-

	Non-alcoholics	24	38.79 ± 6.92	-	-	-
Workers (n=74)	Smokers	38	40.00 ± 6.63	11.76±4.78	-	17.68±7.50
	Non-smokers	36	39.83± 6.57	-	-	17.02±6.84
	Alcoholics	39	39.38 ± 6.54	-	495.12±207.51	17.28±6.91
	Non-alcoholics	35	39.42± 6.68	-	-	17.68±7.16

The results are presented as mean±SD (standard deviation). Two thousand cells per sample were analysed.

Table 2: The mean frequency of micronuclei in exfoliated buccal epithelial cells of foundry sand molding industry workers and controls

Study group		**n=126**	**MN (M ±SD)**
Controls (n=52)	Smokers	31	1.67±0.79
	Non-smokers	21	2.28±1.05
	With drinking habit	28	2.14±1.00
	Without drinking habit	24	1.5±0.72
Workers (n=74)	Smokers	38	5.78±1.11*
	Non-smokers	36	4.08±1.33*
	With drinking habit	39	5.25±0.99*
	Without drinking habit	15	4.74±1.12*

MN=cells with micronuclei *significantly different with their respective controls, $p<0.05$.

CONCLUSION

The results of this study showed increased frequency of micronucleated buccal cells in foundry sand molding workers as compared to non-exposed controls. This indicates that occupational exposure to the many chemicals of foundry environment can have genotoxic effects and these effects increases with the increase in period of exposure. Our findings

conclude that the environment in foundry is genotoxic to human and smoking and drinking habit enhances the genotoxic effects of workers involved in foundry sand molding process. An intervention study with a large sample size would be needed before any definitive conclusions can be drawn.

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