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Research Article

Cytogenetic analysis of foundry molders using the micronucleus frequency in exfoliated buccal mucosa

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ABSTRACT

Foundry workers are exposed to chemical substances such as, PAHs, toluene, phenol, sulphur oxides, etc which can be inhaled, absorbed or possibly ingested. Micronuclei in exfoliated buccal epithelial cells are widely used as biomarkers of cancer risk in humans. We analyzed the frequency of Micronucleated cells (MNC) of 100 foundry workers involved in moulding or melting processes and equal number of age and sex matched healthy volunteers enrolled as controls. Assessment was carried out on the incidence of MNC, binucleated cells (BNC), broken egg cells (BEC), budding cells (BC), and the other anomalies (OA), in 2000 cells per individual. The data were analyzed with SPSS, using the Mann-Whitney U-test, $\alpha = 0.05$. The mean number of anomalies in foundry workers was 2.03 ± 0.13 MNC; 8.55 ± 0.26 BNC; 9.04 ± 0.13 BEC; 0.36 ± 0.09 BC, and 19.92 ± 0.03 OA; in controls it was 0.32 ± 0.03 MNC; 5.17 ± 0.03 BNC; 5.87 ± 0.09 BEC; 0.11 ± 0.13 BC, and 11.56 ± 0.08 OA; the differences for all parameters were significant. The non-occupational factors did not significantly influence the alterations.

Introduction

Foundry is a site where castings are made from molten metal, the foundry work often includes processes like creating casting patterns, making and assembling molds, melting and refining the metal, pouring the metal into molds, and removing adhering sand and redundant metal from the casting [1]. Foundry process consists a complex mixture of chemicals; several of them are recognized carcinogenic and genotoxic agents [2]. The foundry industry workers include; pourers, moulders, core workers and cleaning room operators in addition to crane operators, electricians and welders. There is evidence from the International Agency for Research on Cancer that workers in iron and steel foundries may have an increased risk of developing lung cancer [3].

Foundry workers are often exposed to various metal fumes generated during founding processes, especially during melting and pouring operations. Occupational exposures to airborne polycyclic aromatic hydrocarbons (PAHs) are resulting mainly from the thermal decomposition of carbonaceous ingredients commonly added to foundry sand.

Moulding sand is one of the basic materials often containing organic compounds used to make foundry moulds, necessary in most of the casting manufacturing processes and exposed to the direct contact with

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liquid metal. During casting, harmful compounds are emitted from moulding sand; this mainly occurs during pouring of moulds with liquid metal and during the solidification and cooling of castings.

The emitted pollutants adversely affect the health of the foundry workers. Benzene is a toxic and carcinogenic substance; PAHs are toxic and carcinogenic compounds [4]. Organic binders, coal powder and other carbonaceous additives are the predominant sources of PAHs in iron and steel foundries. In some cases, exhaust gases from engines, furnaces and ovens may increase the exposure of workers to these compounds [3].

This type of exposure can increase the risk to the development of several types of cancer, such as urinary tract, skin, laryngeal and pancreatic cancers, and leukemias. Researchers [5], found an undeviating link between cancer and occupational exposure, and reported that lung cancer is the major cause of death among foundry workers chronically exposed to foundry fumes.

However, this publication addresses only the cytogenetic risks to which foundry moulding workers are most often exposed to carcinogens or mutagens at work. We investigated the possible genotoxic risks among foundry workers, by quantitative determination of cells with micronuclei (MNC) and binucleated (BNC) broken egg cells (BEC) and other anomalies (OA).

Materials and Methods

I. Subjects

The study sample was composed of 100 male foundry moulding workers in Coimbatore, South India, employed to foundry moulding or foundry melting processes and the unexposed group Volunteers matched for age and sex were enrolled in the present study. To obtain necessary data on lifestyles and personal factors (age, working period, diets, etc.), all subjects were interviewed, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens [6].

II. Cytological Preparations

To obtain cells of buccal mucosa, an oral scraping (right and left sides) was performed on every individual, with the help of a wooden tongue depressor, previously washed with saline before the buccal cells were collected. After finishing the scraping, each tongue depressor was transferred to a centrifuge tube containing phosphate, pH 6.8. Subsequently, they were centrifuged for 10 min and then fixed with methanol:acetic acid (3:1), hydrolysis was done with 1 N HCl at 60°C for 10 min and the coloring of the slides was performed with Schiff-fast-green, according to the methodology described by reference number [7].

The analysis of the cells was done under a common optical microscope, binocular, with an objective of 100X and oculars of 10X. Two thousand cells per individual (exposed group and non-exposed group) were observed and the results are reported as number of cells with micronucleus (MNC), broken egg cells (BEC), binucleated cells (BNC), and cells with buds (BC).

Only non-fragmented, non-accumulated, non-overlaid cells, as well as those containing an intact nucleus were considered. Criteria used to identify a micronucleus were established by reference number [8].

III. Statistical Analysis

A total of 2000 cells were inspected per individual (100 exposed workers and 100 controls), scored and the data were stored in the database of the SPSS 11.0 version. Mean and standard deviation (SD) were calculated for each biomarker. The MNC, BNC and BEC distributions of individuals, grouped by each of two-class factors, were compared with the Mann-Whitney test. Associations between independent variables and the occurrence of MNC, BNC BEC and OA were measured by nonparametric Spearman rank correlation coefficients. The null hypothesis was rejected at 5% level of significance.

Results

The averages for demographic characteristics of the exposed group and the control individuals are reported in Table I. Both groups were characterized for gender, age, working time, smoking and alcohol consumption. When examining statistical significance of the relationship between two variables, no significant differences were observed in the general characters reported by workers and controls.

In Table II, the average and standard deviations are shown for the number of MNC, BNC, BEC, BC, and OA of both groups investigated. The evaluation of the frequency of the micronuclei in exfoliated cells of oral mucosa revealed a significant increase ($P = 0.0001$) in the individuals exposed to metal fumes in relation to the non-exposed group (control). The same was observed in relation to BNC ($P = 0.0001$), BEC ($P = 0.0001$), BC ($P = 0.029$), and OA ($P = 0.0001$).

Metal fumes exposed workers showed an increased MN frequency with an increase in duration of work ($P<0.05$). Based on the p-values estimated by Spearman rank coefficients, in our analysis inhalation of metal fumes was the main factor affecting MNC frequency in exfoliated cells.

Discussion

Foundry workers are exposed to numerous emissions in the workplace. Several studies have shown an excessive prevalence of respiratory symptoms, suggesting that chronic bronchitis and airways obstruction may result from inhaling various substances in this industry [9, 10].

Biomarkers have been used in medicine and toxicology for many years to assist in diagnosing and staging diseases [11]. Several studies have revealed an increased risk of lung cancer among iron foundry workers and described that iron founding as a cause of lung cancer in humans, moreover polycyclic aromatic hydrocarbons (PAHs) from heated moulds may be an aetiological factor [12].

In recent years, numerous researchers have investigated a number of genotoxicity parameters in relation to health and diseases [13]. In this population-based cross-sectional study, we observed an association between the increased frequency micronuclei in buccal epithelial cells

and years exposure in workers. Smokers had higher levels of genetic damage in exfoliated oral epithelial cells than non-smokers. No such significant association was found among nonsmokers.

Humans are exposed to PAHs from various occupational, environmental, lifestyle, and therapeutic sources [14], whereas the smoker's subgroup of foundry workers is exposed to higher levels, mainly through their working environment as well as tobacco smoking.

Exposure occurs by breathing air containing PAHs from cigarette smoke and tobacco use is accounting for about 30% of all cancers worldwide, particularly lung cancer, which is the most common malignancy in the world at present. Individual susceptibility to cancer may result from several host factors, such as differences in metabolism, DNA repair, altered expression of regulating genes, and nutritional factors.

An inconsistency between the effect of smoking and occupational exposure to PAHs on the induction of MN and DNA strand breaks has been shown in coke oven workers [15].

In the present study, we observed a modest increase in MNC, BNC and BEC, among exposed individuals with an alcohol habit. However, in the control group alcohol habit was not shown to be associated with any nuclear alterations, which is in line with our previous reports [16 -19].

A study of foundry workers found elevated levels of PAH DNA adducts in circulating lymphocytes of individuals [20]. Similarly, reference number [21] reported a link between carcinogen DNA adducts and somatic gene mutation at the hypoxanthine guanine phosphoribosyl transferase locus in an iron foundry workers exposure to benzo[a]pyrene and other polycyclic aromatic hydrocarbons (PAHs).

Conclusion

Our results showed that smoking increases the pah exposure of occupationally exposed persons by a factor of two, whereas passive smoking does not measurably increase the pahs burden. Our results further indicate that pah exposure from work environment might be of given some importance. The present data add to the growing evidence suggesting that, individuals with the increased micronuclei and other nuclear abnormities have a major chance of several gene mutations which activate pahs from tobacco smoke and occupational exposure and, as a result, are at greater risk for pah-related cancers.

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Table I: Characteristics of workers and controls

	Workers	Controls
Number	100	100
Mean age (years)	37.83 ± 10.22	38.48 ± 9.83
Age range (years)	21-58	22-58
Exposure (years)	5.96 ± 1.90	-
Smokers (Y/N)	45/55	55/45
Alcoholics (Y/N)	48/52	52/48

Table II: Average and standard deviation of the number of cells with micronuclei, binucleated cells, broken egg cells, cells with buds, and other anomalies observed in 2000 cells of workers and controls

Anomalies	Workers	Controls
MNC Mean ± SD	2.03 ± 0.13	0.32 ± 0.03
BNC Mean ± SD	8.55 ± 0.26	5.17 ± 0.03
BEC Mean ± SD	9.04 ± 0.13	5.87 ± 0.09
BC Mean ± SD	0.36 ± 0.09	0.11 ± 0.13
OA Mean ± SD	19.92 ± 0.03	11.56 ± 0.08

SD = standard deviation; MNC = cells with micronuclei; BNC = binucleated cells; BEC = broken egg cells; BC = cells with buds; SA = sum of anomalies

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