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DNA Damage Due to Inhalation of Complex Metal Particulates among Foundry Workers

¹Zorawar Singh and ²Pooja Chadha

¹Department of Zoology, Khalsa College, Amritsar, Punjab, India. 143002.

²Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India. 143005.

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ABSTRACT

Background: The foundry industries are employing a huge number of workers every year. Workers engaged in iron and copper foundries are exposed to a variety of complex compounds, gases, fumes, metal particulates including lead, formed as a result of foundry operation, which are known to have mutagenic effects. **Methods:** In the present study, twenty one foundry operators and five managers were assessed for genotoxicity using comet assay. An equal number of subjects, matched with the exposed subjects with respect to age, sex, alcohol and smoking status, were chosen as controls. **Results:** Significantly higher mean values of damaged cell frequency (DCF: 84.62 ± 1.87 Vs 63.42 ± 2.15 ; $p < 0.05$) and comet tail length (CTL: 33.40 ± 1.89 Vs 2.37 ± 0.27 ; $p < 0.01$) were found in foundry operators as compared to the controls. Significantly higher CTL values were also found in managers than controls ($p < 0.01$). Blood lead levels (BLL) also showed a positive correlation with CTL. **Conclusion:** The present study reflects an increased genotoxic risk among the workers employed in foundry industry.

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INTRODUCTION

Majority of the human cancers are known to arise as a direct consequence of environmental exposure to mutagenic and carcinogenic agents mainly through diet, habit and occupation. Occupational exposures to various chemical agents in different industries pose a major carcinogenic risk. Humans are exposed to a large number of physical and chemical agents which can cause a variety of health hazards [1]. Occupational exposure is one of the aspects that can't be ignored whenever health assessment studies are done. The world's major industries include iron & steel industry. Iron industries are using crude iron in different ways. They are employing a large number of workers for different industrial procedures like melting, welding, cutting, rolling bending etc. Workers who are employed in this field are exposed to a variety of risk factors. Iron overload has been shown to accelerate oxidative DNA damage. Moreover, various kinds of complex mixtures are also released as part of important industrial production processes. There are a number of studies focused on the risk assessment of complex compounds [2]. These elements can mutually influence each other and can cause a variety of health problems.

Foundry operations result in the production of various types of gases including carbon mono-oxide. Workers engaged in foundry operations are also exposed to a silica and number of nascent metal dust particles produced as a part of the industrial operation. Exposure to PAHs in foundry operations is another health hazard for the workers. The introduction of organic binder materials in the late 1950s has resulted in exposures of foundry workers to other chemicals, including phenol, formaldehyde, isocyanates and various amines. Foundry workers are also exposed to respirable metal dust and crystalline silica [3]. Chen *et al.* [4] reported similar respirable dust levels in a study in China. Metal fumes are formed by evaporation, condensation and oxidation of metals in air. Furnace tenders, melters, casters and pourers are exposed to fumes from molten metal [3]. Metal toxicity is well established by various researchers. Metals can lead to oxidative stress which can in turn lead to a number of health disorders including rheumatoid arthritis, asthma, stroke, ageing, atherosclerosis and retinal damage. It is now well established that oxidative stress causes extensive damage to cellular components, which can lead to a number of diseases, including cancer [5].

Corresponding Author: Zorawar Singh, Department of Zoology, Khalsa College, Amritsar, Punjab, India. 143002.
Tel: +91-9417230075 E-mail: zorawarsinghs@rediffmail.com

The workers engaged in foundry operations are also exposed to various compounds including phenol-formaldehyde, furan, urea-formaldehyde and urethane resins used as binders. These ingredients may volatilize into the workplace air during mixing, drying or baking operations. Thermal decomposition of these compounds may lead to the formation of more complex forms of compounds which are released in pouring and shaking processes. As the furnaces operate at 1400°C, the exposure of these binder compounds to high temperatures lead to formation of different gases and smoke aerosols. The resultant compounds can be methane, ethane, ethylene, acetylene, benzene, toluene, xylenes, naphthalenes and a variety of PAHs. Various nitrogen compounds such as ammonia, cyanides and amines may also be formed. When organic solvents are used in sand binders, the vapours may add to the exposure of workers [6]. If phosphoric acid is used as a catalyst, phosphine can be formed in the strongly reducing atmosphere of the hot emissions. In air, phosphine rapidly oxidizes to phosphorus oxide. Westberg *et al.* [7] reported that core-makers had higher exposure to formaldehyde than casters who were more exposed to methyl-isocyanate and isocyanic acid.

Comet assay has also become a reliable tool for the assessment of pre-cancerous risk assessments [8]. Comet assay seems to be a very rapid and sensitive method for detecting DNA damage in individual cells. It has been observed that there is a great lack of regular health assessment of the industry workers. They are exposed to a number of genotoxic substances of which they are not even aware of. Lack of knowledge about the health hazards is also observed. Evaluation of DNA damage may contribute to the early detection of many types of cancer.

MATERIALS AND METHODS

Subject selection:

All the subjects were given the prior information about the objectives of the present study. After taking the consent, twenty six workers (foundry operators, N = 21 and managerial staff, N = 5) were chosen from an iron and copper foundry. Twenty six normal healthy individuals of the same region, matched with the exposed subjects with respect to sex, age, socio-economic status, drinking and smoking habits were chosen as controls (Table 1). All the exposed individuals were extensively working in their respective fields. The foundry operators (N = 21; 32.76 ± 2.20 years) were engaged in making huge machine body parts including sand mouldings. They were also engaged in operating the furnace, melting, pouring and shakeout. The furnaces were operated by the workers at 1400°C. All the subjects were given a questionnaire about their age, sex, diet, alcohol drinking, smoking habits, disorders, medical histories, x-ray treatments, protection equipments, duration of exposure and exposure histories. The chosen control subjects were not having any type of the above said exposures and had not undergone any x-ray therapy, atleast three months prior to the sampling. The exposed and control groups were further divided into smokers and non smokers; drinkers and non drinkers and different age groups to study the effect of these confounders on the DNA damage parameters. The subjects taking more than 5 cigarettes per day were considered as smokers and the subjects taking more than 80 gm (equivalent to 10% abv.) of alcohol per day were considered as drinkers.

Blood sampling:

2ml of the blood sample from each exposed and control subject was procured by venupuncture. 100 µl of the sample was taken in a separate microcentrifuge tube having heparin as an anticoagulant for comet assay. Rest of the blood was put in an ultraclean glass tube having heparin for detecting lead levels. All the glass tubes and microcentrifuge tubes were coded accordingly. Due care was taken during the time of blood sample collection to avoid any kind of injury or infection. Disposable sterilized nitrile gloves were used while blood sampling. The coded samples were transported to the laboratory in a chiller box. The samples were processed within 4 hours of collection for the Single Cell Gel Electrophoresis.

Table 1: Characteristics of subjects.

Group	Sub-group	N	Age	Duration of exposure (years)	Smoking status		Drinking status	
					Smokers	Non smokers	Drinkers	Non drinkers
Exposed	Foundry	21	32.76 ± 2.20 (21-56)	10.47 ± 1.49 (3-25)	12 (57.14%)	9 (42.86%)	10 (48%)	11 (52%)
	Managers	5	42.60 ± 4.35 (28-55)	14.20 ± 2.73 (6-22)	3 (60%)	2 (40%)	3 (60%)	2 (40%)
Control		26	38.74 ± 2.85 (25-62)	-	12 (46.15%)	14 (53.84%)	11 (42.30%)	15 (57.70%)

All values are expressed as Mean ± SE

Comet assay and detection of DNA damage:

SCGE or comet assay was carried out as per the methodology of Singh *et al.* [9] with slight modifications. Slides precoated with 1% normal melting agarose (NMPA) were layered with 20 μ l of blood sample mixed in 0.5% low melting agarose (LMPA). This was followed by another layer of 1% LMPA. Slides were subjected to cold lysing solution at 4°C. Prior to electrophoresis, the slides were equilibrated in alkaline electrophoresis solution (1mM Na₂EDTA and 300mM NaOH) at pH > 13, for 20 minutes and electrophoresis was carried out in the same buffer for 25 minutes at 25V and 300mA. DNA fragments in each cell migrated at a rate inversely proportional to the size of the fragments. Slides were then washed gently 2–3 times, 5min each with 0.4M Tris at pH 7.5. After final wash, the neutral buffer was drained and the slides were washed with distilled water. Each slide was subjected to silver staining. Comet slides were photographed using a digital camera (Olympus, E-520) attached onto a trinocular microscope (Olympus CX-31). The photographs were subjected to computerized image analysis software which analyzed the comet images and determined the comet tail lengths (CTL). 100 comets per slide were scored. Similarly, the images were analyzed to evaluate the percentage of cells with DNA damage as damaged cell frequency (DCF).

Blood lead levels:

The whole blood samples from 15 randomly selected exposed (foundry operators, N = 10 and managers, N = 5) and 15 control subjects were analyzed for blood lead levels using atomic absorption spectroscopy. The blood samples from exposed group were procured during morning hours on the last working day of the week. No such time schedule was followed in case of controls. Preparation of the blood samples for blood lead levels (BLL) was done on the same day of sample collection as per the method given by Palmer *et al.* [10]. The concentrations of blood lead were quantified as μ g/dL.

Statistical analysis:

All statistical analyses were performed using the program Minitab v16.1.0 for windows. Significance of the differences in the means of comet tail length (CTL) and damaged cell frequency (DCF) between the control and exposed groups were analyzed using Mann-Whitney test. Within the groups, analysis of variance (ANOVA) was used to assess the effect of confounders including smoking and drinking habit on different parameters. Pearson correlation coefficient was used to find the relationship between blood lead level and parameters studied. Mean values and standard errors were calculated and $p < 0.05$ was considered as the significant level for the statistical analyses.

RESULTS AND DISCUSSIONS

In the present study, twenty six exposed subjects from an iron and copper foundry and an equal number of controls were subjected to comet assay for the evaluation of genotoxicity induced as a result of exposure to multiple complex compounds produced in foundry operations. The characteristics of the subjects are given in the Table 1. The exposed group had two categories of workers (foundry operators and managers). The mean duration of exposure of managers was higher than in case of foundry operators (14.20 \pm 2.73, 6-22 years vs 10.47 \pm 1.49, 3-25 years). The results (Table 2) of the comet assay revealed a significant difference in comet tail lengths in foundry operators and managers as compared to controls (CTL^f: 33.40 \pm 1.89 and CTL^m: 11.90 \pm 1.54 vs CTL^{control}: 2.37 \pm 0.27 μ m; $p < 0.01$). Damaged cell frequency (DCF) was significantly higher than controls, only in foundry operators ($p < 0.05$). Similar results were found by Basaran *et al.* [11] as they found significant DNA damage in the lymphocytes of foundry and pottery workers. Various other authors have reported cancer mortality and related studies in foundry workers [12-23]. An association between exposure to carcinogens during foundry work and cancer morbidity has been reported by Ahn *et al.* [24]. Main occupational hazards in the foundry are silica, metal fume, noise and heat stress. Zhang *et al.* [25] reported higher risk of silicosis among foundry workers. Zhang *et al.* [26] found significantly different silica concentrations with highest level in melting (4.4 mg/m³). A positive correlation was identified between levels of 1-OHP and 8-hydroxydeoxyguanosine (8-OH-dG), DNA strand breakage and malondialdehyde (MDA) [27].

Table 2: Mean CTL and DCF in foundry operators and control subjects.

Group	Sub-group	N	CTL (μ m)	DCF (%)
Exposed (N = 26)	Foundry operators	21	33.40 \pm 1.89**	84.62 \pm 1.87*
	Managers	5	11.90 \pm 1.54**	43.80 \pm 2.44
Controls		26	2.37 \pm 0.27	63.42 \pm 2.15

All value are expressed as Mean \pm SE

** $p < 0.01$; * $p < 0.05$ as compared to controls

Table 3: Mean CTL and DCF in exposed and control subjects with respect to smoking status.

Group	Smoking status	N	CTL (μm)	DCF (%)
Foundry operators (N = 21)	Smokers	12	32.91 \pm 1.94**	85.0 \pm 1.85
	Non-smokers	9	34.05 \pm 2.14**	84.11 \pm 1.45*
Managers (N = 5)	Smokers	3	11.50 \pm 1.54**	47.33 \pm 1.32#
	Non smokers	2	12.5 \pm 0.85**	38.50 \pm 0.52
Controls (N = 26)	Smokers	12	3.29 \pm 1.43#	74.71 \pm 3.25#
	Non smokers	14	2.03 \pm 1.24	59.26 \pm 2.46

All values are expressed as Mean \pm SE

**p<0.01; *p<0.05 as compared to controls of same smoking status

#p<0.05 as compared to non-smokers of same group

To analyze the effect of smoking habit in exposed subjects, the workers were categorized according to their smoking habit (Table 3). It was found that only DCF in the smoking managers was significantly different from the non-smoking managers ($p<0.05$); whereas, no such difference was found in foundry operators. In our study, we found no association of smoking on the DNA damage parameters (1 way ANOVA; $p>0.05$). On the contrary, Basaran *et al.* [11] found higher DNA damage in smoking foundry workers than non-smokers. In the present study, both CTL and DCF parameters were found to be significantly higher than the non-smokers, but in smoking controls ($p<0.05$).

Table 4: Mean CTL and DCF in exposed and control subjects with respect to drinking status.

Group	Drinking status	N	CTL (μm)	DCF (%)
Foundry operators (N = 21)	Drinkers	10	30.70 \pm 2.11	83.2 \pm 2.97
	Non Drinkers	11	35.86 \pm 2.88	85.90 \pm 3.52
Managers (N = 5)	Drinkers	3	13.66 \pm 1.75*	45.66 \pm 1.67
	Non Drinkers	2	9.25 \pm 1.85	41.00 \pm 2.43
Controls (N = 26)	Drinkers	11	3.39 \pm 1.23*	78.16 \pm 1.67*
	Non Drinkers	15	2.07 \pm 1.12	59.00 \pm 2.52

All values are expressed as Mean \pm SE

*p<0.05 as compared to non-drinkers

Workers and controls were categorized into drinkers and non-drinkers, to find out the effect of alcohol on DNA damage parameters (Table 4). Only the mean CTL in drinking managers was found to be significantly higher than non-drinkers of the same group ($p<0.05$). In control group, both the parameters were significantly higher in drinkers than non-drinkers ($p<0.05$).

Table 5: Mean blood lead levels, CTL and DCF in foundry operators, managers and control subjects.

Group	Sub-group	N	DNA damage parameters		BLL ($\mu\text{g}/\text{dl}$)	R ²
Exposed (N = 15)	Foundry	10	CTL	36.45 \pm 1.73#	54.76 \pm 4.36#†	0.647*
			DCF	86.5 \pm 1.68#		0.437
	Managers	5	CTL	11.90 \pm 1.54	31.80 \pm 0.12†	0.158
			DCF	43.80 \pm 2.44		0.111
Controls	26	CTL	2.37 \pm 0.27	3.76 \pm 2.12	0.751*	
		DCF	63.42 \pm 2.15		0.563	

#Mean values of ten randomly selected foundry operators

*Regression analysis $p<0.05$

†p<0.01 as compared to controls

Various studies have shown the presence of high concentrations of different metals in the sand and working areas of the foundry workers. High levels of Ag, As, Ba, Cd, Cr, and Pb was found in the molding sands via the toxicity characteristic leaching procedure (TCLP) [28]. Lead has been known to cause severe health problems in different industries [29,30]. Thus in the present study, for assessing the correlation of blood lead levels with DNA damage, ten randomly selected foundry operators and five managers were assessed for blood lead levels. The results revealed a significantly higher blood lead levels among foundry operators and managers as compared to controls (Table 5). Regression analysis was performed to analyze the effect of BLL on CTL and DCF. Only the CTL in foundry operators (Figure 1) was found to be under the effect of BLL ($R^2 = 0.647$; $p<0.05$). The correlation between DCF with BLL (Table 2) was found to be insignificant ($R^2 = 0.501$; $p>0.05$). CTL in the control group was also found to be affected by BLL ($R^2 = 0.751$; $p<0.05$).

Conclusions:

Conclusively, the present study points out the elevated health hazard risk among the workers of foundry industries exposed to a number of complex compounds formed as a result of foundry operations. Significantly higher DNA damage in foundry operators and managerial staff indicates higher risk for various health related problems including cancer and silicosis.

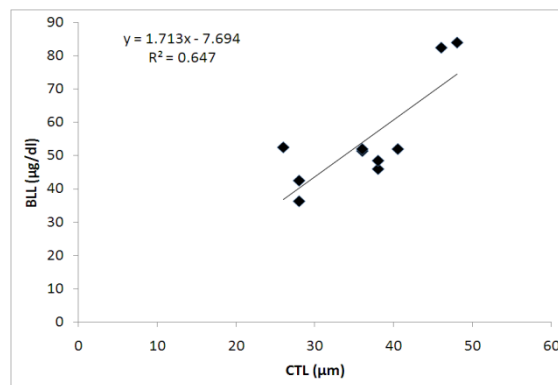


Fig. 1: Correlation of BLL and CTL among foundry operators (N = 10; $p < 0.05$).

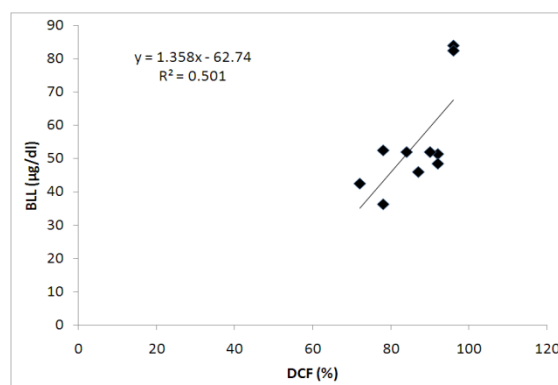


Fig. 2: Correlation of BLL and DCF among foundry operators (N = 10; $p > 0.05$).

Ethical considerations:

The present study was done after taking the prior permission from the ethical committee, Guru Nanak Dev University, Amritsar, Punjab, India. The authors declare no conflict of interest.

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