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Research Article

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Assessment of DNA Damage by Alkaline Comet Assay in Occupationally Exposed Welders of Hyderabad, Telangana

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ABSTRACT

Welding work is one of the pervasive industrial profession in Hyderabad,Telangana. Pin-sized or no attention is usually focused at occupational health risk by local or state authorities in Hyderabad. The present work was aimed at the assessment of DNA damage in welding workers by Alkaline Comet Assay(Single cell gel electrophoresis) in blood lymphocytes. The results revealed that there was statistically significant difference between the level of DNA damage in blood lymphocytes of welders and control group ($p < 0.05$). In addition, the level of damage to DNA in blood of subjects with long term exposure and old age is of serious concern. There is the need to screen occupational activities that can pose serious health risks. The relative ignorance of the welding workers about the health risks they are exposed to as well as the public should be addressed.

Key words: *Welding workers, Occupational exposure to welding fumes, DNA damage*

INTRODUCTION

A worker spends a substantial part of their life in their occupational work. Therefore, the occupation greatly affects the lifestyle and health of the worker. Occupational exposures to various chemical agents in different industries pose a major carcinogenic risk. Previous reports emphasize on DNA damage in occupationally exposed workers of different industries[1,2,3,4]. Welding is one of the key components of numerous manufacturing industries, which pose potential physical and chemical health hazards. Welders are exposed to a number of genotoxic metals, gases, fumes and radiations. Approximately, 800,000 people worldwide are occupationally exposed to metal welding fumes, this increases the generation of reactive oxygen species (ROS) that damage the genetic material. Evidence suggests that DNA damage (genotoxicity), caused by welding-fumes, plays an important role in the development of number of diseases, which incidence is higher in the welder sub-population compared to general population[5,6,7].

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Effective biological survey of welding workers is imperative to determine the genotoxicity and carcinogenicity of exposure[8,9]. Several studies have reported that contamination by heavy metals have induced chromosome/genome mutations and DNA damage using the comet assay[10,11,12]. Most of the workshops are situated either in open spaces or relatively enclosed. As such, the fumes and the dusts are released into the environment (atmosphere) while the workers are exposed directly to most of the metal dust/fume pollutants. The metal fumes produced in the process of welding composed of at least 13 kinds of metals, including manganese (Mn), beryllium (Be), cadmium (Cd), chromium (Cr) cobalt (Cu), iron (Fe), lead (Pb), mercury (Hg), molybdenum (Mo), nickel (Ni), zinc (Zn), antimony (Sb), and vanadium (V)[13]. The chemicals contained in these fumes and gases depend on several factors including; type of welding being performed, material of the electrode, type of metal being welded, presence of coatings on the metal, duration and severity of exposure and ventilation[14]. In addition, the workers only wear glasses to prevent light sparkles from having contact with their eyes and often wore hand gloves as the only protective measure. It is very clear that these workers are excessively and persistently exposed to the contaminants through their skin or accumulate in crevices of the hands and perhaps from food and drink contamination at the work premises. The aim of the study was to assess the extent of DNA damage in blood samples of workers in and around of balanagar area, Hyderabad, Telangana.

MATERIAL AND METHODS

Experimental design:

Welding workers (n = 200) were sampled through random sampling from the industrial area in and around of the balanagar, Hyderabad, Telangana. The control group (n = 200) were selected randomly from the population whose occupations have no relation and history of exposure to welding fumes. However, they were exposed to traffic pollution just like the welding workers but not to welding fumes. Control groups do not differ from welding workers in gender, age, smoking and food habits[15]. All subjects were informed of the objective of the study and their consent was obtained. Selection of test subject, control group and relevant biographical information. Designing a questionnaire which provided valuable information on age, health history, food habits and working conditions of the welding workers.

Blood samples collection:

Blood samples were collected from test subjects in the morning around 7 and 8 am. 5 mL of blood samples were collected through venipuncture. Samples were collected in a green colour tube. The collection site on the subject body was washed with soap and water, followed by alcohol swab. Blood was collected by venipuncture using a phlebotomy needle. Tube was inverted about 8–10 times to prevent clotting. Each specimen tube was attached with an identification label. Specimens were stored at 4◦C[17].

Comet assay:

DNA damage was measured by comet assay in exposed welding workers and control subjects in the Hyderabad, Telangana. Comet assay was performed according to Singh *et al*[18]. Briefly , with using lysing solution (2.5 mol NaCl, 100 mM Na_2EDTA, freshly added 1 % Triton-X100 and 10 % DMSO), electrophoresis buffer (300 mM NaOH, 1 mM Na_2EDTA pH 13.0, 4^0C) and 0.6 % normal melting and low melting agarose. Current for electrophoresis was adjusted to 300 mA by raising or lowering the buffer. The slides were stained by 20 ug/ml ethidium bromide. Cells were observed at a magnification of $40_\times$ by a fluorescence microscope with green light excitation and a 590 nm barrier filter. DNA damage was calculated by randomly counting tailing DNA in 50 cells/sample. Classification of comets was five damage levels according to Tail DNA%, including grade 0, 1, 2, 3 and 4. Grade 0 was defined as no DNA damage and grade 4 was the most serious DNA damage.

Statistical analysis:

Statistical analysis was performed using SPSS 21 statistical software to know the DNA damage was compared between welding workers and control groups using independent sample *t-test*. A p value ≤ 0.05 considered as a statistical significance.

RESULTS AND DISCUSSION

The present study focused on association of socio economic and DNA damage in the workers occupational health hazards in unauthorized small-scale units. We found that workers in welding industry were exposed to a variety of work-related hazards though most of them were using protective equipment. Workers and employers were both unaware of occupational and environmental health hazards. Welding workers are more prone to impaired pulmonary function, chronic bronchitis, interstitial lung disease, asthma, lung cancer, eye burns, short- and long-term injury to the skin, non-melanocytic skin cancer[20]. A similar case study reported frequent inhalation exposures to high concentrations of fumes and gases during welding, interest in potential health problems has centered on respiratory effects, particularly lung cancer, which could be caused by effect of metals in stainless steel welding fumes[21]. The type and amount of welding fumes and respiratory symptoms depend on duration of work, welding method, welding material, ventilation facilities and respiratory protection[26].

The extent of DNA damage at various age groups was shown in the Table 1 for the welding workers and control groups. There was a significant increase in the basal DNA damage in the exposed welding workers when compared to the control subjects. But increasing with age, with in the groups of experimental and control no much DNA damage was observed and haemoglobin levels also not much varied in the control and experimental subjects (Table2). This increase could be explained mainly due to the presence of different types of metals and other chemical compounds present in welding fumes. Addition to the age, studied the correlation between smoking, alcoholic and food habits like vegetarian and non vegetarians with DNA damage. Food habits of vegetarian and non vegetarian shows a significant DNA damage in experimental group compare to control but does not show any DNA damage in the within the group of control and experimental (Table 3) . Smoking and alcoholic habit also shows a significant effect on the basal DNA damage in the exposed group subjects compared to the control group (Table 4 and Table 5) and not much varies between t the smoking and non smoking as well as alcohol and non alcohol . The induction of DNA damage might be because of exposure to welding fumes, which consist of several heavy metals. The welding workers were likely exposed to higher levels of traffic air pollution than the control subjects because welding workstations located in the open area, so Comet effects reported here could at least partly attributable to traffic exposure in addition to welding fumes[27]. Aluminium and zinc metal which are present in welding fumes might produce DNA strand breaks via the oxidative stress induced by metal-fume fever [23,24].Oxidized forms of chromium, nickel[29,30] and include lead[31], manganese[32], cadmium[33], cobalt[34] which also are present in welding fumes, are genotoxic and that could induce DNA damage. Previous work explore that fumes from industrial stainless steel welding processes also increased chromosomal aberrations in few welders[25].However, there was a significant DNA damage levels were observed between control and experimental welding workers and there was no correlation were observed in the food habits smoking and alcoholic with DNA damage in all groups of experimental and control subjects.

Table 1: Level and extent of DNA damage among the various age groups of the experimental (welding workers) and control subjects. Data expressed as Mean ±SD. (*) Indicates a statistically significant from the Controls (P < 0.05)

Group	Age (25-34Yrs)	DNA damage	Age (35-45Yrs)	DNA damage
Control	31.52 ± 2.07	2.67 ± 0.82	39.84 ± 2.91	2.84 ± 1.25
Experimental	31.15 ± 2.28	5.83 ± 1.07*	39.06 ± 1.95	5.89 ± 1.04*

Table 2: Level of DNA damage and Haemoglobin levels in experimental and controls subjects. Data expressed as Mean ±SD. (*) Indicates a statistically significant from the Controls (P < 0.05)

Group	Haemoglobin (mg/dL)	DNA damage
Control	13.93 ± 2.01	2.54 ± 0.92
Experimental	14.10 ± 2.17	5.90 ± 1.13*

Table 3: Level of DNA damage among different food habits of welding workers and Control subjects. Data expressed as Mean ±SD. (*) Indicates a statistically significant from the Controls (P < 0.05)

Group	DNA damage (abitrary units)	
	Control	Experimental
Vegetarian	2.53 ± 0.94	5.65 ± 1.06*
Non Vegetarian	2.54 ± 0.89	5.99 ± 1.04*

Table 4: Level of DNA damage in the Smoking and Non- smoking in experimental and controls subjects. Data expressed as Mean ±SD. (*) Indicates a statistically significant from the Controls (P < 0.05)

Group	DNA damage (abitrary units)	
	Control	Experimental
Smokers	2.37 ± 0.71	6.16 ± 0.96*
Non Smokers	2.74 ± 0.71	5.80 ± 1.18*

Table 5: Level of DNA damage in Alcohol and Non-alcohol of welding workers and Control. Data expressed as Mean ±SD. (*) Indicates a statistically significant from the Controls (P < 0.05)

Group	DNA damage (abitrary units)	
	Control	Experimental
Alcohol	2.30 ± 0.80	5.98 ± 1.01*
Non Alcohol	2.53 ± 0.90	5.92 ± 1.0*

CONCLUSION

Based on the present study, it was concluded that exposed welding workers have significant level of DNA damage. The age of the welding worker had affected the extent of damage.Hence, to identify the susceptible workers in due time and to improve the work efficiency, preventive measures are needed to reduce the risk of welding fumes in industrial workers.

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