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Increased DNA damage among cement industry workers: Single cell gel electrophoresis assay

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ABSTRACT

Cement is a known carcinogen. Large numbers of workers are occupationally exposed to one or more forms of cement. Therefore, the potential carcinogenic hazard to the exposed workers is of great concern. This study examines the genotoxic effect of cement by using Comet assay. The Comet Assay or single cell gel electrophoresis assay is one of the very widely used assays to microscopically detect DNA damage at the level of a single cell. The determination of damage is carried out either through visual scoring of cells (after classification into different categories on the basis of tail length and shape). In this study white blood cells are taken in order to evaluate the genotoxic risk associated with occupational exposure of 15 cement industry workers and 15 age matched controls, in Coimbatore, South India. In the comet assay 100 cells were examined for each individual, both comet tail length and a damage index were calculated. In this present study we found a significantly longer comet tail in a group of workers exposed to cement (35.02±0.186) compared to the control group (30.82±0.154). The comet assay is considered a suitable and fast test for DNA-damaging potential in biomonitoring.

KEYWORDS

Cement dust;
Comet assay;
Tail length;
Blood cells.

INTRODUCTION

Portland cement is a fine powder that is an essential ingredient of concrete. Cement industry is a large industry and it produces dust during cement production. The major pollution problem in cement industries is cement dust emission into the environment from various points of the production process such as the crusher, rotary kiln, cranes, mills, storage silos and packing sections[1]. This has resulted in the exposure of cement dust leading to the impairment of respiratory function and a prevalence of respiratory symptoms amongst workers[2-6]. Occupational exposure to cement dust has been associated with bronchial asthma, reduced respiratory function and cancer of the lungs and the stomach. It has also been reported that cement dust particles could be found in various body organs including liver, spleen, bone, and blood and that they could produce different type of lesions. Exposure to cement dust may increase chromosomal aberrations, lung, bladder and stomach cancer[7]. Cement dust can cause disease due to the chemical nature of cement dust and its irritant, sensitizing and pneumoconotic properties[8]. Inhaled cement dust is suspected to causing bronchial asthma and cancer of the lungs and the stomach[9].

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could be found in various body organs including liver, spleen, bone, and blood and that they could produce different type of lesions. Exposure to cement dust may increase chromosomal aberrations, lung, and bladder and stomach cancer. Cement dust causes chromosomal integrity, cell-cycle progression, DNA replication and repair[10].

Mutagenesis is involved in occupational exposure may contribute to the development of harmful infirmity, many times during means that involve chromosomal changes. In order to evaluate the possible impact of environmental and occupational exposition on health, it is essential to identify the effects of exposure. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposition and to monitor excessively exposed populations[11].

Comet assay is a rapid and sensitive technique to measure sites sensitive to basic pH (alkali-label) and DNA breaks in individual cells. This method was described by Ostling and Johanson in 1984, and in 1988, Singh et al. introduced alkaline conditions to this technique[12]. The assay technique consists of evaluating cells kept in agarose, on a microscope slide, submitted to electrophoresis and dyed with ethidium bromide. Cells with damaged DNA form a comet, consisting of a head (nuclear matrix), and a tail, formed by DNA fragments. The amount of DNA that has migrated is correlated with the damage[13-16]. This assay is extremely versatile, and is used extensively in Biology, Medicine and Toxicology, due to its capacity and sensitivity in demonstrating DNA breaks, both single and double breaks, and alkali-label sites[17-20]. The alkaline conditions cause the separation of the paired bases, enabling the detection of simple chain ruptures[21, 22].

Positive results in the comet assay do not always correspond to positive results in the MN tests, especially when the exposure to genotoxic agents is small. The comet assay usually detects more defects than the MN test. The positives in the comet and MN tests are due to different mechanisms; the MN test detects injuries that survive at least one mitotic cycle, while the comet assay identifies reparable injuries or alkali-label sites[23, 24]. Consequently, Goethem (1997) suggests the use of both MN and comet tests.

Wojewodzka et al. (1998) consider inter-individual variability important; it can be detected by the analysis of parameters in the comet assay. They found considerable intra-individual homogeneity, and high inter-individual variability, suggesting that the extent of the damage, as well as the decrease in the capacity of DNA damage repair, constantly induced by endogenous or exogenous factors, may be involved in the variability of the individual responses found.

MATERIAL AND METHODS

The subjects were selected by random sampling. The study group consisted of 15 cement mill workers and 15 controls. The respective control groups were matched for age, and had no occupational exposition to toxic agents. All the individuals were males and non-smokers. They were about 25 to 55 years old (TABLE 1). All the individuals who agreed to participate in the study were healthy, and they answered a detailed questionnaire according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens[25], which included items about occupational exposure, smoking habit, use of drugs, such as alcohol.

Comet assay

Before slide preparation, 0.2 ml whole blood was centrifuged and the supernatant was removed. After that the cell pellet was resuspended in 1.4ml chromosome medium (RPMI 1640; Gibco BRL) with L-glutamine.

The Comet assay was carried out under alkaline conditions, basically as described by Singh *et al.* (1988).The cell pellet obtained from 60 µl RPMI diluted blood was mixed with 85 µl 0.7% low melting point agarose (LMA) and then placed on fully frosted roughened slides, previously coated with 1 % normal melting point agarose (NMA). When this layer had solidified, a third layer of 0.1 LMA was applied. The slides were immersed for 1 h in ice-cold freshly prepared lysis solution (2.5 M NaCl, 100 mM Na_2EDTA,10 mM Tns-HCl, 1% Na sarcosinate, pH 10) with 1% Triton X-100 and 10%

DMSO added fresh to lyse the cells and to allow DNA unfolding. The slides were then placed on a horizontal gel electrophoresis tank, facing the anode. The unit was filled with fresh electrophoretic buffer (1 mM Na_2EDTA, 300mM NaOH, pH 13) and the slides were allowed to set in this alkaline buffer for 20 min to allow DNA unwinding and expression of alkali-labile sites.

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Electrophoresis was conducted for 20 min at 25 V (300 mA). After that, to remove alkali and detergents, a neutralization buffer (0.4 M Tris-HCl, pH7.5) was added drop wise to the slides and allowed to sit for 5 min, then the DNA was stained with 4,6-diamidino-2-phenylindol (DAPI) (5 Hg/ml). Slides were examined by eye at 400X magnification using a fluorescence microscope. The tail length was measured according to Singh *et al* (1988). The width of the nucleus and the extent of migration of DNA fragments of 50 randomly selected cells per slide were determined. Two parallel replicates were performed per sample and the mean tail length was calculated. Moreover, cells were graded by eye into five categories (A-E) according to the amount of DNA in the tail[26], where A are undamaged cells and E highly damaged cells. To quantify the damage in this scoring, a rank number ranging from 0 (A) to 400 (E) was assigned to each of the categories, in order to calculate a mean of DNA damage grade for all samples.

Statistical analysis

The distributions of mean tail length of the comet and mean of the grade of DNA damage of exposed and control samples were calculated by using two tailed student t test. A differences at $p<0.05$ was considered significant.

RESULTS

The main characteristics of the exposed and control workers were recorded TABLE 1. The individuals were identified in terms of age, years they had worked, smoking and alcoholic habits. The average year of exposure was 12.76yrs for cement industry workers. The mean comet tail length of 100 cells of exposed and control groups were represented in TABLE 2. The comet values were significantly ($P > 0.5$) higher in occupationally exposed group (35.02±0.186μm) than their respective controls (30.82±0.154μm).

TABLE 1 : Characteristics of study groups

S. No	Characteristics	Exposed subjects	Control Subjects
1.	Number of subjects	15	15
2.	Average Age (years)	36.66	34.44
3.	Year of exposure	7	-
	<10 yrs	8	-
	>10 yrs		

TABLE 2 : Size of comet tail in μm of 100 cells analyzed for each individual exposed to cement dust and their controls

Individuals	Cement mill workers			Controls	
	Age	Year of Exposure	Comet tail length (μm)	Age	Comet tail length (μm)
01	29	8	34.2±17.83	26	30.5±7.87
02	40	20	36.3±11.58	24	31.0±6.91
03	31	13	34.4±10.75	30	30.0±4.00
04	45	14	36.8±19.01	43	30.6±6.00
05	41	9	33.9±15.02	47	30.9±5.11
06	44	21	35.3±19.25	26	31.6±6.32
07	40	17	36.0±19.08	40	31.9±7.81
08	27	6	34.8±1.96	29	30.0±1.71
09	35	8	33.5±16.11	35	30.9±6.11
10	37	18	35.7±11.29	41	31.5±5.78
11	32	9	34.6±11.69	32	30.4±3.85
12	35	13	34.9±12.36	40	30.0±5.01
13	38	9	35.6±18.82	36	30.4±5.71
14	37	15	33.6±15.64	37	30.9±4.13
15	39	7	35.8±12.76	30	31.7±6.11
Mean	36.66	12.76	35.02	34.44	30.82
SE	-	-	0.186	-	0.154

DISCUSSION

The Comet assay allows detection of DNA damage and repair at the level of individual cells. The use of this technique is increasing and it has been employed in different *in vitro* studies conducted to detect the genotoxic effects of ionizing radiation, as well as the repair kinetics of such damage in human blood cells[27].

The comet assay has been receiving increasing attention as rapid and very sensitive simple fluorescent microscopy-based method to examine DNA damage and repair at the level of individual cells. This study aims to investigate the genotoxic risk associated with occupational exposure of cement industry workers to complex chemical mixtures. We found a significantly longer comet tail in a group of workers exposed to cement (35.02±0.186) compared to the control group (30.82±0.154). Maciejewska and Cybula (1991) found that cement dust induces chronic exfoliative bronchitis and tissue fibrosis and emphysema[28]. In addition, Oleru 1984 reported that, the most frequently symptoms in cement mill workers were cough, chronic bronchitis, impairment of lung function, chest tightness, restrictive lung disease, skin irritation, conjunctivitis, stomach ache and boils[29].

The present analysis showed an increase in the pro-

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portion of damaged cells in the sample obtained from exposed group than control group. Comet results reported in the present work are based on the response of leukocytes; while our previous results on micronucleus were based on the buccal cells among cement exposed population also gave significant increase in genetic damage[30]. On the basis of such relation our results suggest that cement dust induce DNA damage. We recommended that cement mill workers should regularly use appropriate personal protective equipments at their work site eg:-apparel, mask, goggles and should get periodic medical surveillance. These measures would help to decrease the occupational hazards of cement dust and detect the disease in initial stage when treatment is achievable in cement industrial workers. The study and the standardization of tests for the evaluation of biological damage are essential for public institutions that are concerned with environmental quality and public health. Genotoxic evaluation is necessary to guarantee environmental quality and occupational health, as well as to orient workers to help reduce genetic damage and the risk of serious illness.

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