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Evaluation of genotoxicity through micronucleus assay among individuals exposed to cement dust

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ABSTRACT

In this study, the micronuclei test (MNT) was applied in exfoliated cells of buccal mucosa, in order to assess the genotoxic risk associated with occupational exposure of cement industry workers, construction workers and residents near cement industry. For each individual, 3000 exfoliated buccal cells were analyzed. A statistically significant ($P < 0.05$) increase in the frequency of micronucleai (MN) in the cement industry workers followed by construction workers and residents near cement industry. The mean frequencies of MN in the exposed group were significantly higher ($P < 0.05$) when compared to the control group. These results allowed to conclude that the studied individuals belong to a risk group and should periodically undergo biological monitoring and proper care.

KEYWORDS

Cement dust exposure;
Residents;
Construction workers;
Micronucleus test.

INTRODUCTION

Health suffers the influence of inherited, nutritional, and environmental factors. Populations of industrial areas are intensely exposed to chemical substances that can cause mutations, cancer, and congenital defects. Cement industries discharge cement dust into the environment from various points of the production process such as the crusher, rotary kiln, cranes, industry's, storage silos, and packing sections[1]. Increasing amounts of potentially harmful particles are being emitted into the workplace atmosphere, results human health effects. Building construction workers are occupationally exposed to variety of substances such as natural and man made mineral fibers, cement, quartz, various dust, diesel exhaust, paints and solvents. Many of these substances are known to have adverse health effects on workers[2]. Building construction workers are exposed to high concentration of dust and fumes, mainly the cement dust. The people who are living near cement industries were also expose to cement dust for a long period.

Cement dust contains mixture of calcium oxide, silicon oxide, aluminium-tri-oxide, ferric oxide, magnesium oxide, clay, shale, sand and other impurities. The cement dust particles mainly entered into the body through respiratory and gastrointestinal tracts[3-5]. Inhaled cement dust mainly causes bronchial asthma and lungs and the stomach cancer[6-8]. It has also been reported that cement dust particles could be found in various body organs including liver, spleen, bone, and blood and they produce different type of lesions.

Mutagenesis is involved in the pathogenesis of many neoplasias. Occupational exposure may contribute to

the development of pernicious illnesses, many times through mechanisms that involve chromosomal changes. In order to evaluate the possible impact of environmental exposition on health, it is essential to identity the effects of exposure through epidemiological studies, which also constitute a challenge. Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposition and to monitor populations that are excessively exposed[9].

Micronucleus test of exfoliated cells in epithelial tissue have been used to evaluate the genotoxic effects. Micronucleus is defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus. Micronuclei originated from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in to the daughter nuclei during mitosis. Micronucleus test is the most frequent technique used to detect chromosome breakage or mitotic interference associated events with increased risk for cancer[10]. As micronuclei derive from chromosomal fragment and whole chromosomes lagging behind in anaphase, the micronucleus assay can be used to show both clastogenic and aneugenic effects. Micronucleus formation is undoubtedly an important mechanism for chromosome loss[11].

The use of the micronuclei test (MNT) to detect and quantify the genotoxic action of carcinogenics is well established in several systems, either *in vitro or in vivo*, its sensitivity being compared to the analysis of chromatid breaks and exchanges[12]. This test presents great advantages over other techniques, not requiring cell culture or metaphase preparations, it is applicable on interphase cells, is a good indicator of chromosome mutations[13], is not invasive and has a low cost[14,15].

The present study comprises three different groups of subjects who all exposed to cement dust, a group of workers of cement industry, a group of construction workers and a group of residents living near cement industry. All these groups are frequently exposed to cement dust, where they are exposed to more than one risk factor. The purpose of this study is to evaluate the micronucleus (MN) frequency of different environmental exposures.

MATERIALS AND METHODS

The study group consisted of 15 cement industry workers, 15 subjects who are residing near cement industry and 15 building construction workers in Coimbatore City, South India. They were males, non-smokers, non-alcoholic and did not take any other intoxicants. Volunteers who were not exposed to any known genotoxic agents were used as control group. All individuals were answered a questionnaire about their occupational and non-occupational exposure, habits and diets, according to the protocol published by the International Commission for Protection against Environmental Mutagens and Carcinogens[16]. All subjects participated voluntarily and all provided written agreement before sample collection. None of these study groups showed significant differences with regard to lifestyle and personal factors.

Cell sampling

Before sampling, individuals rinsed their mouth thoroughly with tap water. The exfoliated buccal cells were obtained by gently rubbing the inside of both cheeks with an extra soft toothbrush for 1 minute each. The participant rinsed their mouth with 20 ml of 0.9% saline and expectorated in a 50-ml conical-based tube. The toothbrush was then rinsed in the tube and additional 30 ml saline before the cells were pelleted and washed once with Phosphate buffered saline (pH 7.4).

Micronucleus analysis

The MN analysis was done with a light microscope, at 1000X magnification, using coded slides. Two thousand cells from each individual were examined. Only unfragmented cells that were not smeared, clumped or overlapped and that contained intact nuclei, were included in the analysis. Cells undergoing degenerative processes, such as karyorrhexis, karyolysis and fragmentation of nucleus, broken egg, or pycnosis were excluded from the result, according to Micronuclei had to: (a) be less than 1/3 in diameter of the main nucleus, (b) be on the same plane of focus, (c) have the same color, texture and refraction as the main nucleus, (d) have a smooth oval or round shape, and (e) be clearly separated from the main nucleus. Questionable micronuclei were disregarded[17]. The data were subjected

FULL PAPER

to Students't' test to determine significant difference between the groups. Values are expressed in mean ± S.D.

RESULTS

General characteristics of the study population, including age and duration of employment/stay are given in **TABLE** 1. Results for micronuclei were given in **TABLE** 2. Assessment of MN frequencies in exfoliated buccal cells revealed a significant ($P < 0.05$) increase in exposed group than in control group. The mean number of MN was significantly higher (7.87 ±2.80) in cement industry workers, than residents near a cement industry (7.06±2.37) and building construction workers (7.13±2.19).

DISCUSSION

Exposure to cement dust causes serious health effect. The present result showed an increased frequency of Micronucleus in Buccal mucosa of cement exposures. Genotoxicity analysis using micronuclei (MN) as biomarker proved that asbestos chrysotile gave a maximum damage to the cells at relatively low concentrations[18]. Chrysotile cement has been shown to induce inflammation, oxidative stress and genotoxicity in several *in vivo* and *in vitro* experimental systems. In humans, increased levels of DNA damage (8-hydroxyguanine adducts and strand fragmentation) and higher frequencies of SCE in the blood cells of workers occupationally exposed to asbestos were detected[19]. Chrysotile and asbestos cement powder to induce dose-dependent micronuclei and loss of cell viability *in vitro*[20].

Our previous study reports a direct proportional relationship between the frequency of chromosomal aberrations and the period of exposure to cement dust in cement factory workers[21].

The present result recommended that micronucleus test in buccal mucosa could be used as a biological indicator for evaluating toxic effect of cement during various exposure. Based on our results occupational exposure to cement dust may be the factor that has produce an increased DNA damage, due to the genotoxic action of substances to which they were exposed. Micronuclei are also useful indicator of chemical exposure and toxic response. Therefore, micronuclei may increase the sensitivity of the exfoliated epithelial cell technique in assessment of genotoxity. We recommended that cement exposure should regularly use appropriate personal protective equipments. Extensive studies and standardized tests to evaluate biological damage at different levels are recommended to public agencies concerned with environmental quality and occupational health.

TABLE 1: General characteristics of study population exposed to cement dust

Characteristics	Cement industry workers n=15	Building construction workers n=15	Residents near a cement industry n=15	Controls n=15
Average age (Years)	32.4	35.9	38.7	36.2
Age range (years)	28-53	21-47	29-52	22-54
Average working/Residing Period (years)	10.5	9.4	12.7	-
Range of working/ residing period (years)	3-46	2-25	5-36	-

TABLE 2 : Number of cells with micronuclei (among 3000 cells analyzed for each individual) of the individuals exposed to cement dust and controls

Individuals	Total number of micronuclei/3000cells			
	Cement industry workers	Building construction workers	Residents near cement industry	Controls
1	10	4	8	1
2	14	10	6	2
3	5	9	5	1
4	7	5	6	1
5	8	12	7	3
6	5	9	5	2
7	5	5	10	1
8	12	6	4	4
9	8	7	7	3
10	10	6	5	2
11	7	8	11	4
12	4	5	10	5
13	8	8	6	0
14	6	6	11	3
15	9	7	5	2
Mean± SD	7.87±2.80	7.13±2.19	7.06±2.37	2.27±1.38

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