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Original Research Paper

Sister Chromatid Exchanges in Peripheral Lymphocytes in Shoe Factory Workers Exposed to Organic Solvents

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ABSTRACT

Shoe manufacturing is an age-old profession in India. The genotoxic potential of organic solvents in cultured peripheral lymphocytes has been investigated on 62 male workers in a leather shoe factory located at IDA, Nacharam in Ranga Reddy district of Andhra Pradesh by using one of the recommended cytogenetic biomarkers. The blood samples were collected from male workers and 42 age matched controls. Analysis of sister chromatid exchanges in human peripheral lymphocytes was carried out in control and exposed groups. A significant increase in the percentage of SCEs was observed as compared to control. Thus, the study clearly reveals the mutagenic effect of organic solvents on human beings.

INTRODUCTION

The occupational exposure to organic solvents was typically found in workers of rubber, pharmaceutical, paint and pigment, and shoe making industries. The exposure to small doses may lead euphoria and hallucination, while high doses produce convulsions and coma. The long term exposure may increase the cancer risk (Pitarque et al. 2002), high mortality rates in other cancers (Fu et al. 1996), and an increased risk of leukaemia and nasal cancer (IARC 1987). The workers in shoe industry are exposed to organic solvents particularly toluene and acetone. The glues and gasoline used in the industry may contain benzene, which are responsible for cancers found in workers of shoe factory workers. The genotoxicity of benzene exposed workers showed higher percentage of chromosomal observations in leukaemics such as trisomy and monozymy in human chromosomes (Kim et al. 2004, Bogadi et al. 1997, Rudrama Devi & Jithender Kumar Naik 2010), and micronuclei in human lymphocytes in exfoliated buccal cells of workers exposed to organic solvents (Rudrama Devi & Jithender Kumar Naik 2005, 2010). However, few studies have showed no evidence of chromosomal damage in workers exposed to benzene. Hence, in the present investigation an analysis of sister-chromatid exchanges were carried out in human peripheral lymphocytes of people working in shoe manufacturing factory.

MATERIALS AND METHODS

Study population: Human biomonitoring study was carried out on workers of shoe factory at IDA Nacharam in Ranga Reddy district of Andhra Pradesh. During the study 62 male workers were examined using standard questionnaire taken into account confounding factors such as family history, life style habits, diet, medication, socio-economic status and age, duration of exposure, etc. Simultaneously, control group of 42 age matched subjects belonging to the same socioeconomic status from administrative staff of the factory were also selected using standard questionnaire.

Sample collection: The heparinized blood samples (0.5 mL) were collected in sterile vials and were transported to the laboratory in an ice bath. The samples were brought back to the room temperature prior to the experimental set up and stored at -20°C deep freezer until further experimental use.

Sister-chromatid exchanges analysis: Peripheral lymphocyte cultures were prepared according to the method of Perry & Wolff (1974). Briefly, 5 mL blood was added to 5 mL of RPMI 1640 medium supplemented with 1 mL of fetal calf serum and 0.1 mL of phytohaemoglutanin along with brodeoxyuridine (3µg/mL), which was added at the beginning of initiation of cultures. The cells were incubated at 37°C. Simultaneously, control cell cultures were also maintained. Mitotic arrest was done prior to harvesting by adding 0.2 mL of colchicine to the culture vials. Cells were centrifuged at 2000 rpm for 10 min. The supernatant was removed and 5 mL of prewarmed (37°C) hypotonic solution (0.075 M KCl) was added. Cells were resuspended and incubated at 37°C for 20 mins. After hypotonic treatment the cultures were centrifuged and finally 5 mL of fixative was added to the pellet and slides were prepared. About 50

metaphases per sample/individual were scored and the slides were coded and screened for the presence of sister chromatid exchanges in control and exposed groups.

Statistical analysis: Student '*t*' test was used for finding out the statistical significance of SCEs in controlled and exposed groups. The level of significant was obtained from the standard statistical tables of Fisher (1963).

RESULTS AND DISCUSSION

During the fast few years genotoxic biomarkers have considerable interest as tools for detecting human genotoxic exposure and toxic effects, especially health surveillance programmes dealing with occupational exposure to chemical carcinogens. Currently only cytogenetic end points in peripheral human lymphocytes allow a reasonable epidemiologic evaluation of cancer productivity. In a few years time, uniform data will make possible to reassure the value of SCEs, and now this technique is used in biomarkers of genotoxic exposure as simple alternatives to CA analysis.

Table 1 shows the characteristics of control and exposed groups. The mean age group (in years) ranged from 35.0 to 42.6 in control group and from 36.0 to 42.0 in exposed group. They belonged to same socioeconomic status.

The results of the present study are given in Table 2. There was an increase in the mean SCEs rate per cell in exposed group over the control values. The percentage of mean SCEs rate per cell in exposed group was 5.06 as against 3.20 in the control group.

The suitable method adopted for studying cytogenetic effects induced by suspecting agent in human beings is the micro-culturing of human peripheral blood lymphocytes. Sister chromatid exchange is the cytological manifestation of DNA breakage and rejoining at apparently homologous sites in the chromatids of single chromosome. SCEs can be observed in any cell that has completed two replication cycles in the presence of Brdu. Using the technique several environmental pollutants/chemical substances have been reported in *in vivo* occupational exposed workers (Alexander et al. 2001, Hammer et al. 1998, Pitraque et al. 1997, Madhavi et al. 2008) and *in vitro* mammalian cultures (Laxmi Sowjanya et al. 2008, Vani & Rudrama Devi 1996, Madhavi & Rudrama Devi 2007, Poma et al. 2003, Woniak & Blasiak 2003).

Table 1: Characteristics of study population.

	No of samples	Age in Years	Duration of exposure
Control	42	35.0 to 42.6	
Exposed	62	36.0 to 42.0	20 ± 1.0 yrs

Table 2: Frequency of sister chromatid exchanges.

	Control	Exposed subjects
No of samples	42	62
No of metaphases screened	1260	1860
Total number of SCEs	4032	9416
SCEs/Cell	3.20 ± 1.20	5.06 ± 0.90

30 metaphases were scored for each sample. *P < 0.05

Workers in shoe factory are exposed to mixture of organic solvents, and especially glue and gasoline used may contain benzene which is well known clastogen requiring metabolic activation to be mutagenic. The genotoxicity of workers exposed to benzene has shown increased cytogenetic damage like monosmy and trisomy, which leads to leukaemia (Kim et al. 2004). However, no evidence of genetic damage was observed in benzene exposed workers by Zhang et al. (2002). The combined effect of benzene and toluene induced chromosomal aberrations in shoe factory workers (Bogadi Sare et al. 1997). Further, significant increase in the frequency of micronuclei in peripheral lymphocytes (Jithen Kumar et al. 2005) in exfoliated buccal cells (Gonzalez et al. 2009, Rudrama Devi & Jithender Kumar 2010) has been observed in shoe factory workers exposed to solvents.

There are several reports showing the increased yields of SCEs like in workers of rubber chemicals (Hema Prasad et al. 1986), coal miners (Vijender Reddy & Rudrama Devi) and industrial painters (Madhavi et al. 2006). The overall results indicate occupational exposure to organic solvents causing genetic damage in exposed people, and the effects may lead to carcinogenic as well as genetic defects in man. Thus, in order to prevent health effects, hazards management should take necessary steps to minimize the use of solvents for protection of their workers. Masks should be used to avoid the inhalation of solvent vapours, which are carcinogenic in nature. Thus, preventive measures are necessary to protect the health of exposed individuals.

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