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Evaluation of sister chromatid exchange and chromosomal aberration frequencies in peripheral blood lymphocytes of gasoline station attendants

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Abstract

Petroleum derivatives constitute a complex mixture of chemicals which contain well-known genotoxicants, such as benzene. Thus, chronic occupational exposure to such derivatives may be considered to possess genotoxic risk. In the present study, frequencies of sister chromatid exchange (SCE); aberrant cells, including numerical and structural chromosomal aberrations; and chromosome aberrations were investigated in peripheral blood lymphocytes from 30 exposed workers (15 smokers and 15 nonsmokers) and 30 controls (15 smokers and 15 nonsmokers). The exposed subjects were employed at 12 different petrol pumping stations in the city of Mersin, Turkey. Urinary phenol levels of exposed workers were found to be significantly higher than those of control subjects. Benzene exposure and cigarette smoking decrease the replication index and mitotic index. There is an interaction between benzene exposure and cigarette smoking for replication index and mitotic index. There is no interaction between cigarette smoking and benzene exposure for chromosomal aberrations. The results indicate that there are significant differences in SCE values in the exposed workers compared to the control individuals ($P<0.01$), but there is no difference between smokers and nonsmokers for SCE frequency ($P>0.05$). SCE frequency is higher in smokers than in nonsmokers.

Keywords: Benzene; Gasoline station attendants; Human blood lymphocytes; Sister chromatid exchanges; Chromosomal aberrations

1. Introduction

Genetic monitoring of human populations exposed to potential mutagens/carcinogens is an early warning system for genetic disease or cancer. Induced chromosomal changes in human lymphocytes as well as sister chromatid exchanges are well-established biomarkers of occupational or environmental exposure to genotoxic agents. The most frequently used genetic endpoints are chromosomal aberrations (CAs) and micronuclei (Carrano and Natarajan, 1988; Lambert et al., 1982).

Moreover, people are often exposed to a mixture of different chemical substances and physical factors. It is very difficult to determine the exact cause of an increased frequency of chromosomal damage. The association between exposure to polycyclic aromatic hydrocarbons and certain types of leukemia has been established by epidemiological studies in a number of countries and industries (Tompa et al., 1994; IARC, 1982, 1987).

Benzene is considered to be a human carcinogen; it is clastogenic to rodents and humans and affects the immune response (WHO/IPCS, 1993). Workers can be occupationally exposed to aromatic solvents, such as benzene, as a result of various activities in which the substance is processed, generated, or used (Major et al., 1994; Turkel and Egeli, 1994). Benzene is a ubiquitous industrial and environmental pollutant. It is present in both evaporative and combustive automobile emissions, has been detected in cigarette smoke, and is commonly used as an industrial solvent in the workplace. Several epidemiological and environmental studies conducted on human populations exposed to petroleum exhausts have shown that there is an increased incidence of disease such as lymphopoietic cancer, lung cancer and

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nonmalignant respiratory diseases related to the exposure to such pollutants (Aksoy, 1985; IARC, 1989; Lagario et al., 1993; Gamble et al., 1987). Benzene is an established cause of human leukemia that is thought to act by producing chromosomal aberrations and alterations in cell differentiation. In several recent studies increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially hematological malignancies. Thus, chromosomal aberrations may be a predictor of future leukemia risk. Since the 1960s approximately 50 cytogenetic studies in benzene-exposed subjects have been conducted. Most studies have shown a positive association between benzene exposure and increased chromosomal aberration (Zhang et al., 2002).

Benzene exposure can induce CAs in somatic cells (lymphocytes, bone marrow cells) of human's and of experimental animals in vivo as well as in some in vitro systems. For filling station attendants, exposure to several genotoxic compounds can be expected, and among chemicals measurable in petrol vapor, benzene is one of the most relevant for public health concern, due to its established carcinogenicity (Infante et al., 1990).

In this study we aimed to analyze the possible cytogenotoxic effects of such petroleum exposure on frequencies of sister chromatid exchange (SCE), CA, and aberrant cells, including both structural and numerical chromosomal aberrations in the peripheral blood lymphocytes, in gasoline station attendants. Replication index (RI) and mitotic index (MI) were evaluated in order to determine the cytotoxicity of benzene.

2. Materials and methods

2.1. Subjects investigated

The study was carried out in a group of 30 men (15 smokers, 15 nonsmokers) at 12 different petrol stations in the center of Mersin City, Turkey. All attendants were healthy men whose ages ranged from 25 to 40 years. In all these stations super-grade petrol (leaded and unleaded) and diesel fuel has been sold. The control group consisted of 30 healthy men (15 smokers, 15 nonsmokers) working at the campus of Mersin University, without indication of previous occupational exposure to petroleum derivatives or other agents suspicious of genotoxicity. The subjects were investigated for demographic data; smoking, drinking, and eating habits; exposure to ionizing radiation; diseases; drug consumption; occupational history; and/or exposure to known or suspected mutagens. All attendants were working at the time when the blood samples were collected.

2.2. Cytogenetic method

Two tests were performed: chromosomal analysis and sister chromatid exchange. Blood samples were collected from exposed subjects and controls by venipuncture. For each donor, two lymphocyte cultures were separately set up for both tests. Lymphocyte cultures were prepared according to the developed technique by Moorhead et al. (1960) with slight modifications. Heparinized whole blood (0.8 mL) was added to 5 mL of culture medium F10 (Gibco), supplemented to 18.5% with fetal calf serum (Gibco), with 0.2 mL phytohemagglutinin (Gibco), and with antibiotics (10,000 IU/mL penicillin and 10,000 IU/mL streptomycin). 5-Bromo-2-deoxyuridine (9 μg/mL; Sigma), used for SCE analysis, was added to cultures at the beginning of the 72-h incubation period at 37°C. For both the SCE and, CA analysis, lymphocytes were cultured in the dark for 72 h and metaphases were blocked during the last 1.5 h with colcemid at final concentration of 0.2 μg/mL. The mitotic index was calculated as the proportion of metaphases among the total cell population by counting a total of 1000 cells. In addition to SCEs, cells were analyzed for the relative frequency of first-division metaphases (M1; identifiable by uniform staining of both sister chromatids), second-division metaphases (M2; identifiable by differential staining of the sister chromatids), and third- and subsequent division metaphases (M3; identifiable by nonuniform pattern of staining). RI or proliferation index is the average number of replications completed by metaphase cells and is calculated as follows:

$$\mathrm{RI} = 1 \times (\%\ \mathrm{M1}) + 2 \times (\%\ \mathrm{M2}) + 3 \times (\% \times \mathrm{M3})/100.$$

Chromosomal aberrations were evaluated in 72-h whole-blood cultures according to standard protocol (IAEA, 1986). The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (KCl, 0.075 M) for 15 min, and fixed in methanol/acetic acid (3:1, v/v) solution (Carnoy's fixative). Air-dried preparations were made and the slides were stained by the fluorescence plus Giemsa procedure (Pery and Wolff, 1974) with slight modifications. Generally, for each donor, 100 cells at metaphase were analyzed for chromosome damage using cultures incubated for 72 h, and 100 well-spread metaphases containing 46 chromosomes in their second division that had clearly differentially stained were chosen to be counted for SCE frequency on coded slides. Cells including both numerical and structural chromosomal aberrations were recorded as aberrant cells. The slides were stained with 10% Giemsa dye solution. Chromatid breaks, chromosome breaks, fragments, dicentric and acentric chromosome, deletions, and duplications were evaluated as structural chromosomal aberrations. Because the fre-