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

Ayla Çelik[a,*] and Etem Akbaş[b]

[a] Department of Biology, Faculty of Science, Mersin University, 33342 Mersin, Turkey
[b] Department of Medical Biology, Faculty of Medical Science, Mersin University, 33342 Mersin, Turkey

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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

*Corresponding author. Fax: +90-324-3610047.
*E-mail address:* a.celik@mersin.edu.tr (A. Çelik).

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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-

quency of numerical chromosomal aberrations is very low they were not evaluated as a separate chromosomal aberrations in this study. These aberrations were scored only within aberrant cells.

### 2.3. Urine collection and phenol measurement

Exposure monitoring was performed by the detection of excreted phenol levels in the urine. Samples were obtained from workers immediately after their 8-h work shift. Urinary phenol measurements were performed following the colorimetric quantitative determination method of Yamaguci and Hayashi (1977). The obtained urinary phenol values were corrected for creatinine content.

### 2.4. Statistical analysis

In all calculations, two-way analysis of variance was used. Statistical analysis was performed using the SPSS for Windows 9.05 package program. Type I error rate was accepted as 0.05. The group comparisons were performed by LSD test. Phenol/creatinine values in control and exposed groups were compared by *t* test.

## 3. Results

Table 1 presents the demographic characteristics of the 30 workers exposed to petroleum containing benzene and 30 controls. The monitoring of phenol levels in urine samples revealed the mean values of 7.24 and 19.85 mg/g/L for control subjects and exposed workers, respectively. The mean frequencies and types of chromosomal aberrations are shown in Table 2. Replication index and mitotic index have also been determined in both exposed and control subjects. Table 3 presents the mean frequencies of RI, MI, SCE, and aberrant cells in control and exposed subjects. The results of the replication index (used on a measure of evaluation of cell cycle kinetics) have shown that the proliferation of human lymphocytes exposed to benzene

Table 1
Demographic characteristics of control subjects and of workers currently exposed to benzene

| Study group | *N* | Age (range in years) | Duration of employment (range in years) | Phenol level (mg/g/L creatinine) (range values) |
|---|---|---|---|---|
| Controls | | | | |
| Smoker | 15 | 25–40 | — | 9.03±1.90 (5.25–16.10) |
| Nonsmoker | 15 | 25–40 | — | 5.46±1.76 (1.93–12.10) |
| Total | 30 | 25–40 | — | 7.25±1.36 (1.93–16.10) |
| Exposed | | | | |
| Smoker | 15 | 25–40 | 8–13 | 24.50±7.77 (8.62–52.82) |
| Nonsmoker | 15 | 25–40 | 7–14 | 15.19±4.43 (4.58–31.00) |
| Total | 30 | 25–40 | — | 19.85±4.49 (4.58–52.82) |

*N*, the number of subjects included in the study.

Table 2
Frequencies of chromosomal aberrations in the peripheral blood lymphocytes of exposed and control groups

| Group | *N* | Total number of examined metaphases | Chromatid | | Chromosome | | Fragment | DIC | TSCA | Mean±SE% SCA (excluding gaps) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Gap | Break | Gap | Break | | | | |
| Control | | | | | | | | | | |
| Nonsmokers | 15 | 750 | 3 | 5 | 3 | — | 3 | — | 14 | 0.53±0.23 |
| Smokers | 15 | 750 | 10 | 7 | 7 | 2 | 2 | 1 | 29 | 0.80±0.23[a] |
| Total | 30 | 1500 | 13 | 12 | 10 | 2 | 5 | 1 | 43 | 0.66±0.16[b] |
| Expose workers | | | | | | | | | | |
| Smokers | 15 | 750 | 11 | 11 | 4 | 3 | 6 | 3 | 38 | 1.53±0.23 |
| Nonsmokers | 15 | 750 | 6 | 7 | 4 | 4 | 6 | 3 | 30 | 1.33±0.23[c] |
| Total | 30 | 1500 | 17 | 18 | 8 | 7 | 12 | 6 | 68 | 1.43±0.16 |

TSCA, total structural chromosome aberrations; *N*, the number of subjects included in the study.
[a] In control group, compared to nonsmoker, $P<0.05$.
[b] Compared control group and exposed group, $P>0.05$.
[c] Exposed group, compared to nonsmoker, $P<0.05$.

Table 3
Frequencies of RI, MI, SCE, and aberrant cells in exposed group and control subjects

| | Exposed group | | | Control group | | |
|---|---|---|---|---|---|---|
| | Smoker | Nonsmoker | Total | Smoker | Nonsmoker | Total |
| SCE/metaphase | 6.39±0.36[a] | 5.74±0.31 | 6.06±1.32**[b] | 5.56±0.30[a] | 5.24±0.32 | 5.40±1.18 |
| Aberrant cells | 2.73±0.23**[c] | 2.20±0.30 | 2.46±1.04**[b] | 2.33±0.30**[d] | 1.07±0.15 | 1.70±1.11 |
| RI (%) | 1.79±0.02**[c] | 1.95±0.02 | 1.87±0.01**[b] | 1.93±0.02**[d] | 2.22±0.02 | 2.08±0.01 |
| MI (%) | 3.42±0.16**[c] | 4.51±0.16 | 3.97±0.11**[b] | 4.23±0.16**[d] | 6.06±0.16 | 5.15±0.11 |

Values are expressed as means±SE. RI, replication index; MI, mitotic index.
[a] In both exposed and control groups, compared to nonsmoker, $P>0.05$.
[b] Compared to control group, $P<0.05$.
[c] In the exposed group, compared to nonsmoker, $P<0.05$.
[d] In the control group, compared to nonsmoker, $P<0.05$.

Table 4
Results of pairwise comparison for MI and RI

| Group | MI | RI |
|---|---|---|
| $(Sig^+)(Pet^-)/(Sig^+)(Pet^+)$ | * | * |
| $(Sig^+)(Pet^-)/(Sig^-)(Pet^-)$ | * | * |
| $(Sig^+)(Pet^-)/(Sig^-)(Pet^+)$ | n.s. | n.s. |
| $(Sig^+)(Pet^+)/(Sig^-)(Pet^-)$ | * | * |
| $(Sig^+)(Pet^+)/(Sig^-)(Pet^+)$ | * | * |
| $(Sig^-)(Pet^+)/(Sig^-)(Pet^-)$ | * | * |

$Sig^+$, smoker; $Sig^-$, nonsmoker; $Pet^+$, petrol exposure; $Pet^-$, petrol nonexposure. LSD value for RI, 0.057; LSD value for MI, 0.398. n.s., nonsignificant.
*$P<0.05$.

was significantly inhibited compared to those in the controls (Table 4). Benzene exposure and cigarette smoking lowered the mitotic index.

In the statistical analysis, comparison of SCE, CA, and aberrant cell frequency in peripheral blood lymphocytes showed a significant difference between exposed workers and control subjects, but smoking habits had no effect on SCE frequency in either the control or the exposed individuals. No interaction between smoking and exposure to petroleum was detected for CA. In contrast, there is an interaction between petroleum exposure and cigarette smoking for RI and MI. This interaction was investigated between groups (Table 4). It was determined that both cigarette smoking and petroleum exposure have more effect than only cigarette smoking or only petroleum exposure on the RI and MI. In addition, no aging effects were noted, neither in the exposed workers nor in the controls, on the MI, RI, SCE, or CA.

The difference found between smokers and nonsmokers, in both control subjects and exposed subjects, is statistically equal for SCE, CA, and aberrant cell frequency. When smoking habits were considered, a significant difference was found in both aberrant cell ($P<0.05$) and CA frequency ($P<0.05$). The decrease in cigarette smoking-induced RI and MI was statistically significant for both exposed group and controls.

SCE frequency was higher in smokers than in nonsmokers, but a nonsignificant difference was found for SCE frequency ($P=0.138$) ($P>0.05$).

Urinary phenol levels of exposed workers were found to be significantly higher than those of control subjects.

## 4. Discussion

The aim of this work was to investigate the effects of occupational exposure to petroleum and cigarette smoking on the frequency of SCE, CA, and aberrant cells in the peripheral blood lymphocytes of workers at gasoline stations within the urban area of Mersin City, Turkey.

Filling station attendants are occupationally exposed to a wide range of petrol derivatives and, since contradictory results have been obtained from biomonitoring studies of people exposed to such chemicals, it is necessary to provide more relevant information regarding the possible petrol exposure-related changes. Exposure to petrol components may take place via absorption through the intact skin or by vapor inhalation. According to data obtained from gasoline stations included in this study, each worker pumps an average of 2000 L of petroleum, containing 5% (v/v) benzene, during their 8-h work shift.

Epidemiological studies showed that there is a clear relationship between the increase in SCE and CA and exposure to benzene (Sasiadek et al., 1989; Turkel and Egeli, 1994; Tompa et al., 1994; Pitarque et al., 1997; Bogadi-Sare et al., 1997; Jablonica et al., 1987; Tough and Court Brown, 1965; Tough et al., 1970; Forni et al., 1971; Dean, 1978; Sarto et al., 1984; Yardley-Jones et al., 1990; Gillian et al., 1984; Karacic et al., 1995). However, the data on cytogenetic damage (sister chromatid exchange, chromosome aberrations, and micronuclei) in gasoline station attendants are rather controversial.

In the several studies carried out, investigation of the SCE frequencies in gasoline station attendants revealed that there were no significant differences between controls and experimental subjects (Yardley et al., 1988; Pitarque et al., 1997). It was observed that cigarette smoking induced the chromosomal aberration and sister chromatid exchange in previously published studies (Pitarque et al., 1997; Al-Sabti et al., 1992; Lakhanisky et al., 1993). In the present study, smoking had no effect on SCE frequency in both exposed and control groups but, it was determined that smoking induced the CA and aberrant cells. In some studies, it has been rejected that benzene metabolites, such as hydroquinone, may induce hyperploidy in cells (Sbrana et al., 1993; Zhang et al., 1996). In previous studies performed on workers exposed to benzene, it was determined that benzene exposure lowered the replication index (Tompa et al., 1994). Benzene and its metabolites have been also reported to decrease total bone marrow cellularity (Eastmond et al., 1987) and to interfere with the formation of the mitotic spindle apparatus (Pfeiffer and Metzler, 1996). The results of this study revealed that benzene exposure may be cytotoxic to blood lymphocytes of humans exposed to benzene. Our results revealed a significant increase in frequency of SCE in peripheral blood lymphocytes obtained from gasoline station workers. These data are in agreement with the previous reports obtained on the potential clastogenic and mutagenic effects of benzene (Turkel and Egeli, 1994; Tompa et al., 1994; Pitarque et al., 1997; Bogadi-Sare et al., 1997; Jablonica et al., 1987; Tough and Court Brown, 1965; Tough et al., 1970; Forni et al., 1971; Dean, 1978; Sarto et al., 1984; Yardley-Jones et al., 1990; Gillian et al., 1984; Karacic et al., 1995).

Phenol is the principal metabolite of benzene. Therefore, phenol concentration in the urine of exposed workers can be used as an index of internal dose because the urinary level is highly correlated with external benzene exposure. Biological monitoring of gasoline station attendants showed substantially higher levels of urinary phenol when workers were compared to subjects with no known exposure to either gasoline or benzene (Pandya et al., 1975; Hein et al., 1989). It was determined in epidemiologic studies performed on workers exposed to benzene that benzene exposure increased the urinary phenol levels in those workers (Inoue et al., 1986; Premel-Cabic et al., 1988; Ducos et al., 1992). These researchers found the urinary phenol level in exposed subjects to be higher than that in control subjects. In our study, urine analyses revealed a significant difference between control and exposed subjects. The differences in the phenol concentrations in urine samples of control and exposed subjects reflect that exposed subjects were affected by benzene. However, it is also known that urinary phenol level can be affected by demographic factors, such as diet, smoking, and ingestion of medicine (Waritz, 1985). Since none of the subjects in our study differed in their demographic parameters, the observed difference in the mean urinary phenol levels could be attributed to the occupational exposure to benzene. On the other hand, it should also be emphasized that gasoline station attendants are exposed not only to hydrocarbons present in gasoline vapor, but also to the emissions produced by engines during fuel combustion. It was shown that these emissions may also cause mutagenic and genotoxic effects (Hadnagy and Seemayer, 1988).

## 5. Conclusion

In conclusion, the present study provides some evidence that petrol, including benzene, exposure may be associated with genotoxic effects on the lymphocytes. In addition, cigarette smoking may also cause the increase in SCE frequency in both the exposed group and the control group. That results showed that cigarette smoking can contribute to the effects of petrol derivatives such as benzene. However, further study is needed, involving larger sample sizes, improved assessment of benzene exposure, and use of biomarkers of susceptibility. These results confirm the potent genotoxic effect of petroleum including solvents like benzene as an inducer of chromosome aberration and SCE and also that cigarette smoking has effects on chromosomal aberrations. By monitoring the level of chromosome damage in a population, especially among workers exposed to genotoxic chemicals, it is possible that we may effectively identify individuals or groups with elevated risk of leukemia so that appropriate measures may be taken to reduce their exposure. As work in petrol pump stations appears to involve exposure to genotoxic agents capable of damaging human DNA, workers at petrol stations should take adequate protective measures to prevent long-term effects such as neoplasms.

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