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Assessment of micronuclei and sister chromatid exchange frequency in the petroleum industry workers in province of Vojvodina, Republic of Serbia

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ABSTRACT

Persons who work with petroleum and petroleum derivatives (PPD) are potentially at risk of developing cancer mostly due to the carcinogenity of benzene. Therefore, the aim of this study was to determine in which degree occupational exposure of workers to PPD causes damage to DNA by analysis of micronuclei (MN), sister chromatid exchanges (SCE) and proliferation index (PI). 30 workers of refinery in Novi Sad, participated in the study as exposed and 30 volunteers as control group. Workers exposed to PPD had significantly higher values of MN and SCE in comparison to controls. Exposition time to PPD and type of working place have also significantly effects to DNA damage. The influence of confounding factor such as smoking and age were also evaluated.

1. Introduction

According to the International Agency for Research on Cancer, occupational exposure to petrol and petrol derivatives represents a potential risk of developing cancer in people, largely due to confirmed carcinogenicity of benzene, the main component of petroleum (Fielden and Zacharewski, 2001; McDougal and Garrett, 2007).

Persons working with petroleum and its derivatives (PPD) are by absorption through the skin or through inhalation of petroleum fumes exposed to a number of petroleum products. It is precisely this exposure to the petrol vapors of petroleum derivatives that may cause an increased incidence of the disease, which was confirmed in epidemiological studies of the human population (Lazarov et al., 2000; Carletti and Romano, 2002). Recent studies have shown haematotoxic effect at different concentrations of benzene including the concentration of 1 ppm in the air (McHale et al., 2012). It is known that benzene causes acute myeloid leukemia (AML), myelodysplactic syndrome and other hematologic malignancies such as non-Hodgkin's lymphoma and leukemia in children as well as lymphoproliferative disorders (Tompa et al., 2005; Griego et al., 2008; McHale et al., 2012; Recio et al., 2012).

Peripheral blood lymphocytes in numerous studies are used to determine different genotoxic effects by monitoring the following: the incidence of micronuclei (MN), the frequency of sister-chromatid exchanges (SCE) as well as chromosomal aberrations and DNA damage using different assay (Boess et al., 2003; Navasumrit et al., 2005; Harper et al., 2007; Mondal et al., 2010; Mrđanović et al., 2012; Moeller et al., 2013). However, micronucleus test performed in epithelial cells from bucall mucosa has been shown to be an effective method to detect chromosomal aberrations as well (Celik et al., 2003; Diler and Çelik, 2011).

Therefore, it is important to assess the toxic effect associated with exposure to petroleum and petroleum products not only in accidental situations, but also in exposure to continuous low concentrations of benzene present in the environment (Bindhya et al., 2010). Also, given that smokers may be exposed to benzene 10 times more than non-smokers, smoking is considered to be an important additional factor that need be evaluated in individuals who are occupationally exposed to PPD (Mansi et al., 2012). Age and length of occupational exposure must also be carefully considered as additional factors in the evaluation of DNA damage (Wang et al., 2011).

Abbreviations: AO, antioxidant; AML, acute myeloid leukemia; CBMN, cytokinesis block micronucleus; MN, micronuclei; PPD, petroleum and petroleum derivatives; SCE, sister chromatid exchanges; PI, proliferative index.

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The purpose of the study was to determine toxic effects occupational exposure workers to PPD on DNA damage in refinery in Novi Sad, using the cytokinesis block micronucleus (CBMN) assay and SCE and analyzed in respect to other confounding factors.

2. Material and methods

2.1. Group description

The study included 30 workers from the refinery in Novi Sad (Vojvodina, Serbia) who represented the exposed group and 30 healthy individuals as a control group without previous indication of occupational exposure to PPD or other agents suspicious of genotoxicity. All subjects completed a written questionnaire that included questions including birth date, tobacco and alcohol use, medication use, existing diseases, as well as data related to the length of occupational exposure. Also, from all the individuals informed written consent was obtained before the study. The study was approved by the local ethical committee.

2.2. The peripheral blood lymphocyte cultures

Heparinized whole blood was collected by venous puncture and used for the peripheral blood lymphocyte cultures in CBMN and SCE test. Briefly, 0.5 ml of the whole blood was added to 5 ml of RPMI 1640 cell culture medium (Sigma, USA) supplemented with 2 mM glutamine, 20% of heat-inactivated fetal calf serum (FCS, NIVNS, Serbia) and antibiotics: 100 IU/ml of penicillin and 100 μg/ml of streptomycin (ICN, Serbia). Cell cultures were stimulated for division with phytohemagglutinin (PHA-M, Sigma, USA) at a final concentration of 20 μg/ml and incubated at 37 °C for 72 h in 5% CO_2 atmosphere with 95% humidity.

2.3. Cytokinesis block micronucleus test

CBMN was performed by the standard cytogenetic procedure (Fenech, 2007; IAEA, 2001.) with minor modifications regarding staining. 44 h after stimulation of the lymphocyte culture with PHA, Cytochalasin-B (Sigma, USA) was added at final concentration of 6 μg/ml. After 72 h, the cells were collected by centrifugation, exposed to a cold 0.075 M KCl hypotonic solution and fixed three times. The first fixation was in methanol–acetic acid (3:1) with 1% formaldehyde, but the two following fixations were in methanol–acetic acid (3:1) alone. Drops of a concentrated suspension of cells were placed on dried slides. Cells were stained with Giemsa (2%) in distilled water with three drops of NH_4OH for 9 min.

At least 1000 cells per each sample were analyzed. Standard criteria were used for the identification of MN (Kirsh-Volders et al., 2000). Monitored values included: frequency of micronuclei, micronucleus distribution and proliferation index. MN frequency was presented as a number of micronuclei per 1000 examined binuclear cells (Fig. 1a). Micronucleus distribution was acquired by scoring the binuclear cells containing one or more micronuclei. The PI, that represents a measure of the number of cell cycles that a cell population passes through, was calculated according to formula:

$$\mathrm{PI} = \frac{\mathrm{M1} + 2\mathrm{M2} + 3(\mathrm{M3} + \mathrm{M4})}{N}$$

where M1–M4 represent the numbers of cells with 1–4 nuclei, respectively, and N is the total number of scored cells (IAEA, 2001). The prepared material was observed and analyzed by light microscopy (Olympus BX51, Germany).

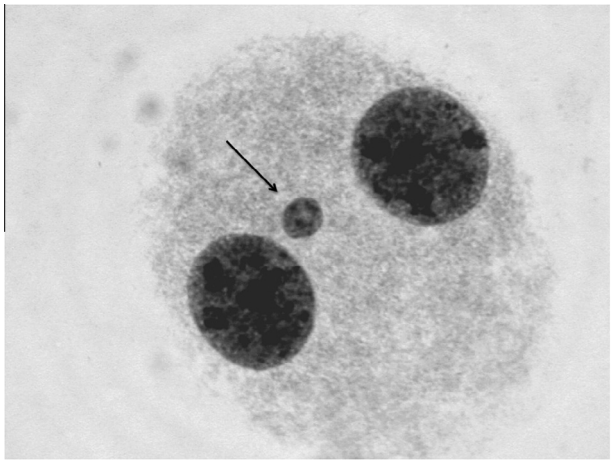

Fig. 1a. Binuclear lymphocytes with micronuclei. Arrow indicates micronuclei.

2.4. Sister chromatid exchanges test

Chromatid differentiation in PBL cultures was initiated by adding of 10 μg/ml 5-bromo-20-deoxyuridine (BrdU, Sigma, USA) to cultures after stimulation with PHA. PBL cultures were stopped 68 h after stimulation with PHA with 0.1 μg/ml of demecolcine (Sigma, USA), which was added 1 h before harvesting. The cells were collected by centrifugation, after which they were incubated at 37 °C in the presence of 0.075 M KCl hypotonic solution for 40 min, and fixed in methanol–acetic acid (ratio 3:1). Cell suspension was placed on wet, cold microslides.

Differential staining of sister chromatids was performed using the fluorescence-plus-Giemsa technique with Hoechst 33258 (Sigma, USA), which was previously described (Verma and Babu, 1995). SCE frequency was presented as the average number of SCE counted in fifty complete metaphase chromosomal sets with well-differentiated sister chromatids (Fig. 1b).

2.5. Statistical analysis

Results are presented as median values. Differences between control and the exposed groups for SCE, MN, PI were analyzed by non-parametric test; Mann–Whitney U test and ANOVA, using legal SPS program version 17. The differences were considered significant when $p < 0.05$.

3. Results

3.1. Characteristics of subjects included in the study

The characteristics of both exposed and control groups are shown in Table 1. The subjects were divided into control and exposed groups, each containing 30 individuals. In relation to the age of the subjects both groups were classified into older and younger, given that the rank of age ranged from 27 to 58 years. Based on the length of exposure (occupational exposure) to PPD, exposed group was divided into two subgroups. The first group consisted of subjects who were exposed to PPD for a shorter time (3–19 years) and the other subgroup for a longer time (20–36 years), while an average duration of exposure was 20 years. Based on the smoking status of both examined groups, both exposed and control group were divided into smokers and non-smokers. The average number of cigarettes in both groups was the same (20). None of the groups had vegetarians.

3.2. The SCE and MN frequency and PI value in the control and exposed group

The SCE and MN frequency in exposed group were significantly higher ($p = 0.03$, and $p = 0.011$, Mann–Whitney U test), compared to control group respectively, while the PI value was significantly

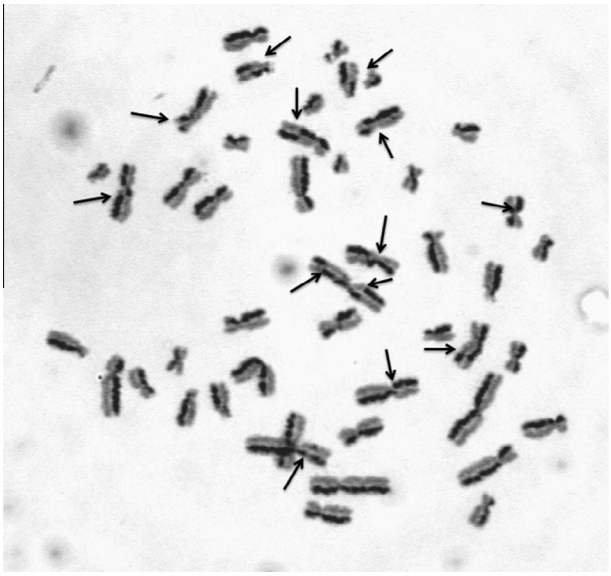

Fig. 1b. Metaphase chromosomes with sister chromatid exchanges (SCE). Arrows indicate SCE.