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Biomonitoring of gasoline station attendants exposed to benzene: Effect of gender

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ABSTRACT

Women are employed in increasing numbers as gasoline station attendants, a work category with risk of exposure to benzene. We have assessed the effect of gender on biomarkers of occupational benzene exposure. Gasoline station attendants (20 men and 20 women) and 40 control individuals (20 men and 20 women) with no history of occupational benzene exposure were evaluated. Benzene exposure was monitoring by environmental and biological measurements. Urinary *trans*,*trans*-muconic acid levels, well-known genetic and hematological alterations linked to benzene exposure, and non-cancer effects on the immune, hepatic, and renal systems were investigated. Our results suggest a potential effect of gender on some effects of occupational benzene exposure, particularly the hematological parameters and *trans*,*trans*-muconic acid levels. Despite limitations of our study, our findings provide important considerations about occupational exposure of women to benzene and may contribute to the development of occupational protection standards.

1. Introduction

Occupational exposure to benzene has been linked to adverse health effects, including hematotoxicity, genotoxicity and carcinogenesis [1–5]. Epidemiological and experimental studies have suggested that benzene exposure can lead to non-cancer health effects [6]. Benzene has been used in several industrial settings [7], especially petrochemical industries [7,8], the chemical industry, and in manufacturing, e.g. rubber, shoes, and printing [7–10]. Transport, distribution, and fueling (gasoline) are also important occupational sources [11].

"Gasoline station attendant" (GSA) is recognized as a job category with risk of exposure to benzene [8]. In Brazil, there are no self-service gasoline stations; fueling is performed by GSA, who are chronically exposed to benzene during work shifts [12]. According to Corrêa [13], among the Brazilian population, GSA are the second largest group of workers potentially exposed to benzene. Historically, this group has comprised mainly male workers. However, recently, the number of women gasoline station attendants has increased, to a ratio of about 2.5:1 men: women [13].

Biomonitoring is a mandatory health protection measure for these workers [2,14]. Tolerance limits have mainly been based on studies of male subjects [15], but exposure can vary substantially between women and men [2,15]. Despite much research on the hazardous effects of benzene exposure, little information is available regarding the possible influence of gender.

In this study, we performed biomonitoring of GSA routinely exposed to benzene, examining the influence of gender on biomarkers of exposure and effect. We measured well-known genetic and hematological alterations caused by benzene exposure, as well as non-cancer effects on the immune, hepatic, and renal systems.

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2. Materials and methods

2.1. Study population

80 subjects participated in this study. The exposed group consisted of 40 GSA from Rio Grande do Sul, Brazil: 20 men (GSA-m) and 20 women (GSA-w). All subjects had been working in their current job for at least 3 consecutive months. The non-occupationally-exposed group (NOEG) consisted of 40 individuals who had no known history of occupational benzene exposure; also 20 men (NOEG-m) and 20 women (NOEG-w). Information about lifestyle, such as smoking status, alcohol drinking habits, age, and history of occupational exposure was obtained through a questionnaire interview. This study was approved by the ethics in research committee of the Federal University of Rio Grande do Sul/RS (No. 21728/11) and informed consent was obtained from each participant.

2.2. Sample collection

Sampling was conducted at the end of the work shift, after 3–4 consecutive days of exposure. Personal monitors were used to collect airborne benzene in the breathing zone of study subjects during the daily work shift, for approximately 8 h. After air sampling, the samples were stored at −20 °C until analysis. Urine samples were collected and stored at −80 °C until analysis of *trans*,*trans*-muconic acid, microalbuminuria, and *N*-acetyl-beta-D-glucosaminidase. Blood venous samples were collected by venipuncture using vacuum tubes. A blood-EDTA tube was collected for hemogram analysis. A blood-heparin tube was collected for comet assay, adhesion molecules, and δ-aminolevulinate dehydratase analysis. A vacuum blood tube without anticoagulant was centrifuged at 1500*g* for 10 min at room temperature. The serum obtained was used for hepatic and renal parameters determination. Buccal cells were collected to perform the micronucleus assay.

2.3. Benzene exposure assessment

Airborne benzene samples were collected using individual passive samplers (SKC 575-002®). Benzene was desorbed with dichloromethane and analyzed by gas chromatography and flame ionization detection (GC-FID). Chromatographic separation was achieved using a GC column Innowax (25 m, 0.2 mm, 0.4 μm), with the following chromatographic conditions: 99.99% purity H_2 was used as carrier gas, pressure = 10,055 psi, initial column temperature = 40 °C. increased at 4 °C min^{-1} until 53 °C and then 40 °C min^{-1} until 200 °C. FID detector temperature was 250 °C. The limits of detection (LOD) and quantification (LOQ) were 0.5 and 1.7 μg mL^{-1}, respectively.

Quantitation of *trans*,*trans*-muconic acid, a urinary metabolite of benzene, was performed according to the method of Ducos et al. [16], with modifications. The LOD and LOQ were 0.05 and 0.1 μg L^{-1}, respectively. The *trans*,*trans*-muconic acid levels were expressed unadjusted (t,t-MA) or adjusted by urinary creatinine (t,t-MA/creatinine). Creatinine concentration was measured by spectrophotometry, using commercial kits (Doles Reagents, Goiânia, GO, Brazil) according to the manufacturer's instructions. As recommended by the World Health Organization (WHO), values between 0.3 and 3.0 g L^{-1} were considered acceptable concentrations of urinary creatinine.

2.4. Genotoxicity biomarkers

The comet assay was performed according to a standard protocol of preparation and analysis, based on previously described methods [17,18].

For the micronucleus (MN) assay, buccal mucosa cells were collected. Cells were fixed with acetic acid: methanol (75:25, v/v), transferred onto clean microscope slides in duplicates, and stained with Giemsa (5%). Scoring was performed according to Thomas et al. [19].

2.5. Hematological biomarkers

Erythrogram, white blood cell parameters, and platelet analyses were performed using a hematology analyzer (Cobas Micros 60 System). δ-Aminolevulinate dehydratase (ALA-D) activity was performed by a spectrophotometric method [20].

2.6. Immunological biomarkers

Expression of CD80 and CD86 in the membranes of monocytes was analyzed by flow cytometry. Erythrocytes were lysed with NH_4Cl solution (0.13 M) and leukocytes were resuspended in PBS buffer. 10^6 leukocytes were incubated with PE-conjugated anti-CD80 and FITC-conjugated anti-CD86 in the dark at 4 °C for 20 min. The cells were analyzed using a FACSC Canto II Flow Cytometer (Becton Dickinson, San Jose, CA) using FlowJo Software (TreeStar). Monocyte cells were identified by manual gating according to side-scatter and size.

2.7. Hepatic and renal biomarkers

Hepatic parameters (albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and classical biomarkers of renal function (serum creatinine, urea, and uric acid) were determined using Cobas Integra 400 plus® (Roche, Indianapolis, IN) laboratory kits.

Microalbuminuria (mALB) and urinary *N*-acetyl-beta-D-glucosaminidase (NAG) were evaluated as early biomarkers of renal function. mALB was quantified in a Cobas Mira® analyzer (Roche, Indianapolis, IN) by an immunoturbidimetric method. NAG was determined by spectrophotometry [21].

2.8. Statistical analysis

Analysis of the data was performed with IBM SPSS Statistics software (version 19). Two-way analysis of variance (ANOVA) followed by multiple comparison tests (Bonferroni post hoc test) were employed to compare the groups. The results were expressed as mean ± standard error of the mean (SEM) or median (interquartile range), according to variables distribution. Correlation tests were performed according to Spearman's rank following the variables distribution. Values of $p \leq 0.05$ were considered significant.

3. Results

Characteristics of the study population, obtained through questionnaire interview, are provided in Table 1. No significant differences were found among the groups with respect to the categorical variables, age and length of occupational exposure, or the continuous variables, smoking and occasional alcohol drinking. Data for benzene exposure assessment are reported in Table 2. According to airborne benzene levels, both GSA groups were exposed to significantly higher concentrations compared to the NOEGs ($p < 0.001$). GSA-m were exposed to slightly higher levels than GSA-w. However, no significant inter-gender difference was observed in the exposed groups ($p > 0.05$) and all the values obtained were within the limits established by ACGIH (American Conference of Governmental Industrial Hygienists).

Both urinary *t*,*t*-MA and urinary *t*,*t*-MA/creatinine levels were significantly elevated in GSA-m and GSA-w compared to NOEG-