INTRODUCTION

Benzene is a known human carcinogenic and a natural ingredient of crude oil and other petroleum products. The burning of biomass also results in a release of benzene. Epidemiological studies show evidence for a causal association between leukemia and exposure to benzene. Therefore, the objective of this study was to evaluate urinary benzene as a biomarker of exposure to environmental benzene. Urine samples were collected from 73 adult males resident in Tehran, Iran. The urinary level of benzene was analyzed using a head space solid phase micro extraction gas chromatograph-mass spectrometry method (HS-SPME-GC-MS method). Median value of U-BEN was 0.94 µg/l (range from 0.2 to 2.9). The results of this study showed that urinary benzene is higher than values shown in prior studies. These high values may have undesirable health effects on the people resident in Tehran. Since urinary benzene behave in a similar way to blood benzene, urinary benzene due to the quick accessibility of urine and a non-invasive option is useful for environmental exposure and for epidemiology studies. As a result, the role of passive smoking and high traffic as the most important contributor to benzene exposure is very important.

Key words: Environmental exposure, Benzene, Urine, Biomarker.
exposure to very low concentrations of airborne benzene, because a correlation has been shown with benzene concentrations ranging from 6 to 478 μg/m³ (8). The small amount of unmetabolized benzene is removed unchanged in the urine. Urinary benzene has been considered as a biomarker of choice at air concentrations under 1 ppm benzene because it is a specific, sensitive and non-invasive method (9-11).

Also, the timing of sampling is not as crucial as for benzene in blood. Benzene in outdoor and indoor air samples have been measured by many researchers (12-16). Benzene have also detected in biological samples of people who were not occupationally exposed to benzene (14). Also, Exposure assessment can be performed by biological monitoring. Biological monitoring is defined as monitoring exposure to toxic pollutants and their effects in the biological liquids of exposed subjects (17).

Generally, Biological monitoring in the general population for exposure to airborne pollutants has been performed in blood (18-20). Blood sampling has ethical and practical limitations. Therefore, finding non-invasive biomarkers for bio monitoring is very important (21,22). The capability of urinary benzene as biomarker of exposure by many researchers in this field have shown (8,23-27).

The aim of this study was to measure the amount of urinary benzene as biomarker of environmental exposure to benzene in non-smoking males (Non-smokers were identified by the questionnaire) in the urban area of Tehran.

Materials and methods

Study population and sampling

The study sample included 73 adult males resident in the city of Tehran, Iran. All males were in good health without occupational exposure to benzene. This study was carried out in 22 areas of Tehran city. In these areas where this study has been performed there are no waste incinerators or major industries that could release benzene to environment. All men filled out a administered questionnaire about personal data, time spent in urban traffic during the environmental sampling, passive smoking. All participants were monitored from January to April 2015, using biological sampling for benzene exposure. The study was limited to the non-smoking men. Urine samples were taken from men at noon. 3 ml of each urine sample was immediately transferred into storage vial for urinary benzene. In the laboratory, the samples for 60 days were stored at at−20 °C until analysis (28).

Measurement of Urinary creatinine and urinary benzene

Jaffe’s colorimetric method was used for measuring of urinary creatinine (U-Cr) (29). The urinary level of benzene was analyzed using by head space solid phase micro extraction–gas chromatograph-mass spectrometry (HS-SPME-GC-MS) (30). At first, 2.0 ml of urine sample was conveyed into a 4.0-ml SPME glass vials containing 1.0 g NaCl. Then, 1 microliter of 1,4-Diethyleneoxide (1,4-Dioxane) was added as the internal standard solution, and the vial was immediately sealed with the magnetic crimp cap. A fiber of 100μm PDMS (Supelco, USA) by manual sampler was applied for headspace solid phase micro extraction sampling, carried out at 50 °C for 30 min with mixing conditions. Analyses were taken from the urine headspace using a fiber of 100μm PDMS for 5 min and thermally desorbed by placing the fiber into the chromatographic injection port for 180 sec. The measurement of urinary benzene was done by using a GC equipped with MS (Agilent Technologies, Agilent 7890N, CA, Palo Alto, USA - Agilent Technologies, Agilent 7890N, CA, Palo Alto, USA). The GC/MS analysis was done at the following conditions: helium as carrier gas with purity 99.999% at a steady flow rate of 1 mL min⁻¹; DB-5 MS analytical capillary column (30 m length, 0.25 mm diameter. and 0.25 μm film thickness); split ratio 2:1; injector temperature 285 °C for 3 min. The retention time for urinary benzene (U-BEN) and internal standard were 2, 3.5 min, respectively. The LOD, computed as the ratio S/N>3, for urinary benzene (U-BEN) was 0.045μg/l. The LOQ for urinary benzene (U-BEN) was 0.149μg/l.

Results

The main characteristics of the study population are shown in Table 1. The mean age of the 73 adult males was 37.36 (range from 19 to 82) years. The mean BMI was 24.66 (range from 18.37 to 32.69) kg/m². All adult males declared to be non-smokers.

Table 2 shows the statistical parameters of benzene in urine samples collected from 73 adult males resident in Tehran. U-BEN had a median value of 0.94μg/l (range from 0.2 to 2.9).
All results for U-BEN were not found below the LOQ. The relationships between urinary benzene and selected characteristics of study participants are shown in Table 3. According to Spearman’s correlation coefficients, BMI, Age, Cr showed no significant correlation with urinary benzene (all p > 0.05).

Discussion

This study applied U-BEN as biomarker to evaluate benzene exposure in males in the general population and to study the effects of high traffic and passive smoking. U-BEN is the good biomarker of benzene exposure for detecting of environmental exposure to benzene by experts in this field, but this study is the first that has measured U-BEN in males in the general population in Iran.

Urinary benzene in this study was measured in 73 males resident in Tehran city.

Our results are different from the a few results of prior studies. Since bio monitoring for urinary benzene in the general population has been restricted (Table 4). U-BEN biomarkers were found in all the analyzed samples. In our study, U-BEN levels ranged from 0.2 to 2.9 ug/l, with a median level of 0.94 µg/l (Table 2), while urinary concentrations by other researchers reported from 0.027 to 2.06 ug/l for U-BEN (median level = 0.069 µg/l) (31). The median level of U-BEN is higher than prior studies performed in 1994–2014 except one study carried out in Italy on bus drivers (32) which median level in our study is lower than this study (Table 4).

This finding is in accordance with results reported in a prior study on general population (33). In this research, the median level of U-BEN in males without proximity with high traffic area and passive smoking is lower than those of others that have passive smoking and proximity with high traffic area; This result verifies prior studies and is related to the high benzene in gasoline and the high dose of benzene in cigarette (8, 28). Prior studies assessed urinary and blood benzene in the general population regarding personal exposure to benzene (18-20).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Country</th>
<th>Smoking Habit</th>
<th>U-BEN (µg/l)</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>73 urban adults males</td>
<td>Iran</td>
<td>Non-smokers</td>
<td>0.94</td>
<td>This work</td>
</tr>
<tr>
<td>83 rural and urban adults</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>0.111</td>
<td>(34)</td>
</tr>
<tr>
<td>58 bus drivers</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>1.155</td>
<td>(32)</td>
</tr>
<tr>
<td>58 urban office workers</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>0.133–0.155</td>
<td>(8)</td>
</tr>
<tr>
<td>137 urban adults</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>0.08</td>
<td>(36)</td>
</tr>
<tr>
<td>65 urban adults</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>0.12</td>
<td>(37)</td>
</tr>
<tr>
<td>86 urban adults</td>
<td>Italy</td>
<td>Non-smokers</td>
<td>0.096</td>
<td>(38)</td>
</tr>
</tbody>
</table>

Table 4: Urinary median levels of benzene (µg/l) in prior studies including the general population.
The result of our study showed the amount of urinary benzene at concentrations comparable to those of blood benzene reported by other researchers. Also, the urinary and blood biomarker of benzene have a similar behavior regarding correlation with smoking and airborne pollutants. Such resemblance in subjects occupationally and environmentally exposed to benzene was seen.

According to Spearman’s correlation coefficients, Table 3, age, BMI and creatinine showed no significant correlation with urinary biomarkers (all p>0.05); however other study performed by Fustinoni showed a better correlation with BMI in the general population.

Conclusions

The determination of urinary benzene in the general population in Iran have not carried out so far. The our results showed that urinary benzene is higher than other prior studies in the general population. These high values may have undesirable health effects on the people resident in Tehran. Also, epidemiological studies show evidence for a causal association between leukemia and exposure to benzene. Thus, our results are a challenge both for the general population and the executives of health politics. Since urinary benzene behave in a similar way to blood benzene, urinary benzene due to the quick accessibility of urine and a non-invasive option is useful for environmental exposure and for epidemiology studies. Also, the role of passive smoking and high traffic as the most important contributor to benzene exposure is very important.

References

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