Biomonitoring of BTEX in primary school children exposed to hookah smoke

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Abstract
Hookah smoking is one of the major indoor sources of benzene, toluene, ethylbenzene, and xylenes (BTEX). This study aimed to investigate the potential exposure to BTEX among primary school children, particularly those exposed to hookah smoke. This cross-sectional study was conducted in Khesht, one of the southwestern cities in Iran, in mid-June 2020. Totally, 50 primary school children exposed to hookah smoke were chosen as the case group and 50 primary school children were selected as the control group. Urinary un-metabolized BTEX was measured by a headspace gas chromatography mass spectrometry (GC–MS). Additionally, a detailed questionnaire was used to gather data and information from the students' parents. The mean levels of urinary benzene, toluene, ethylbenzene, m,p-xylene, and o-xylene were 1.44, 5.87, 2.49, 6.93, and 7.17 μg/L, respectively in the exposed children. Urinary BTEX was 3.93-folds higher in the case group than in the controls (p<0.05). Household cleaning products, the floor on which the house was located, children's sleeping place, and playing outdoors were found to be important factors in predicting urinary BTEX levels. Overall, it was found necessary to avoid indoor smoking to prevent the emission of BTEX compounds via exhaled mainstream smoke and to protect vulnerable non-smokers, especially children, from exposure to second-hand and third-hand smoke.

Keywords Biomonitoring · Hookah smoking · Exposure assessment · Urinary BTEX · Primary school children

Introduction
Since the majority of people spend approximately 80–90% of their time indoors, the concentrations of some pollutants are often higher indoors compared to outdoor environments (Long et al., 2001, Demirel et al. 2014, Amoatey et al. 2018). Thus, household air pollution has been considered one of the most important public health concerns worldwide (Bernstein et al. 2008, Bluysen 2009, Miri et al. 2018, Moradi et al., 2019a, b). One of the challenging concerns in indoor air is the use of tobacco products via hookah (Lin et al. 2007, Tabatabaei et al. 2021). In the recent years, the use of tobacco products via waterpipe, known as hookah, has globally risen among adults and teenagers (Maziak et al. 2016, Leavens et al. 2018). Enhanced tobacco flavoring, the wrong idea that hookah is healthier than cigarette, acceptance by communities, and lower costs are among the effective factors in the prevalence of hookah smoking (Dadipoor et al. 2019). The current use of these devices rises on a large scale within the region, and now more and more is used in western countries (Al-Delaimy and Al-Ani 2021). Therefore, hookah smoking has become a global public health problem (Maziak et al. 2015). In the USA, hookah use increased significantly between 2011 and 2015 among high school students (4.1–7.2%) (Arrazola et al. 2013). Several studies have proven that hookah smoking consists of dangerous chemical substances and a single 45-min hookah session can expose the smoker as much as 48.6 times the amount of smoke from a cigarette (Al Rashidi et al. 2008, Cobb et al. 2010, Eissenberg 2013, Al-Delaimy and Al-Ani 2021). However, hookah has adverse effects on the people exposed to second-hand smoke...
Up to now, various biomonitoring studies have been carried out to evaluate BTEX exposure in different groups of the general population such as workers in cafes serving hookah (Kaplan et al. 2019), beauty salon workers (Moradi et al., 2019a, b), traffic policemen (Manini et al. 2010), and shoes plant workers (Janasik et al. 2010). In addition, two studies were conducted in Zagreb in order to measure BTEX compounds in cigarette smokers’ urine samples (Skender et al. 2004, Brajenovic et al. 2015). However, limited studies have evaluated BTEX exposure among children (Minoia et al. 1996), especially those exposed to hookah smoke. Therefore, the present study aims to determine the potential of the urinary un-metabolized BTEX to be used as a biomarker of environmental exposure to BTEX in the children daily exposed to hookah smoke in southwestern Iran. The main reason for choosing this area was the high rate of hookah smoking (Rafiee et al. 2020).

Materials and methods

Study area

This cross-sectional study was carried out in Khesht, the capital of Khesht District in Kazerun, Fars province, southwest of Iran (29° 33′ 49″ N 51° 20′ 13″ E) (Fig. 1). The city has an area of 30 km² and is 600 m above the sea level. Its population has been estimated to be approximately 11,000 people in 2257 families. In the study area, there are low industrial activities and vehicle traffic, which are the main ambient sources of BTEX compounds emissions (Adamovic et al. 2013), because the houses of this city are located at a distance of 5 km from the main roads, gas stations, and industries.

Data collection and sampling

This study was conducted on the participants aged 7–13 years. Urine samples were collected in mid-June 2020. Overall, 50 children who were exposed to hookah smoke (one or both parents smoked hookah at home on a daily basis) and 50 children as the control group were included in this study. The objectives of the study were briefly explained to all the participants and informed consent forms were signed by the children’s parents. The research was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. It is worth mentioning that the participants did not have any specific diseases that could interfere with the results. Urine samples were taken from children once from 8 to 10 am and were collected in 100 ml polypropylene vials. After coding, the samples were stored in a freezer at −20°C until analysis.
Fig. 1 Map of the location of Khesht
Assessment of the potential confounders

The data were collected from the children and their parents through face-to-face interviews and a comprehensive questionnaire including age, gender (two categories: boys, girls), height, weight, body mass index (BMI), frequency of daily cooking (three categories: less than three times, three times, more than three times), using a ventilator when cooking (two categories: yes, no), floor (two categories: first floor, second floor, and above), main cover of kitchen cabinets (two categories: metal, wooden, and MDF), children’s sleeping place (two categories: bedroom, living room), daily playing outdoor (two categories: yes, no), frequency of weekly use of cleaning agents (three categories: daily, three to four times, less than three times), cooling system (two categories: water cooler, gas cooler), age of the house (three categories: less than 5 years, 5–10 years, more than 10 years), home spray renovation within the past 6 months (two categories: yes, no), home renovation within the past 6 months (two categories: yes, no), and painting the walls of the house during the past 6 months (two categories: yes, no). The following questionnaire items were specific to the case group: type of tobacco (two categories: traditional, fruit), hookah smoking parent (three categories: mother only, father only, both), and number of times of hookah smoking during the day (four categories: once, twice, three times, more than three times).

Sample preparation and chemical analysis

At the first step, 5 mL of the sample was taken from the middle of the urine sample container and was added to a 10 mL vial containing 2 g dried NaCl (Merck Company) (Rafiee et al. 2018). The vial lid was sealed instantly with a septum-sealed polytetrafluoroethylene (PTFE) were provided by Thomas Scientific (Kemtech America) screw cap with spiked with BTEX internal standards (0.25 μg/ml of benzene-d6, toluene-d8, ethylbenzene-d10, o-xylene-d10, and p-xylene-d10 (Merck Company (Darmstadt, Germany)). The un-metabolized BTEX were extracted from the children’s urine samples according to the headspace solid-phase micro extraction (HS-SPME) technique (Rafiee et al. 2019). Prior to extraction, the vials containing the samples were incubated in an agitator at 37°C for 20 min with the mixing speed of 750 rpm. A 75 μm Carboxen-PDMS SPME fiber (Supelco (Belleville, PA, USA)) was used for extraction, which was exposed to the headspace gas for 50 min (Mochalski and Unterkofler 2016, Tsangari et al. 2017). The SPME fiber was then introduced into a gas chromatography (GC) injector where the thermal desorption was performed at 250°C for 5 min. A GC (Agilent 7890N, Agilent Co. USA) combined with a mass spectrometer (MS, Agilent 5975C, Agilent Co. USA) was applied to measure the un-metabolized BTEX compounds in the urine samples. Chromatographic separation was achieved using a 30 m × 0.25 μm × 0.5 μm fused silica (DB-5MS) capillary column (Agilent Co, USA) in the selected ion monitoring (SIM) mode, with helium (purity level of 99.99%) at a flow rate of 1 ml/min (Rafiee et al. 2019). The initial oven temperature was set at 60°C for 1 min. The temperature was then raised to 100°C (at rate of 10°C/min) and was maintained for 1 min. The temperature was subsequently raised to 285°C at 4°C/min and was held for 15 min. Finally, the temperature was set at 200°C for the ion source of the MS (Rafiee et al. 2018). The injector and detector temperatures were set at 290 and 250°C, respectively. SIM analysis was used for each of the species. In this case, instead of measuring the entire m/z, which covers a wide range, only the number of m/z that has the highest frequency and is identified by the user, so it is more sensitive and suitable for quantitative measurements. In order to increase the sensitivity of the device and more accurate analysis of BTEX compounds according to the SIM program, the main ion was selected with the highest frequency according to the Table S1 (supplementary information). The software of MSD Chem Station device is version E.02.01.1177. The creatinine concentration in the urine samples was measured according to the Jaffe’s spectrophotometric method (Tsangari et al. 2017; Shamsedini et al. 2022a, b).

Quality control

In order to verify the proposed method for the analysis of the urine samples, analytical characteristics of the method such as linear dynamic range (LDR), limit of detection (LOD), and limit of quantification (LOQ) were investigated (Table S2). LOD and LOQ, as the quality control methods, were determined using the standard deviation of six consecutive blank signals and 3 × LOD, respectively (Rafiee et al. 2019). Relative standard deviations (RSDs) had been calculated on the basis of consecutive analyses of five independent urine samples received from the same participant. The RSDs vary from 2.7% for toluene to 6.7% for benzene. LDR was within 0.2–500 ng/ml for the method with levels of determination (R²) ranging from 0.990 to 0.996. The acceptable level of urinary creatinine declared by the WHO is between 0.3 and 3.0 g/L (Zhou et al. 2011).

Statistical analysis techniques

R (v. 3.3.1) and SPSS 23 were employed for statistical analysis. The normal distribution of the variables was determined using the Kolmogorov-Smirnov and Shapiro-Wilks tests. In addition, Mann-Whitney U test was used to compare the two groups regarding the urinary un-metabolized BTEX. Moreover, Spearman’s correlation coefficient was used to investigate the correlation between two variables. Furthermore,
multivariate linear regression models were carried out using R (v. 3.3.1). Regression models were designed to estimate the association between the urinary BTEX concentrations and the independent variables. \( P \)-values less than 0.05 were considered statistically significant.

**Results**

**General characteristics of the studied population**

The characteristics of the participants and their houses have been presented in Tables 1 and S3 based on the questionnaire information and data, respectively. The two groups were homogeneous with respect to the mean age and gender distribution. The results also revealed no significant differences between the exposed and non-exposed children in terms of weight, height, and BMI. Since the study was carried out during the COVID-19 pandemic, the children did not physically attend school and the classes were held virtually. The families also spent most of their time at home. Yet, 46% of the parents in the exposed group and 38% of those in the non-exposed group reported that their children cycled or played outside (in the yard or on alleys and streets) for several hours. As mentioned earlier, a part of the questionnaire was intended for the case group, which included questions about the frequency of smoking hookah, number of smokers, and type of tobacco (Table S4). According to the results, 66% of the parents used hookah three times a day, while only 12% used hookah once.

**BTEX urinary concentrations between the exposed and non-exposed children**

The results of the statistical analysis of the urinary BTEX levels between the monitored groups have been presented in Tables 2 and 3. Accordingly, the mean concentrations of benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and \( \sum \) BTEX were respectively 4.11, 3.27, 3.15, 4.47, 4.53, and 3.93 times higher in the urine samples collected from the exposed group compared to the non-exposed group. The results of Mann-Whitney \( U \) test indicated that the differences in the urinary un-metabolized BTEX concentrations were statistically significant (\( p < 0.05 \)). The differences between the exposed and non-exposed groups regarding the urinary concentrations of the BTEX compounds have been presented in Fig. 2.

| Table 1 | Selected characteristics of the participants |
| Exposure type | Exposed group | Control group |
| Number of participants | 50 | 50 |
| Gender (%) | Female (52%) | Male (48%) | Female (52%) | Male (48%) |
| Age (mean±SD, years) | 10.08 ± 2.05 | 9.90 ± 2.09 |
| Height (mean±SD, cm) | 136.9 ± 11.12 | 136.54 ± 11.57 |
| Weight (mean±SD, kg) | 33.54 ± 8.52 | 32.68 ± 8.74 |
| BMI (mean±SD, kg/m²) | 17.60 ± 2.53 | 17.20 ± 2.38 |
| Daily playing outside the house (%) | Yes (46) | Yes (38) | No (54) | No (62) |
| Sleeping place (%) | Living room (44) | Bedroom (56) | Bedroom (78) |

| Table 2 | Statistical analysis and means of the urinary concentrations of BTEX between the case and control groups (μg/L) |
| Exposure type | Case group | Control group | Comparison of the case and control groups |
| Statistical analysis | Mean ± SD (min–max) | Geometric mean ± geometric SD | Mean ± SD (min–max) | Geometric mean ± geometric SD | \( P \)-value |
| Benzene | 1.44 ± 1.09 (0.03–4.92) | 0.78 ± 4.31 | 0.35 ± 0.66 (0.01–2.59) | 0.1 ± 4.31 | <0.001 |
| Toluene | 5.87 ± 4.64 (0.13–24.96) | 3.22 ± 4.01 | 1.79 ± 2.58 (0.03–10.9) | 0.7 ± 3.87 | < 0.001 |
| Ethyl benzene | 2.49 ± 1.85 (0.8–28.24) | 1.52 ± 3.47 | 0.79 ± 1.38 (0.01–6.73) | 0.25 ± 4.45 | < 0.001 |
| m,p-xylene | 6.93 ± 5.98 (0.02–25.24) | 2.88 ± 6.39 | 1.55 ± 3.34 (0.01–13.33) | 0.32 ± 5.48 | < 0.001 |
| O-xylene | 7.17 ± 6.17 (0.09–25.89) | 3.30 ± 5.18 | 1.58 ± 3.47 (0.01–13.81) | 0.37 ± 4.68 | < 0.001 |
| Total BTEX | 23.92 ± 18.78 (0.51–88.01) | 12.79 ± 4.37 | 6.08 ± 10.87 (0.13–44.18) | 1.96 ± 4.22 | < 0.001 |
The correlation between the urinary concentrations of BTEX and the possible influential variables in the exposed group

Spearman rank correlation coefficient was used to determine the relationship between the urinary levels of BTEX in the exposed children and the number of hookah uses per day, type of tobacco, and number of hookah smoking parents. The concentrations of the BTEX compounds in the urine samples of the exposed children were significantly and positively associated with the number of hookah uses per day (CC = 0.504, \( p = 0.000 \)). Among the BTEX compounds, the strongest and weakest correlations were related to o-xylene (CC = 0.542, \( p = 0.000 \)) and ethyl benzene (CC = 0.328, \( p = 0.04 \)), respectively. However, the concentrations of the urinary BTEX in the case group were not linearly correlated to other factors (\( p > 0.05 \)). Furthermore, the mean concentration of \( \sum \) BTEX was approximately 3.75 times higher in the urine samples collected from the children whose parents smoked three times a day (30.47 ± 17.87 μg/L) compared to those who parents smoked only once a day (8.14 ± 11.31 μg/L) (\( p < 0.05 \)).

Linear regression

The results of multiple regression analysis (\( \beta \) coefficient (p-value)) for the association between the urinary concentrations of the target compounds and potentially confounding variables have been presented in Table 4. The floor on which the families lived, the frequency of using household cleaning products, children’s sleeping place, and daily playing outside the house were identified as the effective predictors of urinary un-metabolized BTEX levels. However, the results revealed no significant relationship between the concentrations of the urinary BTEX compounds and other possible influential variables. According to the information provided in the questionnaires, no painting, reconstruction, or spraying was done in any of the houses within the past 6 months. Additionally, all children’s residence places were more than 5000 m distant from the main roads and gas stations.
A statistical summary of the mean levels of the BTEX compounds in the urine samples of the exposed and non-exposed groups based on the effective predictor variables in the multiple regression models has been illustrated in Table 5. Accordingly, significantly lower levels of BTEX were detected in the urine samples of the children who played outdoors daily in comparison to those who did not (Mann-Whitney U test for both groups; \( p < 0.05 \)) (Fig. 3). Moreover, un-metabolized BTEX concentrations were associated with the sleeping place through the night. Based on the results, the urinary concentrations of BTEX were higher in the children who slept in the living room than in those who slept in the bedroom. Nonetheless, this difference was statistically significant only in the case group according to results of Mann-Whitney U test (\( p < 0.05 \)) (Fig. 3). Additionally, the levels of BTEX were lower in the urine samples of the children who lived on the second floor and above compared to those who lived on the ground floor (Fig. 3). Furthermore, the frequency of using household cleaning products during the week was positively related to the increase of the BTEX compounds in the children’s urine samples (Table 5 and Fig. S1).

**Discussion**

The majority of the previous studies have been focused on cigarette smokers (Skender et al. 2004, Brajenović et al. 2015) and no attention has been paid to hookah smokers and individuals exposed to BTEX from hookah smoke, especially children. Additionally, some previous studies measured the BTEX compounds in hookah smoke in café ambient air (Hazrati et al. 2015, Masjedi et al. 2019), but did not address the urinary biomonitoring of these compounds from hookah. Therefore, the results of the current study were compared to those of the studies focused on cigarette smoking, as another type of tobacco consumption. The results were also compared to different groups in other published scientific studies. As shown in Table 6, the profiles of the target compounds were variable in different countries. The results of other studies indicated that people in different countries with various conditions were exposed to different concentrations of the BTEX compounds in the air as well as in other sources.

Hookah smoking is regarded as the main source of BTEX exposure (Hazrati et al. 2015). In the present study, the effect of hookah smoking at home on exposure to benzene, toluene, ethylbenzene, m+p-xylene, and o-xylene was investigated by comparing the exposed and non-exposed groups regarding the differences in the levels of urinary BTEX. Among the monitored target compounds in the urine samples of the exposed children, o-xylene and benzene had the highest and lowest concentrations, respectively in both studies in Zagreb (Fig. S2). This discrepancy might be associated with the differences in the emissions of BTEX compounds from cigarette and hookah. The difference in puffs of hookah (200 puffs) and cigarettes (20 puffs) per session and the charcoal used to

| Factors                      | Benzene (μg/L) | Toluene (μg/L) | Ethylbenzene (μg/L) | m,p-xylene (μg/L) | O-xylene (μg/L) |
|------------------------------|---------------|----------------|---------------------|-------------------|-----------------|
| Floor                        | −0.85 (0.02)  | −1.52 (0.04)  | −0.45 (0.03)        | −3.68 (0.002)     | −3.88 (0.001)   |
| Using a ventilator when cooking | −0.62 (0.11) | −1.87 (0.23)  | −0.82 (0.22)        | 3.26 (0.11)       | 3.33 (0.12)     |
| Number of cooking times during the day | 0.47 (0.27)  | 1.69 (0.35)   | 1.23 (0.11)         | 2.08 (0.38)       | 1.95 (0.43)     |
| The main cover of kitchen cabinets | 0.24 (0.93)  | 1.69 (0.38)   | 0.38 (0.31)         | 1.31 (0.26)       | 1.28 (0.29)     |
| Children’s sleeping place    | 0.68 (<0.001) | 2.99 (<0.001) | 1.25 (<0.001)       | 3.25 (0.000)      | 3.33 (<0.001)   |
| Daily playing outside the house | −0.40 (0.01) | −1.34 (0.04)  | −0.81 (0.04)        | −1.82 (0.03)      | −1.86 (0.04)    |
| Age of the house             | 0.15 (0.48)   | 0.37 (0.96)   | 0.31 (0.42)         | 1.26 (0.29)       | 1.32 (0.29)     |
| Cooling system               | 0.02 (0.92)   | 0.17 (0.83)   | 0.36 (0.31)         | 0.20 (0.85)       | 0.23 (0.83)     |
| Cleaning products*           | 0.72 (<0.001) | 2.58 (<0.001) | 1.16 (<0.001)       | 3.58 (<0.001)     | 3.69 (<0.001)   |
| BMI (kg/m²)                  | 0.004 (0.91)  | 0.02 (0.86)   | 0.1 (0.91)          | 0.06 (0.75)       | 0.07 (0.71)     |
| Creatinine (g/L)             | 0.02 (0.77)   | 0.08 (0.65)   | 0.07 (0.39)         | 0.12 (0.81)       | 0.13 (0.78)     |
| Gender                       | 0.01 (0.93)   | 0.46 (0.43)   | 0.24 (0.33)         | 0.22 (0.77)       | 0.31 (0.69)     |
| Age                          | −0.02 (0.58)  | −0.06 (0.73)  | −0.17 (0.94)        | −0.14 (0.54)      | −0.16 (0.51)    |
| \( R^2 \)                    | 0.659         | 0.627         | 0.643               | 0.620             | 0.619           |

*Frequency of using household cleaning products during the week.
| Story                  | Case group | Control group | P-value | Case group | Control group | P-value |
|------------------------|------------|---------------|---------|------------|---------------|---------|
|                        | 1st        | 2 ≤           |         | 1st        | 2 ≤           |         |
|                        | N          | Mean ± SD     |         | N          | Mean ± SD     |         |
|                        |            | (1–2)         | (1–3)   |            | (1–2)         | (1–3)   |
|                        |            | (2–3)         |         |            | (2–3)         |         |
| Benzene                | 1.69 ± 1.12| 0.64 ± 0.44   | 0.001   | 0.88 ± 0.32| 0.29 ± 0.66   | 0.005   |
| Toluene                | 6.73 ± 4.83| 3.16 ± 2.62   | 0.004   | 4.46 ± 1.5 | 1.50 ± 2.51   | 0.005   |
| Ethylbenzene           | 2.61 ± 1.85| 2.11 ± 1.88   | 0.517   | 3.61 ± 2.07| 0.47 ± 0.85   | 0.001   |
| m+p-xylene             | 8.47 ± 6.03| 2.08 ± 1.85   | 0.002   | 2.46 ± 1.72| 1.45 ± 3.47   | 0.006   |
| O-xylene               | 8.75 ± 6.22| 2.15 ± 1.86   | 0.002   | 2.28 ± 1.93| 1.5 ± 3.61    | 0.005   |
| BTEX                   | 28.26 ± 19.25| 10.16 ± 7.31 | 0.001   | 13.71 ± 4.6 | 5.24 ± 11.06 | 0.005   |
| Sleeping place         |            |               |         |            |               |         |
|                        | Living room| Bedroom       |         | Living room| Bedroom       |         |
|                        | N          | Mean ± SD     |         | N          | Mean ± SD     |         |
|                        |            | (1–2)         | (1–3)   |            | (1–2)         | (1–3)   |
|                        |            | (2–3)         |         |            | (2–3)         |         |
| Benzene                | 2.19 ± 0.98| 0.85 ± 0.77   | 0.000   | 0.86 ± 0.9 | 0.19 ± 0.47   | 0.33    |
| Toluene                | 9.19 ± 4.34| 3.27 ± 2.92   | 0.000   | 3.64 ± 3.38| 1.21 ± 1.99   | 0.21    |
| Ethylbenzene           | 3.77 ± 1.21| 1.48 ± 1.63   | 0.000   | 2.03 ± 2.16| 0.39 ± 0.69   | 0.3     |
| m+p-xylene             | 10.6 ± 6.03| 4.05 ± 4.14   | 0.000   | 3.64 ± 4.77| 0.89 ± 2.48   | 0.23    |
| O-xylene               | 10.9 ± 6.31| 4.24 ± 4.23   | 0.000   | 3.77 ± 5.01| 0.89 ± 2.55   | 0.19    |
| BTEX                   | 36.66 ± 17.53| 13.91 ± 12.85| 0.000   | 13.97 ± 14.78| 3.59 ± 8.06 | 0.27    |
| Playing outdoors       |            |               |         |            |               |         |
|                        | Yes        | No            |         | Yes        | No            |         |
|                        | N          | Mean ± SD     |         | N          | Mean ± SD     |         |
|                        |            | (1–2)         | (1–3)   |            | (1–2)         | (1–3)   |
|                        |            | (2–3)         |         |            | (2–3)         |         |
| Benzene                | 0.67 ± 0.63| 2.09 ± 0.98   | 0.000   | 0.25 ± 0.63| 0.57 ± 0.68   | 0.000   |
| Toluene                | 2.93 ± 2.62| 8.38 ± 4.54   | 0.000   | 1.17 ± 2.51| 3.12 ± 2.26   | 0.000   |
| Ethylbenzene           | 1.48 ± 1.43| 3.35 ± 1.74   | 0.000   | 0.44 ± 0.87| 1.52 ± 1.92   | 0.000   |
| m+p-xylene             | 2.92 ± 3.32| 10.36 ± 5.63  | 0.000   | 1.17 ± 3.32| 2.36 ± 3.32   | 0.000   |
| O-xylene               | 3.05 ± 3.43| 10.67 ± 5.83  | 0.000   | 1.23 ± 3.43| 2.34 ± 3.55   | 0.000   |
| BTEX                   | 11.06 ± 10.5 | 34.87 ± 17.35| 0.000   | 4.27 ± 10.72| 9.93 ± 10.47 | 0.000   |
| Cleaning products*     | 1: daily   | 2–3–4         |         |            | 3:< 3         |         |
|                        | N          | Mean ± SD     |         | N          | Mean ± SD     |         |
|                        |            | (1–2)         | (1–3)   |            | (1–2)         | (1–3)   |
|                        |            | (2–3)         |         |            | (2–3)         |         |
| Benzene                | 1.9±1.1    | 0.8±0.4       | 0.006   | 0.000       | 0.01          | 1.5±0.6 |
| Toluene                | 7.6±4.6    | 4.1±1.8       | 0.01    | 0.001       | 0.006         | 6.6±1.2 |
| Ethylbenzene           | 3.1±1.7    | 2.2±1.5       | 0.106   | 0.002       | 0.004         | 3.9±2.1 |
| m+p-xylene             | 9.4±5.6    | 3.3±2.4       | 0.006   | 0.001       | 0.1           | 6.1±5.1 |
| O-xylene               | 9.7±5.8    | 3.3±2.6       | 0.006   | 0.001       | 0.1           | 6.3±5.4 |
| BTEX                   | 31.7±17.7  | 13.7±6.9      | 0.009   | 0.000       | 0.03          | 24.6±11 |

*Frequency of using household cleaning products during the week.
heat tobacco in hookah can be the reasons for the difference in the amounts of BTEX emissions (Kim et al. 2016). A recent study conducted on the indoor air of waterpipe serving cafés demonstrated that xylenes had the highest concentration among the BTEX compounds emitted from the hookah smoke (Hazarati et al. 2015). This can also explain the higher levels of urinary xylene in the case group in the present study.

In the current research, the mean concentrations of urinary BTEX were higher in the exposed group (1.4, 5.8, 2.9, 6.9, and 7.1 μg/L) compared to the population of other studies. For instance, the mean concentrations of BTEX were 0.2, 0.4, 0.1, 0.3, and 0.3 μg/L in unexposed subjects to smoke and traffic emissions of a study conducted on children (Rafiee et al. 2022). In addition, the median urinary levels of un-metabolized BTEX were 0.11, 0.12, 0.01, 0.03, and 0.03 μg/L in another study conducted on a general population which most of the participants were non-smokers (Tsangari et al. 2017). The difference between the present study and other ones regarding the urinary levels of BTEX might be related to the potential source of release of the BTEX compounds from the hookah smoke. Overall, the previous studies demonstrated that the urinary levels of BTEX were higher in smokers compared to non-smokers and suggested tobacco smoke as one of the major sources of BTEX emissions (Rafiee et al. 2018, Rafiee et al. 2019). In the current work, 66% of the parents in the case group used hookah three times a day, each time lasting for almost an hour. BTEX compounds accumulate easily inside buildings and remain indoors for several hours (Alsbou and Omari 2020). Therefore, children with hookah smoking parents are constantly exposed to BTEX compounds released from the hookah smoke.

Although Khesht is far from the sources of BTEX compounds including gas emissions from traffic and industry, the mean concentrations of urinary BTEX, except for benzene, were higher in the control group (0.35, 1.79, 0.79, 1.55, and 1.58 μg/L) compared to the values reported in a study on a non-smoker of general population (0.09, 0.17, 0.06, 0.14, and 0.06 μg/L) (Brajenović et al. 2015). Moreover, the median concentrations of BTEX in the urine samples collected from the non-exposed children were higher compared to the values found in a prior study on Iranian children (Table 6). The differences between the results of the present study and those of similar investigations could be attributed to the inhalation of more indoor air in this study due to spending most of the day indoors during the COVID-19 pandemic. The negative health effects of BTEX may be even greater at low concentrations over a long period due to continuous exposure to these compounds. In fact, the prevalence of COVID-19 has changed the daily lives of children and adults. For instance, the frequency of cooking, cleaning, and smoking, as the sources of BTEX emissions, has increased at homes (Bolden et al. 2015, Afshari 2020, Hosseini et al. 2020). Researchers have also reported that during this time, excessive use of various types of household cleaning products for eliminating the virus and as well as their evaporation in the absence of suitable ventilation has dramatically increased chemical air pollution indoors (Hosseini et al. 2020). In the present study also, the parents referred to the increased use of cleaning products during the pandemic. According to Table S3, 62% of the participants in the case group and 58% of those in the control group used household cleaning products daily in order to remove contaminants from the kitchen, bathroom, and other surfaces. The frequent utilization of household cleaning products had, in turn, an effect on the levels of urinary un-metabolized BTEX (Fig S1). Based on the results, the BTEX concentrations were significantly higher in the children whose parents used household cleaning products daily compared to those whose parents used cleaning products less than three times a week. Numerous studies have also demonstrated that BTEX are emitted into indoor environments from an extensive variety cleaning products including liquid detergents, disinfectant bathroom cleaners, household cleaners, and furniture polishes (Wallace et al. 1987, Sack et al. 1992, Akland Cerón Bretón et al. 2020).

The current study findings revealed a significant decrease in the urinary concentrations of BTEX in the majority of children who played outdoors daily. In this study, 46% of the children in the case group and 38% of those in the control group played outdoors on a daily basis (Table 1). Regardless of exposure to hookah smoke, the children who did not play outdoors had higher benzene, toluene, ethylbenzene, m,p-xylene, and o-xylene levels (Mann-Whitney U test (p < 0.05)). The lower levels of BTEX in the children playing outdoors could be associated with being away from indoor air, because BTEX compounds pollution is often higher in indoor air compared to outdoors (Werder et al. 2019).

The floor on which the participants lived was found to be a significant predictor of the BTEX urinary concentrations in the current study, exerting the greatest effect on xylenes and the least effect on ethylbenzene (Table 4). Based on the results, the urinary levels of BTEX were higher in the children who lived on the ground floor compared to those who lived on the second and higher floors (Fig. 3). The higher BTEX levels in the houses located on the ground floor could be related to their proximity to garages and vehicles, as noticeable sources of air pollution (Truc and Kim Oanh 2007, Hazrati et al. 2016). On the other hand, lower levels of BTEX in the houses placed on higher floors might be attributed to higher air exchange rate (Hazrati et al. 2016) since air velocity increases with altitude. In other words, air exchange rates may be higher through windows in higher floors. Overall, natural ventilation plays a pivotal role in
diluting the concentrations of indoor air pollutants (Wong and Huang 2004, Hazrati et al. 2016).

In the present study, the urinary concentrations of BTEX were remarkably higher among the children who slept in the living room in comparison to those who slept in the bedroom (Mann-Whitney U test for BTEX in the case group ($p < 0.05$)). One possible reason is that the living room is normally more polluted compared to the bedrooms. Free air diffusion from cooking in the kitchen to the living room may be a reason for the higher concentrations of and exposure to BTEX. A previous study disclosed a strong relationship between cooking habits and the risk of lung cancer, which was attributed to exposure to mutations and carcinogens emitted from oil including benzene (Chiang et al. 1997, Chiang et al. 1998, Lai et al. 2013). In another study, the median BTEX concentrations were equal in the living room and kitchen, both of which were significantly higher compared to the bedroom (Schneider et al. 1999).

In the current study, the urinary concentration of BTEX was not significantly associated with the use of ventilation systems during cooking and the number of cooking times. This result could be due to the participants’ similar conditions regarding the number of cooking times and the use of stove hoods. Totally, 80% of the participants stated that they usually cooked three times a day. Additionally, only 12% of the participants in the exposed group and 10% of those in the non-exposed group used stove hoods for ventilation while cooking (Table S3).

Although the previous studies indicated that fruit-flavored tobacco produced remarkably greater BTEX emissions compared to traditional tobacco (Hazrati et al. 2015), the present study results revealed no significant relationship between the levels of urinary BTEX and type of tobacco in the case group. This might result from the high prevalence of traditional tobacco consumption in the study area (Table S4).

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Overall, the findings of the present study indicated that the children with hookah smokers at their homes were exposed to airborne BTEX. A previous study also showed a significant correlation between exposure to airborne VOCs and urinary non-metabolized VOCs (Fustinoni et al. 2005, Rafiee et al. 2018). Thus, the range of un-metabolized BTEX urinary concentrations in the present study suggested that the
children in the case group were exposed to higher concentrations of BTEX released from hookah smoke. Furthermore, previous studies reported that inhaled BTEX were converted to their metabolites in the body during various reactions and only a small amount of these compounds were excreted non-metabolically (Rappaport et al. 2010, Weisel 2010). Un-metabolized BTEX concentrations measured in children’s urine samples also indicated higher concentrations of the metabolites of the BTEX compounds in children’s blood and urine samples. Unfortunately, benzene metabolites can cause leukemia and bone marrow depression (Snyder and Hedli 1996, McHale et al. 2012). Thus, it is necessary to raise awareness about the risks of hookah smoke for human body, especially in the individuals exposed to second-hand and third-hand smoke, so as to minimize the risk of BTEX exposure.

One of the limitations of the current study was the short half-life of biomarker of exposure. Therefore, the urine samples could only cover the recent exposures and principally the baseline exposures. Moreover, to the best of our knowledge, no study has measured un-metabolized BTEX compounds in the urine samples of children and other individuals exposed to hookah smoke. Hence, no similar study was available to compare the results.

Conclusion

The findings showed that the children whose parents smoked hookah at home on a daily basis were exposed to benzene, toluene, ethylbenzene, m,p-xylene, and o-xylene. A significant difference was also observed between the exposed and non-exposed children regarding the urinary concentrations of un-metabolized BTEX. Hence, hookah smoke was considered a potential indoor source of BTEX exposure. The children who slept in the living room and those who rarely played outdoors were more likely to be exposed to the BTEX compounds. This implied that indoor air could be more polluted than outside, and the living room was probably more polluted than the bedroom. Besides, the relatively higher \( \sum \text{BTEX} \) levels in the urine samples of the non-exposed children in the current study in comparison to the general populations in various locations revealed higher BTEX concentrations in indoor air.

To the best of our knowledge, the present study was the first human urinary monitoring research investigating the BTEX concentrations among the primary school children, particularly those who were exposed to hookah smoke at home on a daily basis. Regarding the effects of hookah smoke on exposed individuals’ health, future studies are recommended to measure the BTEX levels both in the air and urine in order to determine the correlation between the urinary levels of the target compounds and the sources of pollution. Finally, despite the effectiveness of natural ventilation in the concentrations of the target compounds, ventilation and filtration systems could decrease but not eliminate hookah smoke. Therefore, forbidding smoking indoors is the only way to eliminate hookah smoke in indoor environments. In order to maintain children’s health, the use of hookah at homes should be avoided by notifying parents about its adverse effects.

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Data availability The datasets obtained in this study are available from the corresponding author on reasonable request.

Declarations

Ethical approval The research was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Competing interests The authors declare no competing interests.

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