

Exploring Urinary Biomarkers to Assess Oxidative DNA damage Resulting from BTEX Exposure in street children

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Abstract

Children are highly susceptible to environmental contaminants as their physiology and some metabolic pathways differ from adults. The present cross-sectional study aimed to assess whether exposure to benzene, toluene, ethylbenzene, o,p-xylene, and m-xylene (BTEX) affects oxidative DNA damage in street children using a biomonitoring approach. Thirty-five boys (7 to 13 years of age), exposed by working at a busy intersection, and 25 unexposed boys of similar age and living in the neighborhood near the busy intersection were recruited. Urinary un-metabolized BTEX levels were quantified by a headspace gas chromatography-mass spectrometry (GC-MS). Urinary malonaldehyde (MDA) was measured with spectrophotometry. Sociodemographic and lifestyle conditions information was collected by interviews using administered questionnaires. Exposed subjects provided urine before (BE) and after work exposure (AE), while unexposed boys gave a single morning sample. Urinary BTEX concentrations in BE samples were similar to unexposed. Concentrations in AE samples were 2.36-fold higher than observed in BE samples ($p < 0.05$) and higher than those in the unexposed group ($p < 0.05$). In addition, urinary MDA levels in AE samples were 3.2 and 3.07-times higher than in BE samples and in the unexposed group ($p < 0.05$). Environmental tobacco smoke (ETS) increased urinary BTEX and MDA levels in both groups. Our findings confirm that street children working at busy intersections are significantly exposed to BTEX, which is associated with oxidative stress. Implementing protective measures is crucial to reduce exposure and to improve health outcomes in this group.

Keywords: Benzene, Biomonitoring, BTEX, Child labor, Exposure assessment.

1. Introduction

Benzene, toluene, ethylbenzene, o,p-xylene, and m-xylene (BTEX), are among the highest volume produced volatile organic compounds (VOCs) worldwide (Chauhan et al., 2014). Because of their

adverse health effects, BTEX compounds are classified as hazardous air pollutants (HAPs) (Durmusoglu et al., 2010). Health impacts attributed to BTEX exposure mainly depend on concentrations and duration of exposure and include both carcinogenic and non-carcinogenic effects. Previous studies showed that long-term exposure to BTEX could lead to leukaemia, biliary tract cancer, and lung cancer (Rafiee et al., 2018; Song et al., 2017). Direct toxic effects on the kidney and congenital disabilities are among the most important non-carcinogenic effects of BTEX exposure (Taneepanichsku et al., 2018; Zhou et al., 2011).

BTEX mainly enters the human body through inhalation and dermal routes (Hopf et al., 2012). Once inside the body, BTEX are mainly metabolized to their specific metabolites, such as hippuric acid (HA) or *s*-phenylmercapturic (S-PMA). However, a small fraction of inhaled BTEX remains un-metabolized, which along with metabolites, is excreted through the urine (Fustinoni et al., 2011; Wang et al., 2003). The metabolic pathway for BTEX compounds is complex, with various enzymes involved. For instance, benzene in the body is metabolized to its specific phenolic metabolites by cytochrome P-450 2E1 (CYP2E1) (Ross, 2000). Another benzene metabolic pathway is the reaction with glutathione (GSH) to form S-PMA (Nebert et al., 2002). In addition, the human cytochrome P450 (CYP) isozymes and liver microsomal enzymes are involved in toluene, ethylbenzene and xylenes metabolism in the human body (Knecht et al., 2000; Nakajima et al., 1997).

During the oxidation of BTEX in the body, glutathione (GSH) and superoxide dismutase (SOD) protect cells from superoxide toxicity and forms malondialdehyde (MDA) as a result of lipid peroxidation (Tualeka et al., 2020). MDA is thus considered a biomarker of oxidative stress (Xiong et al., 2016) and antioxidant activity (Tualeka et al., 2020).

Human biomonitoring (HBM) has been used widely as a complementary approach in risk assessment to characterize individuals' exposure to pollutants, including BTEX (Faridi et al., 2017; Hoseini et al., 2018; Rafiee et al., 2020). Some previous studies have proposed urinary un-metabolized BTEX levels as a sensitive BTEX biomarker of exposure because it was significantly correlated with airborne BTEX concentrations (Fustinoni et al., 2010; Waidyanatha et al., 2001).

Child labour is a global problem where children aged between 5 and 17 are working in many occupational settings (Hurst, 2007; Shendell et al., 2016). The International Labour Organization reported that globally 152 million children are working and a half (around 73 million) are involved in hazardous work (Office, 2017; Pérez-Herrera et al., 2019). In addition, street children are often involved in child labour, especially in low and middle-income countries (LMICs) (Ansari et al., 2015; Kuimi et al., 2018). Generally, children's physiology and behavioral habits make them especially susceptible to environmental pollutants and occupational hazards, especially in those who live in impoverished families with malnutrition (Moya et al., 2004; Sughis et al., 2014). To date, several studies have characterized the exposure of children working in different occupational settings to various pollutants (Arif et al., 2018; Pérez-Herrera et al., 2019; Sughis et al., 2014). For example, a study in children working in a solid waste disposal facility revealed that the concentrations of polybrominated diphenyl ethers (PBDEs) in the serum of working children were higher than the observed levels in the serum of non-worker children (Cuadra et al., 2006).

Although previous studies attempted to determine the exposure of working children to different environmental contaminants and occupational hazards, exposure of these sensitive populations to chemicals such as BTEX has not been assessed in LMICs. Besides, previous studies reported high BTEX levels in ambient air in Tehran, with annual averages of $7.7 \mu\text{g}/\text{m}^3$ (benzene) and $56.8 \mu\text{g}/\text{m}^3$ (sum of BTEX). These levels exceed Iranian and European benzene guidelines ($5 \mu\text{g}/\text{m}^3$), and are likely related to high traffic emissions (Amini et al., 2017a). Considering the elevated BTEX concentrations in Tehran and the lack of studies characterizing BTEX exposure in street children, we aimed to employ biomonitoring to assess BTEX exposure and oxidative stress, by measuring MDA concentrations, among the sensitive population of street children working at a crowded traffic intersection in the megacity of Tehran, Iran.

2. Materials and methods

2-1. Study area and subject recruitment

This cross-sectional study was performed in Tehran, Iran's capital, with nine million urban residents and over 12.5 million daytime population because of suburbanites traveling into the city (Heger and Sarraf, 2018; Rafiee et al., 2016).

Children aged 7 to 13 years, working as flower sellers at Tohid intersection (Figure 1), one of Tehran's crowded intersections in central Tehran, were recruited as the exposed group. Since most flower sellers were boys, we restricted recruitment to boys. Thirty-five relatively healthy boys were recruited as the exposed group. Subjects in the case group worked in groups of 5-10 children. Although some adults organized the work schedule of the children, the participants spent their working hours working in groups without the company of any adults. An additional 25 boys, not working as flower sellers but living in the area close to Tohid square, were recruited as an unexposed control group. All children's parents or guardians were informed about the study protocol and gave written consent for their children to participate in the project. Information about lifestyle conditions, sociodemographic status, and working situations were collected from participants using interviews and an administered questionnaire.

2-2. Ethical declaration

The present study was carried out according to the World Medical Association (WMA) Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects (Association, 2018). The Ethical Committee at the Shiraz University of Medical Sciences approved this study (no. 98432).

2-3. Urine sampling and preparation

Fieldwork was conducted on Thursday, July 11th, 2019. Thursday is the last weekday in Iran, and traffic is always high at the intersection. Urine samples from children in the exposed group were collected twice as follows: the first urine sample was collected from 8 to 8:30 AM, before starting work; the before exposure (BE) sample. A second urine sample was collected after work (around 8 PM); the after exposure (AE) sample. Unexposed subjects provided single urine from 8:30 to 9 AM). For the case group, urine samples were collected at a public WC close to the children's working area, while control subjects provided the sample at their homes. Two considerations regarding sample collection should be highlighted. First, urinary creatinine levels were analyzed to account for any dilution of BTEX compounds in the urine according to the Jaffé reaction method (Butler, 1975). Second, none of the

children provided first-time morning voids, and the circadian rhythm of urine production is expected to be similar in both groups since the time from waking up until urine collection was similar in both groups. In total, 95 urine samples were collected, including 70 samples from the exposed group and 25 from the unexposed. All samples were gathered into 60mL polypropylene vials, labelled, and stored at 4 °C in a portable fridge before transferring to the laboratory for further analysis. At the laboratory, samples were prepared by adding 2 ml of urine to 10 ml headspace vials containing 1 g of NaCl. The deuterated internal standard for BTEX compounds (benzene d6) was added to the urine samples, and vials were closed tightly by screw caps. Preparation of calibration solutions was carried out by spiking urine samples with appropriate BTEX aliquots.

2-4. Quantification of BTEX in urine samples

Headspace Solid-Phase Micro-Extraction (HS-SPME) was performed to extract un-metabolized BTEX from urine. BTEX quantification methods have been previously reported (Rafiee et al., 2018; Rafiee et al., 2019). Briefly, a gas chromatography (GC, Agilent 7890N, Agilent Co.) coupled to a mass spectrometer (MS, Agilent 5975C, Agilent Co.) was used to quantify BTEX compounds in urine samples. The GC/MS operating parameters are given in Table S1 (Supporting Information). Creatinine levels in all urine samples were in the range 0.3-3.0 g/l recommended by the World Health Organization (WHO); hence, no sample was discarded (Organization, 1996).

2-5. Determination of oxidative stress marker MDA in urine samples

Urine samples were first centrifuged at 1500 rpm for 10 minutes. The MDA equivalents (expressed in $\mu\text{moles L}^{-1}$) were quantified from the formation of thiobarbituric acid reactive substances due to the lipid peroxidation in urine according to the method described in (Chatziargyriou and Dailianis, 2010). In summary, about 500 μL of urine was exposed to phorbol-myristate acetate (PMA) ($10 \mu\text{g mL}^{-1}$). The samples were then centrifuged at 1200 rpm for 10 minutes at 4 °C and the supernatant was removed.

After vortexing for 5 s, butylated hydroxytoluene (BHT) at a concentration of 0.02% was added to prevent further peroxidation of lipids. In the last stage, the samples were incubated at 90-100 °C for 15 min and cooled at room temperature, centrifuged at 10,000 rpm for 10 min, and measured spectrophotometrically at 535 nm. MDA was creatinine corrected, which was determined by the Jaffé reaction method (Butler, 1975).

2-6. Quality control / Quality assurance

A clean urine matrix was prepared by collecting pooled urine samples from volunteer subjects within the general population who lived in rural areas far away from potential BTEX emission sources and diluting them with purified water (4:1 v/v). The mixture was then filtered through a 0.45- μ m filter capsule and divided into three qualities unexposed (QC) pools, including QC low, medium, and high, spiked with BTEX standards of 100, 500, and 1000 ng/L, respectively. A water blank sample and one sample from each QC were processed per 15 samples during the measurement. The levels of blank for each BTEX compound were considerably lower than those observed for the urine samples. In addition, we duplicated 15% of our measurements to evaluate the variance in the measurement and estimate precision. Limit of detection (LOD) and limit of quantification (LOQ) were applied as quality control and were determined for blank samples as the concentration equal to 3 and 10-fold of the quantifier ion's noise (Table S2 in Supporting Information). The calibration curve was plotted by making six samples with different concentrations (from 10 ng/L to 100 μ g/L) by dissolving BTEX solution analytical standard (Sigma Aldrich) in urine with a correlation coefficient of 0.999 or greater.

2-7. Statistical analysis

Statistical analysis was performed using SPSS 21.0 package software (SPSS Inc. Chicago, IL). First, the data distribution was investigated by the Shapiro–Wilk normality test. The skewness of all variables was positive, which indicates the non-normal distribution of urinary BTEX and MDA. The Kruskal-Wallis H test was performed to compare urinary BTEX and MDA levels among the studied groups. In addition, the Mann-Whitney U test was employed to investigate urinary BTEX and MDA concentrations between BE and AE samples in the exposed group.

The association between BTEX determinants and corresponding urinary BTEX levels was analyzed using a linear regression model. Body mass index (BMI) and urinary creatinine were introduced into the model as continuous variables, while self-reported residential traffic exposure and environmental tobacco smoke (ETS) were considered categorical BTEX determinant variables.

Multivariate linear regression analysis was performed to investigate the association between urinary MDA and independent variables potentially affecting its urinary levels. Since benzene is the most toxic BTEX compound and has been used as the most common indicator of exposure to total BTEX, it was selected and included in the regression model as a continuous variable. The concentrations of BTEX and MDA were log-transformed before conducting linear regression. The following variables gathered by the questionnaire were introduced in the model as covariates to adjust for potential confounding: fatigue during work, eating fast foods (2 categories), BMI, and sleep hours per day. BMI and sleep pattern were included as a continuous variable.

Before including variables in the regression models, the collinearity between variables was analyzed by conducting the Spearman correlation test. The pairwise correlation coefficient (r) of < 0.5 was used as the indicator for introducing variables in the model.

3. Results

3-1. Characteristics of the study populations

Selected characteristics of the studied subjects are presented in Table 1. Results showed significant differences between studied groups regarding BMI (Figure 2, $p < 0.05$). Subjects in the exposed group were underweight while the unexposed subjects were in the normal weight. There were no significant differences in other characteristics between the exposed and unexposed groups ($p > 0.05$). Based on the questionnaire, 16 subjects in the exposed group and 10 subjects in the unexposed group were classified as passive smokers.

3-2. BTEX urinary concentrations profile in the studied groups

Urinary BTEX concentrations are summarized in Table 2 and Figure 3. The geometric mean benzene, toluene, ethylbenzene, o,p-xylene, and m-xylene levels in AE samples of the exposed subjects were 0.98, 1.47, 0.55, 1.12, 1.32 $\mu\text{g/L}$, respectively. The geometric mean of BTEX levels in AE samples of the exposed group was 57.8 nmol/L. Toluene and ethylbenzene were observed as the highest and lowest urinary levels in the exposed group, respectively. BTEX levels in the BE samples were comparable to those in from unexposed subjects. Significant differences were found in urinary BTEX concentrations between BE and AE samples in the exposed group ($p < 0.05$), where urinary BTEX concentrations in AE samples were 2.36-time higher than concentrations observed in BE samples. Moreover, the urinary BTEX levels in AE samples of the exposed subjects were significantly higher than concentrations in the unexposed group ($p < 0.05$).

The levels of BTEX compounds in the urine of passive-smoker participants were significantly higher than in subjects who were not exposed to environmental tobacco smoke (ETS), both when comparing the ETS-exposed subjects with the non-ETS exposed within the case group and within the unexposed control group ($p < 0.05$) (Figure 4). Despite the observable effect of the ETS within groups, the effect of occupational exposure to BTEX was still significantly different between the two groups (Figure 4). The median urinary BTEX levels increased with work exposure among passive smokers (2.84-fold) and those without ETS exposure (2.44-fold). In addition, the median BTEX levels in AE samples of passive smokers were 1.54-fold higher than those in subjects who were not exposed to ETS. Likewise, the median urinary BTEX levels of passive smokers in the unexposed subjects were 1.8-fold higher than those who were not exposed to ETS (Figure 4).

3-3. MDA urinary concentrations profile in the studied subjects

Results of MDA urinary levels in the studied groups are provided in Table 3. The AE samples' urinary MDA level was 3.2 and 3.07-time higher than BE samples and in the unexposed group ($p < 0.05$). Besides, Figure 5 shows the distribution of MDA urinary concentrations measured before exposure, after exposure and in the unexposed group. MDA concentrations measured in urine samples collected

after the work shift on the street are higher than the urine samples collected before exposure ($p < 0.05$) and the unexposed group ($p < 0.05$). Likewise, urine samples collected before exposure record higher MDA concentrations than samples collected from the unexposed group ($p < 0.05$).

3-4. Linear regression

Results of linear regression to investigate the association between urinary BTEX levels with determinants of exposure, including passive smoking, BMI, and traffic conditions residence, are presented in Table 4. Only passive smoking showed a significant association with the BTEX levels in the urine of subjects in the studied groups ($p < 0.05$).

Likewise, Table 5 presents the linear regression results assessing the association between MDA with BTEX concentrations, passive smoking, BMI and traffic residence. MDA is associated with higher concentrations of benzene ($\beta = 0.15$; 95% CI: 0.11, 3.2 $\mu\text{g/g creatinine per } \mu\text{g/l}$) and ethylbenzene ($\beta = 0.51$; 95% CI: 4.76, 17.4 $\mu\text{g/g creatinine per } \mu\text{g/l}$). ETS exposure is also a factor affecting the concentrations of MDA measured in urine in the studied population, with concentrations measured in subjects exposed to ETS showing higher concentrations than subjects not exposed to ETS, independent of their occupational exposures (Table 5 and Figure 6). Subjects who were not exposed to ETS experienced a reduction of MDA concentrations ($\beta = -0.219$; 95% CI: -4.29, -1.32 $\mu\text{g/g creatinine per } \mu\text{g/l}$).

4. Discussion

Numerous studies have been conducted to assess BTEX exposure in the general population and occupational settings. In the present study, we employed a biomonitoring approach to investigate BTEX exposure among children working at the Tohid intersection, one of Tehran's crowded traffic intersections. This intersection was selected because it is located between Tehran's two major highways, namely Chamran and Navab. The intersection carries a high traffic density with multiple vehicle types, including cars, taxis, buses, motorcycles, bus rapid transit (BRT), and trucks passing. In addition, the traffic light cycle in this intersection is longer than in many other intersections, allowing longer

continuous traffic movement in one direction, while in the other direction higher emissions from idling traffic, potentially increasing BTEX exposure to street children working there. According to the land use regression (LUR) model for alkylbenzenes developed for Tehran by Amini et al. (2017b), the annual mean benzene concentration in this intersection is expected to be within the range of 12-29 $\mu\text{g}/\text{m}^3$.

Urinary BTEX levels in samples collected from the exposed subjects were significantly higher than those in unexposed. In addition, BTEX levels in AE samples were significantly higher than in the BE samples in the exposed group, consistent with street children's exposure during their work period. Median urinary BTEX levels in AE samples from the exposed group were 10-times higher than the values in BE samples. Our findings are comparable with those reported previously in other occupational settings (Brajenović et al., 2015; De Palma et al., 2012). For instance, Heibati et al. (2018) reported mean levels of benzene, toluene, and xylenes of 11.83, 1.87, and 3.76 $\mu\text{g}/\text{l}$, respectively; 1.2 to 11.5-times higher than our findings in workers at petrol stations. However, the mean level of ethylbenzene in the present study was 1.39 fold higher than the value reported by Heibati et al. (2018).

In the present study, toluene and ethylbenzene were the highest and lowest urinary BTEX levels among the exposed group. Our findings are consistent with those reported previously in Italy's general population (Perbellini et al., 2002) as well as in healthcare waste treatment autoclaves operators (Rafiee et al., 2018). In a study conducted in Japanese workers exposed to solvent, the median level of toluene in the exposed group's urine was 14.8 $\mu\text{g}/\text{l}$, 9-fold higher than those observed levels in our study (Ukai et al., 2007). On the other hand, in a study conducted on paint and footwear factory workers, the highest urinary levels were reported for ethylbenzene (Janasik et al., 2010).

Median urinary BTEX levels among unexposed subjects were 0.2, 0.43, 0.12, 0.31, and 0.3 $\mu\text{g}/\text{l}$, respectively, which is comparable to those reported previously for primary school children in Italy (Minoia et al., 1996). Whereas, Antonucci et al. (2016) reported median BTEX levels of 0.067, 0.081, 0.051, and 0.136 $\mu\text{g}/\text{l}$, respectively, in the urine of children not exposed to ETS who lived in a high traffic area in Italy. On the other hand, in a study performed by Amini et al. (2017b), toluene was reported as the highest BTEX compound in ambient air of Tehran city. Levels of benzene reported by Amini (2017b) were also higher than the Iranian and European guidelines. Overall, this indicates that the inhalation pathway might be the relevant route of exposure to BTEX compounds for street children

in Tehran. Exposure differences observed between children in Tehran and Rome might be attributed to differences in traffic density, size of the city, age of vehicles, and fuel quality between both cities, factors that may lead to higher VOC levels in the ambient air in Tehran (Delgado-Saborit et al., 2009; Miri et al., 2016; Tsangari et al., 2017). The EURO 4 standard was implemented in 2014 in Iran, and 93% of Tehran's vehicles use gasoline and 7% use combinations of gasoline and natural gas (Shahbazi et al., 2016). Emissions from burning these types of fuels are known sources of BTEX. In addition, differences in meteorological parameters such as temperature and wind speed could affect the levels of VOCs compounds, including BTEX, as well as the indoor-to-outdoor air interaction (Sarigiannis et al., 2011). Our findings are consistent with those reported by Tsangari et al. (2017) who reported traffic density, meteorological parameters, and the size of urban areas as factors that can affect urinary BTEX levels in the general population, as reflected in unexposed group and BE samples.

MDA concentrations in the AE samples are 6-fold lower than concentrations reported in rural villagers living near electronic waste recycling facilities in rural areas of China (Yang et al., 2015). Likewise, 2 to 4.5-fold higher MDA concentrations were also recorded in the general population living near metal-polluted soils in Ghana (Bortey-Sam et al., 2018), and 4-fold higher concentrations were recorded in a group of healthy young students in Los Angeles and Beijing (Lin et al., 2016). On the contrary, MDA concentrations were 30 to 35-fold lower in a group of adult subjects participating in the Korean Elderly Environmental Panel study (Han et al., 2016). Similarly, the concentrations of MDA measured in the AE group were 10 to 45-fold higher than concentrations measured in a group of children and their mothers living in China and Korea (Lee et al., 2004). According to Agarwal et al. (2004), urinary excretion of MDA can be observed within 15-30 min after a significant exposure causing lipid peroxidation, returning to normal within 24h after elimination of the oxidative stress source (Agarwal et al., 2004). The increase observed in the present study after exposure to BTEX in street children is consistent with the reported kinetics of MDA metabolism in the human body.

Potentially confounding variables were investigated, including passive smoking, BMI, and traffic status. We identified passive smoking as a predictor variable of urinary BTEX levels in both occupationally exposed and unexposed groups. Numerous studies have reported smoking as one of the most important BTEX sources in indoor environments. In addition, several studies have reported

personal smoking as a major factor affecting urinary BTEX levels (Brajenović et al., 2015; Leusch and Bartkow, 2010; Rafiee et al., 2019). In the present study, urinary BTEX levels among passive smoker subjects in both groups were significantly higher than those in subjects not exposed to ETS. Our result is consistent with previous studies in which ETS was reported as a major predictor of urinary BTEX in children living in low and high-traffic areas (Antonucci et al., 2016; Protano et al., 2012). For instance, Antonucci et al. (2016) reported median benzene, toluene, ethylbenzene, and xylenes levels in a group of children, aged 5-11 years old, who were exposed to ETS and lived in a high traffic area were 0.137, 0.106, 0.069, and 0.153 $\mu\text{g/l}$, respectively compared to 0.067, 0.081, 0.051, and 0.136 $\mu\text{g/l}$ in those who lived in traffic areas and were not exposed to ETS. Differences were also observed in that study when comparing ETS vs non-ETS exposed children living in low traffic areas (Antonucci et al., 2016). The median benzene, toluene, ethylbenzene, o,p-xylene, and m-xylene levels in the urine of children who were passive smokers both in the exposed and non-exposed groups of the present study were 4 to 15-fold higher than the corresponding values reported by Antonucci et al. (2016). This may be due to the fact that half of the children studied by Antonucci et al. (2016) lived in an area with very low traffic density, while the subjects in the present study worked and/or lived in a high traffic density area. In addition, Protano et al. (2012) found significant differences in the excretion of benzene in the urine of children who were exposed to ETS, which was in agreement with our findings. The observed median urinary benzene level in their study was 0.59 $\mu\text{g/l}$, which was 2.36 times higher than the corresponding value in the present study's unexposed subjects. However, median benzene levels in AE samples of passive smokers were almost 2-fold higher than the values observed by Protano et al. (2012).

MDA has been associated with higher levels of benzene, ethylbenzene and exposure to ETS. Associations between MDA and benzene have been reported in a group of petrol station fillers in India (Uzma et al., 2010). However, no association was found between benzene concentrations and MDA levels in a group of Indonesian workers occupationally exposed to benzene (Tualeka et al., 2019). In this study, MDA has also been associated with ethylbenzene, which is consistent with results obtained in animal and model cellular studies (Liu et al., 2013; Wang et al., 2010). Our results are consistent with those reported by Xiong et al. (2016) in a group of occupationally exposed workers, for which BTEX concentrations were associated with MDA urine levels (Xiong et al., 2016). Lastly, exposure to

tobacco smoke has also been associated with elevated concentrations of MDA in urine samples in the current study. This is consistent with results reported in patients hospitalized with acute myocardial infarction in Scotland (Megson et al., 2013) and in brains of murine models (Torres et al., 2012). On the other hand, no association between MDA and exposure to tobacco smoke was found in a group of women undergoing fertility treatment in Iran (Kazemi et al., 2013).

The present study has some limitations that deserve to be mentioned. First, spot urine samples only reflect short-term exposure to BTEX as these compounds have a short half-life. In addition, the small sample size limits the generalizability of the results to other populations. Despite urine samples of the exposed and unexposed groups being collected at slightly different times in the morning, creatinine levels in the urine were analyzed to account for and normalize for any dilution of MDA or BTEX compounds. In addition, the times of urine collection within the subject's circadian rhythm of urine production in the unexposed and exposed groups are expected to be similar since the elapsed time between waking up and urine collection was similar in both groups. Another limitation of the present study was that BTEX levels in ambient air of the intersection or within participants' homes, were not investigated. Last but not least, smoking habits of children cohabitants, such as smoking inside or outside the house and smoking when children are in or out, were not investigated in the present study. However, urinary BTEX levels would reflect all the sources of BTEX of the participants from different exposure routes. Given the elevated BTEX exposures reported in Tehran's roads in previous studies and the nature of the job that the street children conduct, it is expected that a large proportion of the BTEX measured in the urine of street children was related to inhalation of traffic emissions.

To the best of our knowledge, this is the first study in which exposure of street children to BTEX compounds and MDA levels have been investigated. Since our results showed significant amounts of un-metabolized BTEX and MDA in the urine of the exposed group, mitigation strategies should be implemented to minimize street children's exposure to BTEX compounds via inhalation and the damage that might occur by the oxidative stress associated with its exposure.

Conclusion

The present study attempted to characterize BTEX exposure and MDA levels (a biomarker of oxidative stress) among street children who were working at a busy intersection in Tehran, Iran. Overall, our results showed significant differences in urinary BTEX and MDA levels related to occupational exposure. Because of their particular physiology and metabolic pathways, children are uniquely sensitive to environmental pollutants, and their exposure to chemicals like BTEX can lead to adverse effects on their health during their lifespan. Our findings also showed that exposure to ETS could be a significant predictor contributing to high BTEX and MDA concentrations in children's urine. Future studies should investigate the effects of smoking habits of family members on children's urinary BTEX levels.

Finally, children should be protected from BTEX exposure by avoiding working on the street. Unfortunately, street working children are an unfortunate reality, especially among low-income families in developing countries. With this in mind and until the corresponding authorities enforce child labour prevention, stringent regulation and mitigation measures should be implemented to reduce or eliminate street children's exposure to BTEX and subsequent oxidative stress. Reducing children's exposure to ETS can also lower BTEX exposure. Future studies can investigate whether interventional approaches such as diet change or using personal protective equipment, e.g. charcoal masks, can decrease street children's exposure to BTEX compounds and associated oxidative stress MDA concentrations. Such evidence could help define mitigation strategies and define public health policies to reduce exposure to BTEX compounds in street children.

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Figure legends:

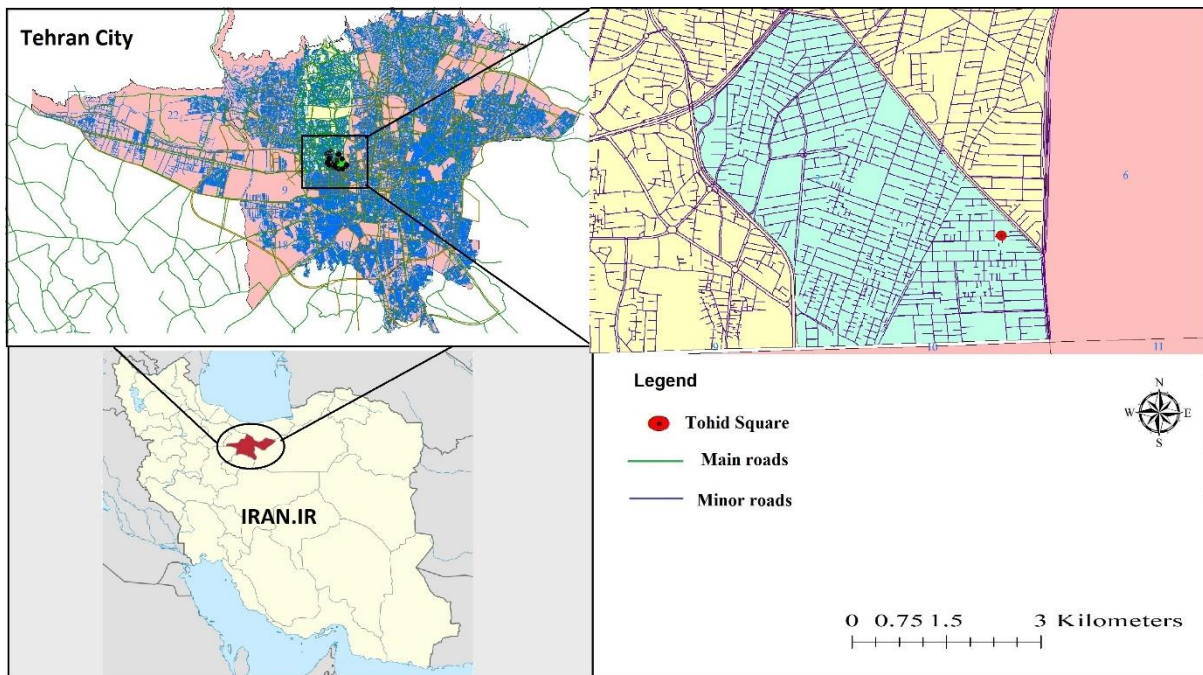


Fig 1. The locations of the study site and the distribution of the unexposed subjects' locations (blue shaded area)

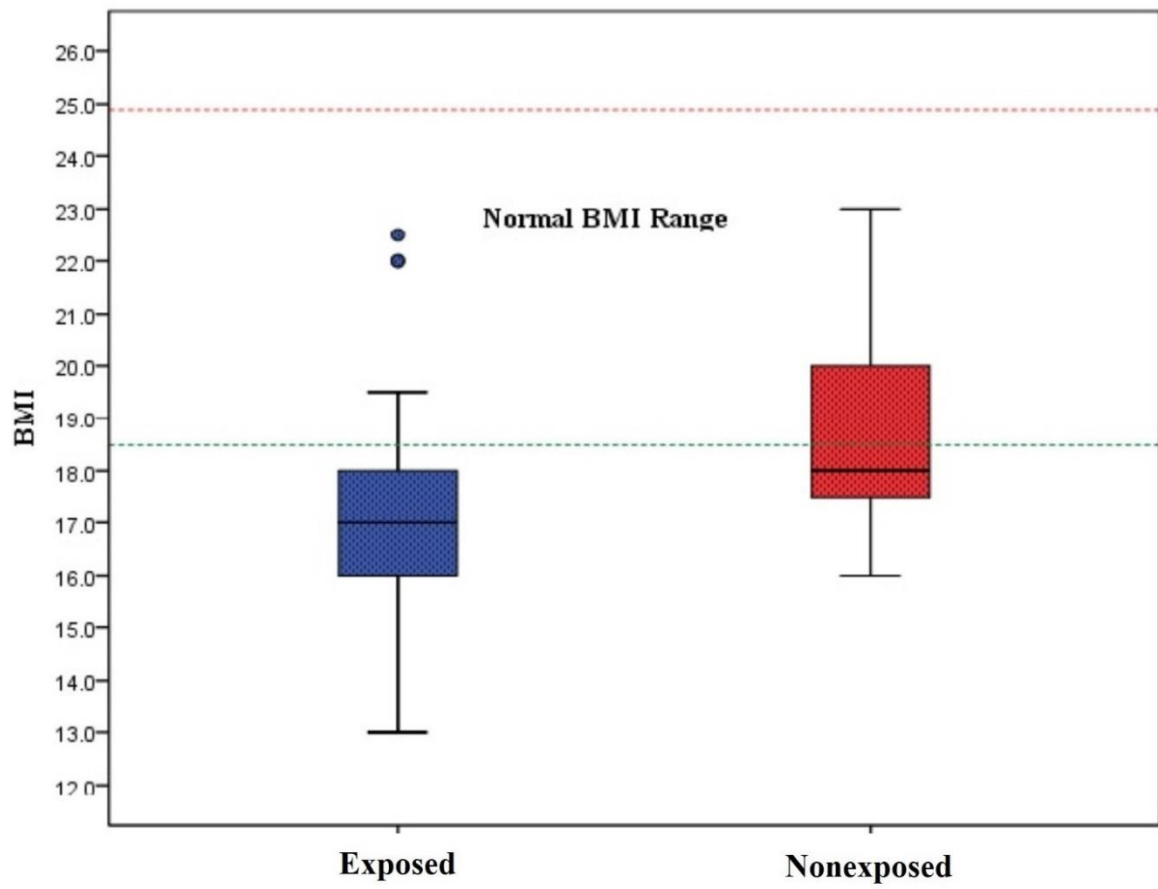


Fig 2. BMI in studied subjects

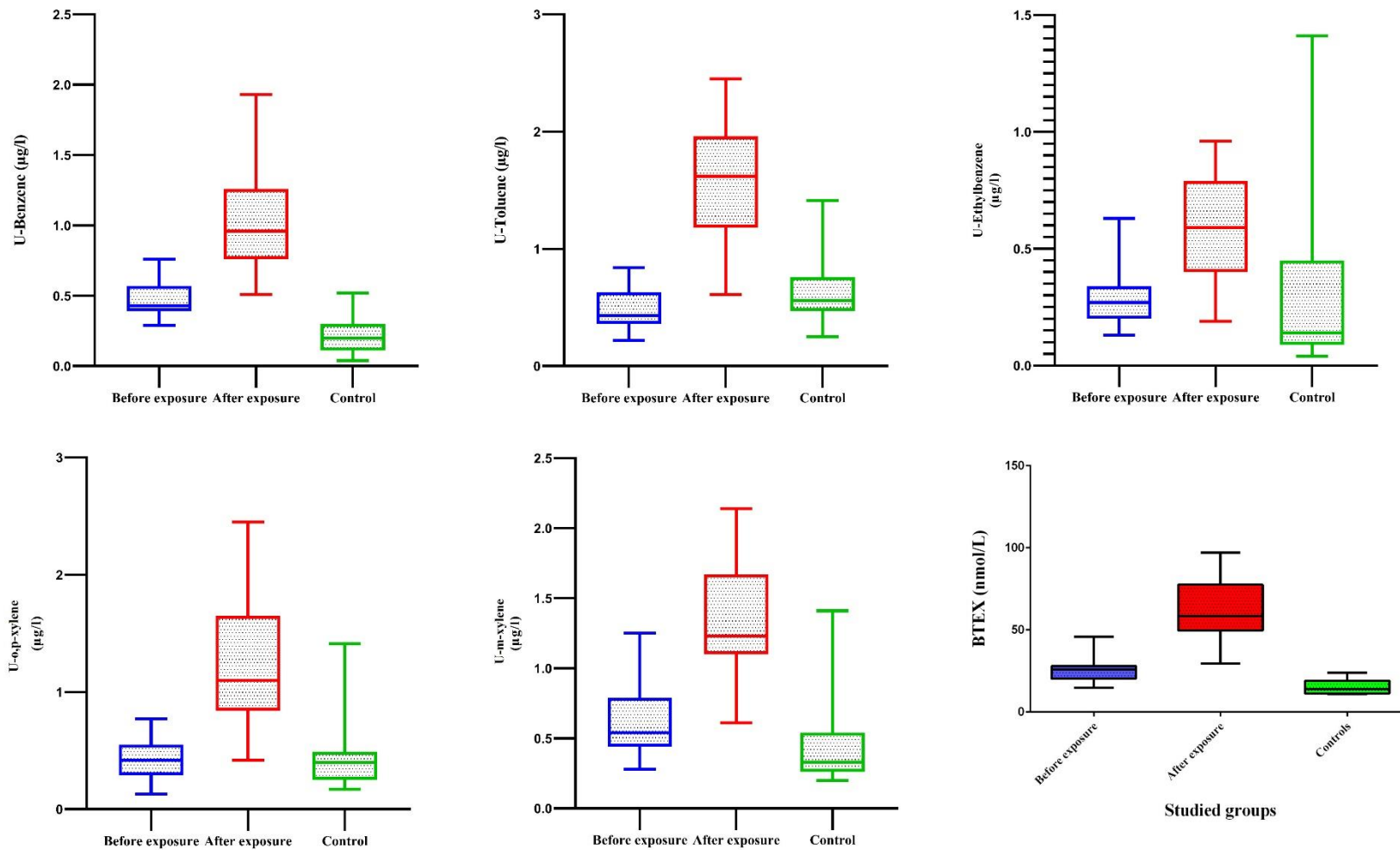


Fig 3. Urinary BTEX levels among study participants

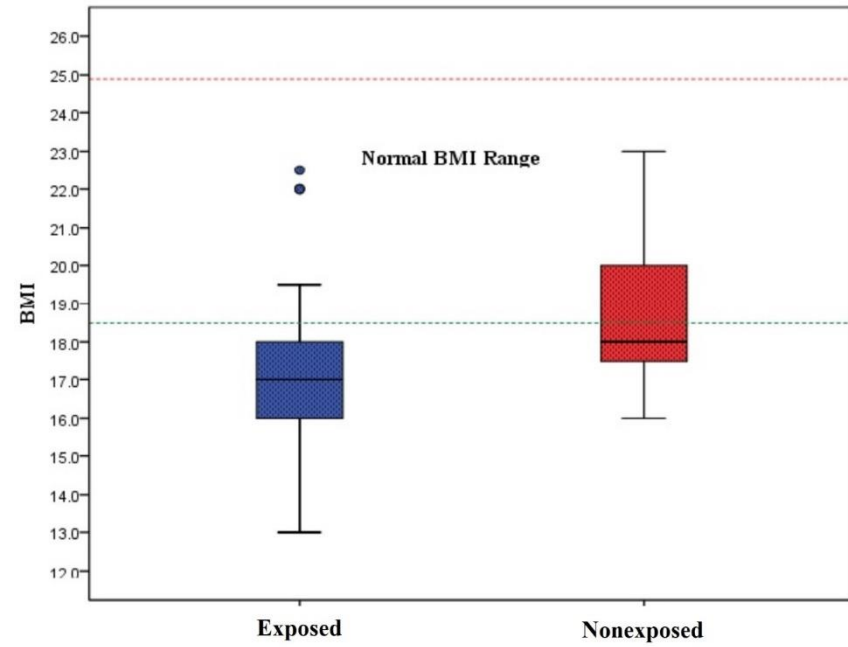
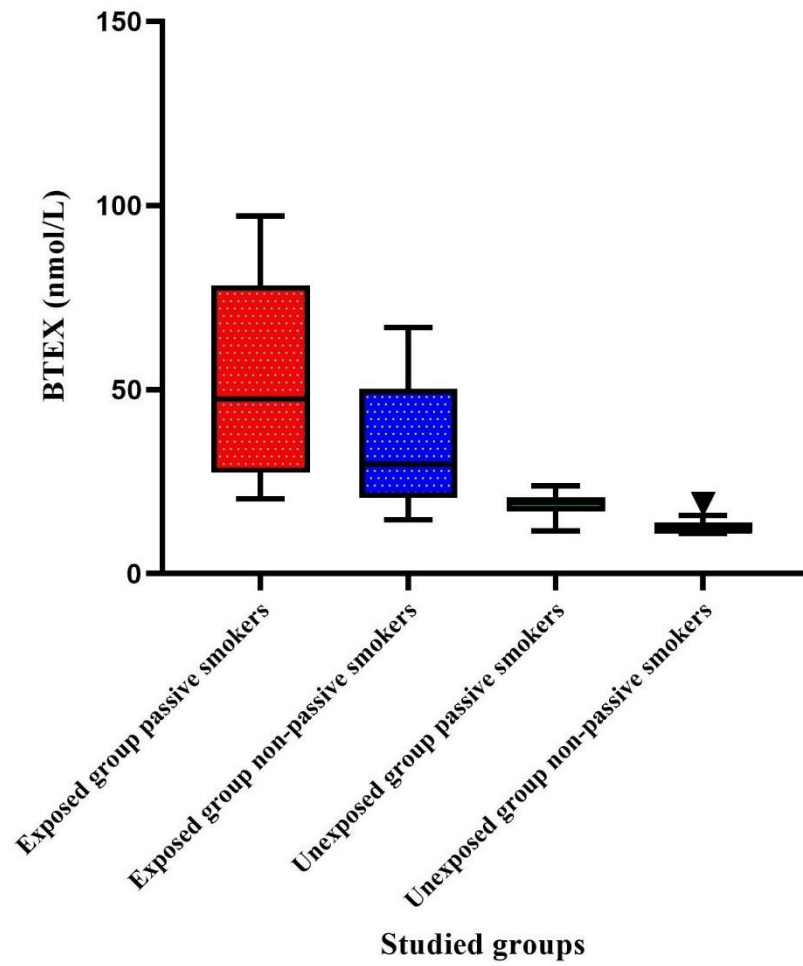


Fig 4. Urinary BTEX levels among passive smokers and non-ETS exposed children

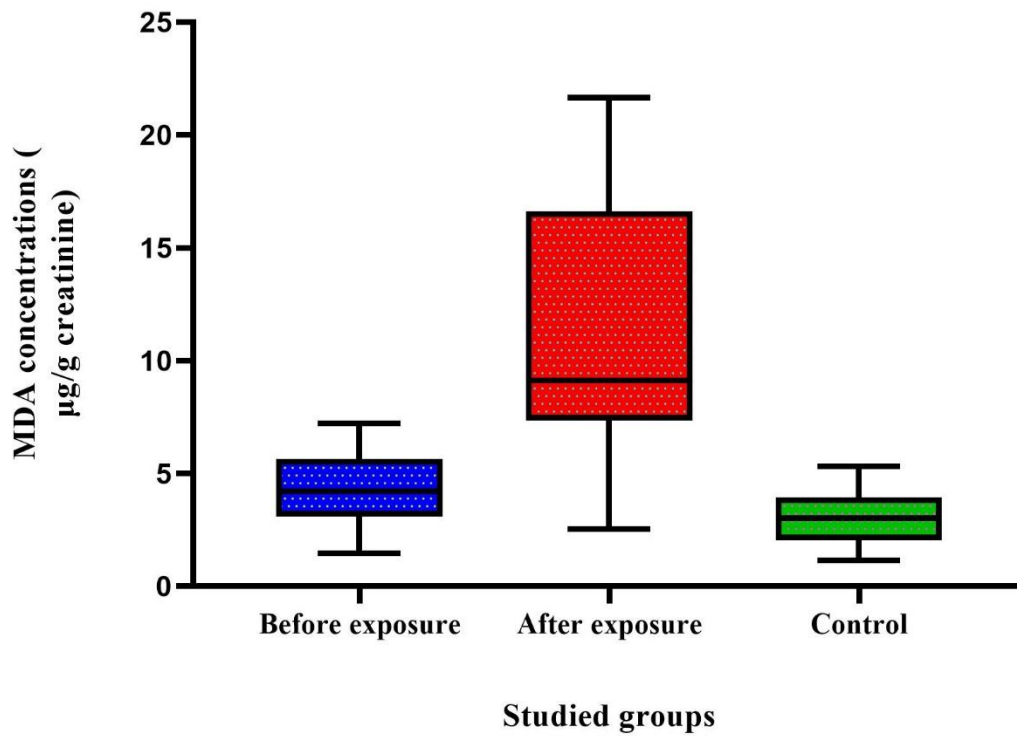


Fig 5. Urinary MDA concentrations among study participants

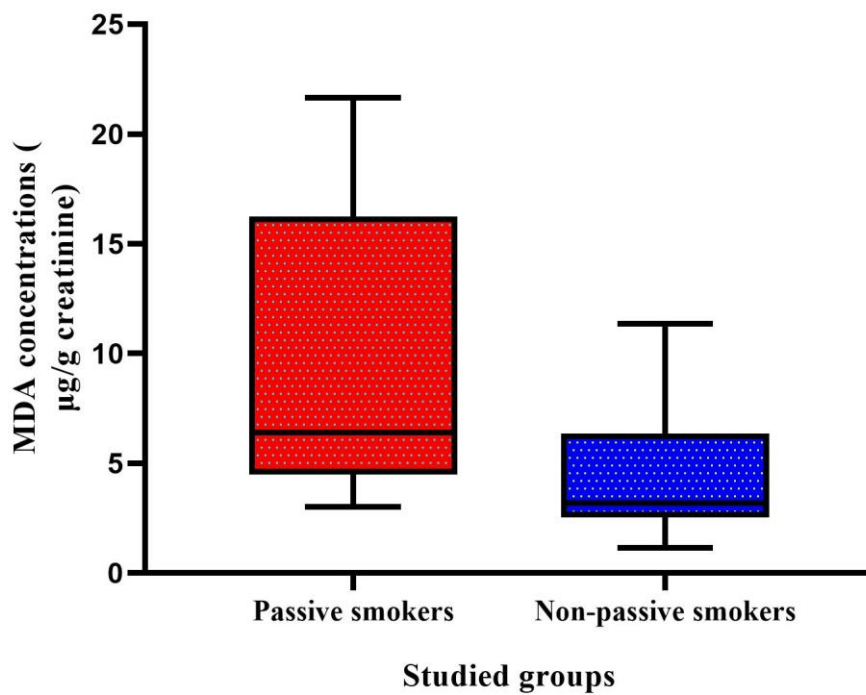


Fig 6. Urinary MDA levels among passive smokers and non-ETS exposed children

Table 1.

Selected characteristics of the participants.

Characteristics	Exposed group	Unexposed group
No.	40	35
	Geo-mean \pm SD	Geo-mean \pm SD
Age (years)	10 \pm 1.75	9.5 \pm 1.95
Height (cm)	142 \pm 12.5	140.5 \pm 11
Weight (kg)	33 \pm 7	36 \pm 9
BMI (kg/m²)	16.2 \pm 1.70	18.5 \pm 2
Working hours / day	10.5 \pm 1.40	-
Working days / week	6.8 \pm 0.41	-
Time spending outdoor per day 48 hours before sampling	14 \pm 0.75	3 \pm 1
Duration of using electronic devices (%)		
<1 hour	71	-
1-3 hours	20	16
3-5 hours	9	16
>5 hours	-	68
House cooling system (%)		
Air conditioner	-	64
Water cooling system	100	36
Eating fast foods (%)		
Once per week	9	56
2-3 time per week	17	28
More than 3 time	74	16
Sleep hours per day	6 \pm 0.5	8 \pm 1
Passive smoking (%)	Yes (43) No (57)	Yes (40) No (60)
Traffic density near the place of residence	Medium (60%) High (40%)	Medium (72%) High (28%)

Table 2.Statistical analysis of urinary BTEX among studied groups ($\mu\text{g/L}$).

Exposure type	Before exposure					After exposure					Unexposed group			
	Mean \pm S.D (Min-Max)	Geo-mean	Comparison with control	Passive smokers Mean \pm S.D (Min-Max)	No ETS exposure Mean \pm S.D (Min-Max)	Mean \pm S.D (Min-Max)	Geo-mean	Comparison with control	Passive smokers Mean \pm S.D (Min-Max)	No ETS exposure Mean \pm S.D (Min-Max)	Mean \pm S.D (Min-Max)	Geo-mean	Passive smokers (Mean \pm S.D)	No ETS exposure (Mean \pm S.D)
Benzene	0.48 \pm 0.13 (0.29-0.76)	0.46	p=0.53	0.52 \pm 0.11 (0.38-0.71)	0.45 \pm 0.13 (0.29-0.76)	1.03 \pm 0.34 (0.51-1.93)	0.98	p<0.05	1.22 \pm 0.35 (0.73-1.93)	0.88 \pm 0.23 (0.53-1.34)	0.22 \pm 0.14 (0.04-0.52)	0.17	0.24 \pm 0.2 (0.04-0.5)	0.2 \pm 0.12 (0.06-0.4)
Toluene	0.66 \pm 0.3 (0.25-1.41)	0.6	p=0.64	0.75 \pm 0.33 (0.31-1.41)	0.56 \pm 0.24 (0.25-1.31)	2.16 \pm 0.42 (0.51-1.93)	1.47	p<0.05	1.84 \pm 0.42 (0.76-2.45)	1.35 \pm 0.42 (6.6-23.5)	0.48 \pm 0.18 (0.22-0.84)	0.45	0.6 \pm 0.15 (0.34-0.76)	0.4 \pm 0.14 (0.2-0.84)
Ethylbenzene	0.28 \pm 0.11 (0.13-0.63)	0.26	p=0.71	0.36 \pm 0.1 (0.25-0.63)	0.22 \pm 0.07 (0.13-0.36)	0.6 \pm 0.23 (0.19-0.96)	0.55	p>0.05	0.8 \pm 0.12 (0.6-0.96)	0.41 \pm 0.11 (1.8-6.4)	0.13 \pm 0.08 (0.04-0.36)	0.11	0.18 \pm 0.1 (0.06-0.36)	0.1 \pm 0.05 (0.04-0.23)
o,p-Xylene	0.42 \pm 0.16 (0.13-0.63)	0.4	p=0.62	0.53 \pm 0.12 (0.31-0.77)	0.35 \pm 0.16 (0.13-0.71)	1.25 \pm 0.57 (0.42-2.45)	1.12	p<0.05	1.67 \pm 0.48 (0.91-2.45)	0.88 \pm 0.28 (0.42-1.47)	0.31 \pm 0.1 (0.17-0.5)	0.29	0.38 \pm 0.1 (0.2-0.5)	0.3 \pm 0.08 (0.17-0.4)
m-Xylene	0.65 \pm 0.30 (0.28-1.25)	0.6	p=0.61	0.81 \pm 0.35 (0.35-1.25)	0.5 \pm 0.16 (0.28-0.79)	1.38 \pm 0.42 (0.61-2.14)	1.32	p<0.05	1.77 \pm 0.22 (1.45-2.14)	1.03 \pm 0.2 (0.61-1.31)	0.32 \pm 0.12 (0.2-0.66)	0.31	0.4 \pm 0.14 (0.26-0.66)	0.3 \pm 0.07 (0.2-0.42)
Total BTEX*	26 \pm 7.7 (14.6-45.8)	25	p=0.81	31 \pm 7.4 (20.3-46)	21.8 \pm 5.4 (14.6-39.8)	60.5 \pm 17.9 (29.5-97)	57.8	p<0.05	75.8 \pm 11.9 (49-97.14)	47.6 \pm 10.2 (29-5.67)	15 \pm 4 (11-24)	14.7	18.8 \pm 3.3 (11.6-24)	12.8 \pm 2.4 (11-19)

- BTEX unit is nmol/L.

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Table 3. Urinary levels of MDA in the studied groups

Marker	Exposed group				Unexposed group	
	Before exposure		After exposure		Mean \pm S.D (Min-Max)	Geometric mean
	Mean \pm S.D (Min-Max)	Geometric mean	Mean \pm S.D (Min-Max)	Geometric mean		
MDA ($\mu\text{g/g}$ creatinine)	3.75 \pm 1.4 (1.45 - 7)	3.5	12 \pm 6 (2.5 - 21.7)	10.5	3.9 \pm 2.1 (1 - 9)	3.3

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5 **Table 4.**
 6 Multivariate linear regression analysis of urinary BTEX ($\mu\text{g/l}$) with factors affecting exposure to BTEX in
 7 the case group [β coefficient (p-value)]
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Factors	Benzene	Toluene	Ethylbenzene	o,p-xylene	m-xylene
Exposure to ETS (n/y)	0.38 (0.03)	0.22 (0.02)	0.21 (0.04)	0.19 (0.02)	0.18 (0.02)
BMI (kg/m^2)	-0.016 (0.6)	-0.006 (0.92)	-0.019 (0.61)	-0.013 (0.5)	-0.019 (0.29)
Traffic density in area of residence (medium regard to low)	0.042 (0.45)	0.027 (0.24)	0.013 (0.12)	0.017 (0.62)	0.014 (0.38)
Traffic density in area of residence (high regard to low)	0.11 (0.12)	0.17 (0.13)	0.091 (0.22)	0.16 (0.22)	0.17(0.33)
Creatinine (g/L)	-0.193 (0.5)	-0.285 (0.02)	-0.211 (0.41)	-0.118 (0.52)	-0.221 (0.81)

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10 **Table 5**
 11 Multivariate linear regression analysis of urinary MDA ($\mu\text{g/g}$ creatinine) with factors affecting urinary
 12 MDA levels in the case group.
 13

Factors	Standardized Coefficients Beta	p-value	95.0% Confidence Interval for B	
			Lower Bound	Upper Bound
Benzene ($\mu\text{g/l}$)	0.18	0.01	0.10	2.8
BMI (kg/m^2)	0.012	0.73	-1.12	1.48
Fatigue during work (n/y)	0.038	0.55	-1.43	0.98
Eating fast foods (n/y)	0.054	0.61	-1.12	1.24
Sleep pattern	0.009	0.72	-1.18	0.081

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