Original Article

Monitoring the Level of Urinary Metabolites of Toluene and Xylene in Pregnant Women in Isfahan and Khansar and their Relationship with Demographic Factors and Air Quality Index

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Abstract

Aim: In this study, the levels of urinary metabolites of toluene and xylene isomers in pregnant women in Isfahan and Khansar cities and their associations with air quality index (AQI) and demographic factors were investigated. **Materials and Methods:** Depending on the population, 55 pregnant women from Isfahan and 15 pregnant women from Khansar, a total of 70 people were enrolled in the study and 140 urine samples were collected during their 1st and 3rd trimesters of pregnancy (autumn and spring). Urinary metabolites of hippuric acid (HA), ortho-, meta-, and para-methyl HA (o-m-p-MHA) were extracted by dispersive liquid-liquid microextraction method, derivatized and analyzed using gas chromatography/mass spectrometer. The results were statistically correlated with AQI level and demographic factors using appropriate statistical methods. **Results:** Mean urinary concentrations of HA, o-m-p-MHA in the 1st trimester of pregnancy were 371.2, 1.14, 2.48, and 1.97 mg/g cr, and those in the 3rd trimester were 582.1, 3.54, 6.93, and 2.43 mg/g cr, respectively. In the 1st trimester, the mean urinary level of ortho-MHA was significantly associated with the outdoor exposure time on workdays and fragrances use ($P < 0.05$). In the 3rd trimester, the mean concentration of HA was significantly correlated to age and outdoor exposure time on workdays ($P < 0.05$). **Conclusion:** The mean urinary concentrations of HA and MHA isomers in the 1st trimester of pregnancy in Isfahan were more than those in Khansar. Because the level of air pollution in Isfahan was higher than Khansar and that in autumn was higher than spring. In the 3rd trimester of pregnancy, the mean levels of MHA isomers in Isfahan were more than those in Khansar. Only the amount of p-MHA was significantly associated with AQI in the 1st trimester of pregnancy. Thus, women who lived in high-traffic urban areas with high AQI were more exposed to toluene and xylene compounds.

Keywords: Air quality index, hippuric acid, Isfahan, methylhippuric acid, pregnant women

Introduction

Air pollution is one of the problems that have affected many communities in the world due to urbanization, increasing motor vehicles, and industrial development. The presence of one or more pollutants in the atmosphere in amounts and for a period that causes adverse effects on humans, animals, and plants is considered air pollution. These pollutants may be a mixture of solid and liquid particles (suspended particles), gases (such as NO_2, NO, SO_2), organic vapors, biological aerosols, and other atmospheric agents that can be dispersed or transferred.[1] Volatile Organic Compounds (VOCs) are 300–1000 carbon-based compounds that emit mainly from the petroleum, petrochemical, oil refinery industries, and motor vehicles. Benzene, toluene, ethylbenzene, and xylene (BTEX) are VOCs that include BTEX isomers having similar physical and chemical properties. Their sources of emissions to the

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atmosphere are motor vehicles, industrial activities such as the petrochemical industry, paints, solvents, and combustion processes. BTEX is one of the main components of gasoline and one of the main solvents used in industry. In urban areas, these compounds make up to 60% of the nonmethane components of VOCs. Overall, 18% of the compounds in gasoline are BTEX, of which 26% is toluene and 52% is xylene.[2] BTEX emits through exhaust, engines and carburetors of vehicles, through braking and tire wear as well as through the evaporation of gasoline from petroleum product storage and distribution system.,[3] BTEX is also used as a fuel additive to increase octane and combustion efficiency as a replacement of lead alkyl.[4]

BTEX also contributes to global warming, stratospheric ozone depletion, photochemical ozone formation, and atmospheric odors.[5] The International Agency for Research on Cancer classified benzene and ethylbenzene as Group 1 carcinogens and toluene and xylene as Group 3 carcinogens.[6] Exposure to toluene can lead to several health effects, including changes in central nervous system function, fatigue, dizziness, lack of coordination, and delayed response and perceptual speed. Direct contact with xylene irritates the skin, leading to dryness, cracking, blistering, and dermatitis.[7] The central nervous system is the first organ to be damaged in acute or chronic exposure to toluene and xylene; however, adverse effects of these compounds on the respiratory system, cardiovascular system, and kidneys have also been reported.[8] Studies have shown that the incidence of cancer in workers in the paint industry increases with prolonged exposure to organic solvents. Chronic exposure to toluene and xylene increases the risk of esophagus, rectum, and colon cancers and also leads to harmful effects on the nervous system, liver, and kidneys.[9] Recent studies have also revealed that exposure to BTEX is associated with an increase in inflammatory biomarkers (C-reactive protein, Interleukin-6 [IL-6], IL-10, IL-1β, and tumor necrosis factor-α) in mid-pregnancy that causes preterm birth.[10]

In a study in Khorasgan district of Isfahan, the mean concentrations of toluene and xylene isomers in the air of fuel stations in autumn were 415.22 and 333.14 $\mu g/m^3$ and those in spring were 422.55 and 486.17 $\mu g/m^3$, respectively. However, the measured concentrations at a distance of 40 m away from the stations were much lower than the stations themselves.[11] In the study of BTEX levels and resources in the air of Ahvaz metropolis, the average concentrations of toluene and xylene were reported to be 5.19 and 1.13 $\mu g/m^3$, respectively, which were higher in traffic areas than in residential areas.[12] In a recent study in a tropical coastal city of Brazil, the average BTEX concentration ranged from 1.6 to 45.5 $\mu g/m^3$ with higher values during the wet period, indicating the effect of meteorological variables.[13] The urinary level of unmetabolized BTEX in pregnant women living near a petrochemical complex in Iran has been reported to be 0.57, 0.83, 0.40, 0.98, μg/L, respectively, which were higher than those in the control group.[14] Urinary levels of BTEX and malonaldehyde, as a biomarker of oxidative stress, in the children who worked near busy streets has been reported to be much higher than those living in the neighborhood.[15]

There have been more or less previous studies on exposure to benzene and ethylbenzene and monitoring of their biomarkers in the human body,[16,17] but so far the exposure to toluene and xylene and the concentration of their metabolites in the body of sensitive groups in Isfahan have not been studied. Since pregnant women are classified as the most sensitive groups in any society, in this study, the extent of exposure to toluene and xylene pollutants by tracking their biological markers in the bodies of pregnant women in Isfahan and Khansar was investigated. First, the concentration of urinary metabolites of toluene and xylene in the urine of pregnant women in Isfahan as a more polluted city, and Khansar as a less polluted city was determined, then the mean values were compared, and the association between their concentrations and demographic and behavioral factors and air quality index (AQI) was evaluated.

Materials and Methods

Study population and sampling method

Isfahan, a large and industrial city with a population of more than 2 million, is located in the center of Iran, 1607 m above sea level. Its average annual temperature is 27°C (max 39°C in summer and min − 18°C in winter) with a low annual mean wind speed blowing predominantly from northwest to southeast. The city of Isfahan with a longitude of 51° 39′ 40″ east, a latitude of 32° 38′ 30″ north, is the third-largest city in Iran, after Tehran and Mashhad. The city's air is mainly affected by industrial and vehicular traffic emissions, which leads to an increase in the concentration of air pollutants. In this study, a less polluted city, Khansar, a city of Isfahan province located 166 km northwest of Isfahan is considered as a less polluted city to compare the exposure levels of two communities to the studied pollutants.

The subjects were selected from the 8 referral health-care centers, 7 from Isfahan and one from Khansar. Each center covers the adjacent population and is located close to the air quality monitoring stations. Based on the statistical data, 70 people were enrolled in the study, 55 from Isfahan and 15 from Khansar. Urine samples of pregnant women were collected in two stages, the 1st and the 3rd trimester of pregnancy, so, a total of 140 spot samples were collected and analyzed for the metabolites of toluene and xylenes. Inclusion criteria included being in the 1st trimester of pregnancy, living in Isfahan and Khansar at least for a year, and willingness to participate in the study and deliver urine samples. Exclusion criteria included incomplete questionnaires, decline to participate in the third trimester, fetal death, and changing the address of residence. At the time of referring to the health centers, with their consent, after explaining the objectives of the study, the participants completed a questionnaire containing the necessary information for the study, then delivered 20 ml of urine samples in sterilized polyethylene containers. 2 ml of the samples were sent to the medical diagnostic laboratory to determine the creatinine level and the rest of the samples were immediately frozen and kept at −20°C until analysis.

Sample preparation, extraction, and analysis of metabolites

For analysis of the metabolites, Agilent 7890A gas chromatograph equipped with Agilent 5975 C mass detector and DB-5 column with dimensions of 30 m × 0.25 mm × 0.25 μm were used. A helium gas flow rate of 1 ml/min and injection temperature of 280°C were selected. The device was set to split mode with a 5:1 ratio. The initial oven temperature was 100°C with an increased rate of 10°C/min until it reached the final temperature of 260°C in 16 min. Furthermore, the temperature of the axillary part of the device was set at 300°C. The standard solutions are dried with nitrogen gas, then 20 μl of N-methyl-N-trimethylsilyl trifluoroacetamide (MSTFA) was added as a derivatizing agent, and finally, 2 μl was injected into the gas chromatograph for analysis. First, using standard solutions, the elution time and the m/z ratio for each of the metabolites were obtained separately. The device was set in selected ion monitoring mode, and a standard solution of 4 metabolites made at a concentration of 1 ppm was injected into the device. After confirming the method of analysis, known concentrations of the metabolites prepared from the standard solutions (0.1, 1, 5, 10, 25, 50, and 100 μg/l) were injected into the device to plot the calibration curve.

Stock solutions of hippuric acid (HA), ortho-, meta-, and para-methyl HA (o-m-p-MHA) were prepared in methanol with a concentration of 1000 ppm. Then, a solution of a mixture of the mentioned analytes with a concentration of 1 ppm was prepared and diluted in 50 ml balloons with deionized water. From each of the standard solutions, 5 ml was taken and poured into a 10 ml flask. Dispersive liquid-liquid microextraction method was used to extract the metabolites from samples and standard solutions.[18] In summary, 50 μl of the beta-glucuronidase solution was added to 5 ml of a urine sample and incubated for 24 h at 37°C. Then, 0.1 g of NaCl was added to each sample and centrifuged for 5 min. The supernatant was transferred to a new 10 ml falcon. Tetrahydrofuran (THF) was used as a dispersant and chloroform as an extractor in volumes of 500 and 60 μl, respectively. To extract the analytes, a mixture of THF and chloroform was injected at once by a microsyringe into a falcon containing standard samples at a specified concentration to form a cloudy solution. The samples were then centrifuged at 4000 rpm for 5 min. Finally, the organic phase formed at the end of the falcon was removed using a syringe and dried using nitrogen gas. Then, 20 μl of MSTFA was added to each sample for derivatization. After a few seconds, the samples were vortexed and finally, 2 μl of the sample was injected into the gas chromatography-Mass Spectrometry for analysis.

Concentrations of the critical pollutants in the ambient air of Isfahan were measured during the study by air pollution monitoring stations located in the city of Isfahan. These are operated by the Isfahan department of environment, which is 7 active stations of traffic and residential types. The data on critical pollutants were used to calculate the AQI.

Results

Demographic characteristics of the subjects and how they are exposed to pollutants are presented in Table 1. In the whole study population, 4.23% were illiterate, 61.97% had a diploma and lower education and 33.81% had a university education. In terms of jobs, 57.75% of the total study population were homemakers, 22.54% were employees, and 19.72% were self-employed. According to the results, 95.77% of the women were nonsmokers and 4.23% smokers; however, about 24% of them were exposed to second-hand smoking pollutants. Most participants used private cars, 32.4% public buses and 21.1% taxi or rented cars for transportation. In terms of the number of pregnancies, 42.86% of mothers were in the first pregnancy, 28.57% in the second pregnancy, and 28.57% in the third pregnancy or more. The mean age of the women was 27.1 ± 5.6 years with a range of 16–44 years [Table 2]. The results of evaluating exposure to the pollutants [Table 1] showed that 64.29% of women in Isfahan, 57.14% in Khansar and 62.86% in total used air fresheners and deodorants. The results also showed that 35.71% of residents in Isfahan, 60% in Khansar and a total, 40.85% of women were exposed to outdoor air for more than 60 min/day on working days and <30 min/day on holidays. Only 31.42% of all women were exposed to the outdoor air for more than 30 min on holidays.

Statistical characteristics of the urinary metabolites and AQI in the 1st and 3rd trimesters of pregnancy are shown in Table 2. The mean of HA, o-, m-, and p-MHA in the 1st trimester in all the participants was 371.2, 1.14, 2.48, and 1.98 mg/g Cr, and those in the 3rd trimester were 58.51, 3.54, 6.93, and 2.43 mg/g Cr, respectively. In 25% of women, the mean of HA, o-, m-, and p-MHA in the 1st trimester was higher than 398, 1.38, 2.94, and 2.24 mg/g Cr, and those in the 3rd trimester were higher than 1067, 2.29, 3.53, and 3.31 mg/g Cr, respectively. As shown in Table 2, the average AQI in Isfahan during the 1st trimester (autumn) was 105.5 ± 5.8, which is unhealthy for sensitive groups. This index was measured at 55.6 ± 5.5 during the 3rd trimester (spring) which is moderately contaminated.

The average concentration of urinary metabolites (mg/g Cr) in pregnant women in the 1st and 3rd trimesters of pregnancy is shown in Table 3. Furthermore, the concentration of HA and the total isomers of MHA in the urine of pregnant women in Isfahan and Khansar in the 1st and 3rd trimesters are compared in Figures 1 and 2, respectively. It can be seen that the average values of these metabolites in the residents of Isfahan in all areas were slightly higher than Khansar, except for HA, which was observed in the residents of Khansar more than Isfahan in the 3rd trimester of pregnancy. The mean of HA, o-, m-, and p-MHA in the 1st trimester of pregnancy in women living in Isfahan was 322.41, 1.15, 2.59, and 2.10 mg/g Cr, and those in Khansar were 263.3, 0.92, 1.96, and 0.51 mg/g Cr, respectively.

Table 1: Demographic characteristics and exposure to pollutants in the subjects

Parameter	Classification	Isfahan, *n* (%)	Khansar, *n* (%)	Isfahan + Khansar, *n* (%)
Level of education	Illiterate	3 (5.36)	0	3 (4.23)
	Primary school	11 (19.64)	2 (13.33)	13 (18.31)
	Secondary school	12 (21.43)	2 (1.47)	14 (19.72)
	Diploma	14 (25)	3 (20)	17 (23.94)
	Associate degree	7 (12.5)	1 (6.67)	8 (11.27)
	Bachelor's degree or higher	9 (16.07)	7 (46.67)	16 (22.54)
Job	Housewife	35 (62.5)	6 (40)	41 (57.75)
	Employee	11 (19.64)	5 (33.33)	16 (22.54)
	Self employed	10 (16.07)	4 (26.67)	14 (19.72)
Smoking	Yes	1 (1.79)	2 (13.33)	3 (4.23)
	No	55 (98.21)	13 (86.67)	68 (95.77)
Second hand smoke	Yes	14 (25)	3 (20)	17 (23.94)
	No	42 (75)	12 (80)	54 (76.06)
Type of Transportation	Private car	25 (44.64)	8 (53.33)	33 (46.48)
	Bus	19 (33.93)	4 (26.67)	23 (32.39)
	Taxi	12 (21.43)	3 (20)	15 (21.13)
Number of pregnancy	1st pregnancy	21 (38.18)	9 (60)	30 (42.86)
	2nd pregnancy	18 (32.73)	2 (13.33)	20 (28.57)
	3rd and more	16 (29.09)	4 (26.67)	20 (28.57)
Use of deodorants	Yes	36 (64.29)	8 (57.14)	44 (62.86)
	No	20 (35.71)	6 (42.86)	26 (37.14)
Exposure time on weekdays (min)	<30	14 (25)	3 (20)	17 (23.94)
	60-30	22 (39.29)	3 (20)	25 (35.21)
	>60	20 (35.71)	9 (60)	29 (40.85)
Exposure time on weekends (min)	<30	39 (70.91)	9 (60)	48 (68.57)
	60-30	13 (23.64)	5 (33.33)	18 (25.71)
	>60	3 (5.45)	1 (6.67)	4 (5.71)

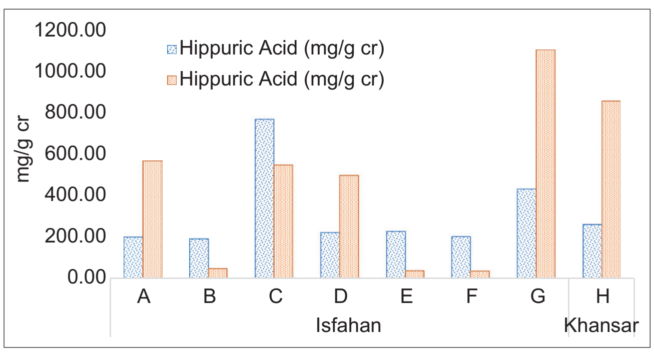

Figure 1: Comparison of hippuric acid concentration in urine of pregnant women in Isfahan and Khansar in the 1st and 3rd trimesters of pregnancy. A: University, B: Khajoo, C: Ahmadabad, D: Parvin, E: Sh.Ratebi, F: Sh.Motamedi and G: Roudaki

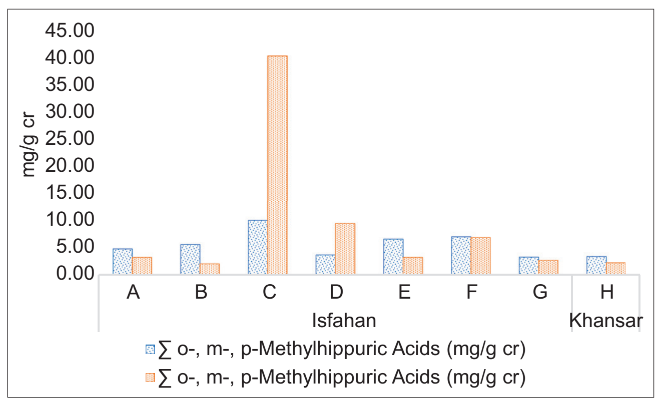

Figure 2: Comparison of total methyl hippuric acid isomers in the urine of pregnant women in Isfahan and Khansar in the 1st and 3rd trimesters of pregnancy. A: University, B: Khajoo, C: Ahmadabad, D: Parvin, E: Sh.Ratebi, F: Sh.Motamedi and G: Roudaki

In addition, the mean of HA, o-, m-, and p-MHA in the 3rd trimester in mothers living in Isfahan were 408.73, 2.54, 5.14, and 2.04 mg/g Cr, and those in Khansar were 860, 0.91, 0.84, and 0.48 mg/g Cr, respectively. Among the areas, women living in area C (Ahmadabad) had the highest values and those living in area B (Khajoo) had the lowest values of metabolites in their urine in both the 1st and 3rd trimesters of pregnancy.

The mean values of AQI in the studied area and their comparison in autumn (1st trimester) and spring (3rd trimester) are shown in Figure 3. The association between the urinary metabolites of toluene and xylenes with AQI in Isfahan in the 1st and 3rd trimesters was statistically analyzed, the results of which are presented in Tables 4 and 5. In the 1st trimester, among the studied metabolites, only p-MHA had a significant relationship with the AQI ($P < 0.05$). However, in the 3rd trimester, none of the metabolites showed a significant relationship with the AQI ($P > 0.05$).

Table 2: Statistical characteristics of urinary metabolites (mg/g cr) and air quality index in the 1st and 3rd trimesters of pregnancy

Statistical characteristics (mg/g cr)	HA		o-MHA		m-MHA		p-MHA		AQI*		Age (years)
	1st trimester	3rd trimester	1st trimester	3rd trimester	1st trimester	3rd trimester	1st trimester	3rd trimester	1st trimester	3rd trimester	
Mean	371.21	582.1	1.14	3.54	2.48	6.93	1.98	2.43	105.5	55.6	27.1
Median	142.76	416.79	0.84	0.92	1.66	1.49	0.88	0.71	105	59	27
SD	934.19	589.72	1.23	9.44	2.93	21.23	5.12	4.26	5.8	5.5	5.6
Minimum	0.08	2.69	0.03	0.02	0.05	0.02	0.07	0.05	98	45	16
Maximum	7410.91	1979.65	8.52	54.41	18.03	113.07	42.7	29.59	115	63	44
25% percentile	43.28	55.06	0.41	0.46	0.9	0.42	0.49	0.38	99	53	23
50% percentile	142.76	416.79	0.84	0.92	1.66	1.49	0.88	0.71	105	59	27
75% percentile	398.08	1067.25	1.38	2.29	2.94	3.53	2.24	3.31	112	61	30

*AQI levels: 0-50=Good, 51-100=Moderate, 101-150=Unhealthy for sensitive people, 151-200=Unhealthy, 201-300=Very unhealthy, 301-500=Hazardous. AQI: Air quality index, SD: Standard deviation, HA: Hippuric acid, MHA: Methyl HA, o-MHA: ortho-MHA, m-MHA: meta-MHA, p-MHA: para-MHA

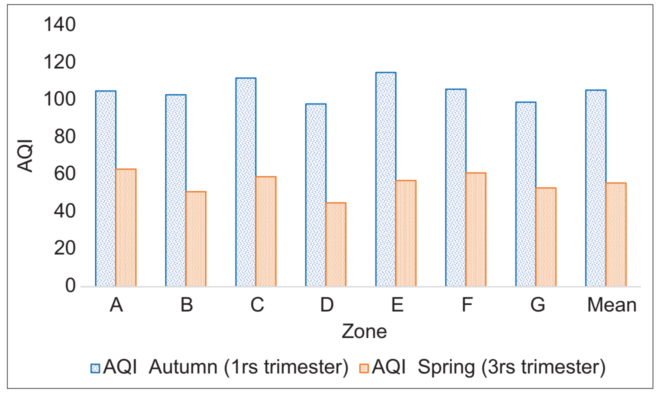

Figure 3: Comparison of the average air quality index in the studied stations in spring and autumn. A: University, B: Khajoo, C: Ahmadabad, D: Parvin, E: Sh.Ratebi, F: Sh.Motamedi and G: Roudaki

The results of evaluating the correlation between the metabolites and demographic and behavioral variables in the 1st and 3rd trimesters are also presented in Tables 4 and 5, respectively. In the 1st trimester, in all subjects (Isfahan + Khansar) the mean concentrations of HA and m-MHA were not significantly correlated with any of the demographic variables (P > 0.05). However, the mean urinary level of o-MHA was significantly associated with the duration of exposure on workdays and with the use of fragrances ($P < 0.05$). In the 3rd trimester, the mean concentrations of HA and m-MHA were significantly correlated to the duration of outdoor exposure on working days ($P < 0.05$). The amount of p-MHA was also significantly associated with smoking ($P < 0.05$). However, the urinary levels of other metabolites did not show a significant relationship with demographic and behavioral factors as mentioned in Table 5.

Discussion

The present study was conducted to investigate the level of urinary metabolites of toluene and xylenes in pregnant women in the 1st and 3rd trimesters of their pregnancy in Isfahan a high-traffic and industrialized city and Khansar as a nonindustrialized and relatively low-traffic city. In addition, the relationship between the level of metabolites and AQI and demographic and behavioral characteristics of individuals were evaluated. Based on previous studies, the metabolites of HA, o-, m-, and p-MHA in urine were examined as biomarkers of exposure to toluene and xylene.[19,20]

Comparison of HA concentration in the urine of pregnant women in different areas of Isfahan and Khansar in the 1st and 3rd trimesters of pregnancy [Figure 1] showed that women exposed to toluene in Isfahan were more than Khansar. As shown in Table 3, the mean urinary level of HA in the 1st trimester in Isfahan residents was 322.41 mg/g Cr, compared to 263.3 mg/g Cr in Khansar residents. However, in the 3rd trimester, the mean amount of HA in Khansar was more than twice in Isfahan (860.3 mg/g vs. 408.7 mg/g Cr). This is even higher than the amount measured in people working in the industry, for example, according to a study in Thailand, the

Table 3: Mean concentration of metabolites (mg/g cr) in urine of pregnant women at different area in the 1st and 3rd trimesters of pregnancy

Trimester	Area*	Creatinine (g/L)	HA	o-MHA	m-MHA	p-MHA	∑o-m-p-MHA	AQI
First trimester (autumn)	A	0.012	202.2	0.87	2.87	1.04	4.78	105
	B	0.011	191.9	0.55	2.8	2.24	5.58	103
	C	0.012	772.5	1.47	3.62	4.91	10	112
	D	0.013	222.8	0.88	1.48	1.32	3.67	98
	E	0.011	227.7	1.29	2.75	2.55	6.59	115
	F	0.011	204.8	1.95	3.25	1.83	7.02	106
	G	0.012	435	1.1	1.36	0.79	3.25	99
	Mean	0.012	322.4	1.16	2.59	2.10	5.84	105.5
	H	0.011	263.3	0.92	1.96	0.51	3.39	-
Third trimester (spring)	A	0.01	569.7	0.65	1.68	0.89	3.22	63
	B	0.01	49.8	0.32	1.25	0.44	2.01	51
	C	0.01	552.7	11.06	22.78	6.66	40.5	59
	D	0.01	502.7	1.66	4.38	3.43	9.47	45
	E	0.009	38.8	0.68	1.68	0.86	3.22	57
	F	0.01	38.5	2.2	3.15	1.56	6.91	61
	G	0.01	1108.9	1.23	1.05	0.43	2.71	53
	Mean	0.01	408.73	2.54	5.14	2.038	9.72	55.6
	H	0.009	860.3	0.91	0.84	0.48	2.24	-

*A: University, B: Khajoo, C: Ahmadabad, D: Parvin, E: Sh.Ratebi, F: Sh.Motamedi, G: Roudaki, H: Khansar. AQI: Air quality index, HA: Hippuric acid, MHA: Methyl HA, o-MHA: ortho-MHA, m-MHA: meta-MHA, p-MHA: para-MHA

level of HA in the urine of paint workers in a steel furniture production industry was reported to be about 800 mg/g Cr.[21] The evaluation of special changes in exposure level in Isfahan showed that in the 1st trimester, women living in area C were more exposed to toluene than in other areas, and in this case, residents in area G ranked the second. However, in the 3rd trimester, the residents of area G had the highest exposure to toluene which is a high-traffic area. Residents of low-traffic areas, including area B, E, and F had lower contact with this pollutant resulting the lower levels of HA in the urine of the individuals.

The results of a study assessing the concentrations of BTEX in residential, industrial, and traffic areas in Nicosia, Cyprus showed a significant difference between these areas. Where, the traffic and industrial areas had higher level of air pollution, resulting a higher level of urinary BTEX in residents of these areas compared to the residential area.[22] These findings are following the results of the present study which showed higher urinary levels of the metabolites in the high-traffic area. Therefore, spatial location can be a good predictor of the level of exposure to these pollutants after correction with other interfering variables. In other words, proximity to the sources of pollutants emission such as industry, highways, or heavy traffic streets leads to more exposure to the pollutants and thus increase their urinary metabolites in individuals. A similar study in Spain found that the air in Barcelona's urban areas compared to Catalonia's rural area, had almost twice as high as benzene and three times as high as toluene and xylene, which were mainly affected by traffic sources.[19] A study measuring aromatic hydrocarbons in the air of three urban areas of Algiers, one in the city center near busy streets, another in a residential area and a third in a semi-rural area with low traffic, found that the main source of BTEX emissions is related to vehicular traffic.[23] In Isfahan, in addition to traffic, the worn-out buses fleet and taxis and low quality fuels are effective in increasing the emission of these pollutants. In addition, the lack of catalytic emission control system on vehicles, the high average age of motor vehicles, the high cost of engine maintenance, diesel and gasoline spills, and their evaporation from refueling stations are some of the factors that contribute to air pollution.[24]

In the study of the Ahvaz metropolis, the average concentrations of toluene and xylene in the air of the city were 5.19 and 1.13 μg/m^3, respectively, and a clear seasonal and spatial variation for these compounds were reported, and traffic was the main source of emissions.[12] In a recent study in a tropical coastal city in Brazil, BTEX levels were higher in the wet season than in the dry season, indicating the impact of meteorological variables.[13] Another important source of BTEX emissions is refueling stations and storage tanks. The results of a study in Khorasgan region of Isfahan showed that the concentration near fuel stations was much higher than 40 meters away from the stations. Thus, it is necessary to control fuel leakage from tanks and refueling pumps and set up a vapors recycling system during fuel transfer, to maintain the health of employees and citizens.[11]

Both in the 1st and 3rd trimesters of pregnancy, the amount of HA as the predominant metabolite of toluene and the total isometries of MHA as the predominant metabolite of xylenes in Isfahan residents was higher than Khansar ($P < 0.05$). In the 3rd trimester of pregnancy, the total amount of MHA in women living in Isfahan was higher than women living in

Table 4: Relationship between the metabolites and air quality index, demographic and behavioral variables in the 1st trimester of pregnancy

Correlation test (1st trimester)	Age	Education level	Job	Smoking	Second-hand smoke	Use of public transport	Number of pregnancy	Use of deodorant	Exposure time on weekdays	Exposure time on weekends	AQI
HA											
Correlation coefficient	0.162	−0.238*	0.001	−0.061	−0.016	−0.079	0.032	0.009	0.055	0.097	−0.002
Significant (two-tailed)	0.183	0.049	0.991	0.62	0.899	0.516	0.795	0.942	0.653	0.429	0.988
No	69	69	69	69	69	69	68	69	69	68	54
o-MHA											
Correlation coefficient	−0.006	−0.012	−0.013	−0.058	−0.151	−0.07	0.051	0.290*	−0.276*	0.022	0.04
Significant (two-tailed)	0.959	0.922	0.916	0.641	0.218	0.568	0.681	0.016	0.023	0.859	0.778
No	68	68	68	68	68	68	67	68	68	67	53
m-MHA											
Correlation coefficient	−0.037	−0.057	0.131	−0.098	−0.037	0.163	0.006	0.107	−0.124	−0.153	0.223
Significant (two-tailed)	0.761	0.638	0.281	0.421	0.76	0.178	0.962	0.379	0.307	0.209	0.102
No	70	70	70	70	70	70	69	70	70	69	55
p-MHA											
Correlation coefficient	0.113	−0.243*	−0.16	0.091	−0.091	−0.021	−0.022	−0.048	0.028	0.085	0.381**
Significant (two-tailed)	0.354	0.045	0.188	0.457	0.455	0.866	0.86	0.697	0.821	0.491	0.004
No	69	69	69	69	69	69	68	69	69	68	54

*The correlation is significant at the level of 0.05 (two-tailed), **The correlation is significant at the level of 0.01 (two-tailed). AQI: Air quality index, HA: Hippuric acid, MHA: Methyl HA, o-MHA: ortho-MHA, m-MHA: meta-MHA, p-MHA: para-MHA

Khansar, which is due to the existence of large industrial sites such as petroleum and petrochemical industry, experiencing heavy vehicular traffic and having inadequate green spaces in Isfahan. In high-traffic urban areas, motor vehicles are the dominant source of air pollutants including BTEX. The results of a study of VOC levels in the atmosphere of industrial and urban areas of La Plata, Argentina showed that most VOCs were created indoors in semi-urban and residential areas, but in urban areas, traffic load was determined as the main source of outdoor VOCs.[25] Although the use of public transportation such as buses reduces the use of private cars and thus reduces traffic, it can increase contact time and consequently exposure to pollutants such as toluene and xylene in case of having to wait a long time in the stations. This may be one of the reasons for the high levels of toluene and xylene metabolites in the urine of pregnant women who used public transportation.

Assessing the correlation between the urinary metabolites and AQI showed that in the 1st trimester, the amount of o- and p-MHA was correlated to AQI on workdays of exposure ($P < 0.05$). However, in the 3rd trimester, the amount of all the studied metabolites had no significant relationship with AQI ($P > 0.05$). Even though the number of smokers in our study was very low, the amount of p-MHA was significantly associated with cigarette smoking ($P < 0.05$). In a study of BTEX levels in urine as biomarkers of exposure to environmental pollutants, it was found that the concentration of benzene and toluene in smokers was 4.6 and 1.2 times higher than nonsmokers, respectively.[26] Thus, according to the results of the present study, women who lived in high-traffic urban areas with high AQI were more exposed to toluene and xylene.

Conclusion

The results of this study showed that pregnant women, as sensitive groups in the society, who live in industrialized and high vehicular traffic areas are exposed to dangerous VOCs such as toluene and xylene. In some cases, the exposure level is as high as those who were in occupational contact with these pollutants. The mean amount of HA in the 1st trimester of pregnancy and total MHA in the 1st and 3rd trimesters of pregnancy in women living in Isfahan is higher than those in Khansar. Assessing the correlation of urinary levels of the metabolites with AQI showed that in the 1st trimester, only the amount of p-MHA has a significant relationship with AQI, but in the 3rd trimester, none of the metabolites has a significant relationship with AQI. This is maybe due to the low level of AQI in the 3rd trimester (spring). The results also showed that in the 1st trimester, the amount of o-MHA has a significant relationship with the outdoor exposure time on weekdays. In the 3rd trimester in all the studied areas, the amount of HA on workdays has a significant correlation with the age of individuals, and the amount of m-MHA is significantly associated with the duration of exposure on workdays. The amount of p-MHA is also significantly associated with smoking. Therefore, it is suggested that women should avoid going to outdoor high-traffic areas of the city as

Table 5: Relationship between the metabolites and air quality index, demographic, and behavioral variables in the 3rd trimester of pregnancy

Correlation test (3rd trimester)	Age	Education level	Job	Smoking	Second hand smoke	Use of public transport	Number of pregnancy	Use of deodorant	Exposure time on weekdays	Exposure time on weekends	AQI
HA											
Correlation coefficient	−0.257*	0.126	−0.156	−0.015	0.226*	0.05	0.003	−0.115	0.028	0.069	−0.182
Significant (two-tailed)	0.044	0.329	0.227	0.906	0.047	0.697	0.979	0.375	0.827	0.598	0.198
No	62	62	62	62	62	62	61	62	62	61	52
o-MHA											
Correlation coefficient	−0.025	−0.123	0.074	0.138	0.06	0.113	−0.02	−0.089	0.156	0.165	0.021
Significant (two-tailed)	0.849	0.341	0.566	0.286	0.641	0.381	0.88	0.493	0.227	0.204	0.884
No	62	62	62	62	62	62	61	62	62	61	52
m-MHA											
Correlation coefficient	0.097	−0.044	0.216*	0.199	−0.058	−0.029	−0.105	−0.130	0.209	0.290*	−0.015
Significant (two-tailed)	0.455	0.736	0.016	0.121	0.653	0.824	0.419	0.315	0.103	0.023	0.914
No	62	62	62	62	62	62	61	62	62	61	52
p-MHA											
Correlation coefficient	−0.023	−0.09	0.166	0.296*	−0.086	−0.065	−0.078	0.001	0.153	0.17	0.073
Significant (two-tailed)	0.858	0.489	0.198	0.02	0.505	0.616	0.552	0.994	0.236	0.19	0.608
No	62	62	62	62	62	62	61	62	62	61	52

*Correlation is significant at the level of 0.05 (2 sequences). AQI: Air quality index, HA: Hippuric acid, MHA: Methyl HA, o-MHA: ortho-MHA, m-MHA: meta-MHA, p-MHA: para-MHA

much as possible during pregnancy and try to use the subway transportation system instead of taxi and bus.

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Conflicts of interest

There are no conflicts of interest.

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