The association between the urinary biomarkers of polycyclic aromatic hydrocarbons and risk of metabolic syndromes and blood cell levels in adults in a Middle Eastern area

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Abstract

Purpose Limited studies have been published on the association between the urinary biomarkers of Polycyclic Aromatic Hydrocarbons (PAHs) and risk of Metabolic Syndromes (MetS) and blood cell levels in adults in the Middle East. The present study aimed to evaluate the exposure to PAHs and the distribution of urinary OH-PAH levels in the general population of Shiraz, Iran, as well as, the association between OH-PAHs and the prevalence of MetS and blood cell levels.

Methods In this study, 200 participants were randomly selected from the adult population, and their first-morning void urine samples were collected.

Results The mean concentrations of 1-OHNap, 2-OHNap, 2-OHFlu, 9-OHPhe, and 1-OHP were 639.8, 332.1, 129, 160.3, and 726.9 ng/g creatinine, respectively. The prevalence of MetS was 26% according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria. The results showed that urinary OH-PAHs, especially 1-OHP, were positively and significantly associated with higher waist circumstance ($p < 0.001$), triglyceride level ($p < 0.001$), systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), number of white blood cells ($p = 0.041$) and red blood cells ($p < 0.001$). It also caused lower levels of High Density Lipoprotein-Cholesterol (HDL-C). In conclusion, the results emphasized the adverse health effects of PAHs on human health, including obesity, hypertension, dyslipidemia, and decreased number of blood cells.

Conclusion Therefore, in order to identify the PAHs sources and to develop methods for decreasing the amount of emissions to the environment, broader researches are needed.

Keywords Organic chemicals · Polycyclic aromatic hydrocarbons · 1-hydroxypyrene · Metabolic disease · Metabolic syndrome

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Introduction

As one of the most important organic pollutants, Polycyclic Aromatic Hydrocarbons (PAHs) can be found in the environment in large amounts [1–3]. They are toxic, mutagenic, and carcinogenic compounds with the highest priority among pollutants, according to the United States Environmental Protection Agency (USEPA), and have been considered as priority pollutants by the United States Environmental Protection Agency (USEPA) [4–6]. They are also known as Endocrine-Disrupting Chemicals (EDCs) [7]. Various epidemiological studies have shown that PAHs could produce Reactive Oxygen Species (ROS) [8–10]. Moreover, a positive and significant relationship has been found between PAHs exposure and the presence of oxidative stress biomarkers in different populations and age groups [11, 12]. The possible

effect of PAHs on Metabolic Syndromes (MetS) (obesity, diabetes, cholesterol, and hypertension) has also been reported in some studies on different age groups [7, 13–15].

However, there is not much information about the effect of urinary OH-PAHs on MetS. Humans are exposed to PAHs in different ways, including breathing in the polluted air, smoking, consuming PAHs through diets, and absorbing them via soil, air, or particles on the skin [16]. Two main routes of public exposure to these compounds include inhalation and dietary PAHs intake [6, 7].

Human biomonitoring is a reliable tool for measuring human exposure to chemicals, and biomarkers can be used to identify the effects in an individual organism [17–20]. In this way, exposure to new chemicals, trends and changes in rates of exposure, exposure distribution among the general population, vulnerable groups and populations at higher exposure, as well as environmental hazards in contaminated areas can be identified. Human biomonitoring programs are often used for metals, phthalates, dioxins, pesticides, volatile organic compounds, and polycyclic aromatic hydrocarbons [20, 21]. Biomonitoring has many advantages compared to environmental monitoring because the total amount of internal dose from all pathways is measured in this method [22]. Different matrices such as urine, nails, hair, and breast milk are used depending on the type of pollutant and the detection limit (LOD) of the analysis method in biomonitoring [23]. Urine is the most common matrix used in human biomonitoring programs [24]. When PAHs enter the human body and after biological transformation processes, they are metabolized through two stages [25] and mainly excreted through urine or stool as hydroxylated metabolites [26, 27]. Thus, these urinary metabolites have been designated as an exposure biomarker or as the substitutes for assessing the exposure. 1-hydroxypyrene (1-OHP) has been utilized as the most usual metabolite among them [16, 28]. Other metabolites used to measure the exposure to PAHs include 1-naphthalene (1-OHNap), 2-naphthalene (2-OHNap), 2-fluorine (2-OHFlu), 3-fluorine (3-OHFlu), and 9-phenanthrene (9-OHPhe) [29].

The global MetS prevalence has been assessed to be around one-fourth of the global population. Put differently, more than one billion individuals experience this syndrome globally [30]. Various studies showed that the prevalence of MetS was about 15.5% in China [31] and 35% in the US [30]. The corresponding value in Iran has been reported to be about 25% of the adult population [32, 33]. MetS is a chronic disease that increases the risk of many diseases, including cardiovascular disease, stroke, and type II diabetes. MetS can be diagnosed based on at least three of the five following medical conditions: high blood sugar (glucose levels >100 mg/dl), hypertension (Systolic Blood Pressure (SBP) $\geq$ 130 mmHg and Diastolic Blood Pressure (DBP) $\geq$ 85 mmHg), abdominal obesity (Waist Circumference (WC) > 102 and 88 cm in males and females, respectively), high serum Triglyceride (TG levels > 150 mg/dl), and High Density Lipoprotein Cholesterol (HDL-C < 50 and 40 mg/dl for males and females, respectively) [34].

As PAHs are mutagenic and carcinogenic [35] they may affect on the prevalence of MetS in exposed individuals in different age groups [15, 36, 37]. Although some researches have been conducted to determine the exposure to PAHs and their health effects, such as diabetes, obesity, and dyslipidemia, in different age groups [38–41], limited data are available to reflect the effects of all urinary OH-PAHs on all MetS parameters. Thus, various urinary OH-PAHs are considered as useful biomarkers for evaluating the prevalence of MetS among the general population. Additionally, this issue has not been completely examined through the simultaneous measurement of exposure biomarkers and all components of MetS. Furthermore, limited epidemiological studies have been performed to evaluate these issues in Middle Eastern countries. The current study aimed to determine the exposure of the general population of Shiraz, Iran to PAHs through measurement of the urinary OH-PAHs and to evaluate the association between exposure to these compounds and the prevalence of MetS and blood cell levels.

Materials and methods

Study population, design, and sampling

This cross-sectional study was performed on adult population of Shiraz in autumn 2020. Shiraz is the capital of Fars province (29°36′37″ N and 52°31′52″ E; 1486 m above the sea level) and the fifth megacity in Iran, with a community over 1869,000 according to the 2016 census and about 17,889 ha. This city is restricted on the west and north by Zagros Mountains. The climate of this city is classified as a cold semi-arid. In the recent years, the air quality of Shiraz has deteriorated due to rapid population growth, increase in fuel consumption, increase in gasoline use, increased utilization of diesel motor vehicles, excessive emissions from their exhausts, and insufficient infrastructures. Besides, industrial development and changes in people's lifestyles have led to changes in dietary habits. Hence, higher amounts of PAHs can be generated through food processing and cooking at high temperatures, such as drying, smoking, grilling, and roasting [42], which can eventually enter human bodies.

The following formula was used to determine the sample size.

$$N = \left(\frac{Z\alpha + Z\beta}{C(r)}\right)^2 + 3 \qquad \text{(Formula1)}$$

Where,

N   number of samples
Cr   regression correlation

Zα 95% confidence level which is equal to 1.96
Zβ power of study which is equal to 80%
β error rate equal to 20%

Sampling was done randomly from the 200 adult population (aging 30–50 years). By referring to the SIB system (an integrated health system under the supervision of the Vice Chancellor for Health of Shiraz University of Medical Sciences), 200 national codes of Shiraz residents were randomly selected and their contact numbers were provided. Then, the selected people were contacted and asked to attend a medical diagnostic laboratory to perform the relevant tests on a specific day. First-morning void samples were gathered at the beginning of the day. These samples were gathered in polypropylene tubes with the volume of 50 ml covered in the aluminum foil that included the date and the ID code of the participants. Then, the samples were immediately transferred to the laboratory at about 0–4 °C and were stored at −18 °C until analysis. The inclusion criteria of the study were A) fasting for at least 12–14 h, B) presence in the town three days before the test, C) not having consumed acetaminophen, adult cold pills, and nutrient supplements (vitamins, minerals, iron, etc.) at least three days before the test, D) not consuming the drugs that affect the metabolism of glucose, lipids, and blood pressure such as steroids, nonsteroidal anti-inflammatory drugs, male or female hormones, thyroid hormone, aspirin, and dipyridamole, E) having no history of chronic diseases such as hypertension, heart, lung, kidney, liver, and thyroid diseases, and recurrent chronic diarrhea, and F) not having the history of occupational exposure to PAHs.

Before the tests, all participants were requested to sign written informed consent forms. The research was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (ethical code: ir.sums.rec.1397.157).

Questionnaire and physical examination

To collect the participants' socio-demographic information, activities, PAHs dietary intake, and smoking history, a questionnaire consisting of 58 questions was given to each person on the day of sampling, which contained information such as age, lifestyle, characteristics of the living environment, occupational exposure, eating habits, Environmental Tobacco Smoke (ETS), and the length of time spent outdoors. The information obtained from this questionnaire was used to examine other effective factors in the increase of OH-PAHs levels in the body. The individuals were asked to enter the information about the past 72 h.

All participants also participated in face-to-face interviews. Blood and urine samples were also collected on the same day. Anthropometric measurements, including body weight, height, and WC, were performed on the sampling day, as well. Weight and height were measured in accordance with the National Health and Nutrition Examination Survey (NHANES) procedure with minimum clothing and without shoes [43]. Blood pressure was measured on the right hand after ten minutes of rest (OMRON, M2 Japan). Blood pressure was measured twice with a ten-minute interval and the mean value was considered as the final blood pressure. Complete Blood Count (CBC) and blood biochemical parameters, including TG, Total Cholesterol (TC), HDL-C, Low-Density Lipoprotein Cholesterol (LDL-C), and Fasting Blood Sugar (FBS), were measured by an automated biochemistry analyzer (PocH-100i, Sysmex, Kobe, Japan) in the clinical laboratory. Finally, the risk of MetS was evaluated according to the NCEP-ATP III criteria [34].

Measurements of urinary OH-PAHs

The samples were analyzed for OH-OHP at the Department of Environmental Health laboratory, Shiraz University of Medical Sciences. In doing so, 12 ml of sodium acetate buffer (0.1 M and pH = 5) was added to 6 ml of the sample. Hydrolysis was performed by adding 80 µl of β-glucuronidase/arylsulfatase to the solution in a water bath at 37 °C for five hours. Then, the mixture was placed on a shaker at 3000 rpm for 15 min, and the metabolite was separated. The separated supernatant was put in a GC vial, and finally, 3 ml of this supernatant was injected into the Gas Chromatography–Mass Spectrometry (GC-MS) device (Agilent, 7890, USA) equipped with the HP5-MS capillary column to measure OH-PAHs. In addition, bisphenol A solution was used as an internal standard to obtain a recovery percentage, which was initially added to the urine samples at a specified concentration. In this study, five urinary metabolites, including, 1-naphthalene (1-OHNap), 2-naphthalene (2-OHNap), 2-fluorine (2-OHFlu), 1-hydroxypyrene (1-OHP), and 9-phenanthrene (9- OHPhe), were measured [29].

The creatinine level in the samples was determined immediately after sampling according to the Jaffe method using a spectrophotometer. Finally, the concentrations were expressed in nanograms per gram of creatinine (ng/g creatinine) and nanograms per mmol of creatinine (ng/mmol creatinine). Creatinine was measured to adjust the effect of urine dilution in different individuals.

Quality control and quality assurance

When the device is set up, a few number of samples were prepared and injected into the GC-MS device to make sure that they are readable. In aggregate, ten samples were made with various concentration (from 10 to 10×10^6 ng/L) through solvating the OH-PAHs standards in dichloromethane. Then, the calibration curve was planned. The Limit of Detection (LOD) was considered as the blank sample's mean

concentration, plus three Standard Deviations (SDs). The Limits of Quantification (LOQ) were specified to be six or ten times the SDs of the blank determinations mean. Values less than the LOD were considered to be half of the LOD. The instrumental LOD, LOQ, Relative Standard Deviation (RSD), and R^2 have been listed in Table 1.

Using the spike sample and the duplicate sample methods, the quality control was done. Specific volumes of the standard solution were added to a sample in the spike sample method. Consequently, the recovery rates for compounds ranged from 73 to 92%. Notwithstanding, out of every ten samples was prepared twice and analyzed in the same way as the duplicate sample method.

Statistical analysis

SPSS version 21 and R software package for Windows were used for all analyses. Descriptive analysis was performed using median, mean, and SD. The normality test (Kolmogorov-Smirnov) was conducted to test the data distribution. Since the excretion of urinary OH-PAHs had a non-normal distribution, the OH-PAHs levels were log-transformed and then statistical analysis were done. Independent t-tests, ANOVA, and Pearson's correlation analysis were used where appropriated. In addition, linear regression models were applied to determine the relationship between PAHs exposure and MetS indicators (i.e., FBS, SBP, DBP, TG, TC, HDL-C, and LDL-C). Both unadjusted and adjusted regression analyses were used for gender.

Results and discussion

Characteristics of the participants

Demographic characteristics and clinical visit results of the participants have been shown in Tables 2 and 3. According to Tables 2, 90 participants (45%) were male and 110 (55%) were female, and their mean age was 40.16 ± 6.89 years. In addition, 85% of the participants were non-smokers and 32.5% were exposed to ETS. Moreover, 68%, 23.5%, and 17.5% of the participants consumed fried, grilled, and burnt food, respectively at least once a week. The results presented in Table 3 showed a significant difference between males and females with regard to Red Blood Cell (RBC), platelet, and TG values ($p < 0.05$).

Distribution of urinary OH-PAHs levels

The distribution of creatinine-corrected urinary OH-PAHs has been presented in Table 4. Accordingly, the mean concentrations of 1-OHNap, 2-OHNap, 2-OHFlu, 9-OHPhe, and 1-OHP were 639.8, 332.1, 129, 160.3, and 726.9 ng/g creatinine, respectively. In addition, among the metabolites, the highest mean concentration were related to 1-OHP (726.9 ng/g creatinine) followed by 1-OHNap. The lowest concentrations was related to 2-OHFlu. The short half-life of 1-OHP (mean about 18–20 h) that results in the rapid excretion of urine might be a reason for the domination concentration [44]. In many studies, 1-OHNap has been introduced as the predominant metabolite [4, 45, 46], because it is rapidly excreted through urine due to its low molecular weight [46]. Motorykin et al. also demonstrated that the concentration of 1-OHP was higher than that of other species [47].

The levels of urinary OH-PAHs were also reported in terms of ng/mmol creatinine and a similar trend was observed for the concentration of urinary metabolites. The mean concentration of urinary ΣOH-PAHs was 223.1 ng/mmol creatinine, which was almost similar to the concentrations reported by Hoseini et al. in Tehran [48]. However, the OH-PAHs concentration was lower than that reported by other researchers [49–51] and was significantly lower compared to the survey performed in the United States [36]. On the other hand, Shahsavani et al. conducted a research on the biological monitoring of PAHs between school children in Shiraz and indicated that the mean 1-OHP concentration was 1460 ng/g creatinine, which was higher than those reported in present study [16]. The higher concentration could be related to the higher susceptibility of children to PAHs in comparison to adults [52].

The distribution of urinary OH-PAHs (ng/mmol createnine) in the study participants has been depicted in Fig. 1. The results demonstrated a significant difference between males and females only regarding the urinary level of 1-OHNap ($p < 0.001$).

The distribution of the studied factors and their relationship with 1-OHP and other OH-PAHs have been shown in Table 5.

Table 1 The LOD, LOQ, RSD, R^2 and % Recovery of the urinary OH-PAHs

Compound	LOD (ng/g)	LOQ (ng/g)	RSD	R2	% Recovery
1-hydroxy naphthalene	0.15	0.5	8.9	0.991	73
2-hydroxy naphthalene	0.18	0.60	6.2	0.996	79
2-Hydroxy Florene	0.21	0.69	3.8	0.994	86
9-Phenanthrenol	0.20	0.66	4.6	0.991	92
1-Hydroxy Pyrene	0.21	0.69	5.1	0.997	88

Table 2 Characteristics of the study participants (n = 200)

Characteristics	Questionnaire
Age (years, mean±SD)	40.16±6.89
Sex (male/female, n, %)	90/110 (45/55)
Education (≤diploma, bachelor's and master, >master, n, %)	103/89/8 (51.5/44.5/4)
Active smoker (yes/no, n, %)	30/170 (15/85)
Passive smoker (yes/no, n, %)	65/135 (32.5/67.5)
Outdoor time in 48 h (hours, mean±SD)	3.24±1.10
Outdoor time in 24 h (hours, mean±SD)	8.18±5.21
Transportation device (bus/taxi/motorcycle/metro/private Car, n, %)	35/42/12/25/86 (17.5/21/6/12.5/43.5)
Living traffic status (low/median or high, n, %)	94/106 (47/53)
Eating fried food frequency (time in a week, never/once/twice/three times and more, %)	42/68/53/37 (21/34/26.5/18.5)
Eating barbecue food frequency (time in a week, never/once and more, %)	153/47 (76.5/23.5)
Eating burnt food frequency (time in a week, never/once and more, %)	165/35 (82.5/17.5)
Exposure to fire (yes/no, n, %)	24/176 (12/88)
Sleeping quality (very good/good/bad or very bad, n, %)	34/126/40 (17/63/20)
Sleeping time (hours, mean±SD)	7.28±2.12
Family life quality (quiet/relatively quiet/sometimes tense/often tense, n, %)	31/83/64/22 (15.5/41.5/32/11)
Metabolic Syndrome (yes/no, n %)	52/148 (26/74)

Based on the results, older, shorter, and less educated people, smokers or those exposed to ETS, individuals who lived in medium to heavy traffic areas, near a bus station, parking lot, restaurant, or repair shop or spent more time outdoors, as well as those who consumed more dietary PAHs were PAHs exposed more than others and had 1-OHP higher levels. The results also showed that high exposure to PAHs could significantly reduce hemoglobin levels.

Figures 2, 3, 4, demonstrated the distribution of OH-PAHs by traffic status, dietary PAHs intake and smoking, respectively. Among the measured urinary OH-PAHs, 1-OHP is the most usual metabolite. Indoor sources of pollution, such as tobacco or cigarette combustion and ETS, significantly increase the urinary 1-OHP [1, 53]. According to the reports, about 0.1 mg benzo[a]pyrene and 0.5 mg pyrene are generated through smoking one cigarette [54]. The results of epidemiological researchs have shown that ETS exposure affects the health of the respiratory system and leads to acute and chronic diseases of the respiratory system [16]. In this study, smoking status (active or passive), total number of cigarettes smoked in active smokers (p = 0.006), and time of ETS exposure (p = 0.016) were significantly associated to the urinary 1-OHP concentrations. Previous studies also confirmed the effect of the number of cigarettes smoked [55], smoking habits, and ETS on increasing the urinary 1-OHP concentration [16, 56].

The results of current study indicated a relationship between the dietary PAHs intake and higher urinary 1-OHP concentrations. Correspondingly, a significant increase in the concentration of the urinary 1-OHP (p = 0.003) was considered in participants with high contents of PAHs in their daily diets. Many researches realized that daily diets and consuming burnt food or grilled meats can lead to an increase in the concentration of 1-OHP [57, 58]. As a matter of fact, diet has been announced as the most critical factor (approximately 90%) for exposure to PAHs [16]. Fried and grilled dishes comprise most of the Iranian diet, one of which is kebab or the grilled meat. Due to the incomplete combustion of charcoal, high amounts of PAHs can be produced as a result of grilling the meats while making kebab [59].

Traffic is yet another potential source of PAHs exposure. The current study findings demonstrated that the 1-OHP concentration was higher in the individuals who lived in a higher level of ambient air pollution and higher congestion of traffics. These results were agree with previous studies in China [52] and Mongolia [57], which showed that traffic emissions could increase urinary 1-OHP levels in exposed population. Moreover, urinary 1-OHP levels were higher in the people who had spent more time outdoors in the last 72 h or lived near restaurants, parking lots, car repair shops, and bus stations.

These results may be partly associated with the relatively higher PAHs exposure because of the higher ambient air pollution produced from these shops. Some researches in developing countries have also exhibited a higher risk of PAHs exposure resulted by the emissions from the vehicle [51, 60].

Distribution of MetS and association between the OH-PAHs levels and risk of MetS

The results of the participants' clinical visits have been shown in Table 2. Accordingly, only the mean level of TG was

Table 3 Clinical visit and distributions of selected parameters in study participants (n = 200)

Clinical visit	sex	Mean	Std. Deviation	P value
Height (cm)	male	174.66	6.65	0.141
	female	159.43	5.98	
Weight (kg)	male	81.41	12.13	0.630
	female	69.69	12.02	
W.C (cm)	male	91.34	8.81	0.740
	female	83.03	9.38	
SBP (mm Hg)*	male	12.39	1.29	0.325
	female	11.38	1.41	
DBP (mm Hg,)*	male	8.41	1.05	0.849
	female	7.86	1.02	
Heart Beat (n)	male	72.26	11.11	0.308
	female	77.04	9.64	
W.B.C (mm^3)*	male	7062.22	1704.82	0.725
	female	6801.81	1487.80	
R.B.C (Mill/mm^3)*	male	5.55	0.56	0.023
	female	4.83	0.430	
Hemoglobin (gm/dl)	male	15.71	1.37	0.074
	female	13.46	1.11	
Platelet (%)	male	238,144.44	48,600.07	0.049
	female	260,209.09	61,928.05	
FBS (mg/dl)*	male	104.20	35.22	0.181
	female	97.60	19.60	
TG (mg/dl)*	male	159.57	83.63	0.028
	female	127.76	74.10	
TC (mg/dl)*	male	184.98	39.10	0.359
	female	181.84	34.76	
HDL-C (mg/dl)*	male	41.55	5.56	0.633
	female	41.73	6.13	
LDL-C (mg/dl)*	male	115.72	27.19	0.543
	female	111.94	24.29	
TG (mg/dl, mean±SD, median, IQR**)		142.08±79.92, 116 (85,176)		
TC (mg/dl, mean±SD, median, IQR)		183.26±36.72, 180 (157.25,205.75)		
FBS (mg/dl, mean±SD, median, IQR)		100.57±27.86, 96 (91,103)		

* WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; WBC, white blood cell; RBC, red blood cell; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; FBS, fasting blood sugar; **IQR, interquartile range for general population

significantly higher in males compared to females. Nevertheless, there was no evidence of any significant difference between the two groups considering other parameter means. Based on the criteria of NCEP-ATP III, the MetS prevalence was 26% [34], which was not significantly different between men and women (p = 0.402, CI = −0.175, 0.070).

The associations between the MetS components and other factors and urinary log-transformed levels of OH-PAHs have been presented in Table 6. Based on the results, people with higher levels of OH-PAHs had significantly higher TG levels, SBP, DBP, and WC, larger number of White Blood Cells (WBC) and RBC, and higher heartbeat. They also had lower levels of HDL-C, but the relationship was not statistically significant. The OH-PAHs were significantly related to the three of the five mentioned medical conditions abut MetS (blood pressure, Waist Circumference and high serum Triglyceride). This implied that urinary OH-PAHs could affect some MetS parameters, and individual exposed to these compounds can increase the MetS prevalence. The results presented in Table 4 also indicated that exposure to increased levels of urinary metabolites could significantly reduce hemoglobin levels and ultimately affect the prevalence of anemia.

PAHs have been identified as being of most significant concern about potential exposure and adverse health effects on humans. Thus, they threaten the health and well-being of humans in a seriously. In the present study, various urinary OH-PAHs had significant deleterious associations with several health risks by considering the interfering factor of sex. However, based on the OH-PAHs and the health factor, these associations were evidently both positive and negative.

Table 4 Urinary OH-PAHs levels of study participants (n = 200)

Parameters		(1-OHNap)** ng/g cr (ng/mmol cr)***	(2-OHNap)** ng/g cr (ng/mmol cr)	(2-OHFlu)** ng/g cr/ (ng/mmol cr)	(9-OHPhe)** ng/g cr (ng/mmol cr)	(1-OHP)** ng/g cr (ng/mmol cr)	Createnine g/l
Mean		639.8 (71.8)	332.1 (37.4)	129 (14.4)	160.3 (18)	726.9 (81.5)	1.62
Std. Deviation		840.2 (94.9)	315.4 (35.7)	234.9 (26.4)	151.8 (17.1)	517.4 (58.3)	0.65
Median		440 (50)	280 (31.7)	10 (1.8)	120 (14.1)	570 (64)	1.65
Minimum		80 (10)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.25
Maximum		829 (940)	316 (360)	210 (240)	122 (140)	262 (300)	3.90
Percentiles	25	320 (35.7)	170 (19.1)	0.00 (0.00)	80 (9.1)	360 (40.4)	1.10
	50	445 (50)	280 (31.7)	15 (1.8)	125 (14.1)	575 (64)	1.65
	75	677.5 (75.8)	410 (46.5)	187 (20.3)	190 (21.3)	937.5 (104.4)	2.10
P value*		<0.001 (<0.001)	0.166 (0.139)	0.978 (0.927)	0.290 (0.222)	0.106 (0.167)	0.007

*Mann-Whitney U test was used to compare non-normal distribution of continuous variables, Grouping variable: sex

**1-OHNap: 1-hydroxynaphthalene 2-OHNap: 2-hydroxynaphthalene; 2-OHFlu: 2-hydroxyfluorene; 9-OHPhe: 2-hydroxyphenanthrene and 1-OHP: 1-hydroxypyrene

***ng/g createnine (ng/mmol createnine)

In this study, the mean ± SD of Body Mass Index (BMI) was 26.51 ± 4.08 kg/m^2. Additionally, 19.5% of the people were obese and 39% overweight. Evidently, higher OH-PAHs concentrations were significantly related to an increase in WC (non-central obesity). In addition, despite the positive correlation between BMI and OH-PAHs, this relationship was not statistically significant (Table 5).

This finding was consistent with other epidemiological researches, indicating an association between the exposure to PAHs and obesity [14, 35, 61]. PAHs are considered as Endocrine-Disrupting Chemicals (EDCs) [61]. Being

exposed to EDCs and other types of chemicals has been mentioned as a notable obesity risk factor. Such chemical can affect adipocytes through changing the metabolism or lipid homeostasis by activating the Peroxisome Proliferator-Activated Receptor (PPAR); it marks the pre-adipocyte cells in the fat tissue as different [7]. In addition, EDCs might affect adipogenesis by acting on the thyroid hormone and hindering lipolysis in adipocytes [62]. Therefore, the exposure to PAHs disables the adipose tissue lipolysis and causes a weight gain increasing and the formation of a fat mass.

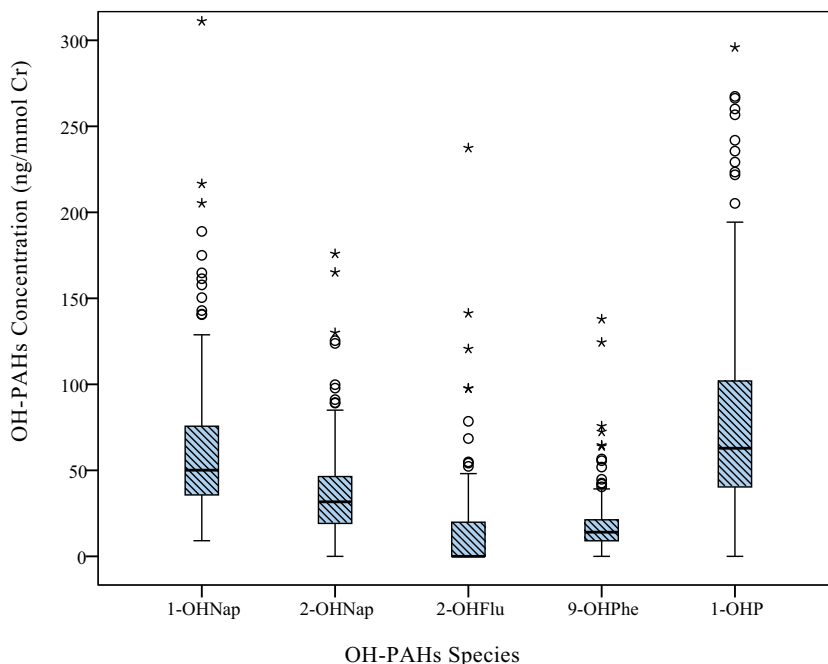**Fig. 1** Urinary OH-PAHs levels in the study participants

Table 5 Distributions of the selected parameters and log-transformed values of OH-PAHs

Parameters		1-OHNap (ng/mmol cr)	2-OHNap (ng/mmol cr)	2-OHFlu (ng/mmol cr)	9-OHPhe (ng/mmol cr)	1-OHP (ng/mmol cr)
Age[c]	Pearson C.	0.189[**]	0.096	0.200[*]	0.128	0.148[*]
	P value	0.007	0.195	0.047	0.074	0.036
Sex (male/female)[a]	0.95% CI	(−0.238) (−0.085)	(−0.175) (−0.005)	(−0.116) (0.113)	(−0.144) (0.056)	(−0.019) (0.149)
	P value	0.000	0.037	0.729	0.390	0.132
Height (cm)[c]	Pearson. C.	−0.301[**]	−0.189[**]	−0.203[*]	−0.150[*]	−0.078
	P value	<0.001	0.010	0.043	0.037	0.273
Weight (kg)[c]	Pearson. C.	0.100	0.115	0.136	0.067	0.098
	P value	0.157	0.120	0.177	0.350	0.170
BMI (kg/m2)[b]	P value	0.224	0.436	0.407	0.590	0.465
Hemoglobin (gm/dl)[c]	Pearson. C.	−0.336[*]	−0.112	−0.012	−0.041	−0.072[*]
	P value	<0.001	0.130	0.909	0.569	0.031
Education[c]	Pearson C.	−0.213[**]	−0.113	−0.017	−0.036	−0.052
	P value	0.002	0.127	0.868	0.620	0.469
number of cigarettes smoked[c]	Pearson C.	0.025	0.013	0.004	0.048	0.194[**]
	P value	0.724	0.866	0.968	0.505	0.006
time of exposure, ETS[c] (passive smoker)	Pearson C.	0.027	0.086	0.019	0.019	0.170[*]
	P value	0.708	0.246	0.848	0.789	0.016[c]
Mean outdoor time in the last 72 h[c]	Pearson C.	0.052	0.158[*]	0.054	0.058	0.167[*]
	P value	0.463	0.031	0.593	0.419	0.018
Type of Transportation device[b]	P value	0.913	0.542	0.515	0.760	0.377
Living traffic status (low/ medium or high)[a]	0.95% CI	(−0.079) (0.080)	(−0.117) (0.054)	(−0.334) (−0.699)	(−0.1680) (0.315)	(−0.074) (−0.095)
	P value	0.988	0.470	0.003	0.179	0.808
Dietary PAH intake in the last 72 h[b]	P value	0.821	0.17	0.727	0.455	0.003
Living Nearby to the bus station (yes/no)[a]	0.95% CI	(−0.119) (0.041)	(−0.065) 0.109	(−0.225) (0.047)	(−0.120) (0.082)	(0.004) (0.174)
	P value	0.343	0.622	0.197	0.713	0.040
Living Nearby to the Car parking (yes/no)[a]	0.95% CI	(−0.022)(0.139)	(−0.003) (0.170)	(−0.152) (0.139)	(−0.084) (0.120)	(0.108)(0.273)
	P value	0.157	0.059	0.928	0.724	<0.001
Living Nearby to the Restaurant (yes/no)[a]	0.95% CI	(−0.074) (0.085)	(−0.091) (0.080)	(−0.182) (0.099)	(−0.146) (0.054)	(0.010) (0.179)
	P value	0.892	0.905	0.562	0.370	0.027
Living Nearby to the car repair shop (yes/no)[a]	0.95% CI	(−0.078) (0.139)	(−0.111) (0.125)	(−0.224) (0.110)	(−0.151) (0.118)	(0.122) (0.344)
	P value	0.582	0.910	0.501	0.805	0.000
Sleeping quality[b]	P value	0.070	0.061	0.554	0.034[*]	0.468
Sleeping time in a day[c]	Pearson C.	−0.064	−0.087	−0.056	−0.016	−0.034
	P value	0.370	0.237	0.578	0.825	0.632
Family life quality[b]	P value	0.036	0.904	0.614	0.914	0.994

*correlation is significant at the 0.05 level

**correlation is significant at the 0.01 level

[a] independent sample t-test: compare differences between the means of two groups based on log transformed OH-PAHs

b ANOVA: compare differences between the means of two or more groups (category variables) based on log transformed OH-PAHs.

[c] Pearson correlation based on log transformed OH-PAHs

According to the regression analysis results, a significant association was observed between hypertension and PAHs (Table 5). Accordingly, higher OH-PAHs concentrations (all five type) were related to a higher hypertension risk (both

Fig. 2 The box plot of urinary OH-PAHs levels distribution by smoking status
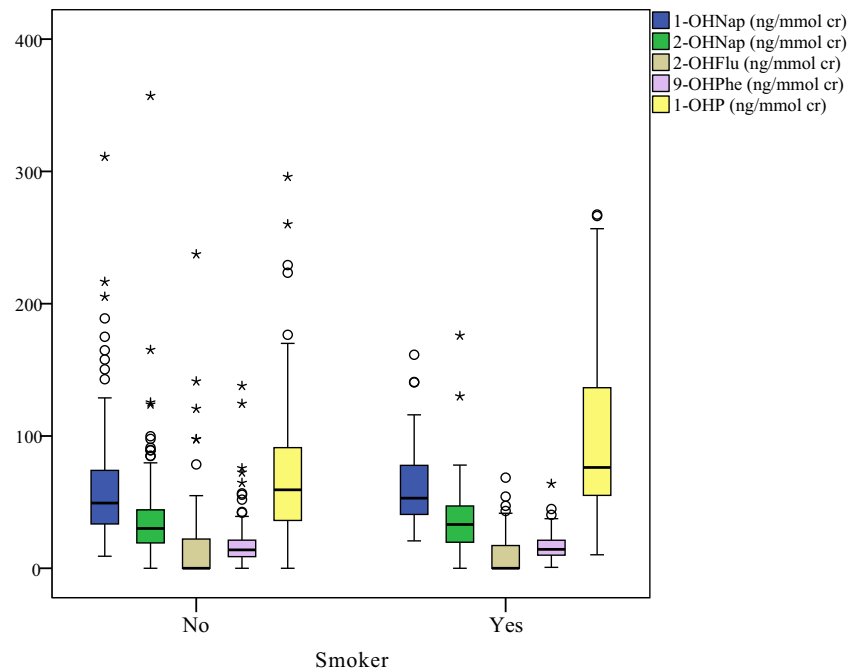
systole and diastole) after modifying the confounding variable; i.e., sex. Numerous studies have found that air pollutants, such as PAHs, tended to increase the risk of hypertension [37, 63, 64], which was consistent with the present study findings. According to the reports, oxidative stress caused by various pollutants also significantly affected hypertension. [13]. Oxidative stress causes vasoconstriction, endothelial damage, and plaque promotion in arteries. It can also act on the autonomic nervous system and significantly influence blood

pressure, which may explain the research findings [13]. A study in rodents suggested that PAHs may cause carcinogenesis and atherogenesis through inflammation and increasing the plaque size [65]. Additionally, this association may be modified by the imbalance of the autonomic nervous system and the arterial vascular dysfunction or vasoconstriction [66].

According to NCEP, dyslipidemia has been defined as serum TG levels ≥200 mg/dl, TC ≥ 200 mg/dl, and HDL-C < 35 mg/dl for males and < 40 mg/dl for females [67]. The

Fig. 3 The box plot of urinary OH-PAHs levels distribution by different dietary PAHs intake
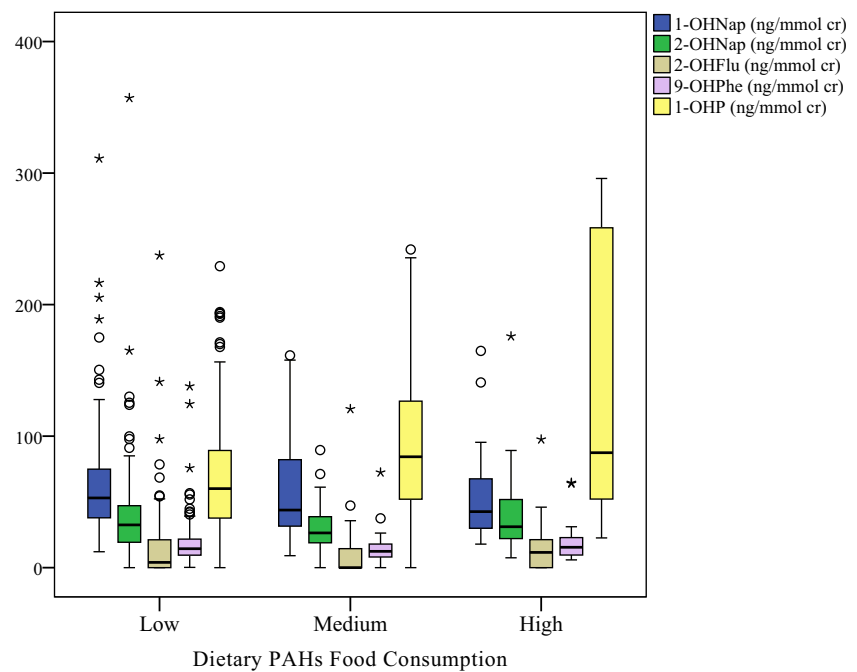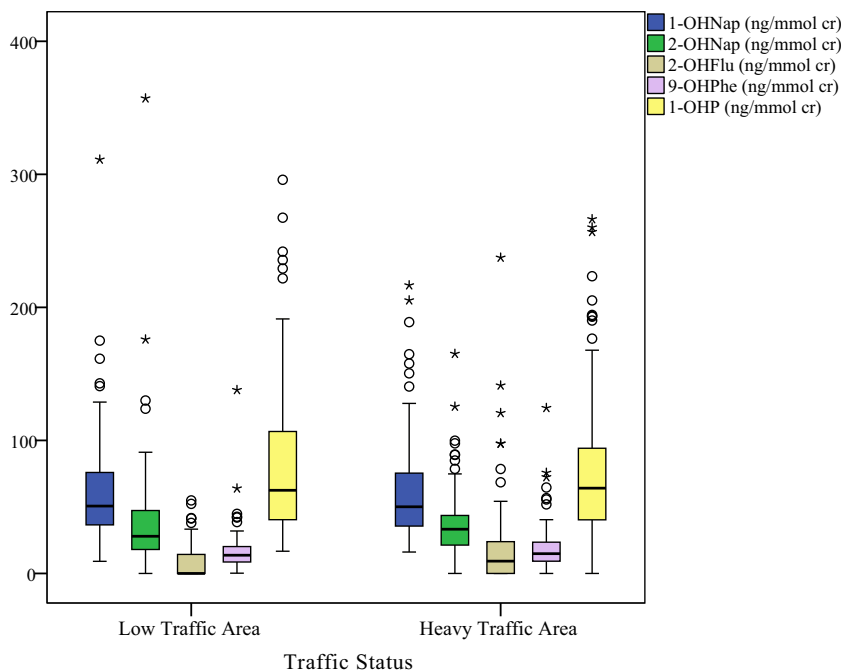
Fig. 4 The box plot of urinary OH-PAHs levels distribution by traffic status

results of this study suggested significant association between the levels of TG and OH-PAHs. They also pointed to a significant positive association between 1-OHP and the level of TC. Furthermore, there was a negative association between the levels of HDL-C and OH-PAHs. However, this association did not have any statistical value. These results were in line with the results of a study done previously, since both indicated that PAHs were related to a disadvantaged lipid profile [65, 68]. Another research argued that the high fluorene biomarkers levels are associated with unfavorable

levels of HDL-C [68]. The fundamental mechanisms related to the effects that exposure to air pollutants, like PAHs, has on HDL–C are yet to be specified. Based on an experimental research, the enzymatic activity of paraoxonase may have an effect related to this. [69]. Furthermore OH-PAHs might affect dyslipidemia by increasing the adipose tissue mass through the cessation of lipolysis β-adrenergic stimulation [70]. Evidently, the increasing of adipose tissue mass affects the insulin resistance, dyslipidemia, and systemic metabolic homeostasis, increasingly [71]. Moreover, several studies have

Table 6 The associations between MetS components and other factors and urinary log-transformed levels of OH-PAHs

parameters	1-OHP (ng/mmol cr)			1-OHNap (ng/mmol cr)			2-OHNap (ng/mmol cr)			9-OHFlu (ng/mmol cr)			1-OHPhe (ng/mmol cr)		
	R	Adj. R[b]	P value	R	Adj. R	P value	R	Adj. R	P value	R	Adj. R	P value	R	Adj. R	P value
FBS [a]	0.013	0.003	0.251	0.084	0.003	0.249	0.023	0.013	0.094	0.014	0.004	0.243	0.017	0.007	0.168
TG [a]	0.069	0.060	<0.001	0.040	0.030	0.017	0.042	0.032	0.014	0.043	0.033	0.012	0.044	0.034	0.011
SBP [a]	0.150	0.141	<0.001	0.156	0.147	<0.001	0.120	0.111	<0.001	0.123	0.114	<0.001	0.122	0.114	0.001
DBP [a]	0.070	0.061	<0.001	0.076	0.067	<0.001	0.065	0.055	0.001	0.101	0.920	<0.001	0.079	0.070	<0.001
HDL-C [a]	0.025	−0.015	0.082	0.000	−0.009	0.930	0.003	−0.006	0.704	0.023	−0.013	0.092	0.014	−0.004	0.234
LDL-C [a]	0.011	0.001	0.320	0.012	0.002	0.285	0.006	−0.003	0.540	0.005	−0.004	0.566	0.006	−0.003	0.535
W.C [a]	0.178	0.170	<0.001	0.176	0.168	<0.001	0.172	0.163	<0.001	0.175	0.167	<0.001	0.171	0.163	<0.001
TC	0.009	0.001	0.408	0.004	−0.005	0.623	0.003	−0.006	0.701	0.003	−0.006	0.699	0.004	−0.005	0.643
W.B.C	0.031	0.022	0.041	0.036	0.026	0.025	0.026	0.016	0.072	0.016	0.006	0.202	0.029	0.019	0.041
R.B.C	0.355	0.348	<0.001	0.357	0.351	<0.001	0.353	0.347	<0.001	0.378	0.372	<0.001	0.366	0.359	<0.001
Heart Beat	0.054	0.044	0.004	0.051	0.041	0.005	0.050	0.041	0.005	0.055	0.046	0.003	0.052	0.043	0.004

[a] MetS components

[b] Adjusted model: linear regression models adjusted for gender

shown that exposure (directly or indirectly) to certain pollutants, such as PAHs, could increase the concentration of harmful elements in the blood, which could potentially increase the risk of cancer (e.g., leukemia) and other inflammatory diseases [46, 72, 73].

As shown in Tables 5 and 6, a significant positive association was observed between OH-PAHs and number of RBC. Besides, 1-OHNap, 1-OHPhe, and 1-OHP had a significant positive effect on increasing the number of WBC. The results presented in Table 5 also indicated a significant decrease in hemoglobin level due to an increase in the levels of 1-OHNap and 1-OHP. These results were consistent with the previous studies disclosing that direct contact with industrial pollution increased the number of white [46, 72] and red blood cells [72]. However, some researches have obtained different results [74]. A study done in 2015 demonstrated that benzene exposure reduced the number of RBC, but had no effects on the number of WBC [75]. The difference between the results could be due to the difference in the nature of the pollutants. Another research indicated that exposure to low-dose benzene caused a decrease in the number of WBC [76]. The fundamental mechanism of the blood cell interaction with air pollution is yet to be revealed. One mechanism might be that PAHs lead to an abnormal bone marrow function, which causes an abnormal increase in the production of WBC and RBC and a decrease in hemoglobin level.

Current study faces some restrictions that must be mentioned. First and major, this research was a cross-sectional study. Whole data were collected at a certain time, the causal relationships between the study parameters and exposure to PAHs could not be determined. In addition, only five OH-PAHs were considered although there are several OH-PAHs metabolites that may cause MetS.

Ultimately, despite the consideration of various potential confounders, other confounders, like genetic factors, were not considered. Current study, however, had several strengths. Current study evaluated five different OH-PAHs in the adolescents (30–50 years old) as an active group. On the other hand, the novelty of the current study was determining the association between OH-PAHs and MetS and blood cell risk factors, which has never been done in Shiraz, Iran. In addition, all multivariate statistical models were adjusted for sex. Furthermore, high concentrations of these metabolites and high prevalence of MetS (in comparison with other researches) can be an alarm accounting for the demand for more stringent laws to be imposed on PAHs compounds in developing countries.

Conclusion

The mean concentration of urinary ΣOH-PAHs in the present study was similar to the concentrations reported in Tehran, Iran, but was lower than those reported in the United States and China. The results indicated that the total number of cigarettes consumed, ETS exposure, dietary PAHs intake, and living in high traffic areas near restaurants, parking lots, car repair shops, and bus stations had a significant positive effect on urinary OH-PAHs and were the major predictors of exposure to PAHs among the participants. Moreover, the prevalence of MetS was 26% according to the NCEP-ATP III criteria, which was higher than the values reported in China and lower than those found in the United States. The study findings demonstrated that some OH-PAHs were associated with a greater risk of MetS parameters. Adults with high levels of OH-PAHS might have a greater risk of obesity, hypertension, and dyslipidemia, higher WBC and RBC levels, and higher heartbeat.

Regarding the harmful PAHs effects on humans' health, broader researches are needed in order to found the sources that help the creation of PAHs and find methods for decreasing the emissions of this compound into the environment. Future studies should also investigate the way that the physiological mechanisms related to every biomarker affects the human health.

Authors' contributions Samaneh Shahsavani: Data curation, Writing-Original draft preparation, Writing-Reviewing and Editing.
   Mansooreh Dehghani: Supervision, Conceptualization, Reviewing and Editing, Project administration.
   Mohammad Hoseini: Supervision, Methodology, Reviewing and Editing, Project administration.
   Mohammad Fararouei: Software, Validation.
   Mahmood Soveid: Investigation.

Data availability   Not applicable.

Declarations

Ethics approval   The Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran, approved the research (ethical code: ir.sums.rec.1397.157).

Consent to participate   All of the authors have read and approved the paper and they are consent to participate.

Consent for publication All of the authors have read and approved the paper and they are consent to publication.

Conflicts of interest/competing interests None declared.

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