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RESEARCH ARTICLE

Monitoring of urinary arsenic (As) and lead (Pb) among a sample of pregnant Iranian women

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Abstract

Purpose Heavy metals, as significant toxic environmental contaminants, can cause serious adverse health outcomes on the human body even in trace concentrations. There is limited evidence on heavy metal concentrations existing in the body fluids of pregnant women. This study aims to evaluate the urinary levels of arsenic (As) and lead (Pb), as two main toxic heavy metals, among pregnant women and their lifestyle determinants.

Methods The study was performed in 2019–2020 in Isfahan, Iran. A number of 140 urine samples of pregnant women who were in their first pregnancy trimester were examined. Inductively coupled plasma optical emission spectrometry (ICP-OES) was applied to analyze the urinary concentrations of As and Pb. Socio-demographic data including age, pre-pregnancy body mass index (BMI), education status, and family income, as well as the use of cleaning products (cosmetic and household cleaning products), and lifestyle habits (food intake, smoking, and physical activity) were collected using a validated questionnaire.

Results The mean concentration of As and Pb were 8.14 ± 10.8 and 9.6 ± 7.1 μg/g creatinine, respectively. The mean urinary concentration of Pb indicated significant differences in the levels of cosmetic usage, second-hand smoking exposure, and the use of Copper, Aluminum, Teflon, Steel, and Enameled utensils for cooking (p-value < 0.05). Furthermore, the mean of urinary Pb concentrations at high levels of physical activity and scratched utensils using was significantly different from the other categories (p-value = 0.02). No significant differences were found between As and Pb concentration with other socio-demographic factors.

Conclusion The lifestyle determinants and cosmetic products use are important predictors of urinary heavy metals in pregnant women, rather than sociodemographic characteristics. Additional research is necessary to determine long-term adverse birth outcomes of exposure to these heavy metals.

Keywords Heavy metals · Prenatal · Trace element · Lifestyle · Socio-demographic

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Introduction

Heavy metals as high-density metals in comparison with organic pollutants are somewhat stable and resistant in the environment and the body tissues at trace concentration [1]. Individuals may be exposed to heavy metals via different routes such as inhalation, ingestion, smoking, and use of cleaning and cosmetic products [2, 3].

Exposure to heavy metal damage nervous system, change the oxidative cell stress, interference with glucose metabolism, and disrupt endocrine [4, 5].

Arsenic and lead are known as two toxic trace elements which cause some serious adverse health outcome on human health especially for vulnerable individuals and pregnant women [6]. Dietary intake is generally considered the most significant way of exposure to heavy metals in human beings. Arsenic in the diet originates from contaminated well water in regions nearby the waste disposal sites, incinerators, electronic manufacturers, mines, and agricultural areas that utilize fertilizer containing arsenic [7, 8]. Previous documents demonstrated that exposure to arsenic can disrupt sugar metabolism and cause gestational diabetes in pregnant women [7]. In addition, chronic arsenic exposure can elevate the risk of developmental disability, mental retardation, and newborn death [9].

Lead also enters the human body through inhalation, ingestion, or absorption through the skin and mucous membrane [10]. It can cause some serious health risks such as disrupting learning ability, growing, and development [10, 11].

Prenatal exposure to environmental toxicants such as heavy metals is a serious public health concern regarding the vulnerability of pregnant women and their fetuses [12]. Heavy metals have been measured in cord blood and amniotic fluid, demonstrating that these metals cross easily through the placenta [13]. So, maternal exposure to these toxic elements may affect fetus growth by altering the homeostatic and metabolic mechanisms [14]. Previous studies have shown increased adverse pregnancy effects when mothers are exposed to heavy metals [15]. Some epidemiological research demonstrated an association between maternal heavy metals exposure and the consequent risks such as abortion, and preterm delivery [16–18].

Numerous factors such as lifestyle and socio-demographic status can influence the exposure rate and subsequently the extent of risk on individuals [19–23]. For example, persons with low socioeconomic situations experience a greater rate of exposure [22, 24, 25]. To the best of our knowledge, no previous publication has evaluated the urinary levels of Arsenic and Lead in Iranian pregnant women with regard to their life-style and socio-demographic variables. Therefore, due to the sensitivity of pregnant women and the direct effect of exposure on birth outcomes, this study was conducted to track the urinary concentrations of Arsenic and Lead in a sample of Iranian pregnant women at their first trimester, as an indicator of exposure to heavy metal, and examine associations between prenatal heavy metals exposures with various factors. The findings of this study may also provide an insight into common sources of exposure to heavy metals among Iranian pregnant women which can help decision-makers to take the appropriate measures.

Materials and methods

Study population

This cross-sectional study was performed in 2019–2020 on pregnant women who lived in Isfahan city, Iran. Exclusion criteria included refusal to participate, history of chronic diseases [including liver and renal diseases, diabetes, malignancy, sickle cell anemia, or seropositive to Human Immunodeficiency Virus (HIV)], sleep deprivation and insomnia, depression/depressive symptomatology, stress, abortion and taking regular medications. A number of 140 women in their first trimester were selected randomly. All parents signed informed consent forms. The distribution of participants' living is demonstrated in Fig. 1. The participants were completely informed about the purposes, procedure, and also voluntary nature of the study. It is noteworthy that the ethical issues related to this study were approved by the ethics committees of Isfahan University of medical sciences. Suggested formula for estimating correlation coefficients was used to compute the sample size. Based on $\alpha = 0.05$, $\beta = 0.15$, and a correlation coefficient of $r = 0.23$ based on the previous study [26], we reached to 140 subjects. The urine samples were gathered in borosilicate containers and then transferred to the laboratory to be preserved at − 20 °C. The validated questionnaires (PERSIAN Birth Cohort questionnaires) were utilized to collect information about socio-demographic variables (maternal age, family income, education status, maternal occupation), lifestyle determinants (pre-pregnancy BMI, physical activity, smoking habit, and food habits (seafood and canned foods consumption, kind of cookware for cooking (zinc, copper, aluminum, enameled, Teflon, cast iron, steel, and glassware), and household cleaning and cosmetic product use [27].

FFQ (Food frequency questionnaire) was applied to gather data about the food intake habits of participants.

IPAQ (International physical activity questionnaire) was used to compute total physical activity (MET-minutes/week) score [28]. A validated food frequency questionnaire (FFQ) was applied to obtain information about food intake habits [29].

Measuring urinary heavy metals

A 3.0 ml of the urine was poured into a polypropylene tube containing 15 µl of 65% (v/v) HNO_3 and then kept in a refrigerator at 5 °C. Two hours before sample preparation, the urine samples were taken to room temperature. One ml of the sample was poured into a 10 ml polypropylene tube and filled up to 5.0 ml with 1.2% (v/v) HNO_3 [30]. Then, the prepared solution was injected into the ICP-OES (Varian 720/730-ES) to analyses heavy metals concentration.

Concentrations of heavy metals were adjusted using creatinine level to reduce the bias of the dilution difference between urine samples.

Quality assurance and quality control (QA/QC)

The quality assurance and quality control (QA/QC) assessments were performed to confirm the reliability

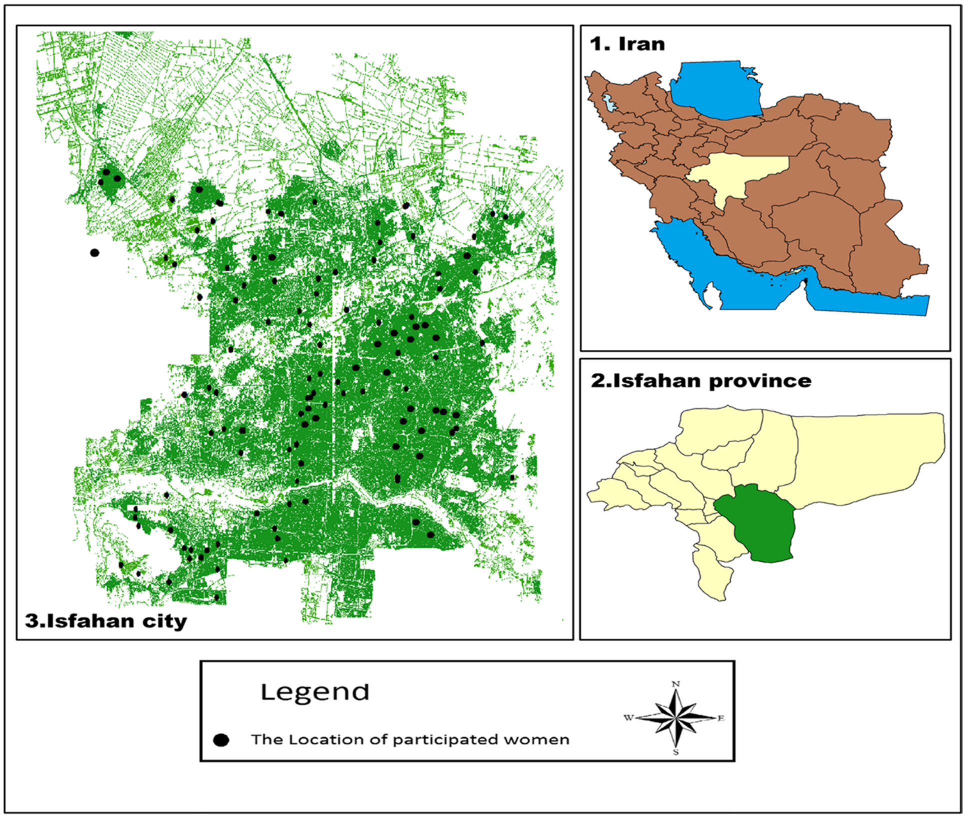

Fig. 1 The distribution of the individuals' living in Isfahan city

of the analytical data and to increase confidence in the relevance of obtained responses. Accordingly, the linear regression gave a good fit ($R^2 \geq 0.98$) with high precision (≤ 13.2% RSD). The limit of detection (LOD) and limit of quantification (LOQ) were based on the signal-to-noise ratio of 3 and 10, respectively. Furthermore, to minimize the bias of the dilution difference between the samples, the heavy metal concentrations were adjusted using creatinine levels. The R2, precision (% RSD), LOD, LOQ, and mean recovery are presented in Table 1.

Data analysis

Continuous determinants have been reported as mean ± SD, minimum, and maximum whereas categorical variables as frequencies (percent). The Kolmogorov–Smirnov test and Q-Q plot were utilized to specify the normality of continuous data. Appropriate transformation approaches were applied for those heavy metal concentrations with the abnormal distribution. Independent samples t-test or analysis of variance (ANOVA) was utilized to compare the mean of heavy metal concentrations across categories of possible lifestyle and demographic variables. Bonferroni post hoc test was used to determine differences between categories after rejecting the null hypothesis in the analysis of variance. All statistical analyses were conducted by SPSS software (IBM SPSS Inc., Chicago, IL, version 23). A p-value less than 0.05 was considered statistically significant.

Table 1 QA/QC parameters of heavy metals

	As	Pb
R2	0.98	0.99
RSD (%)	8	8.9
LOD (µg/L)	1	0.8
LOQ (µg/L)	3	2.4
Recovery (%)	86	100

Results and discussion

The characteristics of the studied population are demonstrated in Table 2. According to the results, 45.7% (n = 64) were less than 30 years old, 54.3% (n = 76) were ≥ 30-years old, 57.1% of women (n = 80) had overweight, while 38.6% of them (n = 54) were classified as normal-weight group, and only 4.3% of women (n = 6) were underweight. A great number of the participants had an academic education (n = 116, 82.8%). Eighty percent of subjects were householder (n = 112) and 63.6% of the total population (n = 89) categorized into the middle- income group.

Based on the obtained results, greater than 50% of pregnant women were the low user of seafood (n = 74)

Table 2 The characteristics of the studied participants

Variables	Categories	n (%)
Maternal age (years)	< 25	16(11.4)
	25–29	48(34.3)
	30–34	52(37.2)
	> 34	24(17.1)
Maternal BMI (kg/m^2)	Underweight (< 18.5)	6(4.3)
	Normal weight (18.5–23.9)	54(38.6)
	Overweight (≥ 24)	80(57.1)
Education level	Academic	116(82.8)
	High school	18(12.9)
	Less than high school	6(4.3)
Family income	Low	43(30.7)
	Middle	89(63.6)
	High	8(5.7)
Maternal occupation	householder	112(80)
	Employed	28(20)
Used Scratched utensils	No	61(43.6)
	Yes	79(56.4)
Seafood consumption	Low (4 > per month)	74(52.9)
	Middle (2–4 per week)	49(35)
	High (daily)	17 (12.1)
Used canned foods	Low (4 > per month)	81 (57.9)
	Middle (2–4 per week)	39(27.9)
	High (daily)	20 (14.3)
Used utensils for cooking		
Zinc	No	90(64.3)
	Yes	50(35.7)
Copper	No	115(82.2)
	Yes	25(17.8)
Aluminum	No	117(83.6)
	Yes	23(16.4)
Enameled	No	128(91.4)
	Yes	12(8.6)
Teflon	No	70(50)
	Yes	70(50)
Cast Iron	No	92(65.7)
	Yes	48(34.3)
Steel	No	83(59.3)
	Yes	57(40.7)
Glassware	No	133(95)
	Yes	7(5)
Cosmetic usage	Yes	88(62.85)
	No	52(37.15)
Using household cleaning products	Yes	140(100)
	No	0(0)
Smoking during pregnancy	Yes	0(0)
	No	140(100)
Passive smoking during pregnancy	Yes	71(50.75)
	Yes	69(49.28)
Physical activity	Low	66(47.1)
	Moderate	49(35)
	High	25 (17.9)

and canned food (n = 81). In addition, 50% of individuals used Teflon utensils for cooking whereas glassware (n = 7, 5%) and enameled (n = 12, 8.6%) containers had minimum consumers for cooking. After using Teflon, steel, zinc, cast iron, copper, and aluminum had respectively the highest consumers for cooking food. Furthermore, more than half of the participants (n = 79, 56.4%) stated that they used scratched containers for cooking.

Moreover, the results showed that household cleaning and cosmetic products were respectively used by 100% (140) and 62.85% (n = 88) of individuals. However, none of the individuals were smokers, about 50.75% (n = 71) were passive smokers.

The majority of the individuals were categorized into the middle (n = 49, 35%) and low (n = 66, 47.1%) physical activity group, though 17.9% of women (n = 25) were classified into the high physical activity group.

The mean, quartile, maximum, and minimum of creatinine-adjusted heavy metals concentration are given in Table 3. Accordingly, As and Pb were respectively detected in 86% and 100% of urine samples. The highest and lowest concentration values were observed with 103 µg/g creatinine and 1.1 µg/g creatinine for As and 41.5 µg/g creatinine and 0.9 µg/g creatinine for Pb.

In the present study, the mean concentration of As and Pb were found to be 8.14 ± 10.8 µg/g creatinine and 9.6 ± 7.1 µg/g creatinine which As was lower than those concentrations reported by other studies conducted in Brazil (As = 11.34 µg/g) [31], and Tokyo (As = 393 µg/g) [32], but Pb was higher than in results reported in Suadi Arabia (Pb = 5.156 ± 0.28 µg/g) [33], Spain (Pb = 4.8 ± 4.3 µg/g) [34], and Tokyo (Pb = 0.483 µg/g) [32]. The inconsistency in results can be explained by the various characteristics of the studied area, lifestyle, and socio-demographic [35]. Furthermore, some researchers announced that the levels of heavy metals detected in the blood population of many low and middle-income developing countries are much higher amount than in developed countries [36, 37].

The distribution of As and Pb concentrations in relation with some influencing determinants including education level, family income, occupation status, seafood, and canned foods consumption, use of the different type of utensils (zinc, copper, aluminum, enameled, Teflon, cast iron, steel, and glassware) and scratched container for cooking, cosmetic and household cleaning products use, smoking

Table 3 Mean concentration of heavy metals (μg/g creatinine)

Creatinine-adjusted heavy metals (μg/g creatinine)	Min	Percentile			Max	Mean ± SD
		25th	50th	75th		
Pb	0.9	5	8.4	12	41.5	9.6(7.1)
As	1.1	2.2	4.5	12	103	8.14 (10.8)

and passive smoking habit, and physical activity is given in Table 4.

Socio-demographic- predictors in urinary heavy metals in pregnant women

According to Table 4, the mean of urinary As and Pb concentrations did not show significant differences in the levels of different variables including maternal education level, family income, BMI, maternal age, and maternal occupation.

Similar results were reported by Zeng [38] and Zaw [39]. However, some studies indicated that family income is directly related to the amount of urinary heavy metals such as Cr, As, Hg, and Cd among pregnant women [40]. Education level, family income, and other sociocultural factors are somehow interdependent which can have different results in developed societies compared with developing countries [41]. A research performed among pregnant women in Myanmar demonstrated no significant difference between maternal education status and heavy metals concentration [42]. In the contrast, a study conducted in China showed that women who had lower education levels showed a higher amount of urinary Cd, Ni, Pb concentration [43]. Various results of maternal education may be rooted in that the influence of education is intensely linked with sociocultural factors [44].

The results of the current study revealed that the mean concentration of urinary As and Pb indicated no significant differences with occupation status and maternal age (p-value > 0.05).Occupational exposure is often considered a risk factor for women fertility, pre-term delivery, and also spontaneous abortion [45]. However, there are some documents that reported a direct association between heavy metal occupational exposure and the increased risk of birth outcome [46]. A recent study carried out in Nigeria revealed that the miscarriage incidence increased by 1.6% in women exposed to some heavy metals [45].

Some studies reported positive associations between heavy metals and matenal age, attributable in part to bioaccumulation [47, 48]. Kasim et al. found that Pb level increased with age, which may be correlated with the release of Pb previously accumulated in tissues [48]. However, numerous studies have been showed inverse results. Esquinas et al. indicated that the older women tended to display lower geometric mean heavy metal levels [49]. This result could be indirectly affected by other co-factors such as lifestyle, genetics, and the metabolism, and sample size which can cause different results. Further intervention studies are needed to elucidate these relationships.

Lifestyle variables- predictors in urinary heavy metals in pregnant women

The mean urinary of Pb concentrations showed significant differences in the levels of the used utensils i.e. Copper, Aluminum, Teflon, Steel, and Enameled (p-value < 0.05). However, the mean concentration of urinary As did not indicate a significant difference with the used utensils by women.

Some studies have revealed that the nature of cookware, cooking process, processing, and storage methods may raise the amount of trace metals in foods [50]. Cabrera et al. [51] reported that some cooking utensils such as Steel, Aluminum, and Teflon can release some substantial trace metals level into foods due to cooking temperatures, heating and acidic condition, damaging and corrosion of container which might consequently lead to disrupting endocrine in the human body [52].

It is must be noted that the scratched cookware increases the risk of leaching heavy metals [53]. According to the statistical results in Table 4, the mean concentrations of Pb indicated a significant difference in the level of the scratched containers for cooking (p-value = 0.02). In developing countries, the majority of people still utilize traditional containers and pots made of Copper, Enameled, and Aluminum for cooking. Unlike modern utensils, these containers do not have any protective layer of inert material to prevent contamination of food [53]**.** Weidenhameretal et al. reported that Pb can migrate from stainless steels and Aluminum utensils during cooking and then enter food at concentrations beyond the recommended public health guidelines [36]. Said revealed high migration of heavy metals with mean values of 30.3 ± 0.56 mg/l, 21.56 ± 0.12 mg/l, 28.98 ± 0.09 mg/l, 14.54 ± 0.15 mg/l, respectively when scratched fold Iron, Aluminum, stainless steel, and Teflon cookware were used [53].

Daily exposure to heavy metals via cosmetic products has been regarded as a negligible source for individuals compared to the main sources such as food, water, and the air. However, due to the cumulative property of trace metal in the human body, cosmetics can be considered as a significant source of heavy metals [54].

Table 4 The mean concentration of urinary heavy metals (μg/g creatinine) across categories of the studied variables

Variables	As		Pb	
	Mean (±SD)	p-value	Mean (±SD)	p-value
Education				
Academic	26.45 (23.8)	0.1	43.2(37.3)	0.9
High school	10.9(9.1)		10.2(8.6)	
< High school	18.2(7.4)		14.2(2.9)	
Family income				
Low	8.13(8.7)	0.9	9.5(5.7)	0.7
Middle	7.13(1.6)		9.1(2.5)	
High	7.33(5.6)		9.6(7)	
Used utensils for cooking				
Zinc				
No	9(2.1)	0.7	9.4(6.9)	0.3
Yes	8.9(0.8)		10.2(7.1)	
Copper				
No	8(1.1)	0.1	7.9(4.7)	**< 0.001**
Yes	9.3(1.5)		18.8(9.4)	
Aluminum				
No	7.9(0.9)	0.6	8.2(5.2)	**< 0.001**
Yes	9.7(2.5)		17.6(9.7)	
Teflon				
No	2(1.6)	0.9	7.5(5.3)	**0.01**
Yes	7.3(0.8)		12(7.9)	
Enameled				
No	8.2(1)	0.7	9.2(7.1)	**0.003**
Yes	8.3(2.1)		15.1(3.6)	
Cast Iron				
No	8.6(1.3)	0.4	9.4(6.3)	0.8
Yes	7.3(1.1)		10.2(8.3)	
Steel				
No	7.7(1.4)	0.3	5.8(2.5)	**< 0.001**
Yes	8.8(1.1)		15.4(7.6)	
Glassware				
No	8.1(1)	0.2	9.5(6.9)	0.1
Yes	9.9(5.4)		13.1(8.8)	
Maternal occupation				
Householder	7.7(6.4)	0.6	10.1(7.3)	0.4
Employed	26.1(9.28)		35.9(21.2)	
Used scratched utensils				
No	7.6(1.1)	0.6	8.1(6.3)	**0.02**
Yes	8.5(1.5)		10.9(7.4)	
Cosmetic use				
Yes	8.5(8)	0.08	12.2(7.3)	**< 0.001**
No	7.6(4.2)		5.1(2.3)	
Seafood consumption				
Low (4 > per month)	7.9(1.5)	0.8	9.2(6.8)	**0.07**
Middle (2–4 per week)	8.8(1.3)		8.8(5.5)	
High (daily)	7.4(1.8)		13.7(10.5)	
Used canned foods				
Low (4 > per month)	8.1(1.3)	0.8	8.8(6.5)	**0.09**
Middle (2–4 per week)	9(1.6)		9.9(6.1)	
High (daily)	6.8(1.6)		12.6(9.9)	
Passive smoking during pregnancy				
Yes	8.6(0.9)	0.07	11.1(8)	**0.01**
No	7.7(1.6)		8.3(5.7)	
Physical activity				
Low	8.4 (4.6)	0.7	7.35(44)	**0.02**
Moderate	7.6(8.1)		8.6(6.9)	
High	8.4 (6.8)		18(8)	

Highlighted value: significant p-value < 0.05

According to the obtained results, the mean concentration Pb show a significant difference in the level of cosmetic usage (p-value < 0.001).

Health Canada revealed that nearly ninety percent of cosmetic products were positively confirmed in terms of the existence of some toxic heavy metals such as Pb, Cd, Ni, and Hg [55]. These heavy metals are extremely toxic and forbidden in cosmetic products as ingredients in the US and EU [56].

A significant amount of Pb was measured by Ajaezi et.al, in skin bleaching agents [57]. Furthermore, the result of a study conducted in Isfahan, Iran indicated 0.08–5.2 μg/l concentration of Pb in lipsticks [58]. However, the Pb range was in the permissible range of FDA, it can be accumulated when used continuously.

The statistical result showed a significant difference between the mean concentrations of Pb and the women who were passively exposed to smoke (p-value = 0.01).

Chiba et al. also detected Cd, Cr, Ni, Pb in smoke/cigarettes and reported also in body fluids of individuals who were smokers. They also announced that individuals who were passively exposed to smoke can absorb more amounts of heavy metals [59]. Some researchers have announced that elevated heavy metals due to smoking and also second-hand smoking may increase spontaneous abortion risk [60], stillbirth, preterm delivery, and smaller infants weight [61].

Bonferroni post hoc test for canned food and seafood consumption showed that the mean urinary of As and Pb concentrations were not significantly different from the other two categories. However, the majority of food-based dietary guidelines insist to consume more seafood during the pregnancy period to provide sufficient iodine and DHA (Docosahexaenoic acid)- a key role in the central nervous system development of fetus [62]. There are some documents detected heavy metals in canned food and seafood. Lin et al. reported that the urinary of Hg and Cd were correlated with canned consumption ($r = 0.124$, p-value < 0.05) [40]. Furthermore, a study conducted in Iran indicated that the mean contents of As and Pb in canned foods were higher than the standard levels [63]. Additionally, Yu et al. showed the rate of fish consumption during pregnancy was correlated with a greater level of some heavy metals such as Hg and Cd in cord blood [64]. The different results may be due to the frequency of canned food and seafood consumption by individuals.

Our results indicated that the mean of urinary Pb concentrations at high levels of physical activity was significantly different from the other two categories (p-value $= 0.02$). In other words, higher Pb concentrations were detected in women who had greater physical activities.

There are only limited publications regards the effect of physical exercise on heavy metal excretion from the body fluids and tissues. In parallel with our findings, Milnerowicz et al. have indicated that a higher level of physical activity is positively associated with a higher level of urinary heavy metals and after 24 h they decrease lower than the initial value. It can be supposed that with greater physical exercise, a higher value of heavy metals permeates from blood to urine [65]. It is probable that heavy metals are additionally released from tissues to blood and urine [66]. It can be concluded that heavy physical activity changes heavy metals reserves in tissues [67]. This may be considered a positive occurrence, especially in terms of the long half-life and the range of its toxicity [65, 68].

Physical activity increases oxygen consumption, and accordingly, the generation of reactive oxygen species (ROS) raises [69]. Regular and moderate physical exercise releases oxidative stress, increases cellular adaptation, and also decreases the quantity of lipid peroxidation products developing within activity performance [68, 70]. In this condition, cells in response to the stress generate various proteins. Metallothionein (MT) is one of them. This protein arrest heavy metals therefore lessening their poisonousness. MT is known as a protein of acute-phase serve as an active free radicals scavenger [68, 71].

Strength and limitation

To the best of our knowledge, it is one of the first documents to determine the probable relation between lifestyle and socio-demographic characteristics of Iranian pregnant women with prenatal heavy metals exposure. However, we found some limitations in this investigation that can affect the findings. The medium or small size of the studied samples with typically similar lifestyle and socio-demographic profiles might affect the statistical results. However, we were able to found a significant correlation between socio-demographic and life style determinants with heavy metals concentrations. In addition, in this study, we collected spot urine samples. We were not able to collect representative urine samples (24-h urine) during the day, although this may not severely affect the total distribution of heavy metals among the studied participants. We attempted to address this by creatinine adjusting of urinary samples.

Conclusion

In this study, As and Pb were detected in 86% and 100% of urine samples of pregnant mothers. The detected mean concentration of Pb had higher values in the Iranian pregnant women's urine compared to other societies. Results demonstrated that the mean concentration of urinary Pb was higher in participants who were second-hand smokers, applied Copper, Aluminum, Teflon, Steel, Enameled, and also damaged cookware for cooking, as well as those who had higher physical activity. The lifestyle characteristics and cosmetic product usage were important predictors for urinary heavy metals in pregnant women than socio-demographic profiles.

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Declarations

Conflict of interest The authors announce that they have no conflict of interest.

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