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RESEARCH ARTICLE

Association between phthalate metabolites in human amniotic fluid and offspring birth size: a sub-study of the PERSIAN birth cohort

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Abstract

As phthalate metabolites might cross the placenta, it is possible to find them in the amniotic fluid. This cross-sectional study aims to investigate the levels of phthalate metabolites in the amniotic fluid of a sample of Iranian women and its association with neonatal anthropometric measures. The other objective was to study the potential sources of maternal exposure to phthalates. This study was conducted from June 2019 to June 2021 in Isfahan, Iran. Pregnant women were recruited from their first trimester of pregnancy and followed up until their delivery. Amniotic fluid samples were collected from those who were assigned to have delivery by cesarean section. Overall, 158 samples of amniotic fluid were collected, of which 139 samples had sufficient volume and were free of blood. Data from 142 newborns were included in this study. Four phthalate metabolites were measured using gas chromatography–mass spectrometry (GC–MS). After extraction of phthalate metabolites, 4 phthalate metabolites including mono-butyl phthalate (MBP; normal: 0.08 ± 0.79; overweight: 0.20 ± 1.11; obese: 0.07 ± 1.07; p-value: 0.405), mono-benzyl phthalate (MBzP; normal: 7.54 ± 6.69; overweight: 7.48 ± 7.16; obese: 8.67 ± 12.75; p-value: 0.729), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP; normal: 4.27 ± 6.36; overweight: 3.03 ± 8.44; obese: 3.53 ± 7.04; p-value: 0.245), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP; normal: 246.18 ± 189.80; overweight: 238.48 ± 200.23; obese: 287.65 ± 206.70; p-value: 0.723) were simultaneously detected in samples of maternal amniotic fluid. Our findings suggest that maternal exposure to phthalate metabolites is positively associated with in utero exposure of the developing fetus. The geometric means and medians of MBP, MBzP, MEOHP, and MEHHP of detected samples were 10.17 (9.52), 6.24 (3.47), 5.03 (11.72), and 174.79 (229.94) (μg/L), respectively. The median anthropometric measures of newborns were as follows: weight 3171.8 g, height 49.6 cm, head circumferences 34.9 cm, chest 33.3 cm, hip 31.5 cm, and arm circumferences 10.8 cm. There was no statistically significant association between phthalate metabolites concentration and newborn's anthropometric measures ($p > 0.05$). Future studies should focus on the collection of amniotic fluid at different trimesters and the corresponding maternal samples to better characterize the association and health impacts of these endocrine-disrupting chemicals during fetal development.

Keywords Amniotic fluid · Phthalate metabolite · Birth outcome · Pregnant women

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Abbreviations

GC-MS	Gas chromatography–mass spectrometry
MBP	Mono-butyl phthalate
MBzP	Mono-benzyl phthalate
MEOHP	Mono(2-ethyl-5-oxohexyl) phthalate
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
MSTFA	N-Trimethylsilyl-N-methyl trifluoroacetamide
IUMS	Isfahan University of Medical Sciences
DLLME	Dispersive liquid–liquid micro extraction
BMI	Body Mass Index
BIC	Bayes Information Criterion
LOD	Limit of detection
LOQ	Limit of quantification

WHO World Health Organization
LCA Latent class analysis

Introduction

Exposure to phthalates as short half-live chemical compounds is now challenging for classifying episodic exposures to them in epidemiological studies. Diesters of phthalic acid or phthalates are widely used in some consumer products including solvents, personal care products, food packaging, toys, paints, building materials, and additives in polyvinyl chloride plastics (Latini 2005). In 2006 and 2015, the production of different types of phthalates was reported 4.7 and 8.0 million metric tons per annum, respectively, and certainly, at the present time, this amount is more than the previous report (Mackintosh et al. 2006; Net et al. 2015). Up to now, more than twenty-five phthalates were applied in different materials, and commonly ten types of them are used more. Figure S1 shows the chemical structure of phthalates and their metabolites studied in literature.

Phthalates are one of the classes of endocrine disruptive compounds, and human exposure to phthalates was monitored in the different matrices such as semen (Specht et al. 2014), urine (Dong et al. 2017; Silva et al. 2007), breast milk (Högberg et al. 2008; Main et al. 2006), and serum (Chen et al. 2017; Nassan et al. 2016). Very recently, we reviewed the association of exposure to phthalates with cardiometabolic risk factors and precocious and delayed pubertal timing in girls and boys in the two separate systematic reviews and meta-analyses (Golestanzadeh et al. 2019, 2020). It is important to describe that one of the important effects of exposure to phthalates is on fetal and child development (Kuo et al. 2013, Le Moal et al. 2015, Sharpe and Skakkebaek 2008, Swan 2008). Therefore, the European Union and the US banned the application of 6 types of phthalates in children's toys (europa.eu/rapid/press-release_IP-05-838_en.htm and www.cpsc.gov/Regulations-Laws--Standards/Statutes/The-Consumer-Product SafetyImprovement-Act). Hence, the exposure to phthalates during pregnancy and the association of phthalates with the birth outcome is one of the important problems that must be more explored today. We searched on the Web of Science (clarivate analytics) and Scopus website to identify studies of phthalates and phthalate metabolites, and we found that more exploration was carried out on the determination of phthalate in the urine, breast milk, and serum. But fewer researches were performed on the biomonitoring of phthalate in other biofluids such as amniotic fluids.

The amniotic fluid is the protective liquid contained by the amniotic sac of a gravid amniote (Bennett et al. 1993). This fluid serves as a cushion for the growing fetus but also serves to facilitate the exchange of nutrients, water, and biochemical products between mother and fetus (Indraccolo et al. 2018). Amniotic fluid is in the amniotic sac. It is generated from maternal plasma and passes through the fetal membranes by osmotic and hydrostatic forces. The human placenta is a complex organ that acts as the interface between the mother and fetus. Although the placenta prevents the transfer of some toxicants from the mother to the fetus, many chemical compounds can pass this barrier (Huang et al. 2018, Jensen and Chernyavsky, 2019).

Various phthalate metabolites are present in different biologic specimens including urine, serum, and amniotic fluid. Very recently, the exploration of the association between exposure to phthalates and the human body are increased among scientific research groups. For instance, in April 2022, Yalcin and co-workers investigated the association between phthalate metabolites and 24-h blood pressure profile in adolescents (Yalçin et al. 2022). They concluded that the mono-benzyl phthalate (MBzP) was associated with adverse blood pressure profiles in adolescents. In a separate research study, Yang and co-workers investigated the association between phthalate metabolites and breast cancer modified by body mass index and hormone receptors (Yang et al. 2022). Their main finding was as follows: the women with BMI < 25 or in BMI ≥ 25 the 3rd quartile of mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) was negatively associated with recurrent breast cancer. Notably, it is shown that the glucuronide distribution pattern of the monoesters phthalates is similar to that of urine (Silva et al. 2003), while generally, the content of phthalate metabolites in serum is lower compared with the excretion of phthalate metabolites in urine (Frederiksen et al. 2007). The important finding of a study was that the concentration of phthalates in amniotic fluid was about four-fold and seven-fold lower than the concentration of phthalate metabolites in urine in adults and children, respectively (Silva et al. 2004). Therefore, the investigation of the presence of phthalate metabolites in human amniotic fluid is of concern. An April 23, 2022, search using the Scopus website for "phthalate metabolites" and "pregnant women" in the article title yielded 226 documents including 206 of research articles, 9 review papers, 4 notes, 3 conference papers, and 3 erratums. Among them, 3 research articles discovered the determination of phthalate metabolites in amniotic fluids in association with cumulative exposure and risk assessment (Katsikantami et al. 2020; Li et al. 2018; Minatoya et al. 2018). Considering that phthalates may be important endocrine disruptor chemicals for humans, particularly in fetal life and early childhood, it seems most important to identify the metabolic pathways of phthalates from the first host, the mother, to the second host, the fetus and infant through the placenta and breast milk (Zhong et al. 2019). To the best of our knowledge, there is no report on the association between phthalate metabolites in human amniotic fluid and offspring birth size. In this study,

the amniotic fluid of pregnant women through the cesarean section was analyzed for four phthalate metabolites to estimate fetal and maternal exposures, biomonitoring data were statistically associated with information on exposure and health problems from questionnaires.

Material and methods

Materials and reagents

Four chemical compounds of phthalate metabolites including mono-butyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gas chromatography (GC) grade solvents including methanol, chlorobenzene, and acetonitrile were purchased from Merck chemical company (Darmstadt, Germany). Finally, *N*-trimethylsilyl-*N*-methyl trifluoroacetamide (MSTFA) and *β*-glucuronidase from *Helix pomatia* were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Study design

This cross-sectional study was conducted on 158 pregnant women from June 2019 to June 2021 in Isfahan, Iran. Isfahan is one of the most industrialized cities in Iran, with a population of about two million inhabitants located in the center of the Iranian plateau. The following inclusion criteria were used for the analyses: having lived in the city of Isfahan for at least 1 year and having no history of chronic disease or long-term drug use. The pregnant women answered a checklist covering questions about occupation, living location, sociodemographic factors, food consumption, use of personal care products, smoking habits, and lifestyle. The questions were answered through face-to-face interviews by a trained interviewer in a clinical lab before providing the amniotic fluid sample. Reliability and validity of questionnaires were approved by content validity (by experts) and re-tested with a correlation of 90%. All questionnaires were checked for irrational answers and errors and cleaned before further analysis.

Amniotic fluid sample collection

This study was conducted as a sub-study of the PERSIAN Birth Cohort in Isfahan. All of the amniotic fluids were collected from 158 participants assigned for delivery by cesarean section. The collaborating gynecologist collected 25–50 mL of clear amniotic fluid at the time of delivery, and collaborating nurses put it in a falcon (50 mL). The collected samples were transferred to the bio-bank of the cohort at Isfahan University of Medical Sciences (IUMS), where it kept frozen at − 75 °C for subsequent analysis.

Laboratory analysis

In this section, details of a laboratory method for the determination of phthalate metabolites in amniotic fluid humans have been reported. At first, the frozen amniotic fluid was transferred to room temperature. Then, 7–9 mL of amniotic fluid and 10-μL *β*-glycosidase enzyme were added to the new falcon (10 mL) along with hard turned. The ready falcons were kept in a shaker incubator instrument for 24 h at 37 °C. Then, all of the falcons were centrifuged for 5 min. at 4000 rpm. Then, by using the pipette, 5.0 mL of amniotic fluid was transferred to the new falcon with the same ID number (See Figure S2).

The pointed phthalate metabolites were extracted from yielded clear supernatant using a dispersive liquid–liquid microextraction (DLLME) method. In detail, a mixture of extraction solvent (chlorobenzene, 80 μL) and dispersive solvent (acetonitrile, 700 μL) was rapidly injected into the falcon for the formation of the cloudy solution. After the formation of the cloudy solution, the pointed falcons were centrifuged for 5 min at 4000 rpm. Then, by using the syringe, 30 μL of the down layer was picked up and injected into the clean Eppendorf for evaporating by inert N_2 gas. Then, 20 μL of *N*-trimethylsilyl-*N*-methyl trifluoroacetamide (MSTFA) was added to the Eppendorf and rapidly injected into the GC instrument (See Figure S3).

SES score

The SES score was calculated, using the principal component analysis (PCA) based on a questionnaire concerning level of education, income, type of home (rented or owned), and family assets (private car and computer). The first tertile of the SES score was considered low socioeconomic status.

Chemical substance exposure

Chemical substance exposure was asked through a questionnaire including how often mothers during pregnancy (daily, weekly, and monthly) used bleaching liquids, fresheners, cleaning sprays, glasswashers, pesticides, cleaning pounders, or liquids. All items were summarized in one score using the principal component approach. Tertiles of obtained scores were labeled as the level of chemical exposure. Using plastic dishes and containers were asked through questions about using a plastic container for keeping food, water, acidic juice, bread, marmalade, or pickles. This level of exposure was extracted using PCA. Using Teflon dishes was asked through one question.

Statistical analysis

Geometric means and specific percentiles were calculated to describe the distributions of phthalates among pregnant women in this study. Natural log (ln) transformation of phthalate concentrations proceeded to produce normal distributions. Pearson correlation coefficients were calculated between different types of phthalate metabolites using ln-transformed concentrations.

Kruskal Wallis's test was used to compare the differences in the concentrations of phthalate metabolites between categories of pre-pregnancy BMI (normal: 18.5–23.9; underweight: < 18.5; overweight: ≥ 24 kg/m^2), maternal height (< 162 and ≥ 162 cm, median as the cut-point), maternal age < 30 and ≥ 30 years), education level (< 6 years; 6–12 year and > 12), gestational diabetes, passive smoking, SES category (extracted through principle component method), and region. The concentration of phthalate metabolites was compared through different exposure levels (cosmetics products, chemical substances, and plastic containers). Association of newborn's anthropometrics measures with phthalate metabolites level was investigated using Spearman correlation.

In this study, LCA was applied to identify clusters of pregnant women from shared exposure patterns across a panel of amniotic fluid phthalate metabolites. The optimal number of classes was determined using goodness-of-fit criteria, which, by definition, must be as small as possible. We used Bayes Information Criterion (BIC), recommended to determine the optimal number of classes. After selecting the best model, we assigned each participant to one class according to his highest computed probability of membership. Statistical analyses were performed using SPSS 20 and Mplus 8. All tests were two-sided and statistical significance was defined as a p-value < 0.05.

Results

Method validation

Before instrumental analysis, the applied method was validated for the phthalate metabolites including MBP, MBzP, MEOHP, and MEHHP. In addition, the evaluated parameters including recovery, linearity, between day precision, and accuracy were evaluated for the pointed phthalate metabolites (Table 1). Then, calibration curves from standard solutions at five concentrations of 0.1, 1, 5, 10, and 50 µg/L were provided to calculate the instrument linearity from the relation coefficient (R^2) which ranged from 0.996 to 0.999 for all of the analytes. Furthermore, the method precision was evaluated for all analysts on different days and it was reported with the factor percentage relative standard deviation (RSD%) which range from 3.1 to 5.8% for analytes in the amniotic fluid matrix. The limit of detection (LOD) and limit of quantification (LOQ) were measured from signal to noise ratio (S/N) which are S/N > 3 and S/N > 8, respectively.

Characteristics of pregnant mother participants

The demographic characteristics of the pregnant women are summarized in Table 2. All pregnant women were Iranian nationals living in Isfahan city. Of the 158 participants studied, 139 pregnant women were enrolled in the current cross-sectional study. The mean (SD) age of the participant population was 31.89 ± 4.69 years (22–38 years), without significant difference in terms of age (p-value < 0.05). The educational level of participants was 1.3%, 6.3%, 11.4%, 43.7%, 12.0%, 23.4%, and 1.9% for illiterate, elementary, secondary, diploma, post-diploma, bachelor, and master, respectively. Other variables, including height, systolic blood pressure, diastolic blood pressure, job status, first marriage age, hip circumference, waist circumference, chest circumference, body mass index (BMI), are presented in this table. The median BMI of pre-pregnancy women (measured as weight in kg per m^2 of height) was 25.80 kg/m^2. According to the BMI categories of the World Health Organization (WHO) (http://apps.who.int/bmi/index.jsp?introPage=intro_3.html), the overall prevalences of obese (BMI > 30 kg/m^2), overweight (BMI > 24 kg/m^2), and underweight (BMI < 18.5 kg/m^2) prepregnant women were 15.5, 36.4, and 48.2% (normal BMI), respectively. More than 75.3% (119 families) of the woman had a total family income between (1–3 milion Toman per month) (1 US dollar ≈ 28,000 Toman). Only 19 (13.2%) women expressed as a passive smoker.

Table 1 Method validation of phthalate metabolites in amniotic fluid samples

Phthalate Metabolites	LOD (µg/L)	LOQ (µg/L)	R^2 (N = 3)	%Recovery (N = 3)	RSD% (N = 3)	%Accuracy
MBP	0.036	0.1188	0.997	98.3	5.8	99.9
MBzP	0.054	0.1782	0.996	85.6	4.2	100.6
MEOHP	0.023	0.0759	0.999	92.5	3.1	104.7
MEHHP	0.088	0.2904	0.997	93.7	3.9	98.6

Table 2 Baseline characteristics of mothers

Number		158
Age (year)		
		31.89 ± 4.69
	< 25	14(8.9)
	25–30	49(31.0)
	30–35	55(34.8)
	> 35	40(25.3)
Education		
	Illiterate	2(1.3)
	Elementary	10(6.3)
	Secondary	18(11.4)
	Diploma	69(43.7)
	Post-Diploma	19(12.0)
	Bachelor	37(23.4)
	Master	3(1.9)
Family income (monthly/Toman)		
	< 1 M	28(17.7)
	1–3 M	119(75.3)
	3–5 M	10(6.3)
	> 5 M	1(0.6)
SES categories		
	Low	56(35.7)
	Moderate	49(31.2)
	High	52(33.1)
Region		
	rural	22(13.9)
	urban	136(86.1)
pre-pregnancy BMI status		
	Normal	53(48.2)
	Overweight	40(36.4)
	Obese	17(15.5)
Passive smoker		
	yes	19(13.2)
	no	125(86.8)
Birth order		
	1	143(97.9)
	2	2(1.4)
	3	1(0.7)
Pre-pregnancy anthropometrics		
	Weight	67.39 ± 16.32
	Hip	105.63 ± 9.06
	Waist	95.33 ± 16.18
	Chest	97.96 ± 11.79
	Height	161.58 ± 5.81
	BMI	25.80 ± 6.04

[a]Mean ± standard deviation, [b]Number (%)

Distribution of phthalate metabolites in amniotic fluids

Table S1 (see supporting information) presents the concentrations of different phthalate metabolites for each participant. The results show that the MEHHP was approximately detected in all of the participants. Of the total pregnant women, 1.4% ($n = 2$) did not exist phthalate metabolites derivatives (MBP, MBzP, MEOHP, MEHHP). But in 98.6% ($n = 137$) of pregnant women, the phthalate metabolite derivatives (MBP, MBzP, MEOHP, MEHHP) have existed. We found that in 2.9% ($n = 4$), 98.6% ($n = 138$), 74.8% ($n = 104$), and 56.11% ($n = 78$) of pregnant women considerable levels of MBP, MEHHP, MBzP, and MEOHP were detected, respectively.

The distribution of phthalate concentrations among the pregnant women in the present study was shown in Table 3. The geometric means and medians of MBP, MBzP, MEOHP, and MEHHP among detected samples were 10.17 (9.52), 6.24 (3.47), 5.03 (11.72), and 174.79 (229.94), respectively. In addition, the mean concentrantion of phthalate metabolite (μg/mL) that was detected in amniotic fluid at a frequency above LOD, was shown in Fig. 1.

Correlations between phthalate metabolites in amniotic fluid samples

Highly significant correlations were obtained between phthalate metabolites in amniotic fluid samples indicating their exposure to pregnant women with common parent compounds including phthalate components. MBzP and MEOHP were moderately correlated ($r = 0.51$, $p\text{-value} < 0.001$), MEOHP and MEHHP were highly correlated ($r = 0.83$, $p\text{-value} < 0.001$) while MEOHP and MBzP were lowly correlated ($r = 0.39$, $p\text{-value} = 0.005$). There was not a statistically significant association between MBP and MBzP, MEOHP, and MEHHP, partly because of the small sample size of detected samples for MBP ($p > 0.05$) (Table 4).

Association of phthalate metabolites in amniotic fluid with maternal characteristics from questionnaires

The effect of maternal exposure on woman's health, and development and infant's health was investigated and different parameters including the age of women, type of region, education level, passive smoker, BMI status, and gestational diabetes were examined. The association of maternal characteristics with phthalate levels was evaluated in Table 5. In according data from biomonitoring and questionnaires, we found that mean of MBzP was significantly higher among older mothers ($p = 0.023$) and low educated ones ($p = 0.032$). The passive smoking of pregnant women

Table 3 Concentration of phthalate metabolites

	Detection rate (%)	Mean ± SD	GM	Min	Percentiles			Max
					25th	50th	75th	
MBP	3.6	20.88 ± 31.12	10.17	< LOD	3.71	9.52	60.43	76.11
MBzP	80.6	8.89 ± 7.22	6.24	< LOD	1.34	3.47	7.25	37.39
MEOHP	46.8	8.75 ± 8.09	5.03	< LOD	3.29	11.72	18.13	43.03
MEHHP	98.6	240.73 ± 191.58	174.79	< LOD	174.68	229.94	284.36	1028.96

Statistics were computed on detected samples. *SD* standard deviation, *LOD* limit of detection, *GM* geometric mean

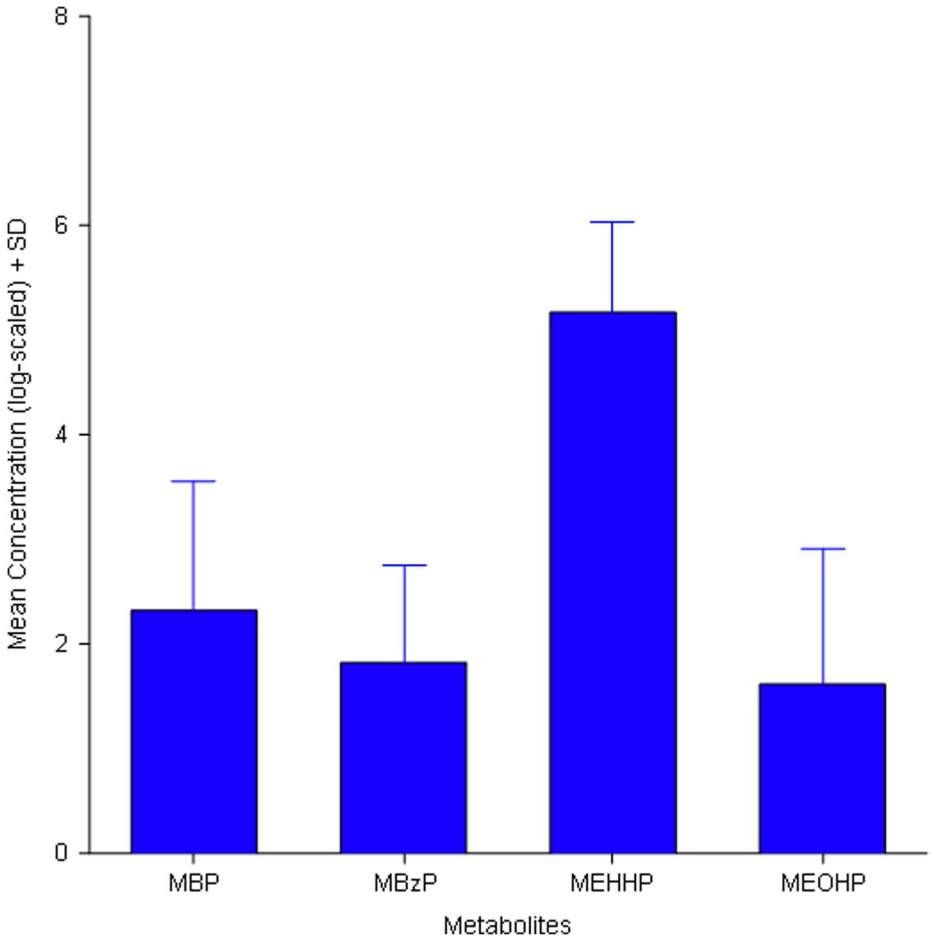

Fig. 1 Mean concentrations (+ standard deviation) (µg/L) of phthalate metabolites that were detected in amniotic fluid at frequency above LOD

Table 4 Correlations of concentration of phthalate metabolites in amniotic fluid samples

	MBP	MBzP	MEOHP	MEHHP
MBP	1	−0.40[a]	−0.60	−0.80
		(0.600)[b]	(0.285)	(0.104)
MBzP		1	0.39	0.51
			(0.005)	(< 0.001)
MEOHP			1	0.83
				(< 0.001)
MEHHP				1

[a]Spearman correlation, [b]2-tailed significance probability

was significantly associated with a higher level of MBP ($p = 0.115$), MBzP ($p = 0.927$), MEOHP ($p = 0.212$), and MEHHP ($p = 0.561$).

Potential exposures of phthalates

Concentrations of phthalate metabolites in relation to exposure to some known sources are presented in Table 6. No significant differences were found for all types of phthalate metabolites with regard to cosmetic products used in the first month of pregnancy, exposure to chemical substances (cleaner liquids, bleaching liquids,

Table 5 Mean ± SD of phthalate concentrations (for MP, EP, and PP, BP) according to different maternal characteristics

	MBP	MBzP	MEOHP	MEHHP
Maternal age (year)				
< 30 year	0.13 ± 0.85	5.87 ± 5.94	3.77 ± 6.80	241.97 ± 218.31
≥ 30 year	0.08 ± 0.69	7.97 ± 7.91	4.04 ± 7.09	238.70 ± 167.63
p[a]	0.584	0.023	0.500	0.134
Living area				
Rural	0.00 ± 0.00	8.51 ± 8.66	4.88 ± 6.32	227.25 ± 142.34
Urban	0.11 ± 0.80	7.02 ± 7.08	3.76 ± 7.05	240.03 ± 192.66
p	0.575	0.498	0.068	0.952
Education				
< 6 year	0.77 ± 2.18	4.52 ± 5.25	2.93 ± 4.42	191.90 ± 134.25
6–12 year	0.08 ± 0.68	7.13 ± 7.39	4.33 ± 7.73	229.16 ± 176.76
> 12 year	0.00 ± 0.00	7.82 ± 7.53	3.56 ± 6.16	263.20 ± 206.00
p	0.032	0.424	0.754	0.638
Passive smoker				
Yes	0.41 ± 1.59	8.43 ± 10.01	5.24 ± 6.56	291.23 ± 241.79
No	0.06 ± 0.57	6.77 ± 6.17	3.86 ± 7.11	232.04 ± 176.02
p	0.115	0.927	0.212	0.561
Pre-pregnancy BMI status				
Normal	0.08 ± 0.79	7.54 ± 6.69	4.27 ± 6.36	246.18 ± 189.80
Overweight	0.20 ± 1.11	7.48 ± 7.16	3.03 ± 8.44	238.48 ± 200.23
Obese	0.07 ± 1.07	8.67 ± 12.75	3.53 ± 7.04	287.65 ± 206.70
p	0.405	0.729	0.245	0.723
SES category				
Low	0.30 ± 1.31	7.26 ± 8.13	4.51 ± 6.00	235.30 ± 180.23
Moderate	0.00 ± 0.00	7.40 ± 7.21	3.41 ± 8.11	226.95 ± 165.73
High	0.00 ± 0.00	6.72 ± 6.52	4.00 ± 6.82	245.41 ± 201.48
p	0.120	0.850	0.069	0.944
Gestational diabetes				
Yes	0.00 ± 0.00	7.01 ± 9.37	3.83 ± 6.85	264.15 ± 182.52
No	0.12 ± 0.85	6.84 ± 6.34	3.84 ± 7.08	229.75 ± 186.03
p	0.564	0.710	0.738	0.299

[a]Kruskal Wallis's test, [b]mean ± SD

cleaning sprays, fresheners; exposure level was extracted using the principal component method) and using plastic containers and dishes. Compared with the using cosmetic product in pregnant women, pregnant women with positive responses had higher concentrations of MBP, MBzP, MEOHP, and MEHHP metabolites. No significant association between high, moderate, and low exposure to chemical substances and pregnant women was observed. Similarly, the pregnant women with high, moderate, and low exposure to plastic containers had no correlation between them and the level of detected phthalate metabolites in amniotic fluid samples. While using Teflon containers caused the pregnant women had higher levels of MBP ($p = 0.564$), MBzP ($p = 0.875$), MEOHP ($p = 0.323$), and MEHHP ($p = 0.932$).

Table 6 Some potential exposures and detected phthalate metabolites level in amniotic fluid

	MBP	MBzP	MEOHP	MEHHP
Using cosmetic products				
Yes	0.14 ± 0.92	6.42 ± 7.46	3.98 ± 7.34	246.15 ± 195.67
No	0.00 ± 0.00	7.78 ± 6.15	3.36 ± 5.87	216.05 ± 155.22
p[a]	0.340	0.061	0.354	0.513
Exposure to chemical substance				
High	0.00 ± 0.00	7.57 ± 8.78	3.83 ± 6.08	208.51 ± 172.48
Moderate	0.14 ± 0.94	7.82 ± 7.90	3.98 ± 6.40	256.89 ± 199.25
Low	0.12 ± 0.83	6.38 ± 5.16	3.91 ± 8.13	244.90 ± 185.41
p	0.656	0.866	0.589	0.495
Plastic containers				
High	0.00 ± 0.00	6.01 ± 7.34	3.76 ± 8.42	232.79 ± 182.29
Moderate	0.17 ± 1.01	6.87 ± 5.43	3.61 ± 5.44	224.80 ± 157.59
Low	0.12 ± 0.82	8.06 ± 8.33	4.12 ± 6.64	247.79 ± 203.35
p	0.311	0.234	0.632	0.890
Teflon use				
Yes	0.15 ± 0.93	6.95 ± 7.08	4.34 ± 6.80	237.50 ± 198.23
No	0.07 ± 0.68	6.77 ± 6.63	3.73 ± 7.00	231.23 ± 168.84
p	0.564	0.875	0.323	0.932

[a]Kruskal Wallis's test, [b]Mean ± SD

Table 7 Newborn's characteristics and birth outcomes

Number		142
Weight (g)		3171.79 ± 518.41[a]
Height (cm)		49.56 ± 1.94
Head circumferences (cm)		34.92 ± 1.37
Chest (cm)		33.30 ± 1.75
Hip (cm)		31.46 ± 2.36
Arm circumferences (cm)		10.80 ± 1.05
Maternal gestational diabetes	Yes	20 (14.2) [b]
	No	121 (85.8)
Low birth weight		
	Yes	10 (7.0)
	No	132 (93.0)
Birth malfunction		
	Yes	4 (2.9)
	No	135 (97.1)

[a]Mean ± standard deviation, [b]Number (%)

Newborn participant characteristics

Characteristics of 142 newborns were available that are presented in Table 7. Participants had a median weight of 3171.8 g and 49.6, 34.9, 33.3, 31.5, and 10.8 cm in height, head circumferences, chest, hip, and arm circumferences, respectively. Approximately, 14.2% of mothers' newborn had gestational diabetes, while 85.8% of them without

Table 8 Association of newborn's anthropometric measures and detected phthalate metabolite levels

	MBP	MBzP	MEOHP	MEHHP
Weight	0.01 (0.890)	0.06 (0.501)	0.06 (0.538)	0.09 (0.342)
Height	−0.01 (0.900)	0.16 (0.102)	−0.05 (0.630)	0.04 (0.656)
Head circumference	0.00 (0.963)	−0.03 (0.724)	−0.07 (0.493)	−0.03 (0.768)
Chest	0.05 (0.673)	−0.01 (0.931)	0.03 (0.775)	0.07 (0.531)
Hip	−0.02 (0.852)	0.11 (0.368)	−0.11 (0.357)	−0.12 (0.313)
Arm	−0.10 (0.415)	0.09 (0.435)	0.08 (0.507)	0.04 (0.733)

Spearman's *r* (two-tailed p value)

Table 9 Fit statistics for latent class analyses

n. classes	n. parameters	LL	BIC
1	4	−252.02	523.77
2	9	−219.86	484.13
3	14	−217.71	504.50
4	19	−217.70	529.16

LL log likelihood, *BIC* Bayes Information Criterion

gestational diabetes. Among 142 newborn participants, only 2.9% had birth malfunction as well as 7.0% low birth weight.

Association between phthalate metabolites and birth outcomes

Table 8 represents the correlation between newborn's anthropometrics (such as weight, height, head circumferences, chest, hip, and arm) and phthalate metabolites level in amniotic fluid samples. Except for a positive association between height and MBzP, there was no statistically significant association between phthalate metabolites concentration and newborn's anthropometric measures ($p > 0.05$).

Latent class analysis

Latent class analysis (LCA) may serve as a useful tool for identifying individuals with shared real-life profiles of chemical exposures. Knowledge of these groupings and their risk of adverse outcomes have the potential to inform targeted public health prevention strategies. In the current study, LCA was applied to identify clusters of pregnant women from with shared exposure patterns across a panel of amniotic fluid phthalate metabolites. Table 9 shows the fit statistics of the LCA model for different numbers of classes. The model with two classes provided the best fit with the smallest BIC (BIC = 484.13). LCA identified individuals with "low exposure" and "high exposure." There were 53 (38.1%) participants in the "high exposure" class, and 86 (61.9%) in "low exposure" class (Fig. 2).

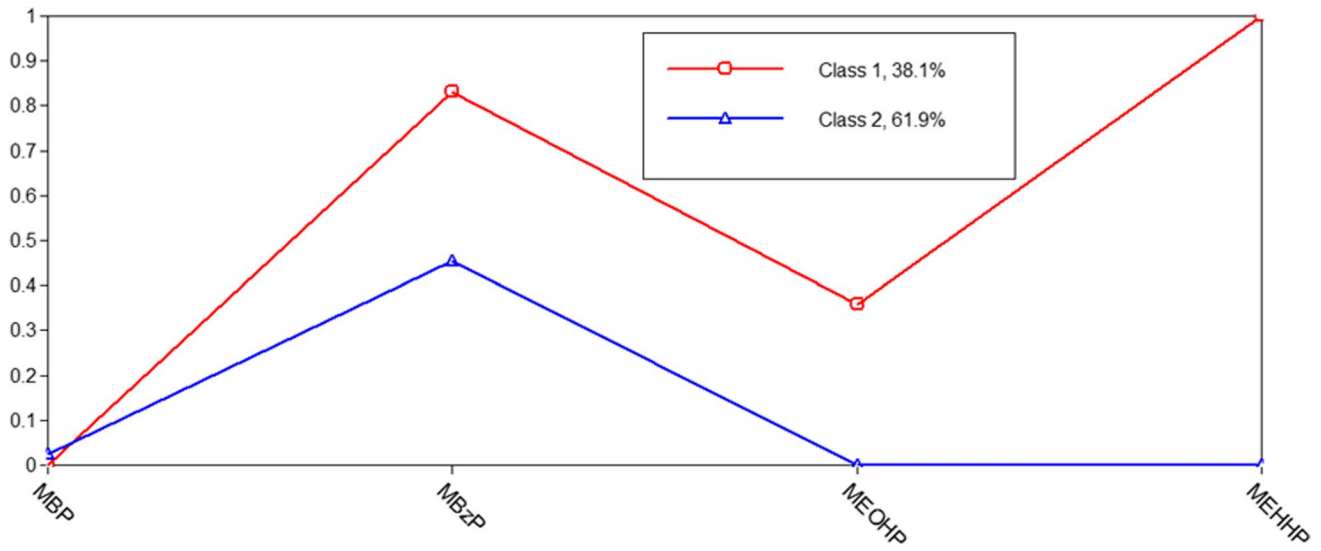

Fig. 2 Frequency of pregnant women with phthalate metabolites upper than median, class 1: "high exposure" group, class 2: "low exposure" group

Table 10 A comparison report of concentrations of phthalate metabolites in amniotic fluid of pregnant women

Country/region	Sampling (*N*)	Phthalate metabolites	Mean ± SD	Median
Europe (Tranfo et al. 2014)	70	MEHP	1.5 ± 5.0	0.7
		MEHHP	0.3 ± 0.2	0.4
		MBzP	0.2 ± 0.1	0.1
		MEP	0.7 ± 0.8	0.5
		MNBP	3.5 ± 2.3	3.2
USA (Silva et al. 2004)	54	MEHP	-	< LOD
		MNBP	-	5.8
		MEP	-	< LOD
Germany (Wittassek et al. 2009)	11	MNBP	9.1	7.8
		MIBP	10.0	4.2
		MBzP	2.1	1.9
		MEHP	2.4	1.6
		MEHHP	< LOQ	< LOQ
		MECPP	0.90	0.53
		MEOHP	< LOD	< LOD
		MCMHP	0.60	0.64
		MHINP	< LOD	< LOD
		MOINP	< LOD	< LOD
		MCIOP	0.51	< LOD
China (Li et al. 2018)	81	MNBP	4.2	3.7
		MCPP	< LOD	< LOD
		MCMHP	1.1	0.90
		MEHHP	< LOD	< LOD
		MEOHP	< LOD	< LOD
		MIBP	2.6	2.2
		MBzP	< LOD	< LOD
		MMP	3.1	2.7
		MEP	0.5	0.2
		MECPP	0.2	0.1
		MEHP	0.8	0.7
Greece (Katsikantami et al. 2020)	100	MEOHP	< LOD	< LOD
		MEHHP	< LOD	< LOD
		MEHP	2.7 ± 1.3	2.3
		MBzP	< LOD	< LOD
		MNBP	12.0 ± 9.8	10.7
		MIBP	39.3 ± 55.1	10.0
Taiwan (Huang et al. 2009)	64	MMP	-	-
		MEP	-	-
		MNBP	-	85.5
		MBzP	-	-
		MEHP	-	24.0

Disscussions

The aim of the present study was to consider the correlation between four phthalate metabolite concentrations measured in the amniotic fluid of pregnant women. The concentrations of phthalate metabolites in human amniotic fluids were measured in a pregnant woman in Isfahan, Iran. To our knowledge, this is the first study reporting on the determination of phthalate metabolites in human amniotic fluid and the association between prenatal exposure to phthalate and the anthropometric indexes of neonates and health characteristics of newborns. We searched the Scopus database for 2000–2021 using the following keywords including phthalate, phthalate metabolites, amniotic fluid, pregnancy, and pregnant women. Table 10 provides the main studies that have measured phthalate metabolites

in the amniotic fluid of pregnant women. The number of participants in our study was higher than those reported methods ($N = 158$) (Huang et al. 2009; Katsikantami et al. 2020; Li et al. 2018; Silva et al. 2004; Tranfo et al. 2014; Wittassek et al. 2009).

According to the literature, the level of phthalate metabolites in urine was higher than in amniotic fluid (amniotic fluid: median 2.3–10.7 $\mu g.L^{-1}$ vs urine: 4.9–46.7 $\mu g.L^{-1}$) (Huang et al. 2009). While the detection rate in amniotic fluid was lower than in urine (amniotic fluid: < LOD-7.8 $\mu g.L^{-1}$ vs urine: 1.3–55.6 $\mu g.L^{-1}$) (Huang et al. 2009; Tranfo et al. 2014). The pointed event indicates that the compounds such as phthalates or phthalate metabolites could not be crossed easily through the placenta tissue barrier (Furugen 2020; Mathiesen et al. 2021).

We observed that higher concentrations of MEHHP in amniotic fluid were related to easy transformation through of placenta (Calafat et al. 2010). Phthalate and antimicrobial agent are frequently used in personal products including lotions, cosmetics, and soaps (Rastogi et al. 1995) and as food preservatives in confectionary and dried meat. Therefore, dermal contact and ingestion are the most common routes of phthalate exposure (Philippat et al. 2013). Examined phthalate levels in amniotic fluid and reported the median concentration of MBP in the analyzed samples to be trace whereas the levels of MBzP, MEHHP, and MEOHP were above LOD. In accordance with the previous study, reports on urinary concentrations of phthalates among pregnant women in Japan (Shirai et al. 2013) and Spain (Casas et al. 2011), the unadjusted total median concentration in the current study was found to be highest for MEHHP (26.21 $\mu g.L^{-1}$), followed by MEOHP (13.02 $\mu g.L^{-1}$), then MBzP (16.97 $\mu g.L^{-1}$) and MBP (0.11 $\mu g.L^{-1}$) in the maternal blood plasma and a similar trend was found in amniotic fluid. MEHHP and MEOHP are the most commonly metabolized in the body that used phthalate in food and cosmetics (Soni et al. 2005); and thus, the concentration of these phthalates was found to be higher in both the matrices when compared to MBP. The potency of phthalate increase with the length of the alkyl chain and hydrogen bonding thus the long-chain phthalate (e.g., MEHHP, MEOHP) is of the highest concern (Boberg et al. 2010; Witorsch and Thomas, 2010). In our study, the phthalates were found in 137 samples, indicating exposure to at least one of the four phthalates (MBP, MBzP, MEHHP, and MEOHP).

According to the literature review, there is little original research for the determination of phthalate metabolites in amniotic fluid, and the most recent of them were compared in Table 10. Li et al. (Li et al. 2018) Silva et al. (Silva et al. 2004) and Wittassek et al. (Wittassek et al. 2009) observed that the oxidative metabolites of DEHP including MEHHP and MEOHP were not detected at concentrations above LOD except one sample in reported research by Katsikantami et al. While in the present study the oxidative metabolites of DEHP including MEHHP was determined in all samples except two amniotic samples. In addition, other DEHP metabolites including MEOHP was detected in 97 samples (61% detected vs. 39% free of MEOHP).

In addition, there is one pregnant woman with a significantly higher concentration of MEOHP (1024 µg/L) relative to other participants. The range of concentration of MEOHP in participants was observed between 1024 and 17 µg/L. Two pregnant women with high and low levels of MEOHP were further considered through their questionnaires. We found that the higher maternal exposure (frequent or daily use) to shampoos, soaps, cosmetics, plastics, paints, enteric coatings in some medications, and pesticide formulations may be caused that the phthalate metabolites with a long carbon chain with hydrophobic characteristics crossed from the placenta. We further explored the obtained level of phthalate metabolites, and it was found that the pregnant women who answered frequent or daily use of personal products had a higher levels of phthalate metabolites in amniotic fluids. A close association was found in the current study and other reported studies (Kalloo et al. 2020; Karzi et al. 2021; Katsikantami et al. 2020; Silinski et al. 2020; Suteau et al. 2020; Zhang et al. 2009).

The provided amniotic fluid consists of fetal urine and is orally taken into the fetus, and then, fetal urine is extracted as a part of the amniotic fluid. The phthalate metabolites are mainly extracted from urine (Marie et al. 2015). Therefore, it is assumed that MBP, MBzP, MEOHP, and MEHHP transfer to the fetus and are accumulated in amniotic fluid again. This fact indicates that during pregnancy, the fetuses are continuously exposed to phthalate metabolites. Based on the phthalates concentrations in the human amniotic fluid, there are several reports showing that there is a strong geographical difference in phthalates concentrations in the amniotic fluid and that phthalates levels in amniotic fluid differ among the gestational weeks (Mitro et al. 2015). Further studies with large and global scales are required.

The presence of MBP, MBzP, MEOHP, and MEHHP in the amniotic fluid indicates that these metabolites can cross the placental barrier after maternal exposure to phthalates. The detection frequencies and the levels of phthalate metabolites in human amniotic fluid were lower than in the urine matrix (Huang et al. 2009; Katsikantami et al. 2020; Wittassek et al. 2009). The pointed event indicates the difficulty of the compounds including liquids, solids, and gases to cross the placenta tissue barrier (Griffiths & Campbell 2014). There are few original research on the determination of phthalate metabolites in amniotic fluid (Anand-Ivell et al. 2018; Katsikantami et al. 2020; Li et al. 2018; Minatoya et al. 2018; Silinski et al. 2020; Suteau et al. 2020). The linear correlations that were found for phthalate metabolites in amniotic fluid indicated the common source of exposure as

they are used as mixtures in the products and also the common parent compound for the MBP, MBzP, MEOHP, and MEHHP metabolites. Similar results have also been previously reported for DiNP and DEHP metabolites in serum and urine (Frederiksen et al. 2010). The influence of maternal exposure on birth outcomes was examined and positive correlations came up between amniotic fluid concentrations of phthalate metabolites and health problems or birth weight. A significant association was observed between high maternal exposure to phthalates and birth weight and similar results for other phthalates are reported in the literature (Huang et al. 2014; Kalloo et al. 2020; Zhang et al. 2009). Based on the use of personal care products and cosmetics, we found that the mothers who reported frequent or daily use of deodorant had higher levels of phthalate metabolites in amniotic fluid. The use of leave-on skin cosmetics has been significantly associated with urinary MBzP and MEP in pregnant mothers (Hsieh et al. 2019). The significant association that was found in the present study for the correlation between phthalate metabolites and birth outcomes has also been reported in other studies (Katsikantami et al. 2016).

Conclusion

The present study focused on the consideration of exposure of pregnant women to phthalates through the biomonitoring of their metabolites in amniotic fluid and its correlation with birth outcomes. Although there are a lot of studies on phthalates in the urine, and blood, there are fewer data about associations between phthalate metabolites in amniotic fluid and data from questionnaires. Our findings add to the evidence that the general population, including pregnant women fetuses, continue to be exposed to the phthalates. Four phthalate metabolites including MBP (normal: 0.08 ± 0.79; overweight: 0.20 ± 1.11; obese: 0.07 ± 1.07; p-value: 0.405), MBzP (normal: 7.54 ± 6.69; overweight: 7.48 ± 7.16; obese: 8.67 ± 12.75; p-value: 0.729), MEOHP (normal: 4.27 ± 6.36; overweight: 3.03 ± 8.44; obese: 3.53 ± 7.04; p-value: 0.245), and MEHHP (normal: 246.18 ± 189.80; overweight: 238.48 ± 200.23; obese: 287.65 ± 206.70; p-value: 0.723) were simultaneously detected in samples of maternal amniotic fluid using GC–MS. Our results suggest that maternal exposure to phthalate metabolites is positively associated with in utero exposure to the developing fetus. Future studies should focus on collthe ection of amniotic fluid at different trimesters and the corresponding maternal samples to better characterize the pharmacokinetics and health impacts of these EDCs during fetal development. In addition, the present study has several potential limitations such as only investigation of amniotic fluid after cesarean section and no data is available for different trimesters of pregnancy.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate Ethics approval was obtained [IR.NIMADREC.1397.410]; all participants gave written informed consent for participation in the study prior to sample collection.

Consent to publish All participants gave written informed consent for participation in the study and its publication.

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

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