AG-2020-256
doi.org/10.1590/S0004-2803.202100000-56

Relationship between lead and cadmium levels in blood and refractory chronic constipation among Iranian children

Nasrin **GHARIBSHAHI**[1], Hazhir **JAVAHERIZADEH**[2], Zahra Nazari **KHORASGANI**[1] and Masoud **MAHDAVINIA**[3]

Received: 11 December 2020
Accepted: 12 April 2021

ABSTRACT – **Background** – There is limited research examining reasons causing refractory chronic constipation (RCC) in children. The effects of lead (Pb) and cadmium (Cd) exposures on this condition have been even less clear. However, some related factors may contribute to evaluation of blood lead levels (BLLs) and blood cadmium levels (BCLs). **Objective** – The present study aimed to examine the relationship between Pb and Cd exposures and RCC in children living in the city of Ahvaz, Khuzestan Province, in Southwestern Iran. **Methods** – This study was performed on a total number of 48 children aged 2–13 years, including 36 medically-diagnosed RCC cases and 12 controls referring to a pediatric clinic in the city of Ahvaz. Their BLLs and BCLs were then determined using a graphite furnace atomic absorption spectrophotometer. The data from the researcher-designed questionnaire were also recoded and the related risk factors were analyzed through Spearman's correlation and logistic regression analysis. **Results** – The findings revealed that the geometric means of Pb and Cd in blood samples in the control group were 58.95 µg/dL and 0.45 µg/dL; respectively. These values in the case group were equally 45.26 µg/dL and 0.26 µg/dL; respectively. A significant difference was additionally observed between BCLs in the case and control groups ($P<0.01$). All children in both groups also had BLLs greater than the permissible limit endorsed by the World Health Organization (WHO) (≤10 µg/dL). On the other hand, 8.3% of the individuals in the case group and 33.3% of those in the control group had BCLs higher than the acceptable range mentioned by WHO (≤0.5 µg/dL). **Conclusion** – Pb and Cd exposures due to environmental pollution and susceptibility to heavy metals may not be associated with RCC in children living in the city of Ahvaz. Although this research was the first one providing data on BLLs and BCLs in children with RCC, the findings could be useful for designing future epidemiologic studies.

Keywords – Refractory chronic constipation; lead; cadmium; children; Iran.

INTRODUCTION

Gastrointestinal (GI) disorders, including constipation, are one of the most common health problems in children[1,2]. So, children with such conditions experience low quality of life which may often lead to high levels of anxiety and depression[3]. The severity of constipation can also range from mild and short-term to severe and chronic type[2]. Such a disorder additionally results in 3–10% increase in physician visits and up to 25% of the referrals to specialist physicians all over the world. Besides, the global incidence rate of constipation in children varies from 0.7 to 29.6. Genetic predisposition, low socioeconomic status, inadequate daily consumption of fibers, low fluid intake inactivity, and environmental factors are thus the most important and effective parameters affecting the incidence of this disease[4]. Moreover, constipation is one of the most expensive diseases, so dealing with its related problems can be very helpful.

Heavy metals including lead (Pb) and cadmium (Cd) are considered as environmental pollutants whose atomic stability in the form of ions can result in their accumulation in the environment. They can even enter into the food chain, causing concerns among health authorities and researchers to meet the purposes of hygienic and health-related problems[5,6]. Lead is a toxic metal that enters into the body through ingestion of dust, water, and food as well as inhalation of particles containing this element[7]. Children, pregnant women, and individuals with low levels of calcium (Ca), iron (Fe), zinc (Zn), and copper (Cu) (due to absorption of high levels of Pb in their bodies) are specifically vulnerable to lead poisoning[8]. Although there is no safe level of Pb exposure in children, the blood lead levels (BLLs) greater than 10 µg/dL cause poisoning symptoms such as anorexia, abdominal pain, tiredness, learning disabilities, mental retardation, reduction in intelligence quotient (IQ) rate, abnormal behaviors, anemia, hypertension, kidney failure, immune deficiency, etc[9]. Another important GI symptom in children created by lead poisoning is chronic constipation[10,11].

Cadmium is also another carcinogenic heavy metal existing in the environment even at low levels. It can enter into the body through food, smoke, and dust exposures. Moreover, elevated levels of Cd in the environment bring about a wide range of undesirable effects in cardiovascular, pulmonary, digestive, regenerative, liver, kidney, and skeletal systems[12,13]. Blood cadmium levels (BCLs) larger than 0.5 µg/dL are thus considered to be toxic and levels below this value can be safe[14].

Declared conflict of interest of all authors: none
Disclosure of funding: This study was supported by Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, with the grant no. TRC-9614. The protocol was also confirmed by the Human Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1396.1086).
[1] Department of Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [2] Alimentary Tract Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. [3] Toxicology Research Center, Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Corresponding author: Masoud Mahdavinia. E-mail: mahdavimasoud@yahoo.com and Nasrin Gharibshahi. E-mail: n.gharibshahi3228@gmail.com

Although BLLs in children with constipation and other diseases have been so far investigated, no studies have ever been reported on examining BLLs in children with refractory chronic constipation (RCC). On the other hand, relationship between children's constipation and their Cd exposures has not been determined. Ahvaz, as one of the most polluted cities in Iran, is highly susceptible to the accumulation of toxic metals such as Pb and Cd in the environment which can enter into the human body[15,16]. Therefore, this study was designed to assess whether RCC in children was associated with Pb and Cd concentrations in children's body or not.

METHODS

Database description

A case-control study was carried out between May 2018 and March 2019 to investigate the association between BCLs and BLLs and RCC in children living in the city of Ahvaz, as the capital of Khuzestan Province in Iran, with a population of approximately 1.5 million, situated in Southwestern Iran. A total of 54 participants (36 cases and 18 controls) were included in the study that the data of six controls were excluded from the analysis due to the high abnormality of BLLs and BCLs and finally, number of 48 children aged 2–13 years including 36 cases (19 males and 17 females) with RCC and 12 healthy individuals (seven males and five females) without any complaints of constipation as control group were accordingly included, RCC was also defined as a poor response to appropriate treatments include lactulose (1–2 mL/kg) and pidrolax (1–2 mL/kg) after three months[17]. As well, all patients met the Rome IV criteria for functional constipation and underwent examinations for Hirschsprung's disease (HD) by barium enema, and for celiac disease, hypothyroidism, spinal disease, as well as possible food allergy using medical history, physical examinations, radiology, and laboratory test results. The data of both groups including gender, age, weight, height, race, parental education, parental smoking habits, parental consanguineous marriage, and socioeconomic status were further recorded through a researcher-designed questionnaire. This study was also approved by the Human Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Once written permissions were obtained from children's parents, 2 mL of the whole blood from each of the participants were collected in a plastic tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. The blood samples were then immediately placed in an ice pack and transferred into a refrigerator at 4°C[18]. The blood samples were also digested for extraction of Pb and Cd using the conventional wet/acid digestion method. Subsequently, 2 mL of blood was transferred into a conical flask and 20 mL of concentrated 10% nitric acid was added to it and kept at room temperature overnight. Afterwards, it was heated on a hot plate until the solution turned colorless. The solution was allowed to cool and its volume reached 6 mL with de-ionized water. Finally, the digested samples were utilized to measure levels of Pb and Cd using a graphite furnace atomic absorption (GFAA) spectrophotometer (Varian AA240 FS) at the Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Statistical analysis

The distribution of demographic characteristics of the participants in the case and control groups was compared through Chi-square test. Geometric means (GMs) were additionally used to describe BLLs and BCLs. The distribution of Pb and Cd levels was also tested by Shapiro-Wilk test. Since BLLs and BCLs were not normally distributed, Mann-Whitney U test or Kruskal-Wallis test was performed to evaluate the relationship between independent variables mentioned in the researcher-designed questionnaire and BLLs and BCLs. The odds ratio (ORs) and 95% confidence intervals (CIs) of heavy metals were correspondingly estimated using unadjusted and adjusted logistic regression models. Spearman's correlation was then used to evaluate the relationships between BLLs and BCLs and some quantitative variables (i.e. age, weight, height, body mass index (BMI)). As well, the data of Pb or Cd concentrations in blood samples were divided into two groups: high-level and low-level groups taking 45 µg/dL as the boundary for BLLs because GI effects such as constipation, abdominal pain, and so on can be seen at BLLs of 45 µg/dL or higher[19] and 0.2 µg/dL as the boundary for BCLs, based on the study by Zheng[20]. To explore some risk or protective factors for high BLLs and BCLs, logistic regression analysis was used. Age, gender, race, parental education, parental smoking habits, parental consanguineous marriage, and socioeconomic status were also adjusted. The data were analyzed using the IBM SPSS Statistics software (version 20). The level of significance was set at 0.05.

RESULTS

TABLE 1 characterizes the general characteristics of the study population. No significant difference was accordingly observed between age, height, weight, and BMI in both groups, ($P>0.05$).

TABLE 1. General characteristics of assessed population.

Variable	Case group	Control group	*P*-value
Age (years)	5.51±3.19	6.41±3.11	0.48
Height (cm)	112.06±20.9	117±20.05	0.49
Weight (kg)	20.19±8.67	23.50±9.07	0.21
BMI (kg/m^2)	15.55±2.29	16.51±1.62	0.189

P-value based on the Mann-Whitney test. BMI: body mass index.

TABLE 2 describes parent-child characteristics of both case and control groups who did not differ in terms of gender, race, maternal education, and parental smoking habits; however, there was a significant difference between paternal education, socioeconomic status, and parental consanguineous marriage in the two groups. TABLE 2 also displays the GMs of BLLs and BCLs corresponding to parent-child characteristics, which were not statistically different with reference to any of the covariates.

The GMs of BCLs and BLLs for the case group were 0.26 µg/dL and 45.26 µg/dL, respectively, while these values in the control group were equal to 0.45 µg/dL and 58.95 µg/dL; respectively. There was no significant difference in BLLs between the two groups but BCLs in the control group were significantly higher than those in the case group (TABLE 3). All the children in both groups had BLLs greater than the permissible limit set by the World Health organization (WHO) (≤10 µg/dL). On the other hand, 8.3% of the individuals in the case group and 33.3% of those in the control group had BCLs higher than the acceptable level mentioned by the WHO (≤0.5 µg/dL).

According to the results of Spearman' correlation, BLLs were negatively associated with age, height, and weight, and correlated with BMI in a positive manner but none were significant. On the

TABLE 2. Participant characteristics in this study.

Characteristic	Case (n=36)	Control (n=12)	BLL (μg/dL) geometric mean	*P*-value	BCL (μg/dL) geometric mean	*P*-value
Gender						
Male	19 (52.8%)	7 (58.3%)	48.35	0.92 [b]	0.30	0.66 [b]
Female	17 (47.2%)	5 (41.7%)	47.87		0.29	
Race						
Fars	16 (44.4%)	9 (75%)	48.27	0.86 [b]	0.31	0.42 [b]
Arab	20 (55.5%)	3 (25%)	48.44		0.28	
Socioeconomic status [a]						
Well	0	2 (16.7%)	40.99		0.43	
Moderate	13 (36.1%)	6 (50%)	45.17	0.62 [c]	0.26	0.37 [c]
Weak	23 (63.9%)	4 (33.3%)	51.35		0.31	
Father's education [a]						
College graduate	9 (25%)	10 (83.3%)	50.79		0.32	
High school graduate	8 (22.2%)	2 (16.7%)	45.62	0.16 [c]	0.29	0.41 [c]
<High school	15 (41.7%)	0	51.98		0.30	
No formal education	4 (11.1%)	0	33.76		0.20	
Mother's education						
College graduate	7 (19.4%)	5 (41.7%)	43.92		0.28	
High school graduate	13 (36.1%)	4 (33.3%)	53.65	0.49 [c]	0.31	0.80 [c]
<High school	14 (38.9%)	3 (25%)	48.24		0.30	
No formal education	2 (5.6%)	0	36.30		0.24	
Parental smoking						
Yes	14 (38.9%)	3 (25%)	52.31	0.42 [b]	0.32	0.44 [b]
No	22 (61.1%)	9 (75%)	46.31		0.28	
Parental consanguineous marriage [a]						
Yes	25 (69.4%)	4 (33.3%)	51.35	0.33 [b]	0.30	0.97 [b]
No	11 (30.6%)	8 (66.7%)	44.11		0.29	

[a] $P < 0.05$ between cases and controls. [b] *P*-value based on the Mann-Whitney test. [c] *P*-value based on the Kruskal-Wallis test. BLL: blood lead level; BCL: blood cadmium level.

TABLE 3. Comparison of mean (mean ± SD and geometric mean) blood cadmium and lead levels within groups.

Groups	N	Blood lead level (μg/dL)			Blood cadmium level (μg/dL)		
		Mean ± SD	GM	*P*-value	Mean ± SD	GM	*P*-value
Case	36	51.02±26.96	45.26	0.054	0.29±0.13	0.26	0.0004*
Control	12	61.18±16.42	58.95		0.47±0.10	0.45	

*Significant *P* value <0.05. *P*-value based on the Mann-Whitney test. SD: standard deviation; GM: geometric means.

other hand, BCLs were positively associated with age, height, weight, and BMI although no significant difference was observed ($P>0.05$). The relationship between BLLs and BCLs were positive and significant ($P<0.05$) (TABLE 4).

To explore some risk or protective factors for elevated BLLs and BCLs, logistic regression analysis was performed, and the results showed no association with BLLs (TABLE 5) and BLCs (TABLE 6) in the unadjusted and adjusted analysis.

DISCUSSION

The present research was one of the rare case-control studies examining exposures to heavy metals and incidence rates of RCC in children. Although extensive research has been thus far carried out on heavy metals, no single study exists reflecting on their effects on RCC. Numerous studies have also estimated risk factors that may lead to RCC in children, but this study set out to investigate

TABLE 4. Spearman Correlation analysis between BLLs, BCLs and some related factors.

Dependent variable	Independent variable	Correlation coefficient	*P*-value
BLL	Age	-0.215	0.14
	Height	-0.163	0.26
	Weight	-0.109	0.46
	BMI	0.118	0.42
	BCL	0.363	0.01*
BCL	Age	0.188	0.20
	Height	0.166	0.26
	Weight	0.220	0.13
	BMI	0.097	0.51
	BLL	0.363	0.01*

*Significant *P* value <0.05. *P*-value based on the Pearson correlation analysis. BLL: blood lead level; BCL: blood cadmium level.

TABLE 5. Logistic regression analysis between BLLs and investigated factors (n=48).

Investigated factors	High or low BLLs[a] Unadjusted OR (95% CI)	Unadjusted *P*-value	Adjusted OR (95% CI)	Adjusted *P*-value
Age	0.94 (0.78–1.13)	0.55	0.99 (0.80–1.22)	0.94
Gender	1.02 (0.32–3.21)	0.96	1.01 (0.28–3.58)	0.97
Race	0.61 (0.19–1.91)	0.39	0.74 (0.19–2.91)	0.67
Father education	1.40 (0.79–2.49)	0.24	1.83 (0.71–4.68)	0.20
Mother education	1.24 (0.63–2.41)	0.52	1.10 (0.36–3.34)	0.85
Socioeconomic status	1.44 (0.53–3.89)	0.46	1.57 (0.35–6.92)	0.55
Parental smoking	0.74 (0.22–2.46)	0.63	0.40 (0.08–1.86)	0.24
parental consanguineous marriage	0.63 (0.19–2.03)	0.44	0.52 (0.09–2.80)	0.45

[a] High or low BLLs, taking 45 µg/dL as boundary. BLL: blood lead level; BCL: blood cadmium level.

TABLE 6. Logistic regression analysis between BCLs and investigated factors (n=48).

Investigated factors	High or low BCLs[a] Unadjusted OR (95% CI)	Unadjusted *P*-value	Adjusted OR (95% CI)	Adjusted *P*-value
Age	1.05 (0.84–1.30)	0.64	1.13 (0.88–1.46)	0.31
Gender	2 (0.51–7.84)	0.32	1.61 (0.35–7.46)	0.53
Race	0.35 (0.09–1.40)	0.14	0.36 (0.06–2.14)	0.26
Father education	1.93 (0.97–3.83)	0.05	3.19 (0.93–10.99)	0.06
Mother education	1.30 (0.60–2.82)	0.49	0.44 (0.09–2.04)	0.44
Socioeconomic status	1.08 (0.35–3.34)	0.88	0.65 (0.10–4.14)	0.64
Parental smoking	1.42 (0.37–5.45)	0.60	0.46 (0.07–3.02)	0.42
Parental consanguineous marriage	0.89 (0.23–3.36)	0.86	0.36 (0.05–2.62)	0.31

[a] High or low BCLs, taking 0.2 µg/dL as boundary. BLL: blood lead level; BCL: blood cadmium level.

the effect of heavy metals on incidence of RCC in children. So, a total number of 36 children with RCC as the case group and 12 healthy children without any constipation were included as the control group and their BLLs and BCLs were evaluated.

The study findings revealed no significant difference in the GMs of BLLs between the case and control groups. The GMs of BLLs in the case and control groups were 45.26 and 58.95 µg/dL, respectively. Furthermore, the given value of BCLs in the control group (0.45 µg/dL) was significantly higher than that in the case group (0.26 µg/dL). Since the case group had taken laxatives, the lower Pb and Cd concentrations in this group might have been due to use of stimulants and increased Pb and Cd excretion. The GMs of BCLs and BLLs in the control group were also higher than those in the case group, although just the BCLs were significantly higher ($P<0.05$). This might be because of high levels of Cd and Pb in both case and control groups as a result of their exposures to heavy metal resources. Considering Ahvaz as a contaminated city, individuals, especially children could be exposed to Cd and Pb through contaminated air, water, food, and consumer products as confirmed in several studies. Moreover, Cd as an important environmental pollutant in the blood samples of Iranian general population is high, according to a systematic review by Ghoochani et al. In addition, Cd contamination has been thus far observed in other Iranian food groups such as rice, cereals, and legumes, canned tuna fish, vegetables, fruit juice, and eggs. A few studies had been also done on BCLs of the general population in Iran. However, comparing the results of this review study with those in other countries revealed that the Iranian population had the highest levels of Cd[(21)].

Urban traffic and roadside soils are thus the most important sources of pollution that are directly related to vehicles and may be simply transferred to other environments by wind and dust. Besides, humans are exposed to potentially toxic metals contained in soil. In a study performed by Neisi et al. found that the risk of cancer in most exposed children was high due to high concentrations of heavy metals in indoor dust in the city of Ahvaz[(16)].

Drinking water is one other source of heavy metal and evaluation of its metals is necessary. In this line, Ali Albaji et al. had reported that, concentrations of Pb and mercury (Hg) in drinking water in the city of Ahvaz were 0.02 and 0.0047 mg/L, respectively, which were larger than standard levels[(22)]. Pourkhabbaz et al. reported that chromium (Cr) and Cd concentrations in Karun River were higher than the allowable limit of EPA3050 standards[(23)].

Furthermore, milk is the main nutritious food, especially for children, thus its contamination with heavy metals is of utmost importance. In this regard, Rahimi had reported mean ± standard deviation (SD) concentrations of Pb and Cd in milk samples collected from the city of Ahvaz (11±5.43 and 2.45±1.57 ng/mL, respectively) that were higher than those in other regions and even the values found in other countries[24].

Rice is also the second most abundant cereal in Iran. Heavy metals can easily accumulate in such crops through the root system and transfer to the human body[25]. For example, Ramezani et al. reported that Pb concentrations in rice samples collected from Khuzestan Province were significantly higher than permissible levels[26].

In another study by Payandeh et al., indicating that the amount of Cd available to the wheat samples (0.31 mg/kg) was above the standard limits set by the WHO (0.20 mg/kg)[27], due to the high use of phosphorus fertilizers containing high levels of Cd.

In agreement with previous studies[28-30], the present study indicated that BLLs had declined as age had increased. It is well recognized that BLLs usually reach at the maximum at 2–3 years of age and decrease after it, so the results of this study corroborated this evidence[31,32]. Children are also more susceptible to Pb than adults for various reasons, such as greater GI absorption, higher probability of nutritional Fe and Ca deficiency states which augment the effects of Pb, and physiological differences. Besides, some painted toys may contain Pb and this is important since children are likely to put them in their mouths[33]. On the other hand, BCLs rise at an older age. These results validate previous findings showing that Cd could not cross the placenta and, gradually accumulate in the body. Since older children go to school and they are subsequently involved in other outdoor activities, they are more likely to be exposed to Cd[28,30,34].

Although there was no significant relationship between BCLs and weight in children, it was positive. Other studies had further found that exposure to Cd could increase lipid accumulation and eventually lead to obesity[35,36]. This study showed a negative association between BLLs and weight in children and a positive association between BLLs and BMI that was not significant. In 2013, Scinicariello, had reported an inverse association between BLLs and body weight in children and adolescents in the United States[37]. In a study performed by Wang et al. in China, it had been demonstrated that BLLs was positively associated with BMI and obesity in women[38], due to changes in the hypothalamic-pituitary-adrenal (HPA) axis with Pb linking[39] which could result in obesity[40] as well as Pb-induced oxidative stress disrupting fat metabolism and causing obesity[41]. There have been also several studies supporting that exposure to Pb was associated with lower BMI[42] and decrease in the growth of infant's height[43], weight[44], along with head and arm circumferences[45].

In the present study, no significant difference was observed between the GMs of BLLs and BCLs of females and males. In the case group, female children had higher BLLs and BCLs than males; however, in the control group, BLLs and BCLs in boys were higher than girls. Previous studies had clearly stated that the concentrations of Pb in males were higher than those in females[46,47], because of higher exposures and as Pb tended to bind to erythrocytes and higher blood hematocrit levels due to increased Pb in them[48,49]. Likewise, Swedish researchers had stated that BLLs in females could reflect hereditary factors to a considerable extent (about 40%), while it could mostly (more than 95%) show environmental exposure in males[50]. Despite the fact many studies had suggested that the concentrations of Cd were higher in boys than girls, for some reasons, such as spending more time outdoors and possibility of more exposure to Cd, especially through smoking[51-53], others had reported that females were more vulnerable than males attributable to their lower Fe levels increasing intestinal Cd absorption, since Cd bioavailability is affected by low Fe status[47,54,55].

Lower socioeconomic status is also associated with elevated BLLs and BCLs. This association is probably due to poor nutrition and location of houses in streets with heavy traffic[56]. In this study, BLLs and BCLs increased as socioeconomic status had decreased, but not significantly, which was consistent with previous findings[57-59].

As no significant difference was observed between parental education and BLLs and BCLs, lower levels of education were associated with a rising trend in BLLs and BCLs as shown previously[58,60]. This was possibly due to unawareness of various sources of Pb and Cd in the environment[57,61].

Tobacco smoking also remains as a substantial source of indoor air pollution and exposure to Pb and Cd in children. In the present study, BLLs and BCLs in children whose parents had very strong smoking habits were higher but not significant compared with another group, which was in line with previous findings[62,63].

The effect of parental consanguineous marriage on Pb and Cd poisoning in children has not been so far investigated, but, with reference to culture and religion in this province, the number of consanguineous marriages was vast[64,65]. In the present study, BLLs and BCLs in children with parental consanguineous marriage were higher than other groups, which needed further investigations.

The study by Maleknejad et al., conducted in Iran on 90, 2–13-year-old children complaining of constipation (case group) and 90 healthy children (control group), indicating that 37.8% of the case group and 8.9% of the control group had a significant difference in serum lead levels of ≥10 µg/dL. They investigated the lead serum level, but we determined lead and cadmium levels in whole blood, furthermore they studied some factors including place of residence, parents' occupation but we studied other factors including race, socioeconomic status, parents' education, parental smoking, and parental consanguineous marriage. On the other hand, Consistent with the present study, they found that lead poisoning was significantly more frequent in children who were less than 7 years old and boys[66].

Some limitations should be considered. First, lack of cooperation and poor compliance of parents was the main reason for the small sample size in the control group although there are few researches which the number of controls is less than the number of cases[29,67]. Since we needed blood samples, it was very difficult to justify and convince their parents to take blood samples from their healthy children. Second, there was no possibility of the colonic transit study. Third, anorectal manometry was not performed for all patients. Fourth, finite time was another limitation that affected the sample size.

To our knowledge, this is the first clinical study demonstrating abnormally high BLLs and BCLs in children living Ahvaz, and so far no studies to measure BLLs and BCLs in this area has been done. The results of this study suggested that, in Iran especially in the city of Ahvaz, definitive plans and regulations needed to be implemented to prevent and control Pb and Cd poisoning in children, because in data article performed by Mahmoudi et al., the high concentration of lead in even healthy Iranian children was reported[68]. Screening and testing BLLs and BCLs should be also improved. In industrial areas, screening programs for BLLs and BCLs are also required to be exercised. Pb and Cd pollution sources such as paints, industrial processes, cosmetics, agricultural activities, and so on should be additionally controlled. Children's hand-washing when they come in from playing outdoors can be effective in this respect. As well, teaching schoolchildren in order to inform them of the sources of heavy metals and protecting them from exposures to heavy metals is recommended.

Evaluation of Pb and Cd levels in other biological samples such as hair and urine and also in parents could be beneficial. Exposure of pregnant women to tobacco smoke should be further minimized and they need to be banned in terms of using Pb-containing cosmetics containing.

Due to high levels of Pb and Cd contamination in the city of Ahvaz, the risk of Pb and Cd poisoning and contamination threatens future generations. Therefore, extensive studies are required to find possible sources of contamination by heavy metals and attempts need to be made to resolve this problem.

CONCLUSION

It was concluded that exposures to Pb and Cd due to environmental pollution and susceptibility to heavy metals may not be associated with RCC in children living in the city of Ahvaz, Khuzestan Province, Iran. Although this research was the first one providing data on BLLs and BCLs for children with RCC, the findings could be useful for designing epidemiologic studies in the future.

ACKNOWLEDGMENTS

This study is part of a Master's Thesis fulfilled by Nasrin Gharibshahi at Toxicology Department, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Special thanks are thus extended to Ahvaz Jundishapur University of Medical Sciences for their financial supports. The abstract of this paper was presented at the "15th Iranian Congress of Toxicology" as a poster presentation with interim findings. The hyperlink of this is https://www.civilica.com/Paper-TOXICOLOGY15-TOXICOLOGY15_150.html with COI: TOXICOLOGY15_150.

Authors' contribution

Mahdavinia M and Javaherizadeh H provided the research idea and funds, Javaherizadeh H is responsible in the diagnosis and classification of patients according their disease activity. Mahdavinia M, Javaherizadeh H, Khorasgani ZN and Gharibshahi N were actively involved in planning and execution of this study. Gharibshahi N is responsible in writing this manuscript and critical analyses of the manuscript. Khorasgani ZN and Gharibshahi N were responsible for organizing the patient's samples in laboratory analyses and interpretation of data. Mahdavinia M, Gharibshahi N, Javaherizadeh H, Khorasgani ZN were responsible for reviewing and editing. All authors read and approved the final manuscript.

Orcid

Nasrin Gharibshahi: 0000-0003-1884-9259.
Hazhir Javaherizadeh: 0000-0001-7898-4589.
Zahra Nazari Khorasgani: 0000-0003-3267-9540.
Masoud Mahdavinia: 0000-0001-9448-1500.

Gharibshahi N, Javaherizadeh H, Khorasgani ZN, Mahdavinia M. Relação entre níveis de chumbo e cádmio no sangue e a prisão de ventre crônica refratária entre crianças iranianas. Arq Gastroenterol. 2021;58(3):329-36.

RESUMO – **Contexto** – Há limitadas pesquisas que procuram razões que causem constipação crônica refratária (CCR) em crianças. Os efeitos das exposições de chumbo (Pb) e cádmio (Cd) nesta condição têm sido ainda menos claros. No entanto, alguns fatores relacionados podem contribuir para a avaliação dos níveis de Pb no sangue (NPbSs) e dos níveis de Cd no sangue (NCdSs). **Objetivo** – O presente estudo teve como objetivo examinar a relação entre as exposições de Pb e Cd e a CCR em crianças residentes na cidade de Ahvaz, província de Khuzestan, no Sudoeste do Irã. **Métodos** – Este estudo foi realizado em um número total de 48 crianças de 2 a 13 anos, incluindo 36 casos de CCR diagnosticados clinicamente, e 12 controles encaminhados a uma clínica pediátrica na cidade de Ahvaz. Seus NPbSs e NCdSs foram então determinados usando um espectrógrafo de absorção atômica do forno de grafite. Os dados do questionário projetado pelo pesquisador também foram recodificados, e os fatores de risco relacionados foram analisados por meio da análise de correlação e regressão logística de Spearman. **Resultados** – Os achados revelaram que as médias geométricas de Pb e Cd em amostras de sangue no grupo controle foram de 58,95 μg/dL e 0,45 μg/dL; respectivamente. Esses valores no grupo constipação foram igualmente 45,26 μg/dL e 0,26 μg/dL; respectivamente. Observou-se diferença significativa entre os NCdSs nos grupos de caso e controle (P<0,01). Todas as crianças de ambos os grupos também apresentaram NPbSs maiores do que o limite permitido endossado pela Organização Mundial da Saúde (OMS) (≤10 μg/dL). Por outro lado, 8,3% dos indivíduos no grupo de casos e 33,3% dos do grupo controle apresentaram NCdSs superiores à faixa aceitável mencionada pela OMS (≤0,5 μg/dL). **Conclusão** – As exposições de Pb e Cd por poluição ambiental e suscetibilidade a metais pesados podem não estar associadas à CCR em crianças residentes na cidade de Ahvaz. Embora esta pesquisa tenha sido a primeira a fornecer dados sobre NPbSs e NCdSs em crianças com CCR, os achados poderiam ser úteis para a concepção de futuros estudos epidemiológicos.

Palavras-chave – Constipação crônica refratária; chumbo; cádmio.

REFERENCES

1. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features, and Rome IV. Gastroenterology. 2016;150:1262-79. e2.
2. Van Den Berg MM, Benninga M, Di Lorenzo C. Epidemiology of childhood constipation: a systematic review. Am J Gastroenterol. 2006;101:2401.
3. Youssef NN, Atienza K, Langseder AL, Strauss RS. Chronic abdominal pain and depressive symptoms: analysis of the national longitudinal study of adolescent health. Clin Gastroenterol Hepatol. 2008;6:329-32.
4. Mugie SM, Benninga MA, Di Lorenzo C. Epidemiology of constipation in children and adults: a systematic review. Best Pract Res Clin Gastroenterol. 2011;25:3-18.
5. Khan A, Khan S, Khan MA, Qamar Z, Waqas M. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environ Sci Pollut Res Int. 2015;22:13772-99.
6. Singh R, Gautam N, Mishra A, Gupta R. Heavy metals and living systems: An overview. Indian J Pharmacol. 2011;43:246.
7. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy Metal Toxicity and the Environment. In: Luch A. (eds) Molecular, Clinical and Environmental Toxicology. Experientia Supplementum, vol 101. Springer, Basel, 2012:133-64. https://doi.org/10.1007/978-3-7643-8340-4_6
8. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003;68:167-82.
9. Hon K, Fung C, Leung A. Childhood lead poisoning: an overview. Hong Kong Med J. 2017;23:616-21.
10. Tait PA, Vora A, James S, Fitzgerald DJ, Pester BA. Severe congenital lead poisoning in a preterm infant due to a herbal remedy. Med J Aust. 2002;177:193-5.
11. Biggs WS, Dery WH. Evaluation and treatment of constipation in infants and children. Am Fam Physician. 20061;73:469-77.
12. Schoeters G, Hond ED, Zuurbier M, Naginiene R, Hazel PVD, Stilianakis N, et al. Cadmium and children: exposure and health effects. Acta Paediatr Suppl. 2006;95:50-4.
13. Bernhoft RA. Cadmium toxicity and treatment. The Scientific World Journal. 2013;2013:394652.
14. Xu J, Sheng L, Yan Z, Hong L. Blood lead and cadmium levels in children: A study conducted in Changchun, Jilin Province, China. Paediatr Child Health. 2014;19:73-6.
15. Heidari-Farsani M, Shirmardi M, Goudarzi G, Alavi-Bakhtiarivand N, Ahmadi-Ankali K, Zallaghi E, et al. The evaluation of heavy metals concentration related to PM10 in ambient air of Ahvaz city, Iran. J Adv Environ Health Res. 2013;1:120-28. DOI: 10.22102/JAEHR. 2013.40133.
16. Neisi A, Goudarzi G, Akbar Babaei A, Niri MV, Naimabadi A, Mohammadi MJ, et al. Study of heavy metal levels in indoor dust and their health risk assessment in children of Ahvaz city, Iran. Toxin Rev. 2016;35:16-23. DOI:10.1080/15569543.2016.1181656.
17. Tabbers MM, DiLorenzo C, Berger MY, Faure C, Langendam MW, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. J Pediatr Gastroenterol Nutr. 2014;58:258-74.
18. Cornelis R, Heinzow B, Herber R, Christensen JM, Poulsen OM, Sabbioni E, et al. Sample collection guidelines for trace elements in blood and urine. J Trace Elem Med Biol. 1996;10:103-27.
19. Hauptman M, Bruccoleri R, Woolf AD. An update on childhood lead poisoning. Clin Pediatr Emerg Med. 2017;18:181-92.
20. Zheng L, Wu K, Li Y, Han D, Zhang B, Gu C, et al. Blood lead and cadmium levels and relevant factors among children from an e-waste recycling town in China. Environ Res. 2008;108:15-20.
21. Ghoochani M, Rastkari N, Yunesian M, Nodehi RN, Mesdaghinia A, Houshiarrad A, et al. What do we know about exposure of Iranians to cadmium? Findings from a systematic review. Environ Sci Pollut Res Int. 2018;25:1-11.
22. Albaji A, Ziarati P, Shiralipour R. Mercury and Lead Contamination Study of Drinking Water in Ahvaz, Iran. Intl J Farm and Alli Sci. 2013;2:751-55.
23. Pourkhabbaz H, Hedayatzadeh F, Cheraghi M. Determination of Heavy Metals Concentration at Water Treatment Sites in Ahwaz and Mollasani Using Bioindicator. Ecopersia. 2018;6:55-66.
24. Rahimi E. Lead and cadmium concentrations in goat, cow, sheep, and buffalo milks from different regions of Iran. Food Chem. 2013;136:389-91.
25. Jalali M, Hemati N. Chemical fractionation of seven heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) in selected paddy soils of Iran. Paddy Water Environ. 2013:299-309. DOI 10.1007/s10333-012-0320-8.
26. Ramezani Z, Khodayar MJ, Nazari Z, Hedayatifar R, Gilani A-a. Cadmium and lead contents of rice (Oryza sativa) grown in Khuzestan, southwest of Iran. J Health Res. 2014;5:65-71.
27. Payandeh K, Jafarnejadi A, Gholami A, Shokohfar A, Panahpor E. EEvaluation of Cadmium Concentration in Wheat Crop Affected by Cropping System. Jundishapur J Health Sci. 2018;10:e14400. DOI: 10.5812/jjhs.14400.
28. Wu K-G, Chang C-Y, Yen C-Y, Lai C-C. Associations between environmental heavy metal exposure and childhood asthma: A population-based study. Scand J Immunol. 2019;52:352-62.
29. Kim S, Arora M, Fernandez C, Landero J, Caruso J, Chen A. Lead, mercury, and cadmium exposure and attention deficit hyperactivity disorder in children. Environ Res. 2013;126:105-10.
30. Kowalska M, Kulka E, Jarosz W, Kowalski M. The determinants of lead and cadmium blood levels for preschool children from industrially contaminated sites in Poland. Int J Occup Med Environ Health. 2018;31:351-59.
31. Olympio KPK, Silva JPR, Silva AS, Souza VCO, Buzalaf MAR, Barbosa-Junior F, et al. Blood lead and cadmium levels in preschool children and associated risk factors in São Paulo, Brazil. Environ Pollut. 2018;240:831-38.
32. Canfield RL, Henderson Jr CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. N Engl J Med. 2003;348:1517-26.
33. McCabe EB. Age and sensitivity to lead toxicity: a review. Environ Health Perspect. 1979;29:29-33.
34. Shah F, Kazi TG, Afridi HI, Kazi N, Baig JA, Shah AQ, et al. Evaluation of status of trace and toxic metals in biological samples (scalp hair, blood, and urine) of normal and anemic children of two age groups. Biol Trace Elem Res. 2011;141:131-49.
35. Tinkov AA, Filippini T, Ajsuvakova OP, Aaseth J, Gluhcheva YG, Ivanova JM, et al. The role of cadmium in obesity and diabetes. Science of The Total Environment. Sci Total Environ. 2017;601:741-55.
36. Green AJ, Hoyo C, Mattingly CJ, Luo Y, Tzeng J-Y, Murphy SK, et al. Cadmium exposure increases the risk of juvenile obesity: a human and zebrafish comparative study. Int J Obes (Lond). 2018;42:1285-95.
37. Scinicariello F, Buser MC, Mevissen M, Portier CJ. Blood lead level association with lower body weight in NHANES 1999–2006. Toxicol Appl Pharmacol. 2013;273:516-23.
38. Wang N, Chen C, Nie X, Han B, Li Q, Chen Y, et al. Blood lead level and its association with body mass index and obesity in China-Results from SPECT-China study. Sci Rep. 2015;5:18299.
39. Braun JM, Wright RJ, Just AC, Schnaas L, Hu H, Wright RO, Tellez-Rojo MM. Relationships between lead biomarkers and diurnal salivary cortisol indices in pregnant women from Mexico City: a cross-sectional study. Environ Health. 2014;13:50 .
40. Kargi AY, Iacobellis G. Adipose tissue and adrenal glands: novel pathophysiological mechanisms and clinical applications. Int J Endocrinol. 2014;2014.
41. Matsuda M, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. Environ Res. 2013;7:e330-e41.
42. Afeiche M, Peterson KE, Sánchez BN, Schnaas L, Cantonwine D, Ettinger AS, et al. Windows of lead exposure sensitivity, attained height, and body mass index at 48 months. J Pediatr. 2012;160:1044-49.
43. Schwartz J, Angle C, Pitcher H. Relationship between childhood blood lead levels and stature. Pediatrics. 1986;77:281-88.
44. Sanı´n LH, González-Cossı´o T, Romieu I, Peterson KE, Ruíz S, Palazuelos E, et al. Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants. Pediatrics. 2001;107:1016-23.
45. Schell LM, Denham M, Stark AD, Parsons P, Schulte E. Growth of infants' length, weight, head and arm circumferences in relation to low levels of blood lead measured serially. Am J Hum Biol. 2009;21:180.
46. Nunes JA, Batista BL, Rodrigues JL, Caldas NM, Neto JA, Barbosa Jr F. A simple method based on ICP-MS for estimation of background levels of arsenic, cadmium, copper, manganese, nickel, lead, and selenium in blood of the Brazilian population. J Toxicol Environ Health A. 2010;73:878-87.
47. Vahter M, Åkesson A, Liden C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. Environ Res. 2007;104:85-95.
48. Becker K, Kaus S, Krause C, Lepom P, Schulz C, Seiwert M, et al. German Environmental Survey 1998 (GerES III): environmental pollutants in blood of the German population. Int J Hyg Environ Health. 2002;205:297-308.
49. Pirkle JL, Kaufmann RB, Brody DJ, Hickman T, Gunter EW, Paschal DC. Exposure of the US population to lead, 1991-1994. Environ Health Perspect. 1998;106:745-50.
50. Björkman L, Vahter M, Pedersen NL. Both the environment and genes are important for concentrations of cadmium and lead in blood. Environ Health Perspect. 2000;108:719-22.

51. Laamech J, Bernard A, Dumont X, Benazzouz B, Lyoussi B. Blood lead, cadmium and mercury among children from urban, industrial and rural areas of Fez Boulemane Region (Morocco): relevant factors and early renal effects. Int J Occup Med Environ Health. 2014;27:641-59.
52. Hossny E, Mokhtar G, El-Awady M, Ali I, Morsy M, Dawood A. Environmental exposure of the pediatric age groups in Cairo City and its suburbs to cadmium pollution. Sci Total Environ. 2001;273:135-46.
53. Farzin L, Amiri M, Shams H, Faghih MAA, Moassesi ME. Blood levels of lead, cadmium, and mercury in residents of Tehran. Biol Trace Elem Res. 2008;123:14-26.
54. Eom S-Y, Lee Y-S, Lee S-G, Seo MN, Choi BS, Kim YD, et al. Lead, mercury, and cadmium exposure in the Korean general population. J Korean Med Sci. 2018;33:e9.
55. Ilmiawati C, Yoshida T, Itoh T, Nakagi Y, Saijo Y, Sugioka Y, et al. Biomonitoring of mercury, cadmium, and lead exposure in Japanese children: a cross-sectional study. Environ Health Prev Med. 2015;20:18.
56. Heinrich J, Popescu MA, Wjst M, Goldstein IF, Wichmann HE. Atopy in children and parental social class. Am J Public Health. 1998;88:1319-24.
57. Gao Z, Cao J, Yan J, Wang J, Cai S, Yan C. Blood lead levels and risk factors among preschool children in a lead polluted area in Taizhou, China. Biomed Res Int. 2017;2017.
58. Meyer I, Heinrich J, Lippold U. Factors affecting lead, cadmium, and arsenic levels in house dust in a smelter town in eastern Germany. Environ Res. 1999;81:32-44.
59. Kim E, Kwon H-J, Ha M, J-A Lim, Lim MH, Yoo SJ, et al. How Does Low Socioeconomic Status Increase Blood Lead Levels in Korean Children? Int J Environ Res Public Health. 2018;15:1488.
60. Morales LS, Gutierrez P, Escarce JJ. Demographic and socioeconomic factors associated with blood lead levels among Mexican-American children and adolescents in the United States. Public Health Rep. 2005;120:448-54.
61. Cao J, Li M, Wang Y, Yu G, Yan C. Environmental lead exposure among preschool children in Shanghai, China: blood lead levels and risk factors. PloS One. 2014;9:e113297.
62. Richter PA, Bishop EE, Wang J, Kaufmann R. Trends in tobacco smoke exposure and blood lead levels among youths and adults in the United States: the National Health and Nutrition Examination Survey, 1999-2008. Prev Chronic Dis. 2013;10:E213-E13
63. Apostolou A, Garcia-Esquinas E, Fadrowski JJ, McLain R, Pat, Weaver VM, Navas-Acien A. Secondhand tobacco smoke: a source of lead exposure in US children and adolescents. Am J Public Health. 2012;102:714-22.
64. Saadat M, Ansari-Lari M, Farhud D. Short report consanguineous marriage in Iran. Ann Hum Biol. 2004;31:263-9.
65. Akrami SM, Osati Z. Is consanguineous marriage religiously encouraged? Islamic and Iranian considerations. J Biosoc Sci. 2007;39:313-6.
66. Maleknejad S, Heidarzadeh A, Rahbar M, Safaei A, Ghomashpasand B. Evaluation of serum lead levels in children with constipation and normal controls in northern Iran. Iran J Pediatr. 2013;23:417.
67. Konofal E, Lecendreux M, Arnulf I, Mouren M-C. Iron deficiency in children with attention-deficit/hyperactivity disorder. Arch Pediatr Adolesc Med. 2004;158:1113-5
68. Mahmoudi N, Latifi AM, Amani MA, Masoumbeigi H, Ghanizadeh G. Data on the environmental exposure to lead in Iran. Data brief. 2018;20:1133-41.