ORIGINAL ARTICLE

A randomized controlled trial on the effects of jujube fruit on the concentrations of some toxic trace elements in human milk

Roya Kelishadi, Najmeh Hasanghaliaei[1], Parinaz Poursafa[2], Mojtaba Keikha[3], Alireza Ghannadi[4], Maryam Yazdi[5], Ebrahim Rahimi[6]

Departments of Pediatrics and [3]Epidemiology, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical Sciences, [1]Department of Pediatrics, School of Medicine, Isfahan University of Medical Sciences, [2]Department of Environmental Health Engineering, Environment Research Center, Research Institute for Primordial Prevention of Non Communicable Disease, Isfahan University of Medical Sciences, [4]Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Sciences, [5]Department of Biostatistics, Faculty of Health, Isfahan University of Medical Sciences, Isfahan, [6]Department of Food Hygiene, Young Researchers Club, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Background: This study aims to investigate the concentrations of lead, cadmium, and arsenic in the human milk, and to assess the effect of jujube fruit consumption by lactating mothers in reducing the concentration of these heavy metals in their milk. **Materials and Methods:** This randomized controlled trial was conducted in 2014 among forty postpartum mothers in Isfahan, the second largest and polluted city in Iran. Mothers were randomized into two groups; the intervention group received 15 g/day of fresh jujube fruit, and the controls received routine care for 8 weeks. **Results:** In the beginning, the concentrations of lead, cadmium, and arsenic were high, without significant difference between groups. The mean (standard deviation) concentrations of lead, cadmium, and arsenic were 29.49 (16.6), 4.65 (3.51), and 1.23 (0.63) µg/L, respectively. The smoothed empirical distribution of environmental pollutants showed that in both groups the mean values and variance of toxic metals decreased after 8 weeks, with a sharper decline in the intervention group. Quantile regression analysis showed that in the intervention group, lead concentration decreased by 2.54 µg/L at the 90th quintile, and cadmium decreased by 0.19 µg/Lat 75th quintile; without significant change in arsenic level. The corresponding figures were not significant in the control group. **Conclusion:** The concentrations of heavy metals were high in human milk, and the consumption of jujube fruit had some beneficial effects in reducing these harmful elements. Pregnant and lactating mothers should be advised to reduce their exposure to environmental pollutants, and consumption of some natural medicinal foods can be useful in reducing the concentration of pollutants in human milk. Because of numerous benefits of breast milk, in spite of the existence of some toxic trace elements, breastfeeding must be encouraged because such contaminants are also found in water and formula. The impact of the current findings on the primary prevention of chronic disease should be determined in future longitudinal studies.

Key words: Health, jujube fruit, pediatric, prevention

How to cite this article: Kelishadi R, Hasanghaliaei N, Poursafa P, Keikha M, Ghannadi A, Yazdi M, Rahimi E. A randomized controlled trial on the effects of jujube fruit on the concentrations of some toxic trace elements in human milk. J Res Med Sci 2016;21:108.

INTRODUCTION

Environmental pollutants and toxic elements have several adverse effects notably in infants and young children.[1] According to the World Health Organization, the presence of environmental pollutants and toxic elements, mainly in air and food, is a global health problem for children.[2,3]

The first contacts of human with these toxic elements are during their fetal period and after birth, through breathing, consuming milk, drinking water, and exposure to various environmental pollutants.[4]

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/1735-1995.193499

Address for correspondence: Prof. Roya Kelishadi, Department of Pediatrics, Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Hezarjerib Ave, Isfahan, Iran. E-mail: kelishadi@med.mui.ac.ir

Received: 19-01-2016; **Revised:** 17-05-2016; **Accepted:** 10-08-2016

Some studies in different countries have shown that human milk contains various pollutants including lead, arsenic, and cadmium. They may enter mother's milk through different sources, mainly air, water, and food.[5-7] The concentrations of these elements in human milk are related to the mother's diet and her exposure to environmental pollutants. Toxic elements as lead, are also transferred to mother's milk through maternal reserves and bloodstream.[4,8]

Although toxic elements have several adverse health effects for all age groups, during neonatal and infancy periods, they pose an extreme risk to the developing nervous system. For instance, lead and cadmium have inhibitory effects on the intellectual and mental development.[9,10] Neurological effects of lead including motor skills disorders, learning, and behavioral problems are well documented.[10-12]

Arsenic exposure of newborns and infants is particularly from the milk that they consume, and may have adverse effects on child growth and development.[13,14]

Some studies in different countries have examined the levels of lead, cadmium, and arsenic in breast milk, and reported concentrations higher than the WHO-recommended reference levels.[15-18]

Worldwide urbanization and industrialization had imposed the hazards of toxic elements for human beings, notably for infants and children. In addition to environmental interventions for controlling the pollutant sources, finding interventional measures for reducing the concentration of toxic elements in the human body may be useful. Some studies have proposed that some foods including fruits, vegetables, and dairy products may reduce the concentration of some toxic chemicals in the human body.[19] This study aims to determine the concentrations of lead, arsenic, and cadmium in the milk of a sample of the Iranian women, and to assess the effects of consuming jujube fruit on reducing these toxic elements in human milk.

MATERIALS AND METHODS

This 8-week clinical trial was conducted in 2014 in Isfahan, the most industrialized and the second most air-polluted city in Iran. Participants consisted of 40 nursing mothers who were randomly selected from those hospitalized for accouchement in Shahid Beheshti Hospital affiliated to Isfahan University of Medical Sciences.

Ethical concerns

This trial was approved by the Research Council and Ethics Committee of the School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. It was registered in the Iranian Registry of Clinical Trials, which is a Primary Registry in the WHO Registry Network (IRCT-ID: IRCT201302061434N8). Written informed consent was obtained from participants; they were reassured about the confidentiality of the data.

Study area

Isfahan is the second largest city and the most industrialized city in Iran with a population of near two millions. It is located in the center of the Iranian plateau, with a latitude of 32°37′N, longitude of 51°40′E, and an average altitude of 1500 m from the sea level bounded by NW–SE mountain range of 3000 m. It has a moderate and dry climate on the whole with sunny weather in most days throughout the year. The air of this city is predominantly affected by industrial emissions and motor traffic.[20] Moreover, it has many industries, including a large iron foundry, a steel plant, an ordnance plant, and a cement plant. The soil of this area is contaminated by different toxic metals.[21]

Study participants

The participants included women who were eligible for the studies who were healthy-looking, with a singleton and full-term pregnancy, and who were living for at least 1 year in areas of Isfahan City with different levels of air pollution that had air pollution monitoring station. Those with a history of any of the following conditions were excluded from the study: tobacco use before or during pregnancy or lactation, having a household member using any kind of tobacco, having any chronic disease, receiving any medications, and following a special diet. These inclusion criteria were considered to reduce the confounding factors, and to increase the similarities in exposure to environmental pollutants. The exclusion criteria consisted of low adherence of mothers to continue the study, occurrence of any health problem, medication use by the mother or not following exclusive breast milk feeding during the trial.

Study procedure

One week after the delivery, 10 mL of breast milk was obtained from mothers and was stored at − 18°C in clean polyethylene containers, which were previously soaked in 1:1 nitric acid for 1 day and well rinsed with double-distilled water (dd H_2O). The study participants were randomly assigned to two groups of equal number (n = 20 in each group). One group received 15 g/day of jujube fruit for 8 weeks, and the other group received usual care. Plant material was collected from Kuhpayeh district in Isfahan Province, Iran. It was taxonomically identified by an academic herbal botanist.

Jujube fruits were purchased from a local provider in Isfahan, Iran. These fruits were authenticated in the herbarium department of the School of Pharmacy, Isfahan University of Medical Sciences. By every 2 weeks of telephonic follow-ups, we ensured the consumption of jujube fruit by the first group. After 8 weeks, the milk samples of participants were collected during a home visit by the project team.

Laboratory methods

First, in the stage of digestion, milk samples were collected and digested according to the wet method. At a temperature of 85°C, 10 mL of each sample was dried to reach a stationary weight, and then 7 mL of pure nitric acid was added, and the samples were stored overnight. Later, they were heated at a temperature of 120°C for better digestion. During digestion, 7 mL of hydrogen peroxide (30%) was added to the samples, and they were stored at the same temperature for an hour. During the time, the samples were all digested. Then each digested sample was 10 mL with deionized distilled water in a volumetric flask of 25 mL. To ensure the accuracy of the experiment, all samples were tested three times, and their mean values were recorded, and to ensure the validity of the test, the recycling percentage was assessed. Standard solutions were added to the samples to evaluate the recycling percentage of lead, cadmium, and arsenic. For the measurement of the three elements, we used atomic absorption with furnace method. Atomic absorption spectrometer (PERKIN ELMER 4100, (FIAS 4100, Perkin Elmer, Foster City, CA, USA)) was used; it is equipped with Graphite furnace system to measure lead, cadmium, and arsenic as the atomization source of elements in the product using HCL production source of the cathode ray. The method of samples preparation for furnace system was as follows: 5.0 g of the samples was weighed and after adding 5 mL of 25% nitric acid, it was kept in Teflon coated containers. They were heated for 5 min with 300 W of power inside the microwave (Berghol MWS-1), and the samples were digested. Thereafter, the samples reached to the volume of 25 mL in volumetric flasks. For measuring elements, initially, 5 mL ammonium pyrrolidine carbamate was added to 10 mL solution of digested samples. The samples were shaken for 20 min until the elements changed to complex as an organometallic form in the product. Then, 2 mL of methyl isobutyl ketone was added to the samples and then shaked for 30 min. After 10 min, they were centrifuged at 2500 rpm, and the elements were moved to the organic phase.

After setting up the furnace, the device lamp and optimizing the atomic absorption spectrometer, we draw the calibration curve of these elements by assistance of standards for these elements, a matrix of palladium modifier and by using the Winlab 32 software (Perkin Elmer Instruments LLC, Shelton). The values of these elements were measured in the prepared solutions.

Statistical analysis

The normal distribution of variables was checked by Shapiro–Wilk test and Q-Q plot. As the concentrations of toxic elements had nonnormal distribution, we applied nonparametric tests. Mann–Whitney U-test was used for comparing the baseline concentrations of toxic elements, and Wilcoxon signed-rank test for comparing them before and after 8 weeks. The effects of jujube fruit on concentrations of toxic elements were assessed by quantile regression test. Quantileregression is used to model the effects of covariates on the conditional quintiles of a response variable.

Ordinary least square linear regression was used for comparison of variables. For data analysis, SPSS (version 20.0, Chicago, IL) and R statistical software (version 2.11.1, R Foundation for Statistical Computing, Vienna) were used. The significance level was set at $P < 0.05$.

RESULTS

The mean (standard deviation [SD]) age of mothers was 27.9 (4.42) years; it was not significantly different in the two groups studied. The number of childbirths was one to three, and the education of most participants was at high school diploma level.

The concentrations of lead, cadmium, and arsenic did not have a normal distribution. As presented in Table 1, at the beginning of the study, no significant difference existed between the concentrations of lead, cadmium, and arsenic in the intervention and control groups. After 8 weeks, the concentrations of all these toxic elements decreased significantly in both groups.

Figure 1 shows the smoothed empirical distribution of lead, cadmium, and arsenic in both groups at baseline and after the intervention. In both groups, toxic elements had skewed distributions, and their mean values and variance decreased after 8 weeks.

Changes in the concentrations of pollutants in each participant are depicted in Figure 2. After 8 weeks, in participants who had low concentrations of lead, arsenic, and cadmium, these levels remained low, and in those who had elevated concentrations of these pollutants, these levels decreased; this decline was sharper in the intervention group.

Table 1: Baseline concentrations of toxic elements in the milk of the intervention and control groups

Toxic trace elements concentration (μg/L)	Intervention group (*n*=20)	Control group (*n*=20)	Total (*n*=40)	*P*
Lead	31.13 (16.08)	27.86 (17.49)	29.49 (16.66)	0.41
Cadmium	4.92 (2.86)	4.39 (4.13)	4.65 (3.51)	0.35
Arsenic	1.32 (0.53)	1.14 (0.72)	1.23 (0.63)	0.12

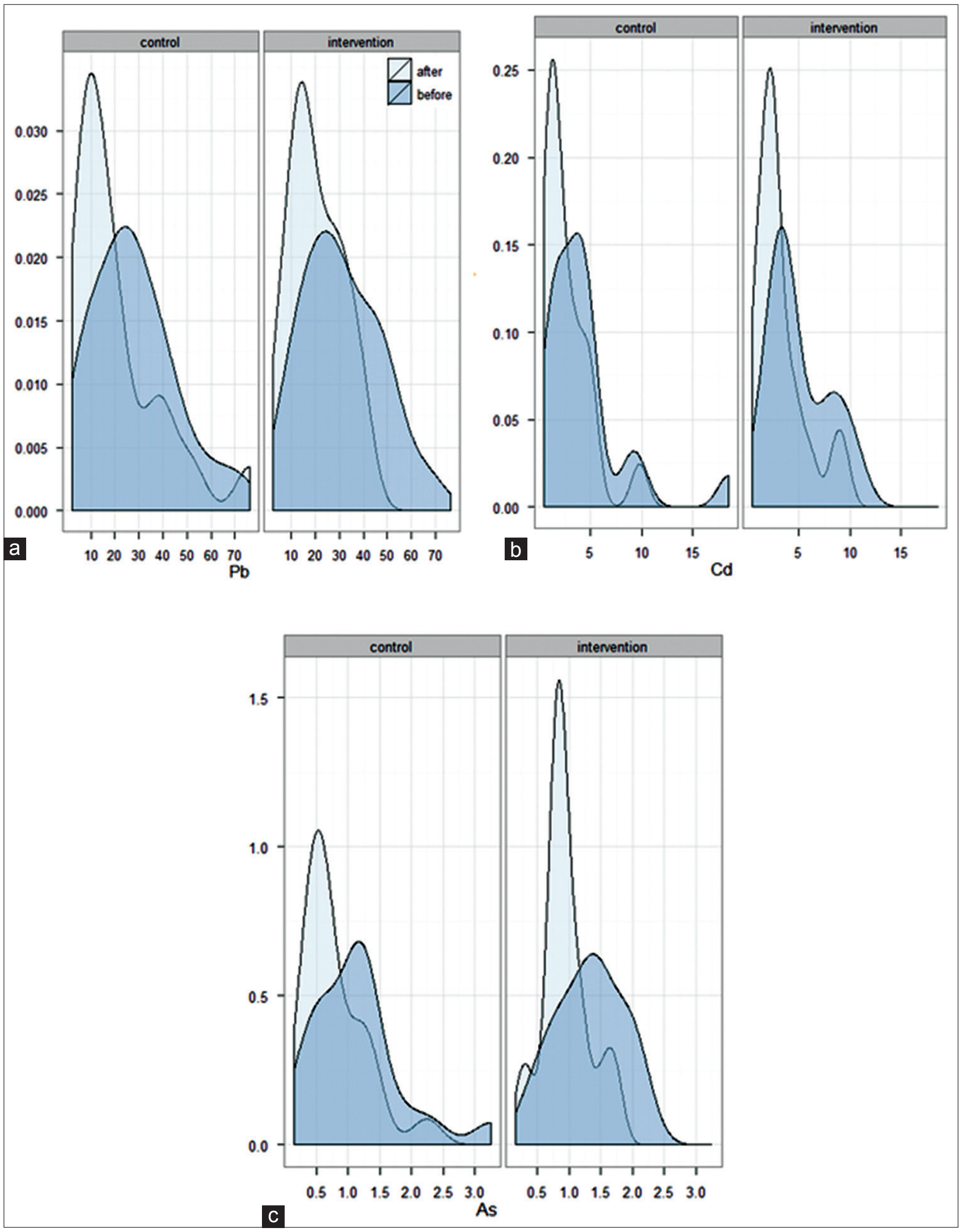

Figure 1: Smoothed empirical distribution by Kernel smoothing of lead, cadmium, and arsenic concentrations (μg/L) of human milk before and after the intervention. (a) Lead concentrations, (b) cadmium concentrations, (c) arsenic concentrations

Table 2 presents the changes in quintiles of pollutant concentrations at baseline and after 8 weeks. In most cases and both groups, these quintiles decreased at the end of the study. We considered the concentrations of pollutants after the trial as the dependent variables, and the baseline levels of pollutants and consumption/not consumption of jujube fruit as independent variables. This table presents the quantile regression coefficients, 95% confidence interval for consumption of jujube fruit on the 10th, 25th, 50th, 75th, and 90th quintiles of toxic elements in breast milk. It shows a significant decrease in lead concentrations, this effect of jujube fruit was observed at the 90th quintile of lead concentration, which decreased lead by 2.54 μg/L. This regression equation is considered as $Q_{0.9}(Pb_2) = 4.64 + 0.82Pb_0 - 2.54$ group, where Pb_0 and Pb_2 are lead concentrations at baseline and after the trial, respectively, the group is considered as 1 for the intervention group and 0 for the control group. This regression equation proposes that at an average level of the lead level (29.49 μg/L), after 8 weeks 90% of the intervention group would have lead levels <26.28 μg/L and the corresponding figure for controls would be 28.82 μg/L.

Likewise, consumption of jujube fruit had a significant association with decreasing the concentrations of cadmium; it decreased cadmium by 0.19 μg/Lat 75th quintile.

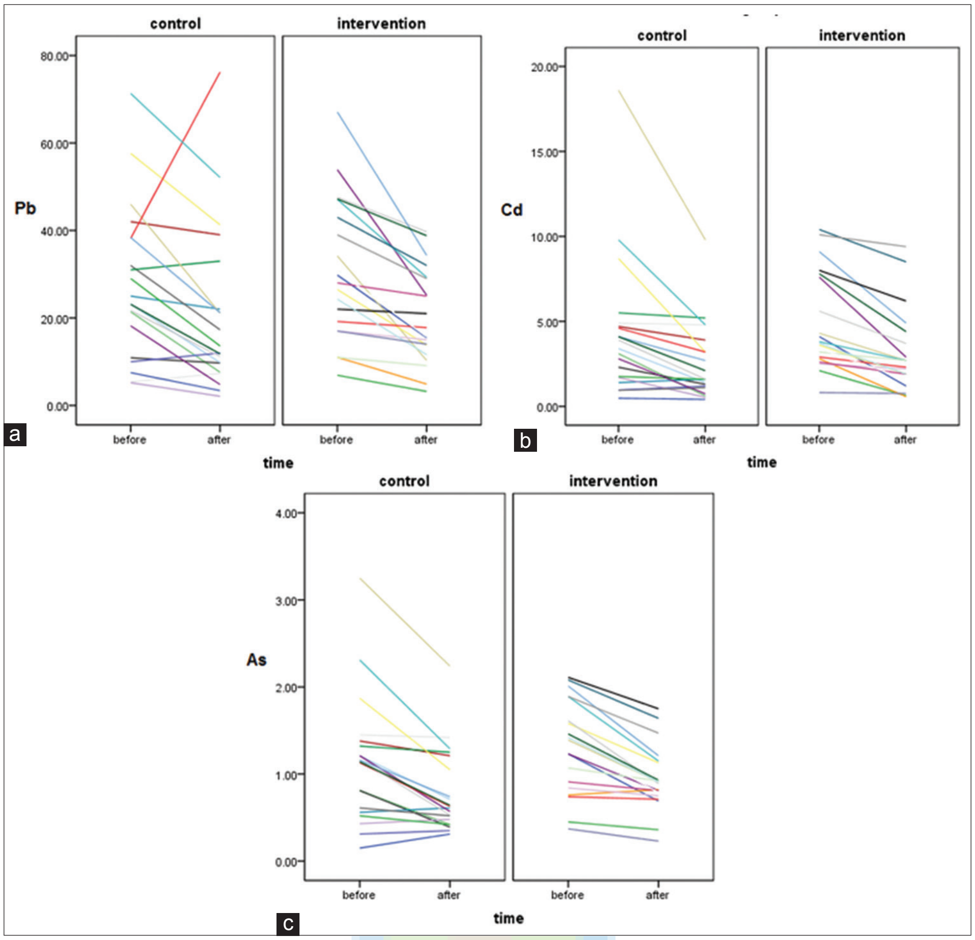

Figure 2: Changes in milk concentration of lead, cadmium, and arsenic (μg/L) in each participant. (a) Lead concentrations, (b) cadmium concentrations, (c) arsenic concentrations

Table 2: Adjusted effect of intervention on quintiles of lead, cadmium, and arsenic of human milk

	Quantile regressions					Linear regression
	10th	25th	50th	75th	90th	
Lead						
Intercept	−1.18 (−7.46-−0.40)	−0.59 (−4.98-−0.43)	−0.89 (−6.79-3.88)	3.25 (0.34-13.55)	4.64 (1.92-52.87)	1.57 (−5.71-8.84)
At birth	0.47 (0.34-0.71)	0.52 (0.46-0.75)	0.58 (0.41-0.79)	0.75 (0.65-0.94)	0.82 (0.61-1.17)	0.69 (0.49-0.89)
Group (control)	0.87 (−6.87-3.80)	0.22 (−0.93-2.40)	3.09 (−4.90-7.03)	0.15 (−4.40-3.50)	−2.54* (−29.87-−1.37)	−2.83 (−9.44-3.78)
Cadmium						
Intercept	−0.43 (−3.19-0.01)	−0.41 (−0.94-0.18)	0.16 (−0.29-0.72)	0.34 (0.01-0.61	0.37 (0.21-0.87)	0.02 (−0.67-0.70)
At birth	0.42 (0.07-0.58)	0.53 (0.33-0.56)	0.52 (0.42-0.91)	0.76 (0.48-0.94)	0.88 (0.80-0.94)	0.59 (0.48-0.69)
Group (control)	0.15 (−0.49-0.44)	0.2 (−0.78-0.58)	0.19 (−0.31-0.74)	−0.20* (−0.65-0.00)	−0.32 (−0.72-1.53)	0.25 (−0.47-0.97)
Arsenic						
Intercept	−0.04 (−0.25-0.01)	0.01 (−0.10-0.12)	0.17 (−0.17-0.24)	0.19 (0.14-0.29)	0.25 (0.19-0.50)	0.09 (−0.05-0.23)
At birth	0.53 (0.43-0.67)	0.56 (0.34-0.60)	0.48 (0.43-0.70)	0.67 (0.62-0.76)	0.76 (0.62-0.86)	0.61 (0.51-0.71)
Group (control)	0.07 (−0.01-0.15)	0.08 (−0.02-0.17)	0.06 (−0.07-0.23)	0.01 (−0.10-0.12)	−0.09 (−0.20-0.06)	0.045 (−0.08-0.17)

Quantile regression coefficients and linear regression and 95% CIs for the effect of the intervention on lead, cadmium, and arsenic levels adjusted on baseline measurements. *Significance level (double-sided) for group variable. CIs = Confidence intervals

According to its regression equation, at an average level of cadmium (4.65 μg/L), 75% of the intervention group would have cadmium level <3.67 μg/L, whereas 75% of controls would have cadmium level of < 3.85 μg/L.

The above-mentioned effects and changes were not significant for arsenic concentrations.

DISCUSSION

In the current study, the human milk concentration of lead, cadmium, and arsenic was relatively high in an industrialized area, and consumption of jujube fruit had some beneficial effects in reducing the concentrations of these pollutants in milk. Although many previous studies have been conducted regarding the level of toxic elements in breast milk,[15-18] limited experience exist on the interventions considering the reduction of these harmful elements in milk.

Jujube fruit or *Ziziphus jujuba* Mill from *Rhamnaceae* family is one of the most important *Ziziphus* species, which is cultivated in several areas including Iran. Fresh and dried fruits have been widely used among Iranians as a functional fruit and remedy for the treatment of constipation, cough, asthma, and atherosclerosis. It is a well-known cooling remedy in the Iranian Traditional Medicine for preparing of several healthy prescriptions.[22,23]

Jujube fruit is one of the useful and healthy fruits of Iran that can be used during lactation. The fruit contains natural substances such as triterpenoids such as betulinic acid, oleanolic acid, and maslinic acid, phytosterols such as beta-sitosterol and stigmasterol, flavonoids such as puerarin and quercetin, alkaloids, tannins and polyphenolic compounds, essential oils, proanthocyanidins, fatty acids, amino acids, trace elements, pectin, sugars, and vitamins. Each of these diverse natural health compounds of the jujube fruit has unique properties.[23-25] To the best of our knowledge, no previous study exists on the effects of this fruit on milk contaminants; we considered it because of its beneficial effects in our previous studies.[22,25] In this study, the effect of consuming jujube fruit by lactating mothers in reducing the concentration of some toxic trace elements in their higher quintiles. Possibly, future studies with longer follow-up might reach better results.

It should be acknowledged that regardless of the effects of jujube fruit, the milk concentrations of lead, cadmium, and arsenic decreased in breast milk after 2 months of lactation in both intervention and control groups. One of the reasons of such reduction might be the change in the composition of human milk over time, for example, its fat and protein content.[26] Moreover, it is suggested that as we conducted the study shortly after childbirth, the contaminants have mainly entered to the maternal body during pregnancy, and the baseline levels of toxic trace elements in breast milk are an indicator of likely prenatal exposures.[27] However, compared to the control group, high concentrations of lead and cadmium, but not that of arsenic, decreased in the milk of those mothers who received jujube fruit; therefore, it can be assumed that in addition to the change in the usual content of human milk over time, jujube fruit had some beneficial effects in decreasing its lead and cadmium content.

In the current study, the mean (SD) concentrations of lead, cadmium, and arsenic were 29.49 (16.6), 4.65 (3.51), and 1.23 (0.63) μg/L, respectively. These levels reflect the maternal-absorbed metals during pregnancy. These values were comparable to the levels reported from some Asian countries,[7,28-30] but higher than those reported from the European countries.[31-33] The WHO has reported wide ranges of heavy metals in human milk samples around the world.[34] In this study, the mean levels of arsenic and cadmium exceeded the range reported by the WHO, and lead was in the upper levels of this range. However, the comparison between different studies has limitations because of differences in geographical location, methods of measurement, and the time of each study.

It is noteworthy to mention that the milk concentrations of lead and cadmium in the current study were substantially higher than the levels reported from the same city 3 years before the current study.[6] These findings suggest that the environmental contaminants have increased in this industrialized city, which along two other cities in Iran are actually included in the top ten air-polluted cities of the world (Iran-times.com/Iran-has-most-polluted-cities-in-world/). Moreover, it is documented that the vegetables grown in this area are contaminated with different toxic heavy metals.[35,36] Our findings are also consistent with high levels of blood cadmium and lead found in a recent study in the Iranian adolescents living in various cities in Iran,[37] which shows such environmental pollutants are not limited to this study area, and studies in other cities of the country might reveal similar results.

In spite of the existence of some toxic trace elements in human milk, because of its numerous beneficial effects, breastfeeding must be encouraged, and it should be taken into account that most of these elements also contaminate drinking water and may exist in formula cans; therefore, their levels might be even higher in formula than in human milk.[38-40]

Although the health effects of toxic trace elements in human milk need to be determined, reducing the concentrations of these pollutants might have long-term beneficial effects. The current study has taken a step toward testing interventions regarding the reduction of some toxic elements in breast milk.

Study limitations and strengths

The main limitations of this study are the short duration of the intervention and follow-up. Moreover, it was a single-center study, further multicentric studies with long-term follow-up might reach better results. The sources of toxic trace elements were not determined in the current trial and should be assessed in the future study. The strengths of the study include its novelty, existence of control group, assessment of elements concentrations by a valid method and providing a new method in reducing the contamination of harmful elements in human milk.

CONCLUSION

The concentrations of heavy metals were high in human milk, and the consumption of jujube fruit had some beneficial effects in reducing these harmful elements. Pregnant and lactating mothers should be advised to reduce their exposure to environmental pollutants. Moreover, consumption of some natural medicinal foods can be useful in reducing the concentration of pollutants in human milk. In spite of the existence of some toxic trace elements, breastfeeding must be encouraged because such contaminants are also found in water and formula, and their health effects remain to be determined. The impact of the current findings on primary prevention of chronic disease should be determined by future longitudinal studies.

Acknowledgments

This study was conducted as a specialty thesis funded by Isfahan University of Medical Sciences.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors have no conflicts of interest.

AUTHORS' CONTRIBUTION

All authors contributed in the study concept and design, conducting the trial, and drafting the paper. All authors read and approved the final version for submission, and accept the responsibility of the paper content.

REFERENCES

1. Koyashiki GA, Paoliello MM, Tchounwou PB. Lead levels in human milk and children's health risk: A systematic review. Rev Environ Health 2010;25:243-53.
2. Available from: http://www.who.int/ceh/risks/cehair/en/. [Last accessed on 2016 May 20].
3. Available from: http://www.euro.who.int/__data/assets/pdf_file/0004/97042/4.4.-Exposure-of-children-to-chemical-hazards-in-food-EDITED_layouted.pdf. [Last accessed on 2016 Mar 15].
4. Örün E, Yalçin SS, Aykut O, Orhan G, Morgil GK, Yurdakök K, *et al.* Breast milk lead and cadmium levels from suburban areas of Ankara. Sci Total Environ 2011;409:2467-72.
5. Moussa W. Determination of lead and cadmium in human milk and measure some of its composition. Aust J Basic Appl 2011;5:236-40.
6. Goudarzi MA, Parsaei P, Nayebpour F, Rahimi E. Determination of mercury, cadmium and lead in human milk in Iran. Toxicol Ind Health 2013;29:820-3.
7. Gürbay A, Charehsaz M, Eken A, Sayal A, Girgin G, Yurdakök M, *et al.* Toxic metals in breast milk samples from Ankara, Turkey: Assessment of lead, cadmium, nickel, and arsenic levels. Biol Trace Elem Res 2012;149:117-22.
8. Sternowsky HJ, Moser B, Szadkowsky D. Arsenic in breast milk during the first 3 months of lactation. Int J Hyg Environ Health 2002;205:405-9.
9. D'Souza HS, Menezes G, Venkatesh T. Role of essential trace minerals on the absorption of heavy metals with special reference to lead. Indian J Clin Biochem 2003;18:154-60.
10. Dietrich KN, Berger OG, Succop PA. Lead exposure and the motor developmental status of urban six-year-old children in the Cincinnati prospective study. Pediatrics 1993;91:301-7.
11. Koller K, Brown T, Spurgeon A, Levy L. Recent developments in low-level lead exposure and intellectual impairment in children. Environ Health Perspect 2004;112:987-94.
12. Tong S, von Schirnding YE, Prapamontol T. Environmental lead exposure: A public health problem of global dimensions. Bull World Health Organ 2000;78:1068-77.
13. Watanabe C, Inaoka T, Matsui T, Ishigaki K, Murayama N, Ohtsuka R. Effects of arsenic on younger generations. J Environ Sci Health A Tox Hazard Subst Environ Eng 2003;38:129-39.
14. Raqib R, Ahmed S, Sultana R, Wagatsuma Y, Mondal D, Hoque AM, *et al.* Effects of in utero arsenic exposure on child immunity and morbidity in rural Bangladesh. Toxicol Lett 2009;185:197-202.
15. Kosanovic M, Adem A, Jokanovic M, Abdulrazzaq YM. Simultaneous determination of cadmium, Mercury, Lead, Arsenic, Copper, and Zinc in Human Breast Milk by ICP-MS/microwave digestion. Anal Lett 2008;41:406-16.
16. Gundacker C, Pietschnig B, Wittmann KJ, Lischka A, Salzer H, Hohenauer L, *et al.* Lead and mercury in breast milk. Pediatrics 2002;110:873-8.
17. Nishijo M, Nakagawa H, Honda R, Tanebe K, Saito S, Teranishi H, *et al.* Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. Occup Environ Med 2002;59:394-6.
18. Koizumi N, Murata K, Hayashi C, Nishio H, Goji J. High cadmium accumulation among humans and primates: Comparison across various mammalian species – A study from Japan. Biol Trace Elem Res 2008;121:205-14.
19. Romieu I, Castro-Giner F, Kunzli N, Sunyer J. Air pollution, oxidative stress and dietary supplementation: A review. Eur Respir J 2008;31:179-97.
20. Kelishadi R, Mirghaffari N, Poursafa P, Gidding SS. Lifestyle and environmental factors associated with inflammation, oxidative stress and insulin resistance in children. Atherosclerosis 2009;203:311-9.
21. Rashidi M, Rameshat MH, Gharib H, Rouzbahani R, Ghias M, Poursafa P. The association between spatial distribution of common malignancies and soil lead concentration in Isfahan, Iran. J Res Med Sci 2012;17:348-54.
22. Ghannadi A, Tavakoli N, Mehri-Ardestani M. Volatile constituents of the *Ziziphus spina-christi* (L.) Willd from Bushehr. Iran J Essent Oil Res 2003;15:191-2.

23. Iranian Herbal Pharmacopoeia Committee. Iranian Herbal Pharmacopoeia. Tehran: Iranian Ministry of Health and Medical Education Publications; 2002. p. 550-7.
24. Mahajan RT, Chopda MZ. Phyto-pharmacology of *Ziziphus jujube* Mill. A plant review. Pharmacogn Rev 2009;3:320-9.
25. Sabzghabaee AM, Khayam I, Kelishadi R, Ghannadi A, Soltani R, Badri SH, *et al*. Effect of *Ziziphus jujube* fruits on dyslipidemia in obese adolescents-a triple-masked randomized controlled clinical trial. Med Arch 2013;67:156-9.
26. Chao HH, Guo CH, Huang CB, Chen PC, Li HC, Hsiung DY, *et al.* Arsenic, cadmium, lead, and aluminium concentrations in human milk at early stages of lactation. Pediatr Neonatol 2014;55:127-34.
27. Mead MN. Contaminants in human milk: Weighing the risks against the benefits of breastfeeding. Environ Health Perspect 2008;116:A427-34.
28. Turan S, Saygi S, Kiliç Z, Acar O. Determination of heavy metal contents in human colostrum samples by electrothermal atomic absorption spectrophotometry. J Trop Pediatr 2001;47:81-5.
29. Al-Saleh I, Shinwari N, Mashhour A. Heavy metal concentrations in the breast milk of Saudi women. Biol Trace Elem Res 2003;96:21-37.
30. Liu KS, Hao JH, Xu YQ, Gu XQ, Shi J, Dai CF, *et al.* Breast milk lead and cadmium levels in suburban areas of Nanjing, China. Chin Med Sci J 2013;28:7-15.
31. Leotsinidis M, Alexopoulos A, Kostopoulou-Farri E. Toxic and essential trace elements in human milk from Greek lactating women: Association with dietary habits and other factors. Chemosphere 2005;61:238-47.
32. Winiarska-Mieczan A. Cadmium, lead, copper and zinc in breast milk in Poland. Biol Trace Elem Res 2014;157:36-44.
33. De Felip E, Bianchi F, Bove C, Cori L, D'Argenzio A, D'Orsi G, *et al.* Priority persistent contaminants in people dwelling in critical areas of Campania region, Italy (SEBIOREC biomonitoring study). Sci Total Environ 2014;487:420-35.
34. Somogyi A, Beck H. Nurturing and breast-feeding: Exposure to chemicals in breast milk. Environ Health Perspect 1993;101 Suppl 2:45-52.
35. Khoshgoftarmanesh AH, Aghili F, Sanaeiostovar A. Daily intake of heavy metals and nitrate through greenhouse cucumber and bell pepper consumption and potential health risks for human. Int J Food Sci Nutr 2009;60 Suppl 1:199-208.
36. Jafarian-Dehkordi A, Alehashem M. Heavy metal contamination of vegetables in Isfahan, Iran. Res Pharm Sci 2013;8:51-8.
37. Kelishadi R, Askarieh A, Motlagh ME, Tajadini M, Heshmat R, Ardalan G, *et al*. Association of blood cadmium level with cardiometabolic risk factors and liver enzymes in a nationally representative sample of adolescents: The CASPIAN-III study. J Environ Public Health 2013;2013:142856.
38. Hilbig A, Kersting M, Sichert-Hellert W. Measured consumption of tap water in German infants and young children as background for potential health risk assessments: Data of the DONALD study. Food Addit Contam 2002;19:829-36.
39. Oskarsson A, Palminger Hallén I, Sundberg J, Petersson Grawé K. Risk assessment in relation to neonatal metal exposure. Analyst 1998;123:19-23.
40. Sadeghi N, Oveisi MR, Jannat B, Hajimahmoodi M, Behfar A, Behzad M, *et al.* Simultaneous measurement of zinc, copper, lead and cadmium in baby weaning food and powder milk by DPASV. Iran J Pharm Res 2014;13:345-9.