



# Breast milk contamination with lead and cadmium and its related factors in Kerman, Iran

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Received: 18 July 2018 / Accepted: 30 September 2018 / Published online: 25 October 2018  
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## Abstract

**Background** Heavy metals may be carcinogenic and have serious health effects in humans. In this study, levels of lead (Pb) and cadmium (Cd) in human milk samples from Kerman, Iran were evaluated.

**Methods** One-hundred breast milk samples were collected from mothers visiting 20 maternal and child care centers. Demographic, diet and smoking data was collected for each mother. Samples were analyzed by Graphite Furnace Atomic Absorption Spectrometry. Statistical analysis was done in Minitab 15, through non-parametric methods, Spearman's correlation and chi-square.

**Results** The mean  $\pm$  sd of Pb and Cd was  $53.6 \pm 64.9$  and  $8.01 \pm 15.5$   $\mu\text{g/L}$  respectively. The median (25–75 percentiles) of Pb and Cd was 34.00 (4.00–81.75) and 5.00 (4.00–7.00)  $\mu\text{g/L}$ ; 6 and 17% of infants were receiving higher than recommended levels of lead and cadmium respectively. Mothers who took fat off their meat before eating and mothers who had a vaginal delivery had higher levels of Cd than others ( $p$  value = 0.04, 0.002). Non-primiparous mothers and those who consumed more yogurt had higher levels of Pb ( $p$  value = 0.011, 0.025).

**Conclusion** Lead and cadmium contamination was considerable among mothers' in Kerman, Iran. There is a need for decreasing the current levels in food, air, water and other consumer products such as cosmetics.

**Keywords** Breast milk · Lead · Cadmium · Infant intake · Iran

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## Background

Breast feeding is a valuable nutrition source for infants [1]. However, recent studies have raised the concern that human breast milk might be contaminated with heavy metals such as cadmium (Cd) and lead (Pb). Lead and/or cadmium have been recently measured in breast milk or colostrum of lactating mothers in many countries including Saudi Arabia [1], Brazil [2], Austria [3], Bangladesh [4] and Japan [5, 6].

There is no doubt that breast milk is an important nutritional source for infants' physiological development, it improves children's immunity and is digested easily [1, 7]. Unlike some chemicals, heavy metals do not preferably bind to lipids and, therefore, do not usually accumulate in higher concentrations in maternal milk than in blood [7]. This means that infants are exposed to higher metals levels as a fetus. However, researchers think high concentrations of heavy metals in a woman's body that apparently does not harm the woman herself can have negative developmental complications for her fetus or the child she's nursing [1]. Meanwhile, researchers

think measuring the levels of heavy metals in breast milk is quite important, because it not only shows exposure levels and contamination with these metals in the mother, but is also an indicator about how much heavy metals the infant was exposed to before birth [1].

Pb and Cd are two toxic heavy metals that have been reported to have negative nephrotoxic, hematotoxic, and neurotoxic effects. Lead has been used for thousands of years around the world from ancient civilizations and for many purposes. Researchers have related elevated blood lead levels in humans in many countries to the lead added to gasoline and also the lead present in soil, dust, paint, water and food [4]. In Iran, rice which is the dominant food group was reported to be highly contaminated with Pb in some studies [8]. Other sources such as cosmetics and traditional remedies might also be the source of lead exposure [1]. There are several reasons for infants being more vulnerable to the adverse effects of heavy metal exposure. Infants grow rapidly, their nervous system is more susceptible, and they have physiologically immature organs [1]. Infants and young children absorb up to 10 times more dietary lead compare to adults [8]. Lead is transferred from the mothers to the fetus through placenta from the 12th week of pregnancy and until delivery. Epidemiological studies have showed that fetuses exposure to Pb can cause spontaneous abortion, low birth weight, early delivery, and congenital malformations [8].

Women like any other living being can be exposed to environmental lead from a very young age, and they therefore start to accumulate lead in their bones from infancy and into their child-bearing age [9]. Pb in breast milk mainly comes from the Pb that was stored previously in the mother's bones. Breast-feeding increases bone turnover, and then Pb is released from mothers' bones and enters breast milk, and this exposures the infant to Pb [1].

Cadmium contaminates humans mainly through food grown on polluted soil or water and tobacco smoking [6]. Cadmium has fetotoxic, embryotoxic [1] and carcinogen effects. Some researchers think Cd increases the risk of premature delivery and causes brain damage in infants [10]. In Iran, cadmium levels were higher among industrial workers exposed to Cd, smokers and people living in industrial areas [8, 11].

Some studies have found strong correlations between Pb in maternal milk and infant blood in infants up to 6 months of age [9, 12] which is actually the time frame that many infants have exclusive breast feeding. Some authors have even suggested that up to 80% of blood Pb in breast-fed infants in their first 3 months of life comes from their mother's milk [10].

Although in most developed countries infant Pb contamination has decreased, in most developing countries it is still a public health concern [13]. In this study, the levels of Pb and Cd was measured in human milk samples from Kerman, Iran;

in order to document the contamination levels of these metals in this city and find out their related factors. This is the first study about this topic done in Kerman, Iran.

Measuring toxic heavy metals in human milk is a non-invasive way to find out their exposure levels in the general population. It can also estimate infants prenatal and post-natal exposure [14]. A few studies are available from other cities in Iran about determining lead or cadmium concentrations in breast milk [15, 16]. In this study, we also investigated the effect of some demographic, diet and smoking factors on the contamination levels of Pb and Cd in lactating mothers.

## Material and methods

This study was a cross-sectional study done in Kerman, Iran in 2012. One hundred (100) human breast milk samples were collected from 20 maternal and child health centers across Kerman city. These health centers were selected randomly from different areas of this city. Each center contributed 5 samples.

The mothers who participated in this study were lactating mothers above 16 years old. These mothers were visiting the health centers for their children's immunization shots on their 8th week. The other inclusion criteria were that the mothers had to be a resident of that area for at least 1 year. None of these mothers worked in occupations that exposed them to toxic heavy metals. All women were living in urban areas of Kerman, and not in industrial zones.

Information about the mothers' age, years of residency, pregnancies, babies' weight, height, and head circumference was taken from their health records. Women were asked about their diet by a questionnaire used in our previous studies [17–19].

The study proposal was approved by the Ethical Committee of Kerman University of Medical Sciences (Code:167/89). The study objectives were explained for the mothers, their questions were answered and then written consent witnessed by a third party was obtained from all of the participating mothers.

A trained research assistant completed the questionnaire for each mother. Mothers were given instructions about how to collect their breast milk samples. Each mother collected 50 cc of breast milk. Samples were kept in a freezer and later chemical analysis was done.

Chemical analysis was done at the International Center of Science, High Technology and Environmental Sciences Laboratory, which is located at Mahan, Kerman, Iran.

## Instrumentation

A spectra-220 atomic adsorption spectrometer and GTA-110 (Graphite Tube Atomizer) and sample dispenser were used in

**Table 1** The demographic characteristics of the participating mothers and newborns in this study

| Variable   | Mean ± Standard Deviation |
|--|---------------------------|
| Mother's Age (years)                             | 26.2 ± 4.4                |
| Time living in the city (years)                  | 17.5 ± 10.2               |
| Baby's weight (grams)                            | 3141.9 ± 463.5            |
| Baby's height (cm)                               | 49.7 ± 2.2                |
| Baby's Head (cm)                                 | 35.0 ± 6.2                |
| Lead in mother's milk (µg/L)                     | 53.6 ± 64.9               |
| Cadmium in mother's milk (µg/L)                  | 8.01 ± 15.5               |
| Number (percent)                                 |                           |
| Birth Route                                      |                           |
| Cesarian   | 54                        |
| Vaginal  | 46                        |
| Baby's Gender                                    |                           |
| Boy  | 53                        |
| Girl   | 47                        |
| Baby Born on time                                | 78                        |
| Baby born more than 10 days sooner than expected | 22                        |
| First Pregnancy                                  | 89                        |
| Not first pregnancy                              | 11                        |
| Diet   |                           |
| More fruits and vegetables than meat             | 39                        |
| More meat than vegetables                        | 31                        |
| Both almost equally                              | 30                        |
| Number of serves of beef a week                  |                           |
| Less than 2                                      | 60                        |
| 2 or more  | 40                        |
| Number of serves of chicken a week               |                           |
| Less than 2                                      | 32                        |
| 2 or more  | 68                        |
| Number of serves of fish a week                  |                           |
| Less than 2                                      | 74                        |
| 2 or more  | 26                        |
| Number of serves of yoghurt a day                |                           |
| Less than once a day                             | 19                        |
| Everyday or more                                 | 81                        |
| Number of serves of lamb a week                  |                           |
| Less than 3                                      | 55                        |
| 3 or more  | 45                        |
| Number of serves of eggs a week                  |                           |
| Less than 2                                      | 42                        |
| 2 or more  | 58                        |
| Number of serves of cheese a week                |                           |
| Less than 4                                      | 42                        |
| 4 or more  | 58                        |
| Number of serves of milk a day                   |                           |
| Less than once a day                             | 76                        |
| Everyday or more                                 | 24                        |
| Do you get fat off meat before eating?           |                           |
| Never, Rarely                                    | 49                        |
| Sometimes  | 12                        |

**Table 1** (continued)

| Variable   | Mean ± Standard Deviation |
|--|---------------------------|
| Always, Usually                                  | 39                        |
| Do you wash fruits and vegetables before eating? |                           |
| Sometimes, Usually                               | 7                         |
| Always   | 93                        |
| Do you peel fruits and vegetables before eating? |                           |
| Never, Rarely                                    | 9                         |
| Sometimes  | 24                        |
| Always, Usually                                  | 67                        |
| Mother's Education                               |                           |
| Under High School                                | 11                        |
| High School or Diploma                           | 56                        |
| University                                       | 33                        |
| Mother's Employment                              |                           |
| Working  | 27                        |
| Unemployed                                       | 73                        |
| Exposed to passive smoking at home               |                           |
| Yes  | 13                        |
| No   | 87                        |
| Total  | 100                       |

this study. The Deuterium background correction was used. All equipment was from Varian Techron Pty. Limited, Mulgrave, Victoria, Australia.

A Varian hollow-cathode lamp was used for Pb. The working current was 5 mA. The spectral line was 283.3 nm and the band width was 0.5 nm. We used pyrolytically coated partition graphite tubes (Varian Canada Inc.) throughout.

### Procedures for determining Cd and Pb

All reagents, glass dishes, and devices were checked beforehand for any kind of contamination with Pb and Cd. The glass dishes were washed and soaked two times in successive diluted nitric acid baths (0.8 mol/L). Then they were completely washed with double distilled water.

Samples are prepared according to the nitric acid (HNO<sub>3</sub>) digestion procedure that has been described by Rahimi et al. [16]. We transferred a 25 mL representative aliquot of the sample to a 100 mL Griffin beaker and 7 mL of concentrated nitric acid (HNO<sub>3</sub> Merck, Germany); we then covered the beaker with a glass; placed the beaker on a hot plate and then cautiously waited until it evaporated to a low volume (5 mL), making certain that the sample does not boil and that no portion of the bottom of the beaker was allowed to go dry. We let the beaker cool and then we added 7 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> Merck, Germany). We then covered the beaker and we returned it to the hot plate. We heated the sample up to 95 ± 5 °C and we refluxed it for 10 to 15 min

without boiling. We continued heating, until digestion was completed and the digestate was light in color.

We removed the beaker from the hot plate and after cooling; we washed the beaker walls and glass with water and we adjusted its final volume to 50 mL with water (ddH<sub>2</sub>O). Particulates in the digestate were then removed by filtration (through Whatman No. 1 filter paper). The samples were then analyzed by GFAA or Graphite Furnace Atomic Absorption Spectrometry. Its detection limit was 1 ppb or 1 µg/L.

Descriptive statistics, and the Mann-Whitney, Kruskal-Wallis and chi-square tests were done in different subgroups. The Spearman correlation test was done as the distribution of

**Table 2** The Spearman Correlations between lead and cadmium in maternal milk and other variables

| Variable                     | Lead                        | Cadmium                     |
|------------------------------|-----------------------------|-----------------------------|
| Mother's age                 | $r = -0.042$<br>$p = 0.677$ | $r = 0.065$<br>$p = 0.524$  |
| Baby's weight                | $r = 0.061$<br>$p = 0.548$  | $r = 0.081$<br>$p = 0.425$  |
| Years Mother lived in Kerman | $r = 0.063$<br>$p = 0.535$  | $r = -0.070$<br>$p = 0.492$ |
| Baby's height                | $r = -0.074$<br>$p = 0.462$ | $r = 0.160$<br>$p = 0.113$  |
| Baby's head circumference    | $r = -0.100$<br>$p = 0.322$ | $r = 0.065$<br>$p = 0.520$  |

**Table 3** Comparison of Cadmium contamination ( $\mu\text{g/L}$ ) in different subgroups

| Variable  | Number | Mean $\pm$ SD     | Mean Rank | *p value |
|---|--------|-------------------|-----------|----------|
| <b>Birth Route</b>                                      |        |                   |           |          |
| Cesarian  | 54     | 5.43 $\pm$ 2.39   | 46.40     |          |
| Vaginal   | 46     | 11.0 $\pm$ 22.5   | 55.32     | 0.117    |
| <b>Baby's Gender</b>                                    |        |                   |           |          |
| Boy   | 53     | 6.08 $\pm$ 4.42   | 49.70     |          |
| Girl  | 47     | 10.2 $\pm$ 22.1   | 51.40     | 0.764    |
| <b>Baby Born on time</b>                                |        |                   |           |          |
| Baby born more than 10 days sooner than expected        | 22     | 8.05 $\pm$ 9.99   | 49.07     | 0.788    |
| <b>First Pregnancy</b>                                  |        |                   |           |          |
| Not first pregnancy                                     | 11     | 5.45 $\pm$ 2.94   | 45.77     | 0.557    |
| <b>Diet</b>   |        |                   |           |          |
| More fruits and vegetables than meat                    | 39     | 6.08 $\pm$ 4.10   | 47.99     |          |
| More meat than vegetables                               | 31     | 12.23 $\pm$ 27.14 | 52.81     |          |
| Both almost equally                                     | 30     | 6.17 $\pm$ 3.35   | 51.38     | 0.763    |
| <b>Number of serves of beef a week</b>                  |        |                   |           |          |
| Less than 2   | 60     | 6.10 $\pm$ 3.94   | 49.31     |          |
| 2 or more   | 40     | 10.9 $\pm$ 24.0   | 52.29     | 0.606    |
| <b>Number of serves of chicken a week</b>               |        |                   |           |          |
| Less than 2   | 32     | 11.1 $\pm$ 25.8   | 54.36     |          |
| 2 or more   | 68     | 6.54 $\pm$ 6.40   | 48.68     | 0.350    |
| <b>Number of serves of fish a week</b>                  |        |                   |           |          |
| Less than 2   | 74     | 8.4 $\pm$ 17.7    | 50.34     |          |
| 2 or more   | 26     | 6.96 $\pm$ 6.10   | 50.96     | 0.923    |
| <b>Number of serves of yoghurt a day</b>                |        |                   |           |          |
| Less than once a day                                    | 19     | 5.42 $\pm$ 2.27   | 48.16     |          |
| Everyday or more  | 81     | 8.6 $\pm$ 17.2    | 51.05     | 0.689    |
| <b>Number of serves of lamb a week</b>                  |        |                   |           |          |
| Less than 3   | 55     | 6.55 $\pm$ 5.84   | 50.13     |          |
| 3 or more   | 45     | 9.8 $\pm$ 22.2    | 50.96     | 0.884    |
| <b>Number of serves of eggs a week</b>                  |        |                   |           |          |
| Less than 2   | 42     | 10.3 $\pm$ 22.8   | 54.43     |          |
| 2 or more   | 58     | 6.36 $\pm$ 6.14   | 47.66     | 0.238    |
| <b>Number of serves of cheese a week</b>                |        |                   |           |          |
| Less than 4   | 42     | 6.45 $\pm$ 4.16   | 52.21     |          |
| 4 or more   | 58     | 9.1 $\pm$ 20.1    | 49.26     | 0.607    |
| <b>Number of serves of milk a day</b>                   |        |                   |           |          |
| Less than once a day                                    | 76     | 6.68 $\pm$ 5.96   | 51.53     |          |
| Everyday or more  | 24     | 12.2 $\pm$ 30.0   | 47.23     | 0.516    |
| <b>Do you get fat off meat before eating?</b>           |        |                   |           |          |
| Never, Rarely   | 49     | 7.16 $\pm$ 7.32   | 50.22     |          |
| Sometimes   | 12     | 4.25 $\pm$ 1.48   | 32.67     |          |
| Always, Usually   | 39     | 10.23 $\pm$ 23.44 | 56.33     | 0.040*   |
| <b>Do you wash fruits and vegetables before eating?</b> |        |                   |           |          |
| Sometimes, Usually                                      | 7      | 5.29 $\pm$ 0.76   | 45.50     |          |
| Always  | 93     | 8.2 $\pm$ 16.1    | 50.88     | 0.624    |
| <b>Do you peel fruits and vegetables before eating?</b> |        |                   |           |          |
| Never, Rarely   | 9      | 7.00 $\pm$ 3.71   | 59.06     |          |
| Sometimes   | 24     | 5.58 $\pm$ 3.17   | 45.40     |          |
| Always, Usually   | 67     | 9.01 $\pm$ 18.79  | 51.18     | 0.441    |

**Table 3** (continued)

| Variable                           | Number | Mean $\pm$ SD     | Mean Rank | *p value |
|------------------------------------|--------|-------------------|-----------|----------|
| Mother's Education                 |        |                   |           |          |
| Under High School                  | 11     | 4.82 $\pm$ 2.86   | 40.32     |          |
| High School or Diploma             | 56     | 6.09 $\pm$ 4.49   | 48.59     |          |
| University                         | 33     | 12.33 $\pm$ 26.06 | 57.14     | 0.175    |
| Mother's Employment                |        |                   |           |          |
| Working                            | 27     | 6.15 $\pm$ 3.25   | 50.85     |          |
| Unemployed                         | 73     | 8.7 $\pm$ 18.1    | 50.37     | 0.940    |
| Exposed to passive smoking at home |        |                   |           |          |
| Yes                                | 13     | 5.23 $\pm$ 3.56   | 38.46     |          |
| No                                 | 87     | 8.4 $\pm$ 16.6    | 52.30     | 0.100    |

\*according to non-parametric tests

lead and cadmium was skewed. Data were analyzed using Minitab15.

**Availability of data and material** All data generated or analyzed during this study are included in this published article. Additional information may be asked from the corresponding author.

## Results

The demographic characteristics of the mothers has been shown in Table 1. The mother's age range was between 17 to 42 and the mean  $\pm$  sd of age was 26.2  $\pm$  4.4. None of the babies had congenital malformations.

The levels of lead and cadmium in breast milk both had non-normal distributions and the Kolmogorov–Smirnov test, *p* value was less than 0.001. Cadmium was detected in all samples, but lead was under the detection limit in 23 (23%) of the samples. The mean  $\pm$  sd of lead was 53.6  $\pm$  64.9  $\mu$ g/L and cadmium was 8.01  $\pm$  15.5  $\mu$ g/L. The median (25–75 percentiles) of lead was 34.00 (4.00–81.75)  $\mu$ g/L; and cadmium was 5.00 (4.00–7.00)  $\mu$ g/L. The highest level of lead was 382  $\mu$ g/L and the highest level of cadmium was 151  $\mu$ g/L.

The Spearman correlation between lead and cadmium was 0.008 and non-significant (*p* value = 0.94). The Spearman correlations between lead and cadmium in maternal milk and other quantitative variables have been shown in Table 2. Lead and cadmium did not show any significant correlation with mother's age, or baby's weight, height or head circumference.

The levels of cadmium in different subgroups have been compared in Table 3. None of the subgroups showed significant differences in their cadmium levels except in one comparison which showed that mothers who always or usually take fat off their meat before eating have higher levels of cadmium than others.

Table 4 shows the levels of Pb in different subgroups. None of the groups showed a significant difference in their Pb levels, except one comparison which showed that mothers, whom it was not their first pregnancy, had higher levels of lead than other mothers.

The analysis was rerun in three exposure groups, based on the 33rd and 66th percentile cutoff point. The results showed that women with more cadmium contamination were more likely to have a vaginal delivery, also women who consumed more yogurt had higher levels of lead contamination (Table 5).

According to WHO the daily permissible intake level of lead for children is 5 micrograms per kilogram a day [20]. Assuming a 2 month old baby weighs about 5 kg; it consumes about 690 mL of breast milk per day in exclusive breast feeding [21]. This means that the daily permissible intake (DPI) level of lead for two-month-old children is about 25  $\mu$ g per day and the permissible level for lead in maternal milk for these 2 month old children is 36.23  $\mu$ g/L.

The daily permissible intake for cadmium according to WHO is 1  $\mu$ g/kg per day for adults [20]. Repeating the above calculations, the daily permissible intake (DPI) level of cadmium for two-month-old children is about 5  $\mu$ g per day and the permissible level of cadmium in maternal milk for these two-month-old children is 7.25  $\mu$ g/L.

According to these calculations in our study, 6 (6%) infants were receiving higher than recommended levels of lead and 17 (17%) infants were receiving higher than recommended levels of cadmium.

**Table 4** Comparison of Lead contamination ( $\mu\text{g/L}$ ) in different subgroups

| Variable  | Number | Mean $\pm$ SD     | Median | *p value |
|---|--------|-------------------|--------|----------|
| <b>Birth Route</b>                                      |        |                   |        |          |
| Cesarian  | 54     | 54.1 $\pm$ 60.5   | 51.34  |          |
| Vaginal   | 46     | 53.1 $\pm$ 70.4   | 49.51  | 0.752    |
| <b>Baby's Gender</b>                                    |        |                   |        |          |
| Boy   | 53     | 39.4 $\pm$ 42.2   | 45.74  |          |
| Girl  | 47     | 69.6 $\pm$ 80.9   | 55.87  | 0.079    |
| <b>Baby Born on time</b>                                |        |                   |        |          |
| Baby born more than 10 days sooner than expected        | 22     | 47.7 $\pm$ 40.3   | 53.41  | 0.592    |
| <b>First Pregnancy</b>                                  |        |                   |        |          |
| Not first pregnancy                                     | 11     | 122.0 $\pm$ 120.0 | 71.32  | 0.011*   |
| <b>Diet</b>   |        |                   |        |          |
| More fruits and vegetables than meat                    | 39     | 47.51 $\pm$ 71.66 | 46.06  |          |
| More meat than vegetables                               | 31     | 61.03 $\pm$ 46.65 | 57.44  |          |
| Both almost equally                                     | 30     | 53.90 $\pm$ 72.60 | 49.10  | 0.248    |
| <b>Number of serves of beef a week</b>                  |        |                   |        |          |
| Less than 2   | 60     | 50.3 $\pm$ 60.7   | 48.45  |          |
| 2 or more   | 40     | 58.5 $\pm$ 71.1   | 53.58  | 0.384    |
| <b>Number of serves of chicken a week</b>               |        |                   |        |          |
| Less than 2   | 32     | 57.4 $\pm$ 59.4   | 52.56  |          |
| 2 or more   | 68     | 51.8 $\pm$ 67.6   | 49.53  | 0.624    |
| <b>Number of serves of fish a week</b>                  |        |                   |        |          |
| Less than 2   | 74     | 49.3 $\pm$ 61.5   | 48.76  |          |
| 2 or more   | 26     | 66.0 $\pm$ 73.5   | 55.46  | 0.308    |
| <b>Number of serves of yoghurt a day</b>                |        |                   |        |          |
| Less than once a day                                    | 19     | 35.2 $\pm$ 44.8   | 41.00  |          |
| Everyday or more  | 81     | 58.0 $\pm$ 68.2   | 52.73  | 0.111    |
| <b>Number of serves of lamb a week</b>                  |        |                   |        |          |
| Less than 3   | 55     | 57.9 $\pm$ 71.0   | 51.12  |          |
| 3 or more   | 45     | 48.4 $\pm$ 56.8   | 49.74  | 0.813    |
| <b>Number of serves of eggs a week</b>                  |        |                   |        |          |
| Less than 2   | 42     | 51.4 $\pm$ 65.0   | 48.86  |          |
| 2 or more   | 58     | 55.2 $\pm$ 65.3   | 51.69  | 0.628    |
| <b>Number of serves of cheese a week</b>                |        |                   |        |          |
| Less than 4   | 42     | 57.0 $\pm$ 85.1   | 46.69  |          |
| 4 or more   | 58     | 51.2 $\pm$ 45.6   | 53.26  | 0.261    |
| <b>Number of serves of milk a day</b>                   |        |                   |        |          |
| Less than once a day                                    | 76     | 56.3 $\pm$ 69.2   | 51.09  |          |
| Everyday or more  | 24     | 45.1 $\pm$ 49.1   | 48.62  | 0.715    |
| <b>Do you get fat off meat before eating?</b>           |        |                   |        |          |
| Never, Rarely   | 49     | 63.84 $\pm$ 72.08 | 53.46  |          |
| Sometimes   | 12     | 41.92 $\pm$ 44.38 | 49.00  |          |
| Always, Usually   | 39     | 44.38 $\pm$ 59.68 | 47.24  | 0.593    |
| <b>Do you wash fruits and vegetables before eating?</b> |        |                   |        |          |
| Sometimes, Usually                                      | 7      | 37.60 $\pm$ 57.13 | 42.21  |          |
| Always  | 93     | 54.8 $\pm$ 65.5   | 51.12  | 0.430    |
| <b>Do you peel fruits and vegetables before eating?</b> |        |                   |        |          |
| Never, Rarely   | 9      | 63.00 $\pm$ 60.47 | 54.17  |          |
| Sometimes   | 24     | 46.79 $\pm$ 51.42 | 46.90  |          |
| Always, Usually   | 67     | 54.81 $\pm$ 70.11 | 51.30  | 0.751    |

**Table 4** (continued)

| Variable                           | Number | Mean $\pm$ SD      | Median | *p value |
|------------------------------------|--------|--------------------|--------|----------|
| Mother's Education                 |        |                    |        |          |
| Under High School                  | 11     | 95.18 $\pm$ 106.18 | 64.91  |          |
| High School or Diploma             | 56     | 45.66 $\pm$ 59.91  | 46.18  |          |
| University                         | 33     | 53.27 $\pm$ 51.17  | 53.03  | 0.119    |
| Mother's Employment                |        |                    |        |          |
| Working                            | 27     | 53.44 $\pm$ 48.60  | 55.07  |          |
| Unemployed                         | 73     | 53.7 $\pm$ 70.2    | 48.81  | 0.335    |
| Exposed to passive smoking at home |        |                    |        |          |
| Yes                                | 13     | 43.23 $\pm$ 58.36  | 44.12  | 0.392    |
| No                                 | 87     | 55.17 $\pm$ 65.94  | 51.45  |          |

\*according to non-parametric tests

## Discussion

In this study, the mean level of lead in breast milk was  $53.6 \pm 64.9$   $\mu\text{g/L}$  and cadmium was  $8.01 \pm 15.5$   $\mu\text{g/L}$ . This amount of lead and cadmium was higher than many other studies and less than some populations [15, 22]. Levels in some other recent world studies have been shown in Table 6.

In this study, mothers' contamination with lead and cadmium was estimated by measuring these metals in breast milk. Researchers have shown strong and significant correlations between the levels of Pb in maternal milk and maternal blood, which means that breast milk levels can well estimate human body contamination levels [7, 13]. Researchers think that Cd concentrations in breast milk also closely reflect Cd body burdens [5].

Maternal age showed a non-significant but positive correlation with cadmium in Brazil [2], but did not show a relation with breast milk Pb or Cd levels in Turkey [14]. No statistically significant correlation was seen between Pb levels in maternal milk specimens versus the age of the mother, in Nigeria [29] either.

Although, in a study from Iran the mean lead levels from mothers older than 24 years was significantly lower than younger mothers and there was an inverse significant correlation between mothers' age and their breast milk Pb [15]; in Taiwan, Pb in breast milk was significantly higher in older mothers [25]. But, in Slovakia, the mean milk concentrations of Cd and Pb in younger women was non-significantly lower than older women [30] and in India older women's blood and milk, was more contaminated with heavy metals as well [23]. In García-Esquinas et al's study [10] and the present study no relation was seen between Cd or Pb contamination and mothers' age.

In Orun et al's study in Turkey, the median of Cd (and not Pb) in maternal milk was significantly higher in unemployed mothers compared to employed mothers. Researchers think

the source of Cd might be dust particles in indoor air or inadequate ventilation and smoking in the house [14]. In our study, the mean of Cd contamination was non-significantly higher in unemployed women but the medians were similar; and Pb levels were not related to employment either.

Also, we did not see an association between education levels and Cd or Pb contamination. In Turkey, education was not related to breast milk Pb and Cd levels [14] and no significant association was found between Pb levels in breast milk and sociodemographic variables in Spain either [10]. However, Pb breast milk levels were significantly higher in mothers who had a higher educational in Taiwan [25].

Some studies found that Cd levels in human milk were significantly related to mothers' smoking status [10] and have even found that there was a direct relation between the amount of cadmium in a woman's breast milk and the number of cigarettes she smoked a day [10, 31, 32]. A study from Austria showed that smokers had 2-times higher concentration of cadmium than non-smokers [3]. In Madrid [10] and in Taiwan [25] smoking mothers were more contaminated with Cd. In Turkey, mothers who actively and/or passively smoked during pregnancy had higher Cd levels in their breast milk, but smoking at 2 months postpartum had no significant relation with breast milk Cd levels [14]. Meanwhile, smoking was not significantly associated with cadmium in other studies [2].

Although some studies report a relation between smoking and maternal milk Pb levels [33], other studies do not show a significant relation [30]. In Turkish mothers, smoking during pregnancy and up to 2 months after delivery had no influence on Pb levels in breast milk either [14]. However, in Egypt, women passively exposed to smoking had more Pb in their breast milk [27]. In the present study, none of the mothers that participated were smokers and those who were exposed to passive smoking were not more contaminated with cadmium or lead. In Spain, there was no relation between Cd or Pb levels in milk and passive smoking either [10].



**Table 5** Comparison of Lead and Cadmium contamination by tertiles in different subgroups

| Variable  | Lead        |             |             | P      | Cadmium     |             |             | P      | Total |
|---|-------------|-------------|-------------|--------|-------------|-------------|-------------|--------|-------|
|   | 1st Tertile | 2nd Tertile | 3rd Tertile |        | 1st Tertile | 2nd Tertile | 3rd Tertile |        |       |
| <b>Birth Route</b>                                      |             |             |             |        |             |             |             |        |       |
| Cesarian  | 19          | 17          | 18          |        | 31          | 20          | 3           |        | 54    |
| Vaginal   | 15          | 15          | 16          | 0.964  | 24          | 8           | 14          | 0.002* | 46    |
| <b>Baby's Gender</b>                                    |             |             |             |        |             |             |             |        |       |
| Boy   | 19          | 21          | 13          |        | 29          | 17          | 7           |        | 53    |
| Girl  | 15          | 11          | 21          | 0.077  | 26          | 11          | 10          | 0.444  | 47    |
| <b>Baby Born on time</b>                                |             |             |             |        |             |             |             |        |       |
| Baby born more than 10 days sooner                      | 4           | 10          | 8           | 0.156  | 12          | 8           | 2           | 0.418  | 22    |
| <b>First Pregnancy</b>                                  |             |             |             |        |             |             |             |        |       |
| Not first pregnancy                                     | 33          | 28          | 28          |        | 49          | 25          | 15          |        | 89    |
|   | 1           | 4           | 6           | 0.145  | 6           | 3           | 2           | 0.994  | 11    |
| <b>Diet</b>   |             |             |             |        |             |             |             |        |       |
| More fruits and vegetables than meat                    | 15          | 14          | 10          |        | 24          | 11          | 4           |        | 39    |
| More meat than vegetables                               | 8           | 7           | 16          |        | 14          | 10          | 7           | 0.546  | 31    |
| Both almost equally                                     | 11          | 11          | 8           | 0.181  | 17          | 7           | 6           |        | 30    |
| <b>Number of serves of beef a week</b>                  |             |             |             |        |             |             |             |        |       |
| Less than 2   | 23          | 19          | 18          |        | 34          | 16          | 10          |        | 60    |
| 2 or more   | 11          | 13          | 16          | 0.463  | 21          | 12          | 7           | 0.914  | 40    |
| <b>Number of serves of chicken a week</b>               |             |             |             |        |             |             |             |        |       |
| Less than 2   | 11          | 9           | 12          |        | 17          | 7           | 8           |        | 32    |
| 2 or more   | 23          | 23          | 22          | 0.822  | 38          | 21          | 9           | 0.296  | 68    |
| <b>Number of serves of fish a week</b>                  |             |             |             |        |             |             |             |        |       |
| Less than 2   | 27          | 24          | 23          |        | 41          | 20          | 13          |        | 74    |
| 2 or more   | 7           | 8           | 11          | 0.536  | 14          | 8           | 4           | 0.924  | 26    |
| <b>Number of serves of yoghurt a day</b>                |             |             |             |        |             |             |             |        |       |
| Less than once a day                                    | 11          | 2           | 6           |        | 11          | 6           | 2           |        | 19    |
| Everyday or more  | 23          | 30          | 28          | 0.025* | 44          | 22          | 15          | 0.697  | 81    |
| <b>Number of serves of lamb a week</b>                  |             |             |             |        |             |             |             |        |       |
| Less than 3   | 20          | 15          | 20          |        | 31          | 17          | 7           |        | 55    |
| 3 or more   | 14          | 17          | 14          | 0.534  | 24          | 11          | 10          | 0.422  | 45    |
| <b>Number of serves of eggs a week</b>                  |             |             |             |        |             |             |             |        |       |
| Less than 2   | 14          | 16          | 12          |        | 21          | 13          | 8           |        | 42    |
| 2 or more   | 20          | 16          | 22          | 0.478  | 34          | 15          | 9           | 0.693  | 58    |
| <b>Number of serves of cheese a week</b>                |             |             |             |        |             |             |             |        |       |
| Less than 4   | 18          | 11          | 13          |        | 22          | 12          | 8           |        | 42    |
| 4 or more   | 16          | 21          | 21          | 0.268  | 33          | 16          | 9           | 0.871  | 58    |
| <b>Number of serves of milk a day</b>                   |             |             |             |        |             |             |             |        |       |
| Less than once a day                                    | 26          | 23          | 27          |        | 40          | 22          | 14          |        | 76    |
| Everyday or more  | 8           | 9           | 7           | 0.771  | 15          | 6           | 3           | 0.670  | 24    |
| <b>Do you get fat off meat before eating?</b>           |             |             |             |        |             |             |             |        |       |
| Never, Rarely   | 17          | 12          | 20          |        | 26          | 13          | 10          |        | 49    |
| Sometimes   | 4           | 4           | 4           |        | 11          | 1           | 0           | 0.075  | 12    |
| Always, Usually   | 13          | 16          | 10          | 0.501  | 18          | 14          | 7           |        | 39    |
| <b>Do you wash fruits and vegetables before eating?</b> |             |             |             |        |             |             |             |        |       |
| Sometimes, Usually                                      | 4           | 1           | 2           |        | 6           | 1           | 0           |        | 7     |
| Always  | 30          | 31          | 32          | 0.370  | 49          | 27          | 17          | 0.215  | 93    |
| <b>Do you peel fruits and vegetables before eating?</b> |             |             |             |        |             |             |             |        |       |

**Table 5** (continued)

| Variable                           | Lead        |             |             | P     | Cadmium     |             |             | P     | Total |
|------------------------------------|-------------|-------------|-------------|-------|-------------|-------------|-------------|-------|-------|
|                                    | 1st Tertile | 2nd Tertile | 3rd Tertile |       | 1st Tertile | 2nd Tertile | 3rd Tertile |       |       |
| Never, Rarely                      | 3           | 3           | 3           |       | 4           | 3           | 2           |       | 9     |
| Sometimes                          | 10          | 6           | 8           |       | 16          | 5           | 3           | 0.746 | 24    |
| Always, Usually                    | 21          | 23          | 23          | 0.902 | 35          | 20          | 12          |       | 67    |
| Mother's Education                 |             |             |             |       |             |             |             |       |       |
| Under High School                  | 2           | 4           | 5           |       | 8           | 0           | 3           |       | 11    |
| High School or Diploma             | 23          | 16          | 17          |       | 33          | 17          | 6           | 0.082 | 56    |
| University                         | 9           | 12          | 12          | 0.519 | 14          | 11          | 8           |       | 33    |
| Mother's Employment                |             |             |             |       |             |             |             |       |       |
| Working                            | 5           | 12          | 10          |       | 14          | 10          | 3           |       | 27    |
| Unemployed                         | 29          | 20          | 24          | 0.106 | 41          | 18          | 14          | 0.387 | 73    |
| Exposed to passive smoking at home |             |             |             |       |             |             |             |       |       |
| Yes                                | 6           | 4           | 3           |       | 11          | 1           | 1           |       | 13    |
| No                                 | 28          | 28          | 31          | 0.554 | 44          | 27          | 16          | 0.069 | 87    |
| Total                              | 34          | 32          | 34          | –     | 55          | 28          | 17          | –     | 100   |

Studies have reported that mother's exposure to Cd might increase early delivery, and consequently cause low birth weight [6]. However, in our study the level of cadmium was similar in mothers with on time and early delivery and we did not see a relation between Cd contamination and birth weight either. In Orum et al.'s study, breast milk Cd levels at 2 months after delivery were significantly and inversely correlated with head circumference; and birth weight of girls [14]. In Austria breast milk Cd levels were not related to gestational age [3] and in Slovakia, researchers did not find a relation between birth weight and Cd or Pb, levels [30] either.

An Iranian study showed that maternal milk lead levels were higher in infants with birth weights under 2950 g, birth heights under or equal to 49 cm and infants with birth head circumferences less than 35 cm. However, the authors were not able to find a significant difference; may be due to the low sample size that was only 27 mothers [15]. In our study, Pb or Cd levels were not related to baby's weight, height or head circumference either.

In this study, women who had a vaginal delivery were more contaminated with Cd than women who had a Cesarean section. We did not find anything about this association in the literature. It is likely that this association is due to the fact that in Iran women from a higher socioeconomic class who live in less polluted areas are more willing to have a Cesarean section instead of a vaginal delivery.

Several studies have reported results on the relation between Cd and Pb in breast milk and diet. In Austria frequent cereal consumption was related to higher Cd levels; and consuming supplements containing trace elements and/or vitamins was related to lower Cd in breast milk of non-smokers

[3]. In Saudia Arabia, not eating fish was related to higher cadmium and lead levels in breast milk [1], but in Spain no dietary food component showed an association with Cd levels in breast milk [10]. In the present study mothers who took fat off their meat before eating had significantly higher levels of cadmium than others, probably because Cd does not accumulate in fat. In Madrid, Spain; Pb concentrations increased with higher potato consumption, but decreased with higher consumption of caffeinated beverages and dairy products; and no type of food was related to Cd levels in breast milk [10].

Researchers in Mexico city found women who eat more dairy products have higher Pb levels in their breast milk [34]. This was similar to our study in which mothers who consumed yogurt everyday were more significantly contaminated with lead than mothers who used less. The reason for this association might be that dairy products can get contaminated with Pb while going through industrial processing or packaging, by containers or vessels [10, 35]. Milk or milk products can also get contaminated when dairy animals consume contaminated food or graze in industrialized areas or areas with heavy traffic [35, 36]. Some studies have reported serious contamination of some brands of butter and cheese with Pb in Iran [37].

In Brazil parity showed a non-significant positive correlation with cadmium [2]. In Norouzi et al.'s study from Iran, the highest mean lead contamination level was seen in mothers with one child which was  $70.64 \pm 24.62 \mu\text{g/L}$  [15] and in another study from Tehran, Iran; the mean value of lead in human milk (2 months post-partum) was  $23.66 \pm 22.43 \mu\text{g/l}$ ; but there was no significant relation between levels of human milk lead and mother's education, age, parity, height or weight [28]. Also, in Spain researchers found no association between

**Table 6** The levels of lead and cadmium measured in human milk samples in some recent world studies and this study

| Reference                         | Country                | Time of sampling                  | Level of Pb  | Level of Cd                                     |
|-----------------------------------|------------------------|-----------------------------------|--|---|
| Khanjani et al., current study    | Iran, Kerman           | 8 weeks after delivery            | Mean: 53.6 ± 64.9 µg/L<br>Median: 34.00 µg/L   | Mean: 8.01 ± 15.5 µg/L<br>Median: 5.00 µg/L     |
| Bergkvist et al., 2010 [4]        | rural Bangladesh       | 2 months postpartum               | Median: 1.3 µg/kg  |   |
| Chien et al., 2006 [9]            | Taiwan                 | colostrum                         | geometric mean:<br>7.68 ± 8.24 µg/L  |   |
| Ettinger et al., 2004 [12]        | Mexico                 | 1 month postpartum                | 0.3 to 8.0 µg/L  |   |
| Counter et al., 2007 [13]         | Ecuadorian villages    | various                           | 1–49 µg/L  |   |
| Gundacker et al., 2007 [3]        | Austria                | second and 14th day post partum.  |  | mean: 0.0867 ± 0.085 µg/L<br>median: 0.063 µg/L |
| Sower et al., 2002                | US                     | 12 months postpartum              | 4.3 µg/L   |   |
| Souad et al., 2006                | Morocco                | Up to 12 months after parturition | Mean: 36.3 µg/l<br>3.1–117.4 µg/l  |   |
| Al-Saleh et al., 2003 [1]         | Saudia Arabia          | various                           | 31.67 µg/l   | 1.732 µg/L                                      |
| Turan et al., 2001                | Turkey                 | 2 months postpartum               | 14.6 µg/l  | 2.8 µg/l  |
| Leotsinidis et al., 2005          | Greece                 | 14 days postpartum                | 0.15 ± 0.25 µg/l   | 0.14 ± 0.12 µg/l                                |
| Vanderjadt, 2001                  | Nigeria                | 2–24 weeks postgestation          | median: 6.7 µg/dL<br>range: 4.6–130, µg/dl   |   |
| Goncalves et al., 2010 [2]        | Brazil                 | colostrum                         | mean: 2.3 µg/L<br>median: 0.9 µg/L   |   |
| Sharma, 2005 [23]                 | India                  | up to 1 week from delivery        |  | 0.1–3.8 µg/l                                    |
| Ursinyovo et al., 2005            | Slovakia               | 4th day postpartum                | Mean: 4.7 µg/kg  | Mean: 0.43 µg/kg                                |
| Orun et al., 2011                 | Turkey, Ankara         | 2 months postpartum               | Median: 20.59 µg/l   | Median: 0.67 µg/l                               |
| Norouzi et al., 2010 [15]         | Iran, Zarrinshahr      | 4th day postpartum                | Mean in mothers with first child: 70.64 µg/L<br>Total not reported   |   |
| Golmohammadi et al., 2007 [24]    | Iran, Tehran and Gilan | colostrum                         | Mean in Gilan: 4.2 ± 2.5<br>Range: from 0.3 to 13.5 µg/ml<br>Mean in Tehran: 5.8 ± 5.5 and varied from 1.1 to 27.5 µg/ml |   |
| Chao et al., 2014 [25]            | Taiwan                 | colostrum                         | 13.22 ± 3.58 ng/mL   | 1.37 ± 0.94 ng/mL                               |
| Ettinger et al., 2014 [7]         | Mexico                 | 1 month postpartum                | 0.8 ± 0.7 µg/L.  |   |
| García-Esquinas et al., 2011 [10] | Mexico                 | third week postpartum             | geometric mean: 15.56 µg/L   | geometric mean: 1.31 µg/L                       |
| Goudarzi et al., 2012 [26]        | Iran, Isfahan          | first to sixth postpartum week    | 7.11 + 3.96 µg/L<br>Range 3.06–19.47 µg/L  | 1.92 ± 1.04 µg/L<br>Range: 0.45–5.87 µg/L       |
| Gurbay et al., 2012               | Turkey, Ankara         | 2–5 days postpartum.              | 391.45 ± 269.01 µg/l   | 4.62 µg/l (measured in only one sample)         |
| Mandour et al., 2013 [27]         | Egypt, Dakahlia        | ?                                 | Range: 0.015–0.049 mg/l<br>15–49 µg/l  |   |
| Winiarska-Mieczan et al., 2014    | Poland                 | Various                           | Mean: 6.331 µg/l   | Mean: 2.114 µg/l                                |
| Soleimani et al., 2014 [28]       | Iran, Tehran           | 2 months post-partum              | Mean: 23.66 ± 22.43 µg/l   |   |

Pb levels in breast milk and the mothers’ previous pregnancies and lactation histories [10]. Gravity, parity, and time period between births, in Turkey, were not related to breast milk Pb and Cd levels [14] and in Slovakia parity, did not affect Cd or Pb, milk levels either; but, primiparous women had more Pb in their breast milk [30]. Pb levels in breast milk and parity were not related in Nigeria [29] and Pb levels in milk were not related with previous pregnancies or lactations in Spain either

[10]. In our study Cd was not related to parity, but Pb was significantly higher in non-primiparous women.

Breast milk Pb or Cd levels were not related to the gender of infants in previous studies [14, 30] or our study.

There were few Iranian studies to compare the results of this study with. In Golmohammadi et al.’s study lead contamination was compared between two regions. The more contaminated region was the central and south of Tehran in which

the mean annual ambient air Pb concentration was more than 0.5 to 1  $\mu\text{g}/\text{m}^3$ . The non-contaminated area was the highland villages of Gilan in which no industry had been established around them. The mean lead concentration in mother's colostrum in the non-contaminated region was  $4.2 \pm 2.5$  and varied from 0.3 to 13.5  $\mu\text{g}/\text{ml}$  and in the contaminated region was  $5.8 \pm 5.5$  and varied from 1.1 to 27.5  $\mu\text{g}/\text{ml}$ . The mean Pb contamination was significantly different between the two regions. There were significant correlations between mothers' blood lead levels and the newborns' blood lead levels, and maternal milk lead levels in both regions [24].

The probable reason for the high Cd and Pb levels in maternal milk in Kerman, Iran; is probably leaded gasoline, the active industries in this province, contaminated food, water, dust and cosmetics. Lead levels in blood and breast milk are high in areas where lead is still used in gasoline, especially in areas with heavy traffic [31]. There are articles about high levels of Pb in dust (over 70 ppm) in Kerman, Iran [38]. Studies have also reported food contaminated with high levels of Pb and Cd in Iran [8]. Dangerous waste is not separated in many areas in Iran and it is disposed along with other waste. These dangerous heavy metals may enter food and water [8]. There are articles about high levels of Pb in surface and ground water in Kerman [38]. The other factor that may contribute to women's lead contamination is cosmetics. Researchers have found high levels of Pb in cosmetics used by women in Kerman [39, 40].

Lead and its compounds are also absorbed through skin, the respiratory and the digestive system. They enter the blood and pass the brain-blood barrier and the placenta and accumulate in different tissues. More than 90% of the total lead in humans accumulates in the skeleton and its half-life is 25 to 32 years [24].

Although, breast milk may contain lead [4], this must not become an excuse for not breast-feeding infants; as formula milk may be contaminated with even more heavy metals [17]. Scientists still believe that breast milk is the infant's best food, and plays an important role in child development and passive immunization [4].

Because of environmental contamination with heavy metal in many world regions, surveillance and periodic measurements of the amount of heavy metals in human milk is necessary. Our results emphasize the necessity of conducting national food and consumer safety programs in order to prevent human contamination with dangerous heavy metals.

The small number of participants was a limitation of this study. This might have prevented some of the variables from becoming significant. However, there were several studies with even less participants in the literature [2, 13, 15, 25–27, 29] and it was difficult to find more interested mothers in Kerman to donate samples. Another limitation was that we were not able to follow-up these children and evaluate the future effects of lead and cadmium contamination on them.

Some researchers have related low IQ (Intelligence Quotient), autism and ADHD (Attention Deficit Hyperactivity Disorder) in children to lead or cadmium contamination in early childhood [41].

## Conclusion

The lead and cadmium contamination levels is considerable among mothers' in Kerman, Iran. The results of the present study emphasize the necessity of surveillance and keeping heavy metal concentrations in food, air, water and other consumer products such as cosmetics at safe limits.

**Acknowledgements** This study was financially supported by Kerman University of Medical Sciences and the International Center of Science, High Technology and Environmental Sciences of Kerman. The authors thank the mothers who contributed to this study.

**Authors' contributions** NK supervised the study, wrote the proposal, conducted the statistical analysis, wrote the initial draft. MJ collected the breast milk samples and completed the questionnaires. EM conducted the chemical analysis and performed the laboratory work. All the authors read, commented on and approved the final manuscript.

**Funding** Kerman University of Medical Sciences, Kerman, Iran and the International Center of Science, High Technology and Environmental Sciences of Kerman, Mahan, Iran.

## Compliance with ethical standards

**Ethics approval and consent to participate** This study was approved by the Ethic Committee of Kerman University of Medical Sciences, Ethics Code: 167–89. All participants signed a consent form before enrolling into the study.

**Consent for publication** Not Applicable.

**Competing interests** None.

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