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# Determination of lead concentration in breast milk and blood of lactanting women in an interior city of Brasil

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## ABSTRACT

**Background:** breast milk offers numerous advantages biological and emotional. However, in some situations, can transfer harmful elements for the health of infants.
**Objective:** to determine lead levels in the blood and milk of mothers whose children frequent a primary mother--child health care. **Methods:** a cross-sectional, observational and descriptive study of 70 healthy women who were breast-feeding babies younger than six months during 01/04/2011 to 30/09/2011. Lead determination was performed by mass spectrometry with inductively coupled plasma. A linear regression model was adjusted to determine the association between lead concentrations in maternal milk and blood. The estimated values of the difference between means and their 95% confidence intervals were obtained. **Results:** mean lead concentration in milk was 1.462 ng/mL and blood lead concentration was 1.801 µg/dL. **Conclusions:** human milk is not a source of contamination for the babies in this area. However, more studies are needed in other regions of the country in view of the growing evidence of foci of lead contamination, which may represent an occult risk for the population.

**Key-words:** human milk - Breastfeeding - Lead - Environmental pollution

## INTRODUCTION

As an essential source of nutrients and defense factors that provide appropriate growth and physical, emotional and immunologic development, human milk is the best food for babies. Although it is offered directly from the breast to the infant, human milk may be the vehicle of some undesirable elements such as environmental contaminants that have eventually reached the maternal organism (1). With the growing industrialization and urbanization started in the past century, the population is increasingly exposed to environmental pollutants. Among them, lead is one of the most important due to its extensive dissemination throughout the environment, its persistence in the organism and its potential toxic effects (2-4).

Children are more sensitive to lead exposure than adults due to their low weight (with an increased proportion of ingested dose/weight unit), the rapid growth and development of their nervous system, and the easier intestinal absorption and motor activity which facilitates the contact of objects with the mouth (5-7). Exposure to even low concentrations of lead can be potentially harmful to the organism mainly by reaching the liver, kidneys, blood, teeth, heart and central nervous system, compromising the

psychological and behavioral functions (8,9). Approximately 90% of the lead present in the organism is located in the bone matrix. Situations that increase bone metabolism such as lactation increase the bone turnover, also increasing the quantity of lead in plasma and consequently in human milk (10,11).

Studies conducted by the World Health Organization (WHO) have pointed out that the concentration of lead in human milk ranges from 2.0 to 16.8 µg/dl in various countries around the world (12). Although serum lead levels are decreasing in populations of developed areas, some countries continue to present a problem. In Jamaica, 44% of children aged 1 to 6 years living in areas at risk for contamination had serum lead levels higher than 25 µg/dl (13) and in China, children residing in highly industrialized regions and with intense vehicle traffic had serum levels of 21.8 to 67.9 µg/dl (14).

In view of the potential risks of chronic lead poisoning, of the evidence that breast-feeding is a mode of transmission to the child and of the directives about exclusive breast-feeding for infants, it is necessary to determine the presence or absence of this metal in human milk as a form of surveillance of the environmental contamination of maternal milk. Thus, the objective of the present study was to determine lead levels in the blood and milk of mothers whose children frequent a service of primary mother-child health care in the city of Ribeirão Preto, São Paulo state, Brazil.

## METHODS

A cross-sectional, observational and descriptive study was conducted on 70 healthy women who were breast-feeding babies younger than six months, enrolled in the Puericulture Program of a primary mother-child health care unit in the city of Ribeirão Preto, State of São Paulo, Brazil, during the period from 01/04/2011 to 30/09/2011. In the first phase of the study a questionnaire was applied regarding the sociodemographic aspects of the mothers such as age, ethnic origin, schooling, economic class, number of children, living in risk areas (sugar cane culture, intense vehicle traffic and proximity to industries), possible exposure to lead on the job (automobile motors and batteries, welding shops, prospecting for gold, foundries, aluminum, glass and plastic products, printing, ceramic, paints in general, and others involving lead), smoking, and alcohol consumption. In the second phase, human milk and blood samples were collected. For the manual collection of 5 ml milk, the breast was first cleaned with cotton wool and distilled water and the woman was instructed to press her breast gently. The milk sample was transferred to a Falcon® BD polyethylene tube and stored at - 20º C. Six mL blood samples were obtained by venipuncture in the left upper limb using metal-free heparinized tubes (Vacuette®) and stored at -20 ºC. For analysis of the two fluids, the samples were thawed to room temperature.

Lead determination in milk was performed by mass spectrometry with inductively coupled plasma (ICP–MS, ELAN DRC II, Perkin Elmer Sciex, Shelton, IL, USA), with the samples being submitted to acid digestion in a microwave-assisted closed system. Lead concentration in blood was determined by ICP-MS.

The analyses were carried out with a mass spectrometer with inductively couple plasma equipped with a reaction cell (DRC-ICP-MS) ELAN DRCII, PerkinElmer, SCIEX, Norwalk, CT, USA) operating with ultrapure argon (99.999%, Praxaair, Brazil). The system for sample introduction consisted of a quartz cyclonic chamber and a Meinhard® nebulizer connected by Tygon® tubes to the peristaltic pump of the ICP-MS (adjusted for 20 rpm). The ICP-MS was operated with a sampling cone and Pt skimmer from PerkinElmer.

High-purity deionized water (resistivity of 18.2 M cm−1) used to prepare the samples and solutions was obtained using a Milli-Q water purification system (Millipore RiOs-DI TM,Bedford, MA, EUA). All reagents used were analytical grade, except HNO3 which was sub-distilled with a quartz distiller (Kürner Analysentechnik) before use. The solutions were prepared in a class 1000 clean room using a laminar flow hood. Rhodium (1000 mg L−1) and multielemental solutions (10 mg L−1) were purchased from PerkinElmer and Triton® X-100 was purchased from Sigma–Aldrich (St. Louis, MO, USA). All materials were previously decontaminated with 15% (v/v) HNO3 for 24 h, washed five times with Milli-Q water, and dried under a laminar flow hood before use.

Blood samples were diluted at a 1:50 proportion in 15 mL Falcon® BD tubes containing 0.01 % (v/v) Triton® X-100, 0.5% nitric acid (v/v) and 10 µgL−1 of the internal Rh standard. Milk samples (100-250 mg) were digested with 4 mL 14 M nitric acid + 2 mL 30 % hydrogen peroxide. The NIST 8435 - Whole Milk Powder reference material and blood reference materials from the National Public Health institute of Canada and of the New York Health Department were used to determine the accuracy and precision of the method.

All procedures were carried out in the laboratories of the Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRPUSP). The study was approved by the Ethics Committee of the University Hospital of the Faculty of Medicine of Ribeirão Preto, USP. The mothers were informed about the purpose of the study and written consent was obtained from all participants.

A linear regression model was adjusted to determine the association between lead concentrations in maternal milk and blood considering maternal milk lead to be the independent variable and maternal blood lead the dependent variable. Thus, the estimates of the parameters of interest and the coefficient of determination (R2) were obtained, indicating the % variability of blood lead concentration that milk lead can explain. The PROC REG feature of the SAS software version 9.2 was used. The Student t-test was applied to determine possible mean differences in blood and milk lead concentrations between the categories of risk factors using the PROC TTEST feature SAS 9.2. software. The estimated values of the difference between means and their 95% confidence intervals were obtained. The concentration of lead in blood was considered in the logarithm form in order to satisfy the assumptions of the test.

## RESULTS

Maternal blood and milk samples from 70 nursing women were analyzed. Of these, 71.4 % were younger than 30 years, 47.1 % were primiparae, 58.6 % did not reside in risk areas, 90 % had no professional exposure to lead, 74.3 % were non-smokers, and 62.8 % did not ingest alcoholic beverages, as shown in Table 1. Table 2 presents the mean, median, maximum and minimum lead concentrations in milk and blood of the women studied. The results of the analyses of maternal milk and blood are presented in Table 3 and the regression models in Table 4.

## DISCUSSION

Due to the universal spread of lead in the environment and its importance as a factor affecting health, the WHO has reported 2-5 ng/g to be acceptable lead levels in human milk, although it is known that there is no safe level of lead exposure at present (9,14).

Low lead concentrations were detected in the present study both in maternal blood and milk, showing that the subjects of the population sample studied are not exposed to contact with this metal. Similar results have been obtained in studies conducted in Mexico, the United States and Austria, where the lead concentrations in human milk were within the normal limits established by the WHO (15-17). These findings, however, differ from those reported in other studies conducted in different parts of the world which have shown high lead concentrations in human milk. In Germany, Sternowsky & Wessolowsky analyzed milk samples from mothers residing in the urban and rural zone and detected concentrations of 13.3 and 9.1 µg/L, respectively, i.e., values above the normal range accepted by the WHO (18).

In a study conducted in Italy on 34 breast-feeding women residing in the rural zone, the authors detected mean concentrations of 45.6 µg/L, ranging from zero to 425 µg/L, as opposed to mean concentrations of 126.5 µg/L, ranging from 1 to 472 µg/L, in 20 mothers residing in the urban zone (19). Similar results were obtained by Isaac et al in India, where lead concentrations in human milk samples were higher among mothers residing in urban regions (20). Less elevated lead concentrations in human milk samples were detected in Turkey (14.6 µg/L) among middle class mothers and in two Saudi Arabia regions, with mean values of 31.7 µg/L (21,22).

Two brazilian studies detected concentrations of 2.8 and 2.9 µg/L, respectively (23,24), while other authors analyzing colostrum of women residing in environmental risk areas detected a mean value of 154.4 µg/L (25).

In Slovakia, a study conducted on 158 mothers with no history of lead exposure residing in eight different regions of the country, milk samples collected on the fourth day postpartum revealed concentrations of 0.9 µg/L, showing a strong relation between lead levels in human milk and both active and passive smoking habit (26). Kirel et al observed a correlation between lead concentrations in maternal blood and milk in a study of mothers residing in the central region of Turkey, whose mean lead concentration in milk was 2.3 µg/L (27).

A study of 177 Chinese children, 93.8% of whom were breast-fed, demonstrated that blood lead levels were significantly correlated with lead levels in maternal milk and blood, indicating that breast-feeding was the main route of lead ingestion by the babies (28). Another study conducted in China detected lead concentrations in human milk ranging from 6.8 to 8.6 µg/L (29).

More recently, Goudarzi et al, in a study conducted in Iran, analyzed 37 samples of human milk collected from healthy mothers during the first six weeks after delivery and detected a mean concentration of 7.1 ± 3.9 µg/L (range: 3.0 to 19.4 µg/L), which was considered to be elevated and worrisome for the population studied (30).

Although biomonitoring can be used to identify potential risks for human health – and in the case of maternal milk both the mother and the baby are being monitored – it should be pointed out that there are factors that may impair the interpretation of the various studies published thus far, such as differences in methods and in the age when the samples were collected, as well as the type of analysis used. This impairs a comparison of the results, especially considering that there is no longitudinal study evaluating the behavior of lead concentration throughout the lactation period (31,32).

Despite the limitations due to the small number of samples and of mothers residing in a given geographic area, the present results lead us to conclude that human milk is not a source of contamination for the babies in this area. However, it is important to point out that more studies are needed in other regions of the country in view of the growing evidence of foci of lead contamination, which may represent an occult risk for the population.

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**Table 1 -** Distribution of sociodemographic variables of 70 nursing mothers from Ribeirão Preto

| **Maternal variable** | **n** | **%** |
|---|---|---|
| Age (years) | | |
| > 20 | 14 | 20.0 |
| 20 - 30 | 36 | 51.4 |
| > 30 | 20 | 28.6 |
| Parity | | |
| 1 | 33 | 47.1 |
| 2 | 14 | 20.0 |
| 3 | 14 | 20.0 |
| ≥ 4 | 9 | 12.9 |
| Resides in a risk area | | |
| no | 41 | 58.6 |
| yes | 29 | 41.4 |
| Professional exposure to lead | | |
| no | 63 | 90.0 |
| yes | 7 | 10.0 |
| Smoking | | |
| no | 52 | 74.3 |
| yes | 18 | 25.7 |
| Alcohol consumption | | |
| no | 44 | 62.8 |
| yes | 26 | 37.2 |

**Table 2** - Distribution of lead concentrations in the blood and milk of 70 nursing mothers from Ribeirão Preto

| Variable | mean ± SD | median | maximum | minimum |
|---|---|---|---|---|
| **Blood Pb (µg/dL)** | 1.801±1.065 | 1.59 | 7.56 | 0.55 |
| **Milk Pb (ng/mL)** | 1.462±1.282 | 0.94 | 4.82 | 0.01 |

**Table 3** - Results of the Student t-test for comparison of the means

| Site | Variable | Difference between means | 95% CI | | *p* |
|---|---|---|---|---|---|
| | | | LL | UL | |
| **Blood*** | Age | -0.2 | -0.45 | 0.05 | 0.11 |
| | Ethnicity | -0.15 | -0.38 | 0.08 | 0.19 |
| | Children | -0.2 | -0.42 | 0.03 | 0.09 |
| | Risk | -0.06 | -0.29 | 0.18 | 0.63 |
| | Job | -0.15 | -0.53 | 0.24 | 0.45 |
| | Origin | -0.06 | -0.19 | 0.31 | 0.61 |
| | Smoking | -0.1 | -0.36 | 0.17 | 0.47 |
| | Alcoholism | 0.09 | -0.15 | 0.36 | 0.46 |
| **Milk** | Age | -0.18 | -0.5 | 0.87 | 0.54 |
| | Ethnicity | -0.34 | -0.96 | 0.27 | 0.27 |
| | Children | 0.16 | -0.46 | 0.78 | 0.51 |
| | Risk | 0.06 | -0.57 | 0.69 | 0.85 |
| | Job | -0.94 | -1.94 | 0.06 | 0.07 |
| | Origin | -0.28 | -0.94 | 0.38 | 0.4 |
| | Smoking | 0.002 | -0.71 | 0.72 | 1 |
| | Alcoholism | -0.02 | -0.66 | 0.61 | 0.94 |

*logarithm LL - lower limit UL - upper limit

**Table 4** - Results of the regression models - association between blood and milk

| Parameter | Estimate | 95% CI | | *p* | R2 | Sperman correlation |
|---|---|---|---|---|---|---|
| | | LL | UL | | | |
| Blood | -0.06 | -0.35 | 0.23 | 0.66 | 0.003 | 0.12 |

LL - lower limit UL - upper lim