Original Article

Iran J Pediatr
Dec 2014; Vol 24 (No 6), Pp: 692-696

Breast Milk Concentration of Rubidium in Lactating Mothers by Instrumental Neutron Activation Analysis Method

Seyedeh-Fatemeh Khatami*[1], MD; Pouya Parvaresh[2], MD; Parviz Parvaresh[3], PhD; Sara Sadat Madani Kouchak[3], MSc, Jamshid Khorsandi[4], PhD

[1]Department of Pediatrics, Mashhad University of Medical Sciences, [2]Department of Internal Medicine, Szeged Medical Faculty, Szeged Medical University, Szeged, Hungary, [3]Department of Physics, Payame Noor University, Mashhad, [4]Esfahan Nuclear Technology Center, Atomic Energy Organization of Iran, Isfahan, Iran

Received: *Dec 05, 2013*; Accepted: *Jul 05, 2014;* First Online Available: *Oct 28, 2014*

Abstract

Objective: Relatively little is known about the trace elements content of human milk from different countries. This has not been fully investigated especially among Iranian women. This study aimed to assess the concentration of Rubidium (Rb) as a poisonous trace element in transitional breast milk of lactating mothers living in Mashhad.

Methods: Forty nursing mothers in early lactation 3 days to 15 days postpartum, free from any medical disorder and/or medication were randomly selected. We have applied Instrumental Neutron Activation Analysis (INAA) to assess the long-lived isotope trace element Rb in transitional milk of these economically moderate 18-39 year old Iranian women.

Findings: The average concentration level of Rb was 32.176 ppm dry weight (min 8.660, max 107.210 ppm). No significant correlation was observed between Rb concentration and maternal weight and age (P=0.06, P=0.05 respectively) and newborns' weight, age and sex (P=0.07, P=0.2, P=0.2 respectively).

Conclusion: Although the Rubidium concentration found in this study is among the highest reported in the literature, it could not be compared to other studies because of differences in analytical performance, state of lactation, and unavailable reference ranges, so this finding needs further investigations.

Iranian Journal of Pediatrics, Volume 24 (Number 6), December 2014, Pages: 692-696

Key Words: Human milk; Toxic Trace Elements; Rubidium; Newborn

Introduction

Breast-feeding is an integral part of the reproductive process, the natural and ideal way of feeding the infant, and a unique biological and emotional basis for child development. It has been known for quite some time that milk is deficient in minerals. Currently no fewer than 16 elements are thought to be essential for humans[1]. Possibly required trace minerals include fluorine, arsenic, rubidium (RB), tin, niobium, strontium, gold, silver and nickel[2], although Rb has been reported as potential toxic element in literature. Total body content of Rb (ICRP reference) is 0.32 g, 0.00046 per cent of total body weight[1].

Rubidium was discovered by a German scientist named Gustav Kirchhoff with Robert Bunsen in 1861. Rubidium competes with potassium ions for entry into the body, also activates and mobilizes lithium. Very high rubidium partnered with low

*** Corresponding Author;**
Address: Department of Pediatrics, Mashhad University of Medical Sciences, Mashhad, Iran
E-mail: f_khatami@yahoo.com

potassium can put muscles into a state of semi-paralysis. Rubidium will take the place of potassium in the sodium-potassium pump. The absorption function best takes place in the jejunum of the small intestine. Rubidium affiliates with the amino acid methionine[3].

Rossipal and Krachler examined content of 19 trace elements of 79 milk samples taken from 46 healthy mothers on days 1–293 of the lactation. It was established that concentration of poisonous trace elements such as cesium, rubidium, and strontium decreases the sucking, which can mean even a difference of 60% that must be taken into consideration when collecting the mother's milk samples[4]. The results of study indicate that an active transport mechanism for Rb from mother to newborn exists[5].

The studies have adopted the Instrumental Neutron Activation Analysis (INAA) due to its advantages of low detection limit, multi elemental capability, a non-destructive method[1,6].

The reliable reference ranges for most trace elements in human milk throughout the lactation period are not available. A general trend toward lower concentrations in the milk with continuing lactation for essential elements and almost constant concentrations for not essential elements has been reported[7].

This is to our knowledge the first study on Rb in transitional milk of Iranian women. This study aimed to assess the concentration of Rubidium of breast milk in lactating mothers living in Mashhad, to estimate the status of infant with breast feeding by using a sensitive analytical method (INAA).

Subjects and Methods

The study was carried out in the national laboratory of Payame Noor University (PNU) and Esfahan Nuclear Technology Center of AEOI, Mashhad University of Medical Sciences and Razavi hospital which provides full care for neonates. This is a prospective, descriptive and analytical study in Iranian mothers' breast milk from January through September 2011. A total of 40 lactating mothers who had singleton, healthy, appropriate for gestational age, term (37-40 weeks gestation) infants were randomly asked to participate. All participating mothers were apparently well nourished, free from any medical disorder and/or medication who delivered at Razavi hospital in Mashhad, Iran. The women were instructed to collect milk sample at the beginning and the end of the breastfeeding session. The transitional milk samples were collected at neonatal care unit, in early lactation 3 days to 15 days postpartum. The breast was cleaned with de-ionized water and breast milk was hand expressed. 10-15 ml breast milk from each mother was obtained.

Contaminated milk samples were discarded. The mothers with history of disease, smoking, drug abuse, vegetarians and those with medical treatment were excluded from the study. The local institutional ethics committee approved it and informed written consent was obtained from mothers. Data on neonatal weight, sex, age and maternal age, and weight were obtained via interviews and questionnaires within the neonatal chart, and previous records.

All samples were collected in sterilized polyethylene tubes and within few minutes transferred to special glass bottles. All glassware and bottles used for collection and for analysis were pre cleaned with analytical grade nitric acid solution and rinsed with ion free water at PNU national library, all samples were stored at -20 °C until freezing. After drying by drier (oven) Philip Harris Ltd, Scheneton, England (in Pyrex dishes with 5 cm diameter in an oven at 80 °C for 48 h), the dried milk samples were powdered by a porcelain mortar, and weighted using a Sarterius TE124s balance model-Japan, 300 mg from each sample sealed in cylindrical polyethylene capsules, the samples were placed in the rabbit capsules and sent to the reactor through a pneumatic transport system and distributed in irradiation tubes. Samples were irradiated in Esfahan Nuclear Technology Center of AEOI miniature neutron source reactor laboratory by swimming pool research reactor facilities in thermal neutron flux $5\times10^{11}n.cm^{-2}.s^{-1}$ for 5 hours. The measurements were done after decay period of 30-38 days for Rb, counting performed for 10000 sec. The counting of the samples and certified sample was carried out using the gamma-ray data acquisition system which consists of high-purity germanium (HPGe) detector and ADC analyzer (MCA) card. The standard reference for solid materials used for

verification and quality control was B01 (apple leaves, contains: Hg, Mo, Ni, Rb, Se, Na, Sr, V, Zn), supplied by Esfahan Nuclear Technology Center AEOI, Isfahan, Iran. The irradiated standard reference materials were measured in the same conditions and the net area of peaks were corrected according to samples and standard reference elements. The data were analyzed by Gamma-2000 and EMCA PLUS software.

Statistical analyses were performed using SPSS (PASW Statistics 16).Quantitative variables were expressed as mean±SD and were compared using student t-test, and one sample Kolmogorov-Smirnov test. The independent sample t-test and Pearson correlation tests were used in the statistical analysis. The threshold of significance was set at $P<0.05$.

Findings

40 women of medium socio-economic status from urban area, in early lactation continued the study 3 days to 15 days. The mean maternal age and weight were 26 (range 18-39) years, and 48 (range 45-75) kg respectively. No significant correlations were observed between Rb concentration and maternal weight and age ($P=0.06$, $P=0.05$ respectively). The mean weight of the neonates was 3299.5 g (±383.0) and mean age 5.1 days (±2.6). No significant correlations were found between Rb concentration and newborns' weight, age and sex ($P=0.07$, $P=0.2$, $P=0.2$ respectively).

Rubidium concentrations in dry weight of the samples are shown in Table 1. The average, median, standard deviation, variance, minimum, and maximum concentrations of Rb were 32.176, 28.195, 21.193, 437.905, 8.660 and 107.210 ppm in dry weight, respectively.

Discussion

In current study, concentration of Rubidium was measured in transitional human milk samples, from Iranian lactating mothers by INAA method. The average Rubidium concentration with 32.176 ppm dry weight was found to be the highest among reported values in the literature. The

Table 1: Rubidium concentrations in dry weight of samples

No.	Concentration (ppm)	No.	Concentration (ppm)
1	24.413	21	24.918
2	28.210	22	21.635
3	46.917	23	8.659
4	31.296	24	18.778
5	11.423	25	9.449
6	35.612	26	13.852
7	48.578	27	73.564
8	47.256	28	12.123
9	28.179	29	107.210
10	41.879	30	43.938
11	39.836	31	13.702
12	17.042	32	65.640
13	64.177	33	9.558
14	34.867	34	19.059
15	39.619	35	13.706
16	27.215	36	45.651
17	9.076	37	66.799
18	22.004	38	13.396
19	33.043	39	15.708
20	29.563	40	29.497

certified reference materials Lichen International Atomic Energy Agency (IAEA) -336 supplied by IAEA and the study in some Nigerian commercial infant food and cereal formula showed the Rb levels as 1.76±0.22 ppm and 1.75±0.21 ppm by INNA method respectively[6]. The concentrations for Rb in human milk are between the concentration ranges for serum (78 to 317µg/l) and for whole blood (900 to 4145 µg/l)[7]. The Grimanis,Vassilaki study showed higher range of concentrations of most of the trace elements were observed in colostrum,transitional and mature milk than in half cream and humanized milk. No significant differences for Rb were found between colostrum, transitional and mature human milk (by INNA method). Range and averages for Rb in colostrum are 0.42-1,0.71µg/g wet weight, transitional milk 0.6-1.1, 0.81, and mature milk (0.6-0.66, 0.63[8]. Although the analytical method was INAA, preparation method of samples was different of ours. The value reported for the purpose of verification and quality control, is mean Rb intake of 2.2±0.3 mg/day for most countries. Since the necessity of this element for humans remains unproven, the intake level could not be compared to a recommended range for a safe and adequate dietary intake[9].

The knowledge regarding most element concentrations, especially those of toxic metals in breast milk, and how they are regulated, interact or are affected by maternal exposure, is limited. Comparisons of breast milk concentration of elements across studies are hampered by differences in analytical performance, state of lactation and factors related to dietary habits and environmental concentrations and genetic factors[10,11].

The mean concentrations of Rb were different according to state of lactation. Krachler et al reported that concentrations of Rb in human milk ranged from 440 to 1620µg/kg dry mass by inductively coupled plasma mass spectrometry method (ICP-Ms). Fujisawa et al reported concentration of Rb in breast milk from mothers of low birth weight infants do not differ from the literature standard (55.9 ±14.1 µg/dl by ICP-Ms)[12], although Alimonti et al found higher Rb concentrations in term newborns by the same method compared to preterm newborns[5].

Therefore, literature data are inconsistent for many elements, and it is often not possible to conclude whether the differences are real or artifacts arising from analytical difficulties[10]. As mentioned above, wide variations influenced the results, so comparison between our results and other studies is not possible.

We have not analyzed maternal serum, cord blood and newborn levels of Rb. To corroborate this result and make this applicable to clinical decisions, and to avoid supplementation of mothers with this non essential trace element preparations, or suggesting food items specially low in Rb, trace elements must be simultaneously determined in milk samples from individual mothers throughout their lactation period and whenever possible, in the sera of mothers and infants, which are limitations of our study.

Conclusion

Although the Rubidium concentration found in this study is among the highest reported in the literature (Average 32.176 ppm dry weight), this Rb level could not be compared to other studies because of differences in method of preparation, processing, analytical performance, state of lactation, and unavailable reference values, so this finding needs further investigations in relation to health consequences for mothers and infants.

Acknowledgment

We are grateful to the women who participated in this study, and the field workers who helped us with sampling. Financial support was provided by AEO.

Authors' Contribution

S-F. Khatami: Design and concept of the study, provide the background data

P Parvaresh: Design and concept of the study, provide the background data, the statistical analysis and drafted the manuscript, sample collection and handling

S. Madani: The statistical analysis and drafted the manuscript, carry out the sample preparation and element analyses

J. Khorsandi: Carry out the sample preparation and element analyses.

All authors read and approved the final version of the manuscript.

Conflict of Interest: None

References

1. Parr RM. Trace elements in human milk. *IAEA Bulletin* 1983;25(2):7-15.
2. Wilson L. Toxic metals and human health. In: Wilson L and Eck PC (eds.) Toxic metals in human health and disease. 2nd ed. Mexico: Eck Institute Nutrition and Bioenergetics; 2012; Pp: 1-37.
3. Walter Ch. Rubidium: the sleeper mineral. *ACRES, USA, the Voice of ECO-Agriculture* 2008;38(1):23-24.
4. Salmon, S Csapo. Composition of the mother's milk III. Macro and micro element contents. A review. *Acta Univ Sapientiae Alimentaria.* 2009;2(2):235-75.
5. Krachler M, Rossipal E, Micetic-Turk D. Trace element transfer from the mother to the newborn - investigations on triplets of colostrum, maternal and umbilical cord sera. *Euro J Clin Nutr* 1999;53(6): 486-94.
6. Joseph E, Nasiru R, Ahmed YA. Trace elements pattern in some Nigerian commercial infant milk and infant cereal formulas. *Annals of Biological Research.* 2011;2(2):351-60.
7. Joseph E, Shi Li F, Rossipal E, Irgolic KJ. Changes in the concentrations of trace elements in human milk during lactation. *J Trace Elements Med Biol* 1998; 12(3):159-76.
8. Grimanis AP, Vassilaki-Grimani M, Alexiou D, et al. Determination of seven trace elements in human milk, powdered cow's milk and infant foods by neutron activation analysis. In: Nuclear Activation Techniques in the Life Sciences. International Atomic energy Agency. *IAEA-SM227/26*. 1979; Pp: 241-53.
9. Hendrix P, Van Cauwenbergh R, Robberecht H, et al. Daily dietary rubidium intake in Belgium using duplicate portion sampling. *Eur Food Res Technol* 1997;204(3):165-7.
10. Björklund KL, Vahter M, Palm B, et al. Metals and trace element concentrations in breast milk of first time healthy mothers: A biological monitoring study. *Environ Health* 2012;11:92.
11. Cashman KD. Milk salts, trace elements, nutritional significance, *Encyclopedia of Dairy Sciences.* ISBN: 9780123744074, Jan 2011.
12. Fujisawa Ch, Iura A, Ohta H, et al. The serum levels of calcium and trace elements in low weight infants. *Biomed Res Trace Elements* 2008;19(1):97-100.