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Longitudinal Variation of Trace Elements Concentration in Human Milk in Jeddah, Saudi Arabia

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Abstract. Information on the secretion of trace elements in human milk is needed, not only in order to estimate the intake by the breast-fed infant, but also as a starting point for recommendations of intakes from other types of infant foods and minerals diets during lactation. Duration of lactation, particularly in the first few weeks, markedly affects concentrations of some elements, including iron, zinc and manganese. Zinc, copper, iron, chromium, cadmium, manganese and lead were measured in breast milk samples collected from 11 healthy lactating mothers. Samples were collected from day of delivery to 15 days postpartum. The mean (±SD) concentration values in ppb of Zn, Cu, Fe, Mn, Cr, Cd and Pb in the morning samples are 2330.8±3.1, 491.9±1.8, 164.3±1.4, 18.2±0.3, 7.1±0.3, 2.03±0.01 and 1.47±0.01 respectively while those in the afternoon samples are 2434.6±2.9, 562.1±2.1, 299.9±2.0, 24.5±0.5, 20.2±0.7, 4.6±0.1 and 1.76±0.1 respectively. The concentrations of the elements in milk samples collected in the afternoon are higher than those in the morning samples. There is an apparent decline in the mean concentration levels as the stage of lactation progressed. Our results are also compared with the corresponding values of different countries available in literature. It is observed that the concentration levels of the elements studied in breast milk of Saudi mothers are well comparable with the values reported elsewhere in the world.

Keywords: Atomic absorption spectrometer; human milk; trace elements, zinc, iron, copper, chromium, cadmium, manganese, lead.

1. Introduction

Human milk is considered to be the optimal source of nutrition for the infant. Nutrient levels in milk-based formulas and milk substitutes for infants are generally modeled on the composition of human milk. However, composition of human milk is not constant[1-5]. It has long been known that human milk composition varies widely among women and that variations in quality even occur within the same woman[6].This complicates the problem faced by pediatricians and nutritionists in their attempts to assess nutrient intakes of healthy breast-fed infants. One of the main factors affecting nutrient levels in human milk is the stage of lactation. Accurate information is needed about nutrient concentrations in breast milk at different stages of lactation and under variety of conditions [6-10].

Duration of lactation, particularly in the first few weeks, markedly affects concentrations of some elements, including iron, manganese and zinc, but other elements, such as chromium, show little change [2,6,10].

The effect of mother's diet on the concentration of nutrients in breast milk is also important [6,7,9,10]. Only a few longitudinal studies have examined the relationship between diet and concentrations of major and trace elements in human milk and these have yielded controversial results. Feeley *et al.*[1] found no significant relationship between the concentration of zinc in breast milk and maternal intake of zinc but these findings are in contrast to those of Kerbs *et al*.[11]. There is also controversy about the efficiency of high levels of calcium intake to increase its concentration in breast milk[12].

There is extensive literature on the longitudinal variations of trace element concentrations in mature milk. The content of early lactation human milk has been less frequently studied [6,7]. However, the most dramatic changes in milk components take place during the first 2 weeks after parturition [6,13,14]. At the same time, the volumes of milk produced increase from ~ 0 – 500 ml in 1 week. Reports on changes in trace element concentrations in human milk during early lactation and on trace element concentrations in human milk from Saudi mothers are scarce. Again since the neonatal period is one of the most critical with respect to nutrition, the purpose of this investigation was to assess the copper, iron, manganese, zinc, chromium, cadmium and lead concentrations in colostrum and transitory human milk from healthy Saudi lactating women and to observe the diurnal variation in concentrations of these trace elements in mothers' milk available in and around Jeddah City. A total of 156 human milk samples were analyzed after 'wet digestion' for seven trace elements using Graphite Atomic Absorption Spectrometer (AAS). The obtained metal concentrations are compared with the corresponding values of different countries available in the literature.

2. Materials and Methods

2.1 Collection of Milk Samples

The human milk samples were collected from lactating mothers in King Abdulaziz University Hospital, Jeddah. A doctor and a nurse were assigned to collect human milk samples. The mothers were hospitalized before delivery and they were given food from the hospital. All the milk samples were collected during their stay at the hospital. The milk samples were collected twice a day – one in the morning before breakfast and before feeding and a second sample in the afternoon after lunch before feeding to investigate the effect of diet on the elemental concentration for the same subject. We used sterilized plastic bottles to collect human milk samples. All milk samples were put in the freezer until the analysis was carried out.

The women, who gave oral consent, were enrolled during the first week postpartum. The total of 11 lactating mothers were selected. The mean age of mothers was 25 years. All the mothers were healthy and apparently well nourished women, based on clinical observation and has no history of any serious disease. The mothers were not taking vitamin supplements and/or medications during pregnancy and after giving birth. All had uncomplicated pregnancies and delivered a single full term infant (39 ± 2 weeks). All infants were healthy and growing well. The mean birth weight of the infants was 3128 g. Most of the milk samples were collected from day 1 (day of delivery) to 7 days postpartum. Samples from two lactating mothers were also collected from day 1 (day of delivery) to 15 days postpartum. The protocol for milk collection was designed to minimize trace element contamination. The breast was cleaned with de-ionized water and breast milk was hand expressed, into a 30 ml trace element free polystyrene flask. Milk samples were transported to the laboratory on ice. Aliquots of the milk were transferred into 15 ml trace element free polystyrene tubes, then frozen to - 20^0C until analysis. The entire collection procedure was checked for copper, iron, zinc and manganese and was found to be free from contamination.

2.2 Reagents and Glass Wares

Atomic Absorption spectroscopic standard solutions for Zn, Cd, Cr, Cu, Fe, Mn and Pb were purchased from Fisher Scientific Company, USA. Working standard solutions were prepared by diluting the stock solution. Sulfuric acid, perchloric acid and nitric acid were all of AR quality (BDH, England). All glass wares (Conical flask, volumetric flask, watch glass, pipette, measuring cylinder, etc.) were of borocylicate (England). De-ionized water has been used where required.

2.3 Sample Digestion and Preparation of Analytic Solution for AAS

The milk sample needs to be brought into clear solution for analysis by Atomic Absorption Spectrometer(AAS). For this reason the milk sample was first digested with chemicals where the organic matrix of milk was destroyed and left the element into a clear solution. 'Wet Digestion' method (*i.e.*, digestion with nitric, sulfuric and perchloric acids) has been used in the present study. The detailed procedure is available in the Ref. [4,15,16].

2.4 Calibration Curve

The VARIAN Atomic Absorption Spectrometer (AAS), Model spectra AA 30P consisting of a double beam, four lamp Turrent Spectrometer with a Deuterium background corrector and a temperature programmable Graphite Tube Furnace Assembly (GTA 96) was used in this study. Temperature program of the furnace was optimized to obtain the best signal during the atomization process. The drying time and ashing temperature for each element was determined earlier. All these data were fed into the computer associated with the AAS machine. The range of linearity of the concentration vs. absorbance curve is of great importance in determining accurate concentrations. Standard aqueous solutions of different elements obtained from Fisher Scientific Company, USA were used to calibrate the AAS machine. The calibration curves were drawn for Zn, Cd, Cr, Cu, Fe, Mn and Pb by Machintosh Microcomputer using linear regression analysis of the concentrations of the standard solutions versus absorbance values.

A new calibration curve was plotted for each element every time a new batch of milk was arranged for analysis. Each standard solution was measured at least three times and the mean was plotted.

The sensitivity of the AAS machine was tested by using 10 ppb standard lead (Pb) solution. The mean absorbance value of several measurements was found to agree well with the manufacturer's stated value with a Relative Standard Deviation (RSD) of 1.6 %.

2.5 Measurement of Elemental Concentration in Milk Samples

A 10 µl aliquot of each milk sample, obtained after wet digestion, was injected into the graphite tube of the AAS with the help of an auto-sampler, and the elemental concentration was read from the output of the printer connected to the computer. Each sample was repeated several times for each element and the average was recorded. The concentrations of Zn, Cd, Cr, Cu, Fe, Mn and Pb were determined for each sample of human milk. A total of 156 samples of human milk were analyzed in the present study.

2.6 Quality Control

The reliability of method for estimation of Zn, Cd, Cr, Cu, Fe, Mn and Pb concentration in milk samples by AAS technique has been checked by analyzing standard reference Milk Sample (A-11) obtained from the International Atomic Energy Agency (IAEA). Measurements were taken several times and the average result agreed within ±7% of the certified values. Our values agree well with values published by different authors [10,17].

3. Results and Discussions

The results of our measurements of the elemental concentrations in standard reference milk sample (A – 11) obtained from IAEA are presented in Table 1.

The range of linearity of concentration versus absorbance graph is of great importance in determining the elemental concentration of the

Table 1. Concentrations of Zn, Cu, Fe, Mn, Cd, Pb and Cr in standard reference milk sample (A-11) from IAEA.

Reference sample	**Metal**	**Unit**	**Certified Concentration (IAEA Values)**	**Average observed concentrations**
Milk sample IAEA (A-11)	Zn	µg/g	38.9 (36.6-41.2)	37.1 (34.8-39.2)
	Cu	µg/g	0.38 (0.35-0.41)	0.40 (0.37-0.42)
	Fe	µg/g	2.1 (2.0-2.4)	2.2 (1.9-2.4)
	Mn	µg/g	0.30 (0.26-0.32)	0.32 (0.29-0.34)
	Cd	µg/g	1.7 (1.2-2.2)	1.6 (1.3-2.1)
	Pb	µg/g	54 (29-79)	50.9 (48-55)
	Cr	µg/g	0.27 (0.22-0.29)	0.25 (0.24-0.28)

milk samples. The calibration graphs obtained for Fe, Cu, Mn, and Pb are shown in Fig. 1 and 2. Similar graphs were also drawn for Zn, Cd and Cr but are not shown here.

The adjusted linear equations and correlation coefficients are:

Iron: $y = 0.0008\,x + 1.0431$, $r = 0.9959$

Copper: $y = 0.0009\,x + 0.0978$, $r = 0.9982$

Manganese: $y = 0.037\,x + 0.0861$, $r = 0.9986$

Lead: $y = 0.0201\,x + 0.037$, $r = 0.9965$

Zinc: $y = 0.0005\,x + 2.233$, $r = 0.9919$

Chromium: $y = 0.0054\,x$ $r = 0.9997$

Cadmium: $y = 0.0569\,x + 0.121$, $r = 0.9879$

The observed correlation coefficients were also assessed using student’s t – test at 5% level of significance. The results showed that there is significant correlation between the variables for each element under study.

The results of the present study for seven trace elements in human milk samples are given in Table 2. The mean of 9 milk samples collected in the morning from 9 lactating mothers and the mean of 9 milk samples collected in the afternoon from the same lactating mothers are presented for each element for different days of collection of the milk samples. In both types of samples, the mean concentration of Zn is the highest followed by copper, iron, manganese, chromium, cadmium and the last lead. It is clearly evident from Table 2 that the concentration of all the elements in the afternoon samples are higher than those in the morning samples. Picciano *et al*.[18] and Feeley *et al*.[1] also reported that the elemental contents of the am samples were significantly lower than those of the pm samples.

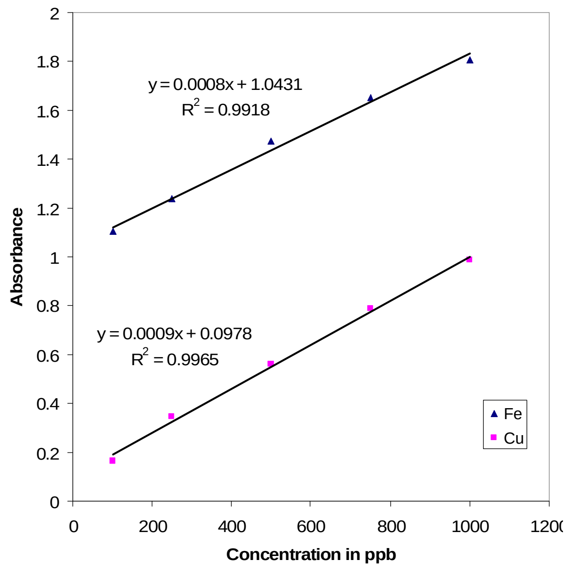

Fig. 1. Concentration versus absorbance calib- ration curves for Fe and Cu.

Milk Samples from two lactating mothers were also collected in the morning and afternoon from day 1 (day of delivery) to 15 days postpartum. The mean of the samples for each element with day of collection are presented in Table 3.

The longitudinal variation in concentrations of the elements are shown in Fig. 3 – 9. It is observed that there are large and apparently irregular changes occurring in the early postpartum period. There is an apparent decline in the mean levels as the stage of lactation progressed. More experiments are needed to explain such changes. The declination in trace element concentrations in human milk as the stage of lactation progressed has also been reported by Casey *et al.*[2] in USA and Arnaud and Favier[6] in France and by other investigators in many parts of the world [3,9,11,13,14].

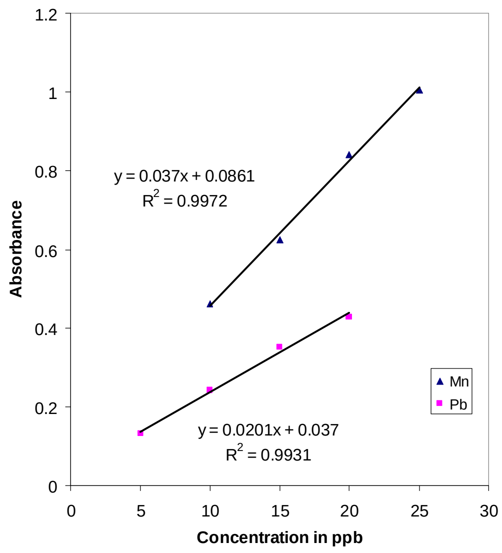

Fig. 2. Concentration versus absorbance calibration curves for Mn and Pb.

In order to convert ml of milk to g and to determine the daily intake of these metals by Saudi infants, a specific gravity of 1.01 was used which agrees well with value used by different authors [2,7,11,14]. For better statistics we used the mean value of the morning and afternoon samples for each element from Table 2. The elemental concentrations in human milk in µg/l were determined and presented in Table 4. It is observed that the concentration levels of the elements in breast milk in samples investigated are well comparable with those reported elsewhere. Although the concentration of Zn is high in breast milk in samples investigated, this level is much lower compared to the values observed in some western countries such as USA [1,19] and Poland[4]. The concentration of Mn in Saudi Arabia mother's milk is found to be highest compared with the corresponding values found in the literature. Again the concentrations of Cd and Pb are higher in breast milk in Saudi Arabia but these values are lower compared to the values observed in Poland[4].

Table 2 . Elemental concentration in human milk collected from day 1 to day 7 postpartum.

Day of collection of milk sample	Time of collection of milk sample	Elemental Concentration (ppb) Mean ± SD						
		Zinc	Copper	Iron	Manganese	Chromium	Cadmium	Lead
1	Morning	2086.3 ± 11.2	495.7 ± 4.7	211.0 ±7.7	19.3 ± 0.4	7.1 ± 0.7	2.0 ± 0.01	2.4 ± 0.01
	Afternoon	2340.1 ± 10.1	575.4 ± 5.1	302.1 ± 8.7	21.7 ± 1.1	17.7 ± 1.4	2.3 ± 0.4	3.0 ± 0.01
2	Morning	2474.3 ± 6.2	480.6 ± 4.5	143.5 ± 3.9	19.6 ± 0.4	7.3 ± 0.6	2.0 ± 0.01	2.0 ± 0.01
	Afternoon	2607.7 ± 5.0	561.2 ± 5.1	309.7 ± 8.8	22.0 ± 1.2	19.0 ± 1.8	3.3 ± 0.4	2.0± 0.01
3	Morning	2569.6 ± 13.2	496.2 ± 5.3	160.6 ± 1.1	17.0 ± 0.7	7. 7 ± 0.9	5.0 ± 0.01	1.9 ± 0.01
	Afternoon	2601.8 ± 8.7	554.4 ± 5.8	340.0 ± 1.5	27.0 ± 1.3	18.0 ± 1.0	6.0 ± 0.01	2.0 ± 0.01
4	Morning	2590.3 ± 5.6	481.6 ± 3.9	142.0 ± 1.3	21.3 ± 1.1	7.0 ± 0.7	2.0 ± 0.01	1.0 ± 0.01
	Afternoon	2724.7 ± 3.8	571.6 ± 5.5	322.4 ± 1.2	35.7 ± 1.6	23.0 ± 2.1	7.3 ± 0.4	1.6 ± 0.4
5	Morning	2289.2 ± 4.8	486.2 ± 4.2	151.0 ± 1.7	20.4 ± 1.0	6.8 ± 0.98	1.2 ± 0.1	1.1 ± 0.01
	Afternoon	2371.3 ± 4.2	578.4 ± 6.1	330.6 ± 4.6	27.8 ± 1.5	40.0 ± 2.1	7.0 ± 0.6	1.7 ±0.01
6	Morning	2246.0 ± 6.7	503.3 ± 5.7	171.3 ± 2.7	15.3 ± 1.1	6.4 ± 0.6	1.0 ± 0.01	1.0 ±0.01
	Afternoon	2294.3 ± 9.9	546.1 ± 5.7	249.3 ± 3.5	19.7± 0.4	13.7 ± 1.8	4.0 ± 0.01	1.0 ± 0.01
7	Morning	2060.2 ± 6.2	499.7 ± 4.3	170.4 ± 2.6	14.8± 0.9	7.1 ± 1.0	1. 0 ± 0.01	1.0 ± 0.01
	Afternoon	2102.4 ± 8.2	547.7± 5.1	245.6 ± 3.3	17.4 ± 1.1	10.3 ± 2.1	2.6 ± 0.01	1.0±0.01

Table 3. Elemental concentration in human milk collected from day 1 to day 15 postpartum.

Day of collection of milk sample	Elemental Concentration (ppb) Mean ± SD						
	Zinc	**Copper**	**Iron**	**Manganese**	**Chromium**	**Cadmium**	**Lead**
1	2132.6 ± 10.6	549.5± 6.2	337.2 ± 6.8	14. 8± 1.6	12.6 ± 1.7	1.8 ± 0.01	2.5 ± 0.01
3	2302.4 ± 10.2	544.2 ± 5.8	323.4 ±6.3	15.1± 1.9	18.8 ± 1.9	2.0 ± 0.2	2.4± 0.01
5	2426.5± 11.2	545.8 ± 6.1	315.9 ± 5.9	16.3 ± 2.2	20.6 ± 2.1	3.4 ± 0.4	2.2 ± 0.01
7	1632.6 ±11.1	551.2 ± 5.3	320.8 ± 5.1	20.2 ± 2.4	23.2 ± 2.8	4.1 ± 0.5	2.0 ± 0.01
9	1377.8 ± 9.6	545.2 ± 5.6	315.1± 5.4	15.1 ± 2.1	15.3 ± 2.3	3.2 ± 0.2	2.0 ± 0.01
11	1354.7 ±8.9	535.4 ± 6.3	310.4 ± 5.2	14.8 ± 1.8	10.4 ± 2.1	3.0 ± 0.2	1. 5 ± 0.01
13	1308.6 ± 8.2	531.1 ± 6.1	300.7 ± 4.6	14.5 ± 1.7	9.8 ± 1.8	2.4 ± 0.2	1.4 ± 0.01
15	1239.3 ± 6.4	527.9 ± 5.8	296.2 ± 4.2	13.6 ± 1.6	7.6 ± 1.6	2.1 ± 0.1	1.3 ± 0.01

Table 4. Comparison of the elemental concentrations in human milk samples from different countries.

Type of Milk	Country	Concentration (µg/l)							Reference
		Cr	**Cu**	**Zn**	**Fe**	**Mn**	**Cd**	**Pb**	
Human milk	USA	-	200-900	500-5000	-	-	-	-	[19]
	Germany	-	350	2300	-	-	-	-	[21]
	Sweden	-	-	-	-	-	0.1	2.0	[27]
	Sweden	-	-	-	-	-	0.06	0.7	[23]
	55 Countries (Mean value)	-	290	1680	-	-	1.3	-	[28]
	Germany	-	270	1400	-	-	-	-	[24]
	Sudan	-	117	1300	-	-	-	2.6	[5]
	India	-	195	1772	-	-	0.09	1.9	[17]
	Bangladesh	-	120-250	280-1800	330-700	-	-	-	[22]
	Poland	14.2	1100	5017	-	-	6.2	5.4	[4]
	USA	-	1009	5067	936	-	-	-	[1]
	Spain	12.4-22.3	210-1191	-	237-860	5.9-22.8	-	-	[20]
	Cited values	6.4-18.5	30-750	-	260-730	4.8-17.8	-	-	[20]
	Saudi Arabia	15.2	540.9	2685.6	259.1	28.8	5.6	2.7	Present study

The daily intake of metals depends on both the concentration and the amount of food consumed. It is observed from Table 2 and Fig. 3 that the secretion of Zinc is highest on day 4 postpartum. Again the secretion of Cu, Fe, Mn, Cr, Cd and Pb are highest on day 1, day 1, day 4, day 4, day 2, and day 1 respectively. The only food of breast feed infants (age less than 1 month) is human milk. The mean of 24 h output of breast milk of an Indian mother is 550 ml for first 3 months, 680 ml for 4 to 6 months and 700 ml for 6 to 12 months[17,25]. According to results published by Feeley *et al.* [1] and Casey *et al.* [2] ,the mean daily outputs of breast milk of an American mother are 400, 500 and 600 ml at the early transitional, transitional, and mature stages of lactation, respectively.

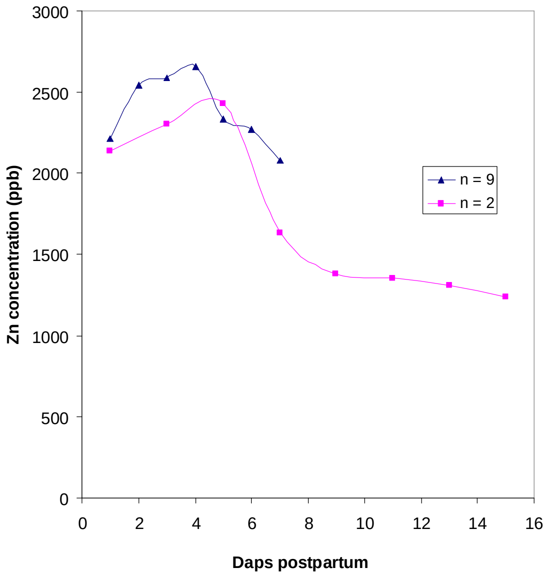

Fig. 3. Longitudinal changes in Zn concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 samples (n=2).

Assuming a value of 500 ml/day for the milk consumption by the infants in Saudi Arabia, the maximum daily intake of these metals by Saudi infants are determined and are depicted in Table 5. The Recommended Dietary Allowance (RDA)//Tolerable /Recommended Safe and Adequate value as set by different international organizations, such as NCR and FAO/WHO are also given in the same table. It is evident that the intakes of essential elements (Zn, Cu, Fe, Mn and Cr) are well below the recommenced/tolerable values but the breast milk of Saudi mothers is a very poor source of Zn, Cu, Fe and Mn. The intake of toxic elements Cd and Pb is also well below the recommended/ tolerable levels[17,26].

Table 5. Comparison of daily intakes of metals from 500 ml of breast milk by Saudi infants with recommended values.

Milk	Elements	Maximum daily intake from breast milk (μg/day)	Recommended/Tolerable value (μg/day)	Reference
Human Milk	Cr	7.6	10 -120	[29&10]
	Cu	270.5	500-1000	[17&29]
	Zn	1342.8	3000-5000	[17&29]
	Fe	129.6	5000-6000	[1&29]
	Mn	14.4	500-700	[10&29]
	Cd	2.8	2.8-3.5	[17&26]
	Pb	1.4	12.5-17.5	[17&26]

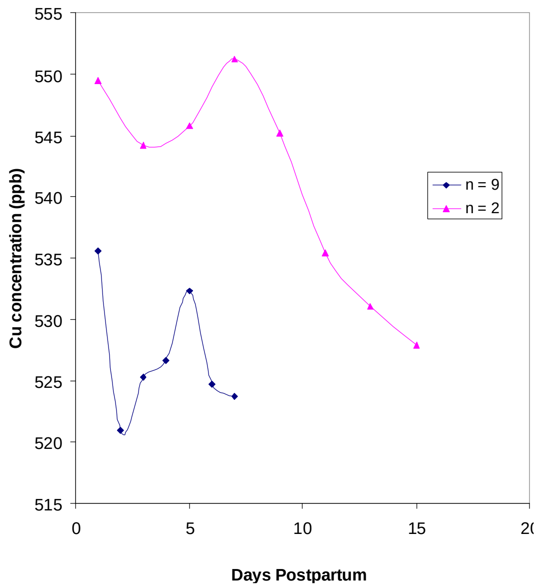

Fig. 4. Longitudinal changes in Cu concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 samples (n=2).

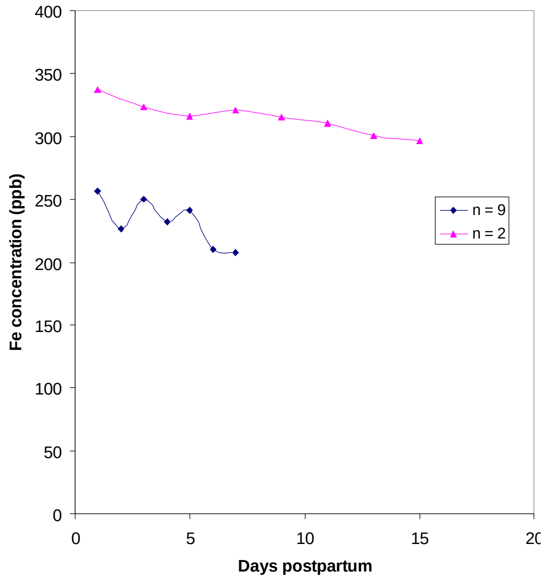

Fig. 5. Longitudinal changes in Fe concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 (n=2).

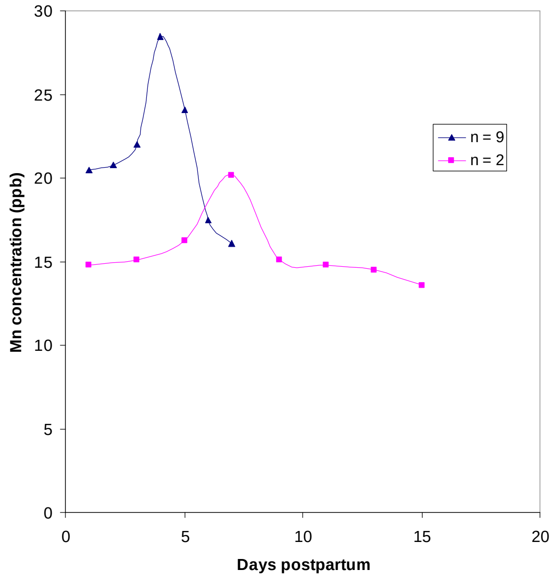

Fig. 6. Longitudinal changes in Mn concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 (n=2).

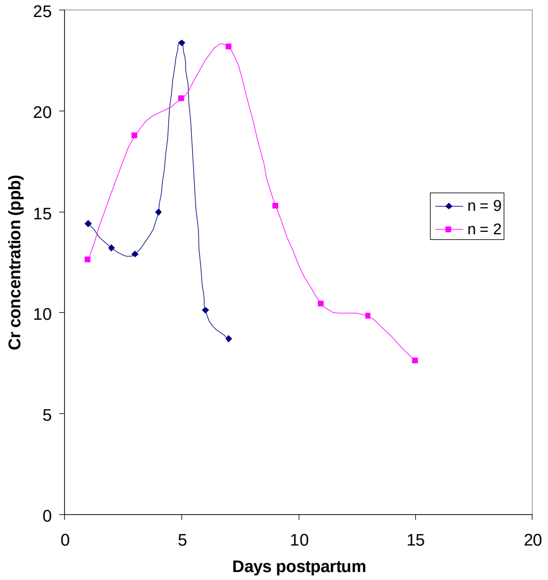

Fig. 7. Longitudinal changes in Cr concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 (n=2).

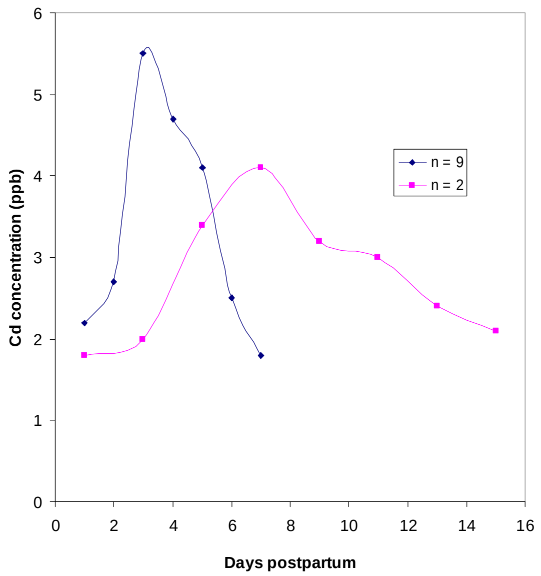

Fig. 8. Longitudinal changes in Cd concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 (n=2).

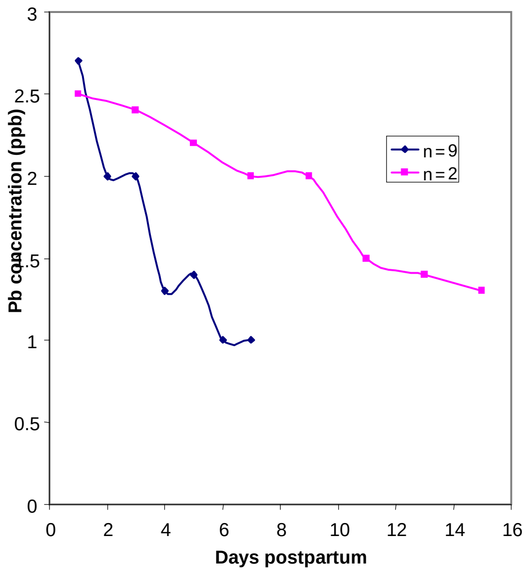

Fig. 9. Longitudinal changes in Pb concentration of milk samples from Saudi women, average of 9 samples (n=9) and average of 2 (n=2).

Human milk is supposed to be an ideal source of nutrients for human infants and meet the requirements of the newborn. Therefore, it is important to examine the contribution of infant gender, term and birth weight to the trace element variation in mother's milk. But we did not examine the effects of these factors because of small number of lactating mothers participating in the study. However, there are few reports which indicate that none of these factors influence the trace element concentrations [6,8]. Sources of variation of human milk composition also include maternal trace element intake and status, maternal age, residing area, family income and length of gestation[6,8,9,18]. We have planned for more experiments to investigate the effect of these factors on the variation of human milk composition.

Further studies are obviously needed to understand the biological mechanism involved in the regulation of the secretion of trace elements in human milk. The understanding of the biology of human milk is essential for developing milk-formula substitutes.

4. Conclusions

The present study was performed to determine the concentrations of Zn, Cu, Fe, Mn, Cr, Cd and Pb in breast milk samples collected from lactating mothers in Jeddah and also to observe the longitudinal variation in concentrations of these elements as the stage of lactation progressed.

The mean elemental concentration values in ppb of Zn, Cu, Fe, Mn, Cr, Cd and Pb in the morning samples are 2330.8±3.1, 491.9±1.8, 164.3±1.4, 18.2±0.3, 7.1±0.3, 2.03±0.01 and 1.47±0.01 respectively while those in the afternoon samples are 2434.6±2.9, 562.1±2.1, 299.9±2.0, 24.5±0.5, 20.2±0.7, 4.6±0.1 and 1.76±0.1 respectively. The afternoon samples contain higher concentrations of the elements than those in the morning samples.

The longitudinal variation in concentrations of the elements show that there are large and apparently irregular changes occurring in the early postpartum period. There is an apparent decline in the mean concentration levels as the stage of lactation progressed.

It is observed that the concentration levels of the elements in breast milk in Saudi Arabia are well comparable with those reported elsewhere by different authors.

It is evident from the results obtained in this study that the intakes (µg/day) of the essential elements (Zn, Cu, Fe, Mn and Cr) are well below the recommended/tolerance values but the breast milk of Saudi mothers are very poor sources of Zn, Cu, Fe and Mn. The intake of toxic elements Cd and Pb are also below the safety limit for the infants.

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التغير الزمني لتركيز العناصر النادرة في الحليب البشري بمدينة جدة - المملكة العربية السعودية

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المستخلص. تعد المعلومات التي يجب توفرها عن وجود عناصر تتبع في الحليب البشري شيئاً ضرورياً ليس فقط لتقدير الكميه التي يحصل عليها الطفل من خلال الرضاعة, بل أيضاً لكونها نقطة بداية لإعداد توصيات عن أنواع أخرى من الغذاء الذي يجب أن يتناوله الرضيع خلال فترة الرضاعة.

خلال فترة الرضاعة, وخاصةً خلال الأسابيع القليلة الأولى, يلاحظ أثر مرحلة الرضاعة على تركيز بعض العناصر بما فيها الحديد والزنك والمنجنيز. وقد تم سابقاً قياس تركيز كل من الزنك والنحاس والحديد والكروم والكادميوم والمنجنيز والرصاص في عينات من حليب الأمهات جمعت من حليب ١١ مرضعة متمتعة بالصحة. وقد جمعت العينات في فترات مختلفة بدءً من يوم الولادة وحتى ١٥ يوم بعد الولادة. أظهرت الدراسة أن معدل تركيز العناصر بوحدة الجزء من البليون من عنصر (Pb , Cd , Cr , Mn , Fe , Cu , Zn) في حليب الأم الذي تم تجميعه في الفترة الصباحية هو :

2330.8±3.1, 491.9±1.8, 164.3±1.4, 18.2±0.3, 7.1±0.3, 2.03±0.01, 1.47±0.01

على التوالي. بينما كان التركيز لنفس العناصر في العينات التي جمعت من حليب الأم في الفترة المسائية هو:

2434.6 ± 2.9 , 562.1 ± 2.1 , 299.9 ± 2.0 , 24.5 ± 0.5 , 20.2 ± 0.7 , 4.6 ± 0.1 , 1.76 ± 0.1

ويعد تركيز هذه العناصر في العينات التي جمعت منتصف النهار أعلى من تركيزها في العينات التي جمعت في الصباح الباكر, ووجد انخفاض واضح في مستوى تركيز العناصر الرئيسية خلال تقدم فترة الرضاعة. و تمت مقارنة هذه النتائج مع تراكيز أخرى مماثلة لعدة دول مختلفة ورد ذكرها في مقدمة هذا البحث.

ولوحظ أيضاً أن مستويات التراكيز للمواد التي تم دراستها في الحليب الموجود في صدور الأمهات السعوديات لا تختلف مع القيم المدونة و المسموح بها عالمياً في أي مكان أخر.