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Vitamin C Content in Human Milk (Colostrum, Transitional and Mature) and Serum of a Sample of Bangladeshi Mothers

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ABSTRACT

The vitamin C content of human milk was determined among twenty-six newly delivered mothers. Milk samples (colostrum, transitional and mature) were obtained at different stages of lactation. Mean vitamin C concentration varied from 3.52 ± 0.56 mg/dl for colostrum to 3.03 ± 0.67 mg/dl for mature milk with the advancement of time, but was in agreement with the infant's daily requirement. Serum vitamin C of a sub-sample of mothers (n=7) was also estimated for a comparison of vitamin C concentration between maternal breastmilk and serum. Vitamin C level estimated in the serum was 0.44 ± 0.29 mg/dl, revealing an eight-fold lower concentration compared to the corresponding milk samples.

INTRODUCTION

Breastfeeding is an unequalled way of providing ideal food for the healthy growth and development of infants, and has a unique biological and emotional influence on the health of both the child and mother (WHO/UNICEF, 1989). The protective effect of breastfeeding against acute infection has been addressed extensively. Human milk (colostrum, transitional and mature milk) can transfer specific or non-specific immunities to the external mucosal surface of the intestine and possibly to the respiratory tract of the newborn. Anti-oxidant vitamins viz. vitamins A, E, C play an important role in immunomodulation. Vitamin C present in human colostrum and milk has a number of biochemical functions linked to the function of the immune system. It helps in the maintenance of a natural barrier against infection, stimulates leukocytes for their phagocytic and anti-microbial activity, augments antibody production and complement levels (Thomas & Holt, 1978) and also enhances synthesis of interferon (Siegel, 1993). For growth, development and survival, infants need an optimum supply of vitamin C.

In Bangladesh, breastfeeding is universally practised, but exclusive breastfeeding is rare. Percentage of low birth weight babies is one of the highest in the world, about 50% of the total birth (WHO, 1997). Infant mortality rate is estimated at 57 per 1000 live births, which is also very high in the region (Ministry of Health, 1988-1999). Most deaths are due to infections such as diarrhoea, respiratory infection etc. Poor maternal dietary intake further complicates child survival. Data from the recent National Nutrition Survey reveals very poor dietary intake of vitamin C by urban pregnant and lactating mothers (Jahan & Hossain, 1998). They were reported to take only 37mg and 33mg per day respectively, which could only fulfil half of the estimated requirement.

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Based on these considerations, this study planned to estimate the vitamin C content in breast milk of Bangladeshi mothers to determine whether the amount satisfies the infant's need. Blood samples were also collected from a sub-group for the estimation of vitamin C, and to correlate the difference in concentration between the two biological tissues i.e. blood and milk.

MATERIALS AND METHODS

Collection of breast secretions and blood

Twenty-six samples of human colostrum and milk were collected by manual expression from 26 apparently healthy mothers, who were admitted to the Dhaka Medical College Hospital for delivery. Informed consent was obtained from the participating mothers. Colostrum (2ml) was collected on second post-partum day during their stay in the post-natal ward, and thereafter the milk samples (2ml) was collected on the second and fourth weeks in their home. Blood (1ml) was collected from a sub-sample (n=7) on the second post-partum day. Women suffering from any breast or systemic diseases were not included in the study. All the mothers had full term pregnancy.

Estimation of vitamin C levels

The vitamin C content of colostrum, milk and blood samples was determined by the method developed by Lowry, Lopez & Bressey (1945). Blood samples were kept undisturbed at room temperature for 60 mins and were then centrifuged at 3000 rpm for 10 mins for extraction of serum. Colostrum and milk samples were centrifuged at 3000 rpm for 20 mins. On centrifuge, the fat component of colostrum and milk separates from the fluid part and moves upwards leaving a clear mid-zone, while the cells settle at the bottom. From the clear mid-zone, 0.3 ml sample was taken in a test tube to which 1.2 ml TCA was added (trichloro acetic acid) and mixed well by a vortex for 15 secs. The mixture was then centrifuged at 3000 rpm for 10 mins. From this, 0.9 ml supernatant was taken and 0.4 ml DTC (2,4 dinitrophenyl hydrazine thiourea copper sulphate) was added. It was then covered with aluminium foil and incubated at 60°C for 60 mins in a water bath. Immediately after incubation, the sample was chilled in ice-cold water and 1.6 ml of 65% sulphuric acid was added gradually. Finally the treated sample was stored at room temperature for 30 mins. A similar procedure was applied for serum analyses. Absorbance was measured against a reagent blank at 520 nm by a spectrophotometer (UV-1201, UV-vis, Shimadzu, Japan}. Every sample was analysed twice to obtain duplicate readings.

Assessment of nutritional status of mother

Besides the age and parity of the mothers, weight and height were measured to assess the nutritional status of the mother. For each subject, BMI (weight in kg / height in m^2) was determined. A BMI of less than 18.5 was classified as undernourished.

Statistical analyses

Data analyses were performed with Stastistical Package for the Social Sciences (SPSS version 10.0). Values were expressed as percentage and mean± sd. Appropriate test statistics (ANOVA) were done to determine the effect of health and other maternal characteristics on vitamin C content in breast secretions.

RESULTS

The subjects ranged from 18 to 32 years in age. The majority of the mothers (21 women) were between 20–29 years. Thirty-five percent of mothers were primi para. Based on the calculated BMI, twenty-three mothers were found to be well nourished (BMI: 18.5-25) while three were under-nourished (BMI: <18.5).

The mean vitamin C content in the breast secretions for all mothers (n=26) were 3.52 ± 0.56 mg / dl for colostrum, 3.25 ± 0.53 mg / dl for transitional milk and 3.03 ± 0.67 mg / dl for mature milk (Table 1). For the sub-samples (n=7), mean vitamin C content in breast secretion were 3.50 ± 0.88 mg / dl for colostrum, 3.41 ± 0.49 mg / dl for transitional milk, 3.49 ± 0.53 mg / dl for mature milk and 0.44 ± 0.29 mg / dl for serum. Analysis of one way ANOVA showed no significant group difference in colostrum, transitional milk or mature milk (Table 2).

Table 1. Vitamin C content (mg/dl) in human milk and serum

Samples analyzed	Mean ± sd (n = 26)	Mean ± sd (n = 7)
Colostrum	3.52 ± 0.56	3.50 ± 0.49
Transitional milk	3.25 ± 0.67	3.41 ± 0.49
Mature milk	3.03 ± 0.67	3.49 ± 0.53
Serum	- -	0.44 ± 0.29

Table 2. Differences in vitamin C content in breast secretions according to BMI, parity and family income

Parameters	Subjects		Colostrum	Transitional Milk	Mature milk	P-value
	n	%				
BMI						
< 18.5	3	12	3.49±0.59	3.52±0.58	3.55±0.62	0.990
18.51-24.99	9	73	3.61±0.73	3.26±0.53	3.00±0.27	0.304
25 & above	4	15	3.46±0.63	3.01±0.72	2.82±0.31	0.462
Parity						
One	9	35	3.58±0.55	3.65±0.43	3.21±0.77	0.312
Two	11	42	3.32±0.65	3.24±0.48	3.17±0.51	0.869
Three & more	6	23	2.82±0.71	3.17±0.64	3.09±0.69	0.514
Income (*Tk/month)						
Less than 4000	6	24	3.56±0.49	3.39±0.67	3.63 ± 0.52	0.634
4000-7999	10	38	3.41±0.73	3.31±0.51	3.10±0.43	0.521
8000 & above	10	38	3.36±0.69	2.94±0.68	2.93±0.65	0.414

*1 US $ = Tk 58.00

DISCUSSION

We report here the concentration of vitamin C in the human milk samples of twenty-six post partum mothers, and in the serum of sub-samples (n=7). An attempt was also made to compare the vitamin C concentrations in breast secretions with maternal BMI, parity and income.

Results showed that the concentrations of vitamin C in the milk samples (3.03-3.52 mg/dl i.e., 30-35 mg/l) of the studied subjects was in agreement with the reference value (National Research Council, 1989). Vitamin C content in colostrum, transitional and mature milk showed little differences. A gradual decline in the concentration of vitamin C in the milk samples over the period was observed, but the difference was insignificant. Other researchers also showed a similar observation (Udipi *et al.*, 1985; Salmenpera, 1984). The RDA of vitamin C for infants through 6 months of age, 35mg/day, is based on an assumed intake of 850 ml/day of milk, which contains approximately 30-55mg of vitamin C/L i.e., 3.0-5.5 mg/day (Byerly & Kirskey, 1985). Average milk production of the marginally nourished women of Bangladesh, who constitute the majority of lactating women in the country, is 750 ml/d (Brown *et al.*, 1986). So if the infants remain on the breast fully for the first 6 months after birth, the available vitamin C in breastmilk (for 750ml) will be around 41-42 mg, which is enough to fulfil the child's daily requirement.

A striking difference in concentration of vitamin C in breastmilk and serum was observed. Vitamin C level in normal serum is 0.4-1.5mg/dl (Jelliffe & Jelliffe, 1978). Higher tissue concentration in breastmilk is probably due to unique secretory activity in the mammary glands. It also suggests nature's choice to replenish the newborn's requirement. Possibility of placental synthesis in pregnancy has also been suggested (Jellife and Jelliffe, 1978). Both the placenta and breasts may be able to actively secrete ascorbic acid.

One way ANOVA showed that the vitamin C concentration in milk was not influenced by the nutritional status of the mother as measured by BMI, the number of pregnancies or economic condition of the mother.

The findings of the present study support the belief that in developing countries like Bangladesh, where most of the women are poor and nutritionally undernourished, continued proper breastfeeding, would not only help to fulfil the infant's nutrient requirement but also protect them from falling sick.

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