## Immunonutrient Constituents of Human Colostrum

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## Abstract

The immunonutrient (Cu, Zn, Fe, vitamin E, C, A) constituents of colostrum from twentyseven post-partum mothers were analysed and examined in relation to maternal age, parity and BMI. HPLC was employed to analyse the colostral vitamin E and A, and spectrophotometric method was used for estimation of ascorbic acid level. The Cu, Zn and Fe contents of colostrum were determined by atomic absorption flamme spectrophometry. The colostrum concentrations of vitamin E, C and A were  $25.51 \pm 11.21 \mu mol/L$ ,  $216.96 \pm 87.72 \mu mol/L$ ,  $0.65 \pm 0.29 \mu mol/L$  respectively. The Cu, Zn and Fe contents of colostrum were  $10.94 \pm 5.94 \mu mol/L$ ,  $65.82 \pm 20.63 \mu mol/L$ ,  $21.54 \pm 7.30 \mu mol/L$  respectively. No significant relationship was found between maternal age, parity, BMI and the elemental content of colostrum. Significant correlation was found between ascorbic acid and parity (P>0.05).

Key words : Human Colostrum, Immunonutrients, Antioxidant Micronutrients

## Introduction

Human colostrum contains all of the constituents that are required for optimal growth and development of a neonate. They support development of immune system providing both specific and non-specific immunities<sup>1</sup>. Colostrum is the first defence for the newborn. It stimulates the newborn's immune system, and affords immunity to the external mucosal surface of the intestine and to the respiratory tract of the newborn<sup>1</sup>. It has also unique protective effect against acute infections<sup>2-5</sup>. In addition to the non-nutritive bioactive components (like the immunoglobulins), colostrum contains a large number of immunoactive nutrients including antioxidant micronutrients. Antioxidant vitamins and minerals play an important role in immunophysiological functions acting as potential immunoenhancers and antioxidants<sup>6,7</sup>, deficiencies of which down-regulates immune function, but their overload is immunotoxic<sup>8-11</sup>. In view of their important role in immunophysiological

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system, colostrum concentrations of vitamin E, C, A, and minerals like copper, zinc, iron, and the correlation between maternal characteristics and nutrient content of colostrum were investigated among selected Bangladeshi mothers.

## **Materials and Methods**

## Study population

The study was conducted amongst twentyseven post-partum mothers of age 16 to 40 years, who delivered their babies at the Maternity and Child Health Training Center, Azimpur, Dhaka. They belonged to low to mid socioeconomic group. Those, who voluntered as participants selected randomly irrespective of their biological condition. Women suffering from any breast or systemic diseases were not included in the study. Informed consent was obtained from the participating mothers. Colostrum (2ml) was collected by hand expressed into an acid washed glass vials on the 2<sup>nd</sup> post-partum day during their stay in the post-natal ward. Privacy of the mothers was maintained. Anthropometric data, age and parity of each of the mothers were recorded. To conduct the study, ethical permission was taken from Bangladesh Medical Research Council (BMRC), Dhaka.

## Colostrul analysis

Retional and  $\alpha$ -tocopherol. Colostrum samples were preserved at -20°C for analysis of retinol and  $\alpha$ -tocopherol. Reversed phase HPLC (LC-10AD, SHIMADZU, HPLC 1991, Model-7125, Japan) was used for simultaneous determination of retinol and  $\alpha$ tocopherol in the colostrum as described by Islam et al<sup>6</sup>. The retinol and  $\alpha$ -tocopherol extracted from the colostrum was injected into chromatography on a C<sub>18</sub> shim pack column with methanol:water (95:5) mobile phase. Retinol and  $\alpha$ -tocopherol were detected spectrophotometrically at 291nm. To verify the assay accuracy, standard analytes retinol and  $\alpha$ -tocopherol (Sigma Chemical Co, USA) were injected for every 10 test samples.

Ascorbic acid. The concentration of ascorbic acid in the colostrum was determined by spectrophotometric method using phenyl hydrazine indicator (Sigma Chemical Co, USA) as described by Islam et al<sup>6</sup>. Absorbance was measured against a reagent blank at 520nm by a Spectrophotometer (UV-1201, UV-VIS, Shimadzu, Japan).

Zinc, Copper and Iron. To analyse the colostral mineral contents, 1ml colostrum was acid digested using an acid mixture in block heater to make a clear solution. Concentrations of copper, zinc and iron in the colostrum were then determined by an

atomic absorption flamme emission spectrophotometer (AA-6200 Series, Shimadzu Corporation, Kyoto, Japan) as described by Picciano and Guthrie<sup>12</sup> using standard copper, zinc and iron (Sigma Chemicals Co, USA). Absorbances of copper, zinc and iron were read at 324.8nm, 213.9nm and 248.3nm respectively. To verify the assay accuracy and quality, the standard mineral preparations were run for every 10 test samples. A software package (AA-6200, ver 1.1, Shimadzu Corporation, Kyoto, Japan) was used to calculate the minerals concentrations.

#### Statistical analysis

SPSS software package (version 10.0, SPSS Inc. Chicago, USA) was used to analyse the data. Descriptive statistics were calculated for all variables. Values were expressed as percentage and mean±sd. Non-parametric analysis ( $\chi^2$  test) was performed to assess the association of colostral micronutrients with maternal characteristics. Linear regression analysis was performed to find the extent of influence of maternal characteristic on the immunonutrients (if any).

#### Results

Maternal charateristics of the study population are shown in the table1. The mean maternal age, parity and BMI were  $25.04 \pm 5.43$  years,  $1.52 \pm 1.01$  and  $23.44 \pm 3.73$ respectively. Most of the mothers were young (16-24 years) having normal BMI (18.5-25) and were primi parae. Table 2 shows the immunonutrient profile of colostrum. The concentrations of vitamin E, C and A in colostrum were 25.51  $\pm$ 11.21 $\mu$ mol/L, 216.96 ± 87.72  $\mu$ mol/L, 0.65 ± 0.29  $\mu$ mol/L respectively. The Cu, Zn and Fe content of colostrum was  $10.94 \pm 5.94 \mu$ mol/L,  $65.82 \pm 20.63 \mu$ mol/L,  $21.54 \pm$ 7.30µmol/L respectively. Non-parametric analysis showed no significant relationship between maternal age, parity, BMI and the elemental content of colostrum (table 3). Significant association was found between ascorbic acid and parity ( $\chi^2 = 21.55$ ). Though  $\gamma^2$  values did not indicate any significant association of age with vitamin E, and parity with Zn, but their 'r' values (-0.97, -0.32) showed certain negative correlation. Based on these findings, linear regression analysis was done, which exhibited regression co-efficient  $\beta$ = -0.645 with P=0.003 and R<sup>2</sup>=0.307 for ascorbic acid and parity. But regression co-efficient between age and vitamin E ( $\beta$ = -0.112 with P=0.632 and R<sup>2</sup> =0.009), and that between parity and Zn ( $\beta$ = -0.211 with P=0.211,  $R^2=0.062$ ) were found to be insignificant.

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Characteristic	Number (%)	Mean ± sd	Total Mean ± sd	
Age in year				
16-24	14(52)	$25.57 \pm 2.03$	$25.04 \pm 5.43$	
24-32	10(37)	$28.30 \pm 1.82$		
32-40	3 (11)	$35.00 \pm 0.00$		
Parity				
Primi	19(70.4)	$1 \pm 0.00$	$1.52 \pm 1.01$	
Multi	8(29.6)	$2.75 \pm 1.01$		
BMI				
16-18.5	4 (15)	$18.34\pm4.10$	$23.44 \pm 3.73$	
18.5-25	16 (59)	$21.94 \pm 1.69$		
>25	7 (26)	$28.32 \pm 2.52$		

Table 1: Socio-demographic characteristics of the mothers under study
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# Table 2: Micronutrient profile of human colostrum

Micronutrient in µmol/L	Number (%)	Mean ± sd	Total Mean ± sd
Vitamin E		L	<u>المنابعة المنابعة ا</u>
≤18.8	9(33)	$13.67 \pm 2.49$	$25.51 \pm 11.21$
18.8-30.00	10(37)	$24.69 \pm 1.76$	
30.00 and above	8(30)	$39.84 \pm 6.31$	
Vitamin C			
≤116.00	1(4)	$95.96 \pm 0.00$	216.96 ± 87.72
116.00-162.00	6(22)	$143.46 \pm 16.81$	
162 and above	20(74)	$245.06 \pm 84.53$	
Vitamin A			
≤0.81	19(70)	$0.51 \pm 0.16$	$0.65 \pm 0.29$
0.81-1.34	7(26)	$0.90 \pm 0.01$	
1.34 and above	1(4)	$1.65 \pm 0.00$	
Copper			
≤15.00	19(70)	$8.05 \pm 4.53$	$10.94 \pm 5.94$
15.00-27.00	8(30)	17.821.25	
Zinc			
≤62.00	11(41)	$44.95 \pm 10.14$	$65.82 \pm 20.63$
62.00-77.00	6(22)	$65.69 \pm 5.35$	
77.00 and above	10(37)	87.82 ± 53.46	
Iron			
≤10.60	2(7)	$8.91 \pm 0.00$	$21.54 \pm 7.30$
10.60-12.50	1(4)	$11.14 \pm 0.00$	
12.50 and above	24(89)	$23.03 \pm 6.28$	

Maternal	$\chi^2$					
characteristic	Vitamin E	Vitamin C	Vitamin A	Copper	Zinc	Iron
BMI	5.94	1.86	0.86	0.56	3.14	1.92
	(0.08)	(0.19)	(0.09)	(0.27)	(0.09)	(0.10)
Age	6.43	1.86	8.88	3.59	0.29	1.30
	(-0.97)	(0.19)	(0.17)	(0.27)	(0.04)	(0.09)
Parity	1.89	21.55*	(6.98)	2.26	3.80	1.43
	(-0.05)	(-0.57)	(0.15)	(0.27)	(-0.32)	(-0.02

 Table 3. Correlation of colostral micronutrient profile with maternal characteristics

<sup>a</sup>  $\chi^2 \ge 9.488$  at 4 df (degree of freedom) is significant. \*Significant Figure in parenthesis are r-values.

### Discussion

The mean concentration reported in the study for vitamin E in colostrum was found to be much higher than the values reported for milk<sup>13-16</sup>, but is in agreement with the concentration claimed elsewhere for colostrum<sup>14</sup>. Concentrations of vitamin C and A in the colostrum were similar to those reported for Bangladesh<sup>16</sup> and India<sup>17</sup>, but these values are lower than those reported for the developed and developing countries<sup>18,19</sup>. However, the mineral values obtained in the present study are consistent with the data reported in other studies <sup>20,21</sup>. Correlation between maternal characteristic (i.e. age, parity and BMI) and the concentrational values of colostral micronutrients, except for ascorbic acid with parity, could not be ascertained. This outcome is matched up to some extent with the findings of other researchers<sup>22</sup>. The reason for positive association of vitamin C with parity ( $\chi^2 = 21.55$ ) is not clear. However, it is reported that breasts may be able to actively secrete ascorbic acid in pregnancy<sup>23</sup>.

It is to be concluded that human colostrum is rich in antioxidant immunonutrients, which play an important role in the growth, development and immunity of infant's life. It further revealed that colostral concentration of micronutrients is universal and is independent of nutritional status, age or parity of mothers. This finding is of considerable public health significance. Promotion of colostrum feeding will ensure child survival, growth and development irrespective of social strata.

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