IMMUNOLOGICAL STATUS OF HUMAN BREAST MILK AND ITS IMPORTANCE IN THE EARLY POSTNATAL PERIOD

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Introduction

Breast fed newborns are shown to be less susceptible to infections and allergies when compared to artificially fed neonates. These differences become more exaggerated in developing areas of the world due to poor sanitation (Head, 1977). A lower morbidity and mortality in breast fed newborns is due to the presence of various immunological factors in the breast milk, which provide antibacterial and antiviral protection, specially to the gastrointestinal tract (Hanson and Winberg 1972). It therefore appears that breast feeding during the early neonatal period plays a crucial role in providing the immunologically naive infant with some protection.

The present study was undertaken to determine the immunological status of human breast milk and immunological difference if any, between the neonates given breast or formula milk.

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Material and Methods

This study was conducted on 80 full-term healthy neonatal maternal pairs. Thirty neonates were breast fed by their mothers (Group A), 30 were separated from their mothers and given formula milk (Group B) and 20 neonates were separated from their mothers and given a combination of colostrum (20 ml. three times day) and formula milk (Group C).

Paired samples of blood from cord and corresponding mothers were collected. Subsequently, samples of blood from the neonates were obtained between the fourth to seventh day after birth. Colostrum and transitional milk were collected by manual expression into sterile containers. Undiluted toned milk from Delhi Milk Scheme acted as formula milk. The milk samples were defatted by centrifugation at 500 g. for 15 minutes. The sera and milk samples were stored at —20°C till analysis. The samples were analysed for total protein by Biuret method (King and Wooten, 1959), protein fraction by paper electrophoresis (Block, *et al* 1958) and immunoglobulins IgG, IgM and non-secretory IgA by single radial immunodiffusion technique (Mancini, et al 1965) using WHO standards Batch No. 67/97).

Observation

Breast milk: The levels of total protein and protein fractions were significantly higher ($P < .001$) in the colostrum as compared to the transitional milk. Beta globulin was not detected in the breast milk (Table I).

The levels of IgA, IgG and IgM were highest in the first day samples of colostrum, showing a rapid decline thereafter (Table II). Colostrum contained a high concentration of IgA, the first post-partum day sample having approximately five times the levels in the mother's serum. The IgA in the transitional milk was comparable to the serum levels. The concentrations of IgM and IgG in colostrum and transitional milk were significantly low and even lower than the levels observed in the serum (Table II).

Formula Milk: The levels of total proteins and protein fractions in the samples of D.M.S. milk were significantly lower ($P < 0.001$) than the breast milk. Unlike human milk, beta globulin was detected in formula milk. The level of albumin was significantly higher ($P < 0.001$) (Table I).

Serum: The total gamma globulin level in the sera of neonates (1-7 days old) given exclusive breast milk (group A) was significantly higher ($P < 0.05$) than the other two groups (Table III). However, the differences in the levels of individual immunoglobulins in the three groups were not statistically significant.

Conjunctivitis was observed in 3 cases of Group A, 7 cases of Group B and 2 cases of Group C. However, no major infections were observed in any of the groups. Rhinitis, possibly due to reagin mediated allergy was observed in one infant in Group C.

Discussion

The present study shows that human breast milk, both colostrum and transitional, have a high level of total proteins, particularly rich in globulins. High levels of gamma globulins is believed to be due to their local production and selective concentration in the mammary glands just before and at the time of parturition (Goldblum, *et al* 1975). It has been postulated that IgA is produced locally in the human breast (Goldblum, *et al* 1975). The level of IgA in transitional milk is comparable to that of maternal blood; therefore it is possible that the high levels found in the colostrum represent the concentration of IgA during the latter part of pregnancy.

It is also possible that like IgA, local production of IgG and IgM may occur in the mammary glands at the time of parturition, and thus, whatever is present in the milk may be what has been present locally (Ogra and Ogra 1978). The small amounts of these immunoglobulins in breast milk may be because of a blood and acinar barrier preventing passive transfer of these immunoglobulins from the maternal blood to the milk ducts (Yadav, *et al* 1980).

The amount of colostrum secreted on the first post-partum day is generally limited to a few ml. as opposed to the abundant secretion of milk in the transitional period as well as during later phases of lactation. The decline in the immunoglobulin concentration during the first week of the onset of lactation may reflect a dilutional phenomenon (Ogra and Ogra, 1978; Ammann and Stiehm 1966). However, the immunological benefits available to the infant are probably not significantly altered, as the fall in the immunoglobulin concentration is com-

TABLE I

Protein Analysis of Mo her's Blood, Human Colostrum (Day 1-4), Transitional Milk (Day 5-8) and Formula Milk (g/dl). Values expressed as mean ± S.D.

	Total protein	Albumin	Total globulins	Globulin fractions			
				$Alpha_1$	$Alpha_2$	Beta	Gamma
Mother's blood	6.46 ± 0.54	2.48 ± 0.63	3.98 ± 0.34	0.69 ± 0.30	0.72± 0.29	1.52± 0.42	1.15± 0.28
Colostrum	9.30 ± 3.00	0.70 ± 0.66	8.60 ± 2.34	0.64 ± 0.58	1.06± 0.56	—	7.95± 2.28
Transitional milk	4.36 ± 1.67*	0.25 ± 0.17*	4.11 ± 1.88	0.36 ± 0.14*	0.48± 0.24*	—	3.27± 1.50
Formula milk	2.50*	1.69*	0.81*	0.11*	0.08*	0.06	0.50*

*—P <0.001.

TABLE II

Immunoglobulin Levels in Colostrum (Day 1-4), Transitional Milk (Day 5-8) and Mother's Blood (mg/dl). Values expressed as mean ± S.D.

Immuno-globulin	COLOSTRUM				Transitional Milk	Mother's blood
	Day 1	Day 2	Day 3	Day 4		
IgA	1159.66 ± 168.28	** 954.81 ± 185.47	* 737.6 ± 176.50	* 505.20 ± 142.60	** 248.98 ± 83.08	214.40 ± 25.39
IgG	52.86 ± 7.84	45.12 ± 9.01	37.30 ± 6.16	* 22.84 ± 5.46	** 17.63 ± 3.88	1221.12 ± 260.63
IgM	71.33 ± 8.06	62.29 ± 11.28	43.52 ± 9.54	38.55 ± 8.32	24.01 ± 10.13	188.16 ± 16.55

*—P <0.05 or P <0.01, compared to the preceding days value.
**—P <0.001, compared to Day 1 value.

TABLE III

Protein Analysis of Cord Blood and Bood of 4-7 Days Old Neonates (g/dl). Values expressed as mean ± S.D.

Blood	Total	Albumin	Total globulin	Globulin Fractions			
				$Alpha_1$	$Alpha_2$	Beta	Gamma
Group A							
Cord	5.82±	3.23±	2.59±	0.39±	0.46±	0.77±	1.15±
	0.57	1.01	0.41	0.24	0.20	0.79	0.41
Neonate	5.90±	3.22±	2.68±	0.32±	0.49±	0.67±	1.39±
	0.53	0.75	0.25	0.18	0.21	0.34	0.46*
Group B							
Cord	5.60±	2.91±	2.69±	0.23±	0.44±	0.76±	1.16±
	0.62	0.70	0.42	0.15	0.21	0.36	0.28
Neonate	5.58±	3.02	2.56±	0.30±	0.41±	0.70±	1.15±
	0.60	0.86	0.29	0.21	0.18	0.29	0.41
Group C							
Cord	5.84±	3.18±	2.66±	0.28±	0.53±	0.73±	1.12±
	0.73	0.90	2.26	0.15	0.23	0.50	0.42
Neonate	5.88±	3.30±	2.58±	0.26±	0.58±	0.67±	1.07±
	0.60	0.82	0.26	0.15	0.24	0.29	0.35

* — Change significant ($P < 0.05$) compared to corresponding cord blood (Group A) and blood of neonates in Groups B and C.

pensated by the increase in the volume of milk secreted (McClelland, *et al* 1978).

The albumin in formula milk is abundant and comprises of both lactalbumin and albumin derived from serum (Mata, 1978), thus accounting for the observation that its level was significantly higher than that of the human milk. The gamma globulins in formula milk were negligible in comparison with human milk. Formula milk has a low concentration of gamma globulins, moreover those that exist are destroyed by the process of pasteurization (Mata, 1978). Hence the infant fed exclusively on formula milk does not get much of globulins and lack of this immunological protection is provided by breast milk. Unlike breast milk, beta globulin was found to be present in the formula milk. Beta globulin is a common antigen implicated in food allergy in the newborn infant (Mata, 1978).

The evidence for absorption of colostral immunoglobulins from the neonatal gut is conflicting. Ammann and Stiehm (1966) found no difference in the immune globulin levels between artificially fed and breast-fed infants after four days of feeding. Yap *et al* (1979) also found no significant difference between mean IgA concentrations on the sixth day of life in the sera of two groups of infants given exclusive breast and formula milk respectively. On the other hand, Iyengar and Salvaraj (1972) reported significantly higher levels of immune globulins on the fifth day life in colostrum-fed infants. Ogra *et al* (1977) reported a rise in serum IgA levels in three infants, who were fed colostrum 18-24 hours after birth.

In the present study, a significant increase was noted in the serum gamma globulin levels exclusively in the breast-fed neonates as compared to the other two

groups, suggesting that some amount of colostrual proteins are absorbed into the infant's circulation via the gut. However, the differences in the individual immunoglobulin levels between the three groups of infants were not statistically significant.

Some authors (Ammann, *et al* 1966) believe that small molecules of the breast milk proteins such as transfer factor (M.W. 10,000) or migration inhibition factor (M.W. 25,000) are preferentially absorbed from the neonatal gut. The heavier molecules such as colostral secretory IgA, IgM and IgG may not be so easily absorbed acrose the neonatal-intestinal mucosa (Ammann, *et al* 1966). It is possible that the colostral lipid-associated proteins are handled differently by the neonatal gastrointestinal mucosa and detectable amounts of these find their way into the circulation (McClelland, *et al* 1978). In the present study some amount of colostral proteins do appear to be absorbed across the gastrointestinal mucosa resulting in an increase in the serum gamma globulin concentration of the exclusively breast-fed neonate in comparison to the other two groups. The maternal milk immunoglobulins ingested by the infant do not, however, appear to significantly influence the blood immunoglobulin levels. The breast milk definitely is in a position to provide immunological security to the infant at the level of the gastrointestinal mucosa, though the contribution may be relatively low towards the body as a whole. This is of great clinical significance as gastrointestinal infections of the infants are the commonest infections.

Summary

The present study was conducted on eighty healthy full-term neonatal maternal pairs, during the first week of the postnatal period. Levels of total protein, protein fractions, IgG, IgM and IgA were determinated in the colostrum (Day 1-4) and transitional milk (Day 5-8) samples obtained from the mothers during the first week of lactation, and serum samples of the mother, cord serum and serum obtained from 4-7 days old neonates given exclusive breast milk (30), exclusive formula milk (30) and a combination of breast and formula milk (20). The concentration of total proteins, protein fractions and immunoglobulins was significantly higher in the colostrum than in the transitional milk. A significant increase ($P < 0.05$) was detected in the level of serum gamma globulins of neonates given exclusive breast milk as compared to the formula and breast + formula fed neonates. No significant difference was observed in the mean serum IgG, IgM and IgA levels of the three groups of neonates.

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