

Original Research Article

A study on the failure of breast feeding during the first month of life with effect on immunological level

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ABSTRACT

Background: In the critical phase of immunological immaturity of the newborn, particularly for the immune system of mucous membranes, infants receive large amounts of bioactive components through colostrum and breast milk. Breastfeeding provides unsurpassed natural nutrition to the newborn and infant. Study was done to know the effects of breast milk feeding versus formula feeding in early infancy on the development of serum IgA, IgM and IgG.

Methods: The present study investigated 100 cases of failure of breast feeding. The cases included both complete and partial failure. Values of immunoglobulin levels (IgA, IgM and IgG) in the serum of eleven breast fed and eleven artificially fed infants (all aged one month) were determined using Tripartigen plates.

Results: Mean level of IgA in artificially fed infants was $20.72 \pm 3.82 \mu\text{g}/100 \text{ ml}$. The diameter of precipitin ring using sample number 7 was 3.9 mm. The mean level of IgA in breast fed infants was $25.94 \pm 3.89 \mu\text{g}/100 \text{ ml}$. The mean level of IgM in artificially fed infants was $31.690 \pm 3.504 \mu\text{g}/100 \text{ ml}$. The mean level of IgM in breast fed infants was $36.81 \pm 5.13 \mu\text{g}/100 \text{ ml}$. The mean level of IgG in artificially fed infants was $480.25 \pm 52.23 \mu\text{g}/100 \text{ ml}$. The mean level of IgG in breast fed infants was $517.59 \pm 56.72 \mu\text{g}/100 \text{ ml}$.

Conclusions: It is evident from the results of immunoglobulin estimation (Ig A, Ig M and IgG) in infants with artificial milk and in infants with breast milk (vide table 5, 6, 7, 8, 9 and 10) that though the mean serum levels (Ig A, Ig M and IgG) in breast fed infants were slightly higher than that of artificially fed infants. There was no statistically significant difference in the serum immunoglobulin levels between these two groups.

Keywords: Artificial feeding, Breast feeding, Human milk, Immunoglobulins, Infants

INTRODUCTION

Colostrum is the most potent natural immune booster known to science. Breastfeeding protects infants against infections mainly via secretory IgA (sIgA) antibodies, but also via other various bioactive factors. It is striking that the defense factors of human milk function without causing inflammation; some components are even anti-

inflammatory. In the early stages of lactation, IgA, anti-inflammatory factors and, more likely, immunologically active cells provide additional support for the immature immune system of the neonate.¹

For the fetus and newborn, immunologic defences are present, but immature. To compensate, the mother's immunoglobulin (Ig) G antibody moves across the

placental barrier to provide some protection. After birth, these maternal antibodies wane in the first 6 to 12 months of human life. The neonate and infant can receive additional maternal protection from breast milk, however. Human breast milk contains large quantities of secretory Ig A (sIgA). These antibodies, which have formed as a consequence of the mother's previous exposure to infectious agents, can bind to potential pathogens and prevent their attachment to the infant's cells.²

METHODS

The present study investigated 100 cases of failure of breast feeding. The cases included both complete and partial failure. Authors also estimated the serum immunoglobulin levels of eleven completely artificially fed and same number of completely breast-fed infants- all aged one month and all healthy, to find out any difference in the levels.

Authors conducted present study in the well-baby clinics mainly, and also in the neonatal nursery of a tertiary care teaching and referral hospital, Kolkata after taking approval from institutional ethics committee. Authors collected present cases from those babies who were delivered within one year's time before present investigation started and also from those babies who were delivered during present investigation. The study included mothers of all age groups from 19 to 20 years up to 40 years on an average. All of them who have delivered in the same hospital were from middle socioeconomic class group with a few exceptions.

In this present study 22 babies were investigated for immunological studies, 11 being fed completely at the bottle and the other 11 completely at the breast. Out of 100 cases of breast feeding failure, 11 babies were selected, whose mothers had complete failure of breast feeding during the first postnatal growth, who were aged exactly one month (plus or minus one to two days on either side), were full term, of average weight (2.5-3 kg at birth) completely healthy on clinical examination with no clinical evidence of infection in any part of the body and their weight gains during the first months were within normal limits (300 to 350 gm). All the babies in the present study were fed with tinned milk.

Another 11 babies were selected from the Well Baby Clinics (as controls) who were fed entirely with breast milk during the first month of their lives. They were of same age, of similar birth weight and gestational age, having same health and nutritional status.

At least 2 c.c. of venous blood was drawn by puncture of femoral vein under aseptic precautions. After collection of 22 serum samples, estimation of immunoglobulin levels (IgG, IgA, IgM) of each of them were done.

For immunoglobulin estimation, authors selected the method of immune-diffusion and precipitation in

Tripartigen plates (of Hoechst Pharmaceuticals Ltd.). The single radial immuno-diffusion (SRID) method had been utilized in these plates. For immunoglobulin estimation by using Tripartigen plates, because the method was comparatively easy, and no prior preparation of the plates was necessary. The readings were acceptable only when the diameter of precipitation rings was within 4-9 mm. That was equivalent to following concentrations of immunoglobulins, for example IgA 27 to 350 µg/100 ml (16 to 208 IU/ml), and for IgM 34 to 430 µg/100 ml (39 to 494 IU/ml).³

As IgA and IgM concentrations of the most biological fluids were within this range, undiluted fluid (serum here) could be used for IgA and IgM estimation. But, as the IgG concentration of most biological fluids were large, if used undiluted, they would produce precipitation rings greater than 9 mm in diameter. So, the readings would not be acceptable. For that reason, before IgG estimation, the control serum and the serum samples were diluted 1:10 with isotonic saline. The readings obtained were multiplied by 10 to find out exact IgG concentration of undiluted fluids.^{3,4}

Assay range for IgG

16-197 µg/100 ml [1.8 -22.6 IU/ml]-undiluted sample or 160-1970 µg/100 ml [18-226 IU/ml]-sample diluted 1:10.

Serum samples-22 in number.

Tripartigen plates

These were glass plates-coated with a mixture containing gel solution and specific antibody against specific antigen (e.g. IgG, IgA and IgM). Thus, separate plates were available for estimation of IgG, IgA and IgM. Each of the plates had a glass cover. On each plate, 12 wells were there, each of 2 mm diameters, and they were numbered from 1 to 12 in a clockwise manner. Six plates were used- 2 for IgG, 2 for IgA and another 2 for IgM estimation.

Controls

Serum (manufactured by Hoechst Pharmaceuticals Ltd.), and it contains antigens (IgG, IgA and IgM) in such concentrations which are compatible with the assay ranges of the plates. So same control serum could be used in all three types of tripartigen plates (e.g. for IgG, IgA and IgM) in the present study. The method was to fill the well number 12 of each plate for IgA and IgM estimation with undiluted control serum and those for IgG estimation with 1:10 diluted control serum. Purpose of using the control serum was to examine the functional status of the plates.

Partigen ruler

Partigen ruler (manufactured by Hoechst Pharmaceuticals Ltd) was made of transparent material. At its centre there

were one horizontal line and two diverging lines on either side of it. All the three were running from one end of the scale to the other end. There was vertical graduation at right angles to the central horizontal line. The figures written below the lines indicated diameter of the rings in millimetre and the figures written above, indicated diameter of the rings in millimetre-the second figures were not of any use in present study.²

Function of it was to measure the diameter of precipitation rings on the tripartigen plates. For measurement of diameters, the transparent ruler was placed upon the closed plate after precipitation rings have been formed. The plates were inspected regularly at intervals of 24 hours. After about 50 hrs, the plates for IgG and IgA estimation showed round precipitation rings around the wells (from 1 to 11) and after about 80 hrs, the plates for IgM showed round precipitation rings around the wells (from 1 to 11). From the ring diameters in the plates for IgA and IgM estimation, IgA and IgM concentrations were obtained directly from the table of reference values.^{3,4}

For accurate estimation of concentrations of IgG in serum samples, the concentrations mentioned in the table for reference values against the diameters of precipitation rings were first found out. Thereafter, each of the values was multiplied by 10 (as 1:10th diluted sera were used for IgG estimation). In this way, by using the Tripartigen plates, authors found out IgG, IgA and IgM concentrations of each of the 11 samples of sera of bottle fed infants and the same of 11 samples of sera of breast fed infants (as controls) thus making a total of 22 samples.

RESULTS

On investigating 100 cases of failure of breast feeding, authors found that causes of breast feeding failure fall mainly into three groups (Table 1). Table 1 show that highest number of cases falls into Group A (which were due to cause in the mother).

Table 1: Circumstances interfering with breast feeding.

Groups	Causes of breast feeding failure	Total no. of cases	Percentage
A	Maternal causes	71	71
B	Causes in the baby	19	19
C	Other causes	10	10
	Total	100	100

It shows increased incidence of breast feeding failure in the age group above 25 years (66%) and much less incidence of breast feeding failure in below 25 years age group (34%). It shows more failure of breast feeding in primigravida (47%) than in second gravid (33%). Less incidence of failure in third gravid (15%) and least incidence in women with four or more children (5%)

(Table 2). Study revealed that increased incidence of success of breast feeding with increased levels of maternal education.

Table 2: Relation of breast feeding failure with maternal parity.

Parity	No. of cases	Percentage
Para 1	47	47
Para 2	33	33
Para 3	15	15
Para 4 and above	5	5
Total	100	100

Values of immunoglobulin levels (IgA, IgM and IgG) in the serum of eleven breast fed and eleven artificially fed infants (all aged one month) were determined using Tripartigen plates.

Diameters of precipitin rings produced in the plates for IgA level estimation, using the serum of eleven artificially fed infants are expressed in Table 3.

Those with the serum of eleven breast fed infants are expressed in Table 4. The serum IgA levels of these two groups are also expressed in Table 5.

Table 3: Diameters of precipitin rings in Tripartigen plates and serum IgA levels of eleven artificially fed infants.

Diameters of precipitin rings (in mm)	IgA concentration in µg/100ml
4.1	21.08
4.0	17.11
4.3	29.32
4.1	21.08
4.1	21.08
4.0	17.11
3.9	Inconclusive
4.2	25.15
4.0	17.11
4.0	17.11
4.1	21.08

Mean level of IgA in artificially fed infants were $20.72 \pm 3.82 \mu\text{g}/100 \text{ ml}$. The diameter of precipitin ring using sample number 7 was 3.9 mm.

The Tripartigen plates, if any precipitin ring diameter is less than 4 mm, no conclusive value of immunoglobulin level can be estimated. So, IgA concentration of sample number 7 could not be determined (Table 3).

The mean level of IgA in breast fed infants were $25.94 \pm 3.89 \mu\text{g}/100 \text{ ml}$ (Table 4).

The diameter of precipitin rings using serum of same two groups of infants, in the Tripartigen plates for serum IgM

level estimation and corresponding IgM levels are expressed in Table 5 and 6.

Table 4: Diameters of precipitin rings in Tripartigen IgA immune-diffusion plate with resultant IgA levels in serum of eleven breast fed infants.

Diameters of precipitin rings (in mm)	IgA concentration in $\mu\text{g}/100\text{ml}$
4.3	29.32
4.1	21.08
4.1	21.08
4.2	25.15
4.1	21.08
4.2	25.15
4.2	25.15
4.3	29.32
4.4	33.59
4.3	29.32
4.2	25.15

Table 5: Diameters of precipitin rings in Tripartigen IgM immune-diffusion plate with resultant IgM levels in serum of eleven artificially fed infants.

Diameters of precipitin rings (in mm)	Ig M concentration in $\mu\text{g}/100\text{ml}$
4.0	28.86
4.1	33.93
4.0	28.86
4.2	39.13
4.0	28.86
4.0	28.86
4.1	33.93
3.8	Inconclusive
4.1	33.93
3.9	Inconclusive
4.0	28.86

Table 6: Diameters of precipitin rings in Tripartigen IgM immune-diffusion plate with resultant IgM levels in serum of eleven breast fed infants.

Diameters of precipitin rings (in mm)	Ig M concentration in $\mu\text{g}/100\text{ ml}$
4.1	33.93
4.2	39.13
4.2	39.13
4.1	33.93
4.3	44.45
4.1	33.93
4.2	39.13
4.0	28.86
4.3	44.45
4.0	28.86
4.2	39.13

The mean level of IgM in artificially fed infants was 31.690 ± 3.504 $\mu\text{g}/100\text{ ml}$. As the diameters of precipitin

rings using sample number 8 and 10 were less than 4 mm, serum IgM of these samples could not be estimated (Table 5).

The mean level of IgM in breast fed infants was 36.81 ± 5.13 $\mu\text{g}/100\text{ ml}$ (Table 6).

The diameter of precipitin rings using serum of same two groups of infants in the Tripartigen plates for serum IgG level estimation and corresponding IgG levels are expressed in Table 7 and 8.

The mean level of IgG in artificially fed infants was 480.25 ± 52.23 $\mu\text{g} /100\text{ ml}$ (Table 7).

Table 7: Diameters of precipitin rings in Tripartigen IgG immune-diffusion plate with resultant IgG levels in serum of eleven artificially fed infants.

Diameters of precipitin rings (in mm)	Value in reference table \times dilution factor	Ig G concentration in $\mu\text{g}/100\text{ml}$
4.9	38.77×10	387.7
5.2	47.15×10	471.50
5.4	53.01×10	530.1
5.1	44.30×10	443
5.5	56.02×10	560.2
5.4	53.01×10	530.1
5.4	53.01×10	530.1
5.3	50.05×10	500.5
5.2	47.15×10	471.5
5.1	44.3×10	443
5.0	41.5×10	415

Table 8: Diameters of precipitin rings in Tripartigen IgG immune-diffusion plate with resultant IgG levels in serum of eleven breast fed infants.

Diameters of precipitin rings (in mm)	Value in reference table \times dilution factor	Ig G concentration in $\mu\text{g}/100\text{ml}$
5.4	53.01×10	530.1
5.6	59.09×10	590.9
5.3	50.05×10	500.5
5.4	53.01×10	530.1
5.6	59.09×10	590.9
5.6	59.09×10	590.9
5.4	53.01×10	530.1
5.3	50.05×10	500.5
5.2	47.15×10	471.5
5.1	44.3×10	443
5.0	41.5×10	415

The mean level of IgG in breast fed infants was 517.59 ± 56.72 $\mu\text{g} /100\text{ ml}$ (Table 8). It is evident from the results of immunoglobulin estimation (Ig A, Ig M and IgG) in infants with artificial milk and in infants with breast milk (vide table 3, 4, 5, 6, 7 and 8) that though the mean serum levels (Ig A, Ig M and IgG) in breast fed

infants were slightly higher than that of artificially fed infants. There was no statistically significant difference in the serum immunoglobulin levels between these two groups.

DISCUSSION

In the present study of 100 cases of breast feeding failure, it was found that some cause in the mother was responsible in the great majority of cases which constituted 71% cases in the present series. In this group, some obvious causes were found in only 26 (36.6%) mothers of which 25.3% mother had sickness and 11.3% mothers had breast and nipple abnormalities. Painful breast congestion was the major (37.5% cases) breast and nipple abnormalities causing lactational failure. Major causes of sickness of mother causing lactational failure were caesarean section in 44.5% cases and post-partum tubal ligation operation in 28% cases.

Study by Mathur GP et al shown that 75 mothers with lactation failure were studied, whose less than 4-month-old babies were admitted to the hospital. Partial lactational failure (94.7%) was noted more often than complete lactational failure (5.3%). Initiation of breastfeeding was delayed for 2 to 5 days usually for traditional reasons (77.3%) and because the mothers felt that the milk output was inadequate (92%). The various causes of lactation failure were determined and the relationship to various factors was analyzed. The commonest cause of lactation failure was insufficient milk or no milk (80%).⁵

Breastfeeding early or immediately after birth

A longitudinal study estimated that 22 percent of newborn lives could be saved if breastfeeding were initiated within the first hour.⁶ Early initiation of breastfeeding provides warmth, promotes bonding, and helps the mother by reducing the risk of postpartum haemorrhage. During the first days of life, breastfeeding helps to prevent low blood sugar (hypoglycaemia) and low temperature (hypothermia), which are important contributors to newborn deaths. Most newborns are ready to find the nipple and latch on to the breast within the first hour of birth, if provided with immediate skin-to-skin contact. Colostrum, the thick and yellowish or clear breastmilk produced in the first few days, provides the baby with high levels of antibodies, immune cells, vitamin A, and other protective factors.⁷

Colostrum (milk produced during the first days after birth), in addition to being a rich source of nutrients, contains high concentrations of various protective factors with anti-infective action, such as enzymes (lysozyme, lactoferrin etc), immunoglobulins, cytokines, complement system components, leukocytes, oligosaccharides, nucleotides, lipids, and hormones that interact with each other and with the mucous membranes of the digestive and upper respiratory tracts of infants,

providing passive immunity as well as stimulation for the development and maturation of the infant's immune system.⁸

IgM antibodies are the second most abundant immunoglobulin in human colostrum, at concentrations of up to 2.5 mg/ml. High avidity IgM antibodies reactive with viruses and bacteria may play an important role in protecting the mucosal surfaces of infants. IgG is found at low concentrations in human milk, around 0.1 mg/ml (10% of serum values) and, in addition to neutralizing activity, has opsonizing activity that can activate the complement system and antibody-dependent cytotoxicity, which is not thought to be strongly present on the infants' mucosal surfaces.⁹

Study by Cheng MM et al showed that the average concentrations of serum IgA and IgM in all infants were 1.171 ± 1.079 and 256.2 ± 165.8 µg/ml, respectively. There were significantly higher concentrations of serum IgA in the FF group than MF group at 3, 4 and 6 days of age and BF group at 5 and 6 days old. Paired serum IgA concentrations revealed that IgA significantly decreased in the BF group, but not in the FF and MF groups. Meanwhile, paired serum IgM concentrations revealed that IgM increased significantly during early infancy in all groups. However, the IgM levels had no difference among the 3 groups within 7 days of age.¹⁰

Study by Chndra RK revealed concentrations of total protein and immunoglobulins A, G and M were determined in milk samples collected from 21 mothers who delivered preterm and 21 mothers who delivered at term.¹¹ Levels of protein and of immunoglobulins, particularly IgA, were significantly higher in the preterm group samples. There were no differences in concentrations of protein or individual immunoglobulins related to mode of expression of manual, manual or mechanical, rate of milk flow or time of collection during the 24-hour period.

The world health organization (WHO) recommends that newborns should be fed exclusively with maternal milk during the first six months of life, and the American Academy of Pediatrics recognizes the benefits of human milk for preterm newborns.^{12,13} Industrialized milk formulas can supply the nutritional necessities of preterm and term newborns; however, the protective properties of maternal milk are unique and cannot be reproduced in the laboratory.¹⁴

Maternal milk contains some defense components, among them immunoglobulin, which has quantitative and qualitative differences when compared with the immunoglobulin in blood serum. Immunoglobulin concentrations are very elevated in colostrum, constituting most of the protein content of this secretion, reaching more than 90% of the pool protein during the first day of the lactation, decreasing in the next days.^{13,15,16}

Various studies have demonstrated that the colostrum and milk produced by mothers of preterm and low-weight newborns have more elevated secretory IgA levels than that of the mothers of term newborns. Considering that the immunological system is physiologically more immature in preterm newborns, it is possible that the milk and colostrum of the mothers of preterm newborns have higher levels of secretory IgA in order to provide for the necessities of these fragile children.^{17,18,19}

CONCLUSION

Breastfeeding is well known to provide immune protection and prevent various diseases in the perinatal period. Human breast milk is also accepted as the best nutritional source for the neonate and infant, and it provides other widely accepted benefits to the mother and child. The expansion of knowledge about the immunological composition of breast milk reinforces the importance of many components present even in small amounts in this secretion, which is a perfect food and a supplement with increasingly recognized immunological value.

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