Evaluation of the Secretory Immunoglobulin A Levels in the Colostrum and Milk of Mothers of Term and Pre-Term Newborns

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Objective: To determine and to compare the levels of secretory immunoglobulin A in samples of colostrum and milk of mothers of term and preterm neonates. Material and Methods: The levels of secretory immunoglobulin A of 10 mothers of term neonates and 10 mothers of preterm neonates were determined from 5mL of colostrum or milk collected on the 1st, 4th, 10th and 15th days of the puerperal period, using the radial immunodifusion technique. We employed anamnesis, as well as physical and gynecological exams in women in the puerperal period. All the patients were attended at the Januário Cicco Maternity College. Results: The secretory immunoglobulin A levels were significantly higher in the colostrum and milk of mothers of preterm neonates when compared with the levels found in colostrum and milk of mothers of term neonates (Mann-Whitney test, p<0.0001). There was a significant decline in the secretory immunoglobulin A levels of the colostrum and milk of the mothers of term and preterm neonates during the four periods (Kruskal-Wallis test, p<00001). Conclusions: The secretory immunoglobulin A levels in colostrum and milk of mothers of preterm neonates were significantly higher than in the mothers of term neonates, demonstrating immunological adaptation in preterm neonate breast-feeding.
Key Words: Secretory immunoglobulin A, milk, neonates.

Natural feeding constitutes the best form of newborn (NB) alimentation, in view of its nutritional and immunological properties, and its low cost. Maternal milk (MM) is constituted of proteins, lipids, mineral and vitamin elements with adequate quality and quantity for the development of the newborn [1]. In the initial phase of life, human milk (HM) is unquestionably the food that best congregates ideal nutritional characteristics, with adequate nutrient balance, besides offering innumerable immunological and psychological advantages that are important for the reduction of infantile morbidity and mortality [2]. Amongst the immunological properties, MM transfers antibodies, defense cells and enzymes, so that breast-fed infants are relatively protected from infectious diseases, which constitute one of the main causes of infantile mortality in developing countries [1]. Maternal milk diminishes the incidence and the gravity of gastrointestinal, urinary and respiratory infections, besides providing a protective effect against bacterial meningitis, botulism, Crohn disease, ulcerative colitis, lymphomas, allergic and other chronic digestive diseases [3]. An ample study of the non-random character of hospitalized preterm newborns indicated that the incidence of necrotizing enterocolitis is significantly lower in those fed with human milk, either exclusively or partially, than in those fed with infant formula [4].

During the decades of 1940 to 1960, there were controversies about the use of maternal versus industrialized milk for preterm newborns, because artificial milk gave the largest weight gains. In the 80's, studies began to show the importance of the preterm newborn mother's milk, particularly during the two first weeks after childbirth, as it contained more calories, a

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greater fat concentration, more protein, sodium and secretory IgA (sIgA) than the milk of newborn term mothers [5,6]. Consequently, there was a consensus for the recommendation of the use of the mother's milk to feed the preterm during the first weeks of lactation [3]. Currently, the American Academy of Pediatrics recommends breast feeding during the entire first year of the term newborn's life and recognizes the benefits of human milk for preterm newborn feeding [3]. The metabolic and gastrointestinal immaturity, immunological damage, and associated medical conditions in the preterm newborn can affect nutritional support. Human milk is capable of satisfying the necessities of the preterm and can provide for similar growth rates when compared to term newborns [7]. Human lactation proceeds through three well defined periods: colostrum, intermediate milk and mature milk. Colostrum provides the necessities of the newborn during the first week of life, supplying on average 67 Kcal/dL. It is rich in proteins, minerals and in immunological factors, facilitating *Lactobacillus bifidus* growth in the gastrointestinal tract and meconium elimination by the newborn [8-10].

The composition of colostrum changes between the sixth and the 14th day of lactation, with a reduction of the concentration of immunoglobulin and proteins and an increase in the lactose levels, fat and energy content, until it has the characteristics of mature human milk [10]. Human milk is divided into two types of elements; these are nutritional factors: proteins (casein, lactoalbumina and lactoglobulina), carbohydrates, fat, minerals, iron, vitamins (B and K), enzymes (lipase and amylase) and disease protection factors: immunoglobulin (IgA, IgM, IgG, IgD and IgE), unspecific defense cells (epithelial cells and macrophages, neutrophils, eosinophils, lymphocytes, and other cells) and immunological factors (bifidus factor, lysozyme, lactoferrin, interferon, complement and lipids) [11]. All five classes of immunoglobulins are present in human milk; they are at higher concentrations in colostrum, constituting a large part of the protein content of this secretion. During lactation, there is a reduction in the concentrations of immunoglobulin; however, the absolute quantity of what is ingested by the child remains unchanged because of an increase in milk volume [12].

IgA type secretory antibodies predominate (SIgA) in human milk; however, they are not absorbed by the newborn gastrointestinal tract. These antibodies protect the infant's mucosal surfaces. Secretory IgA has a peculiar structure that impedes adhesion and posterior establishment of pathogenic microorganisms on the mucous surfaces [13].

Antigens enter in contact with the maternal gastrointestinal or respiratory tract mucous surfaces, where immunocompetent cells are stimulated to produce immunoglobulin, mainly secretory IgA, which is passed on to the newborn by passive immunization or immunity transference [13,14]. Due to the immaturity of the immune system, the preterm newborn is more vulnerable to infectious processes, making it dependent on the immunological support provided by breast-feeding.

We examined and compared the levels of total secretory IgA in the colostrum and milk of mothers of preterm and term newborns.

Material and Methods

This study respected the norms indicated by the "Declaration of Helsinki" (WORLD, 1996) and by Resolution 196/99 of the National Council of Health (BRAZIL, 1996). The spontaneous agreement of the patient was expressed by signature of an informed consent form. This research was approved by the Committee of Ethics in Research of the Januário Cicco Maternity College of the Federal University of Rio Grande do Norte.

Sample processing

Samples of colostrum and maternal milk were collected by manual expression into empty containers on the 1st, 4th, 10th and 15th days of lactation from 20 puerperal women at the Januário Cicco Maternity College. Ten of them were mothers of preterm newborns and 10 were mothers of term newborns.

After collection, the samples were transported on ice to the laboratory and centrifuged at 1800 X g for 10 min at 4ºC in a refrigerated centrifuge. Three distinct phases resulted: the superior layer contained the lipid portion, the intermediate layer had the liquid (aqueous) phase and the bottom layer was a "pellet", which consisted of the cellular elements of human milk. The lipid layer was removed with the aid of Pasteur's pipette and the liquid phase was divided into aliquots of 1.5 mL in eppendorf tubes and stored at –20ºC until the analyses [16,13]. The total secretory IgA was determined by a quantitative radial immunoassay (I.D.R.Q.), using the technique of Mancini et al. (1965), with specific anti-human alpha-chain IgA antibodies (SIGMA). A primary Standard for these assays was made with a pool of colostrums with a known concentration of SIgA of 3.4 g/L [13]. These were read on a 1% agarose gel in PBS (pH 7.4), with 65 µL of anti-IgA human alpha chain antibody incorporated into the 10 mL of gel on a 8 by 8 cm glass plate. After solidification of the gel, it was perforated with 25 2-mm diameter holes. Aliquots (5 µL) of serial dilutions of the standard, colostrum and human milk were placed in each orifice. The plates were incubated at 4ºC in a humid chamber for 48 hours. At the end of the incubation period, the plates were stained and the halo diameters determined, to quantify the concentrations of secretory IgA in colostrum and human milk. After incubation, the plates were wrapped with three layers of gauze and washed with saline solution three times, in order to remove the excess protein contained in agarose gel. After the last washing, filter paper was placed on top of the still humid plate and incubated overnight (16-18 hours). After this period, the filter paper that covered the plate was removed carefully, humidifying it with salt water. The plate was taken to an oven for complete drying. After that, it was stained with Ponceau "S" solution for 20 minutes and then discolored with distilled water [13]. The data on the colostrum and milk of the 20 women were processed using Graphpad InStat software.

Results

The levels of total secretory IgA in samples of colostrum and milk of mothers of term newborns varied significantly between the 1st, 4th, 10th and 15th days of lactation (Friedman test, $p < 0.0001$). The levels of secretory IgA were significantly more elevated on the 1st day when compared with the other days (Wilcoxon test, $p=0.005$); there were higher levels of secretory IgA on the 4th day than the 10th (Wilcoxon test, $p = 0.008$) and the 15th day (Wilcoxon test, $p = 0.009$). There were no significant differences between the 10th and the 15th day of lactation (Wilcoxon test, $p = 0.2026$, Table 1). The levels of total secretory IgA in samples of colostrum and milk of mothers of preterm babies were significantly different between the 1st, 4th, 10th and 15th days of lactation (Friedman test, $p < 0.0006$). The levels of secretory IgA were significantly higher on the 1st day when compared with the 4th (Wilcoxon test, $p = 0.007$), the 10th (Wilcoxon test, $p = 0.005$) and 15th day (Wilcoxon test, $p = 0.005$).

There were no significant differences between the 4th and the 10th day (Wilcoxon test, $p = 0.1141$), between the 4th and the 15th day (Wilcoxon test, $p = 0.2411$) and between the 10th and 15th day (Wilcoxon test, $p = 0.7671$, Table 2). The levels of total secretory IgA were significantly higher in the colostrum and milk of mothers of preterm newborns, when compared with the levels found in the colostrum of mothers of term newborns (Mann-Whitney test, $p < 0.00001$, Table 3).

Discussion

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 [15,3]. 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 [1].

Table 1. Total secretory IgA (mg/dL) by quantitative radial immunoassay in colostrum and milk samples of mothers of term newborns

	Lactation days			
Mean ± sd	**1º (2835 ± 956)**	**4º (129.7 ± 33.5)**	**10º (88.4 ± 16.5)**	**15º (88.4 ± 16.5)**
Minimum value	1,194	68	63	53
Median	2,831	129	89	75.5
Maximum value	4,140	179	114	130
Friedman variance analysis, p < 0.0001				
Wilcoxon Test				
(day 1) x (day 4)			p = 0.0050	
(day 1) x (day 10)			p = 0.0050	
(day 1) x (day 15)			p = 0.0050	
(day 4) x (day 10)			p = 0.0080	
(day 4) x (day 15)			p = 0.0090	
(day 10) x (day 15)			p = 0.2026	

Table 2. Total secretory IgA (mg/dL) by quantitative radial immunoassay in colostrum and milk samples from mothers of preterm newborns

	Lactation days			
Mean ± sd	**1 (23234 ± 13.6)**	**4 (2283 ± 3499.7)**	**10 (702.6 ± 529.2)**	**15 (723.8 ± 565.3)**
Minimum value	8,855	473	200	143
Median	21,389	813	545.5	576.5
Maximum value	46,808	11,889	1,764	2,065
Friedman variance analysis, p = 0.0006				
Wilcoxon Test				
(day 1) x (day 4)			p = 0.0070	
(day 1) x (day 10)			p = 0.0050	
(day 1) x (day 15)			p = 0.0050	
(day 4) x (day 10)			p = 0.1141	
(day 4) x (day 15)			p = 0.2411	
(day 10) x (day 15)			p = 0.7671	

Table 3. Total secretory IgA concentrations (mg/dL) by quantitative radial immunoassay in colostrum and milk samples from mothers of term and preterm newborns

Lactation days								
	Day 1*		Day 4*		Day 10*		Day 1*	
	Term	Pre-term	Term	Pre-term	Term	Pre-term	Term	Pre-term
Mean	2,835.6	23,234.3	129.7	2,282.7	88.4	702.6	79.9	718.7
± sd	956.13	13,646	33.5	3,499.7	16.5	529.2	22.0	561.7
Min.	1,194	8,855	68	473	63	200	53	143
Median	2,832	21,389	129	813	89	545.5	130	2,065
Max.	4,140	46,808	179	11,889	114	1,764	75.5	576.5

*Mann- Whitney test p <0.00001; Min. = minimum. Max. = maximum. sd: standard deviation.

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 [3,16,17].

Though the concentrations decrease during the lactation period, the total amount of immunoglobulin ingested by the child practically remains unchanged during the first two to three months of life due to an increase in milk volume [12].

We observed a significant decline in the secretory IgA levels from the 1st to the 15th day of lactation of term newborn breast-feeding and from the 1st to the 10th day of preterm newborn breast-feeding. We believe that the maintenance of elevated levels of secretory IgA in the milk provide by the mothers of preterm newborns due to their need for greater immunological support.

The secretory IgA protects the newborn intestinal mucosa against foreign microorganisms [16]. During infection, SIgA inhibits pathogens from adhering to the mucous, intervenes in bacterial mobility, and neutralizes toxic products. Other mechanisms include elimination of antigens and intracellular viruses by transcytosis [18, 19]. Perhaps the secretory IgA action against infections, mainly diarrheas and colitis, are essential for the immunological protection of newborns, especially in the case of preterm infants.

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 [20, 21]. 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. In our study, the SIgA levels in the colostrum and milk of mothers of preterm newborns were higher than for mothers of term newborns, demonstrating an immunological adaptation in lactation for the preterm newborn.

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