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Evaluation of Sialic Acid in Human Breast Milk and Infant Formula Food

Avidime Ohunene Makoju[1, *], Olorunshola Kolawole Victor[2], Goji Anthony Donatus Teru[1]

[1]Department of Human Physiology, Kaduna State Universty, Kaduna, Nigeria
[2]Department of Human Physiology, University of Abuja, Abuja, Nigeria

Abstract

Background: Breast milk is the best nutrient source for infants. It contains all elements needed for a normal growth and development of infants. Human milk contains a large amount of sialic acid compared with bovine milk. Sialic acid contained in oligosaccharides, glycolipids and glycoproteins in milk is considered to play important roles in physiological functions in infancy. Objective: Sialic acid (N-acetylneuraminic acid, NANA) is an essential component of mucins, glycoproteins and gangliosides and therefore important for the function of cell membranes, membrane receptors and the normal development of the brain. The aim of this study is to measure and compare the concentration of sialic acid in breast milk and some selected infant formulae in Zaria, Nigeria. Method: In the present study comparison was made between the concentration of free and bound sialic acid in milk from lactating mothers at the different stages of lactation and in a range of infant formulae using Thiobarbituric Acid Assay method. A total of 67 samples of human breast milk was obtained (18 colostrum, 19 transitional, 18 mature and 12 (involutional) and five different commercial infant formulas. Result: Bound sialic acid was highest in colostrum (8.33 ± 1.77mg/ml) and more than 9% higher than in infant formulas (P value < 0.05) and this concentration decreased gradually from colostrum to the involutional stage of lactation. There was a similar pattern for free sialic acid content. Conclusion: It was concluded that human breast milk is a rich source of bound sialic acid, which contrasts to the relatively small amounts found in infant formula food. This may contribute to a normal development of brain functions.

Keywords

Breastmilk, Lactation. Sialic Acid, Infant Formula

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1. Introduction

Sialic acids (N-acetylneuraminic acid, NANA) are terminal compounds in the oligosaccharide chains of many glycoproteins and glycolipids found in the cell membrane and human breast milk is a very rich source of sialic acids. Infant formula also provides a source of sialic acids for the infant but not in sufficient quantity required by the growing infant [1]. Sialic acid is an essential component of mucus, glycoproteins and gangliosides and therefore important for the function of cell membranes, membrane receptors and the normal development of the brain and the best source for obtaining it is from breast milk other sources are saliva, gastric juice, serum, urine and tears. Other physiological roles of sialic acid include transport of positively charged molecules as well as in the attraction and repulsion of cells and molecules thereby contributing to the high viscosity of mucins lining and protecting endothelia, for instance, in the intestine or on the surface of frog eggs [2]. Sialic acids have also been revealed to be important in cell differentiation, neuronal development and brain development, non-immunogenic protection and immuno reactivity such as in cancer progression and virus invasion [2]. Though a cause

* Corresponding author
E-mail address: nenemakoju@yahoo.com (A. O. Makoju)

and effect relationship has not been established between the dietary intake of sialic acid and memory and learning [3] studies have revealed that sialic acid supplementation in animals is associated with increase of gangliosides in the brain and improved learning ability, while formula fed infants obtain far less sialic acid than is present in human milk and so higher brain ganglioside and glycoprotein sialic acid concentrations in infants fed human milk suggests increased synaptogenesis and differences in neurodevelopment [4]. Recombinant glycoprotein therapeutics produced in nonhuman mammalian cell lines may be relevant to improving the efficacy of glycoprotein therapeutics [5], also sialic acid (N-acetylneuraminic acid, NANA) is an essential component of mucus, glycoproteins and gangliosides and therefore important for the function of cell membranes, membrane receptors and the normal development of the brain and the best source for obtaining it is from breast milk. Sialic acids are one of the most important molecules of life, since they occupy the terminal position in macromolecules and cell membrane. In this exposed position, they contribute significantly to the structural properties of these molecules and are involved in many biological and pathological phenomena [2, 6]. Sialic acid has a unique structure, which has a negative charge owing to a carboxyl group enabling it to play roles in cellular functions [7] like the transport of positively charged molecules as well as in the attraction and repulsion of cells and molecules, antimicrobial factors, digestive enzymes et cetera. Sialic acids, docohexanoic acid and many other components of the infant's diet are found in small amounts and absent in some infant formula brands [1]. Deficiency of sialic acid leads to poor brain development, mucin deficient disorders and reduced immunological response [6]. Sialic acids rarely occur fee in nature [4] but the normal range of total sialic acid (TSA) level in serum/plasma is 1.58-2.22 mmol L-1, the free form of sialic acid only constituting 0.5-3 mumol L-1 and the lipid-associated (LSA) forms 10-50 mumol L-1. [9] found total sialic acid concentrations were highest in colostrum (x +/- SEM: 5.04 +/- 0.21 mmol/L in full term) and decreased by nearly 80% over the next 3 months in their study.

Research has been carried out using bovine milk [8] however some studies have also attempted to show the variation in sialic acid concentration at different stages of lactation and compared with a range of commercial infant formulas in Germany and in Australia [1, 9] but there is still limited data available on the sialic acid content of human milk and infant formulas in Africa. Due to existing high parity, malnutrition, high HIV/AIDS prevalence and poverty, it has become pertinent to study the concentration of sialic acid in breast milk and some selected infant formulae which would encourage mothers breast feed their infants if sialic acid content is higher than in infant formulas

2. Objective

To measure and compare the concentration of sialic acid in breast milk and some selected infant formulae in Zaria, Nigeria.

3. Methodology

Subjects and Investigative Design

This study was conducted in Zaria, Northern Nigeria between September and October 2010. Breast milk was collected from sixty-seven volunteer lactating mothers at different phases of lactation (colostrum, transitional, mature and involutional) after obtaining their informed consent. These mothers attend various clinics at Salama Hospital, Zaria, Nigeria and Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Ethical Consideration

The study recruited subjects only after detailed explanation as to the nature and benefit of the study had been given, and verbal and or written consent obtained.

Approval for this study was obtained from the Ethical Committee on Human Research of the Ahmadu Bello University Teaching Hospital, Shika, Zaria, Nigeria.

Collection of Breast Milk

2ml of breast milk was collected from the lactating mothers into sterile bottles and immediately refrigerated at -4°C. Eighteen samples were collected from mothers at the colostral phase of lactation, nineteen at the transitional phase, eighteen at the mature phase and twelve samples from mothers at the involutional phase of lactation.

Infant Formula

The infant formula used were SMA Gold, batch number OT114T10, SMA Nutrition, Ireland, Frisocreme batch number: 325383A, Friesland Campina, Holland; Nan batch number: 11760346AA, Nestle Nutrition, Amsterdam, Nederlands; Cerelac, batch number: L10480291A, Nestle Nutrition, Spain

Determination of Total Sialic Acid

Breast milk and infant formula samples were heated at 80°C for one hour in 0.1N H_2SO_4 [10] to release bound sialic acid without degradation, subsequently, the total sialic acid content was estimated quantitiatively by the thiobartituric acid method developed by Aminoff (1961) as follows:

Periodic acid (0.25ml) reagent was added to each sample (2ml of sample in test tubes) and then incubated at 37°C for

20 minutes. Sodium arsenite (0.1ml) was then added. Followed byThiobarbituric acid (im). The mixture was heated for ten minutes in boiling water and cooled at room temperature. Colour extraction was done using *N*-butanol-HCI (2.5ml) and mixed (19.1), the extract was centrifuged at 600rpm for five minutes. Absorbance of the butanol layer was determined at 549nm using spectrophotometer.

Determination of Free Sialic Acid

This was determined by the same method for total sialic acid but without prior heating of samples.

Determination of Bound Sialic Acid

This was done by taking the difference between the total and free sialic acid. The sialic acid concentration is given in mg/ml.

4. Statistical Analysis

Results were expressed as mean SEM. The difference between the amount of sialic acid in breastmilk and infant formula was determined using ANOVA and the difference between free and bound sialic acid was determined using student t-test. Results were deemed significant at $P<0.05$.

5. Results

The concentration of bound sialic acid and free sialic acid found in breastmilk at the different stages of lactation and in infant formula is as shown in tables 1 and 2.

Table 1. Sialic acid concentration in breast milk.

	Colostrum	Transitional	Mature	Involutional
Bound	8.33 ± 0.18	4.13 ± 0.15	1.16 ± 0.26	0.10 ± 0.12
Free	2.09 ± 0.18	2.37 ± 0.11	1.38 ± 0.15	1.90 ± 0.21

Table 2. Sialic acid concentration in infant formula.

	FRISOCREME	CERELAC	NAN	SMA	SIMILAC
Bound	0.17 ± 0.01	0	0.90 ± 0.03	0.38 ± 0.01	0.9 ± 0.01
Free	1.43 ± 0.02	1.43 ± 0.04	1.22 ± 0.02	1.53 ± 0.01	1.53 ± 0.02

Bound Sialic Acid (BSA)

Table 1 shows the mean BSA in colostrum, transitional, mature and involutional milk.

Tables 1 and 2 show a gradual decrease from the colostral stage to the involutional stages of lactation with statistical significance of $P<0.05$. The difference between the means of the mature and involutional stages of lactation was not statistically significant at $P<0.05$.

The difference between the means of the mature and involutional stages of lactation was not statistically significant ($P>0.05$). Table 2 shows the concentration of BSA in the infant formulas

Table 3. Percentage difference of breastmilk and infant formula.

Infant formula	Bound Sialic Acid %	Free Sialic Acid %
Frisocreme & Colostrum	Colostrums – 98	59.34
Frisocreme & Trans. Milk	Transitional Milk – 96.05	62.37
Frisocreme & Mature Milk	Mature Milk – 87.22	49.11
Frisocreme & Invol. Milk	Involutional Milk – 37.04	57.04
Cerelac & Colostrum	Colostrum – 100	59.38
Cerelac & Trans. Milk	Transitional Milk – 100	62.37
Cerelac & Mature Milk	Mature Milk – 100	49.11
Cerelac & Involutional Milk	Involutional Milk – 100	57.06
Nan & Colostrum	Colostrums – 90.25	63.14
Nan & Transitional Milk	Involutional Milk – 100	66.02
Nan & Mature Milk	Mature Milk – 56.31	53.08
SMA & Colostrum	Colostrums – 95.64	57.74
SMA & Transitional Milk	Involutional Milk – 10	60.90
SMA & Mature Milk	Mature Milk – 75.33	47.42
SMA & Involutional Milk	Involutional Milk – 20.83	55.39
Similac and Colostrum	Colostrums – 90.25	57.74
Similac and Transitional Milk	Transitional Milk – 82.11	60.77
Similar and Mature Milk	Mature Milk – 56.31	47.42
Similac and Involutional Milk	Involutional – 10	55.39

Table 3 shows colostrum contains more than 90% more BSA than any of the infant formulae ($P<0.05$) transitional milk more than 50% ($P>0.05$). Table 3 shows involutional milk contained lower BSA than most of the infant formulas except for Cerelac, which had none.

Free Sialic Acid (FSA)

Table 2 shows the mean FSA showing a slight decrease from colostrum to mature stages and then slight increase at the involutional stage.

The only difference statistically significant was between colostrum and mature milk and transitional and mature milk ($P<0.05$).

For colostrum and transitional; colostrum and involutional; transitional and involutional and; mature and involutional, the values obtained were not statistically significant ($P>0.05$).

Table 3 shows colostrum contained between 57-63% more FSA, transitional 60 — 66%, mature milk 47-53% and involutional milk 55-60% more FSA than the infant formulas.

6. Discussion

The Nervous System is at its highest level of activity when a child is born. This activity depends solely on neuronal cells whose function depends on the presence of gangliosides, which have sialic acid as a component of their terminal chains [9].

Though limited data are available on the sialic acid content of human breast milk and infant formulas [9], have shown that human breast milk contains a very rich source of bound sialic [4, 9]. Another study by [1] also showed similar trends.

From the results obtained, compared with that of [1] and [9] shows normal trend and it is obvious that colostrum contains the highest concentration of bound sialic acid. The results also show that colostrum within the first few hours contained higher levels of bound sialic acid than colostrum produced later on hence breast milk should be given to the newborn from the onset, contrary to beliefs that the initial milk produced is bad for the baby [12].

The transitional and mature stages of lactation also contain high concentration of sialic acid with further indication to continue breast-feeding at least for the first six months of life. The free sialic acid present in both breastmilk and infant formula are of no use to the infant because, this is sialic acid that has been cleaved off from the glycoconjugates in the breastmilk [6]. This free sialic acid is easily degraded, not easily absorbed from the gastrointestinal tract and thus excreted in urine and faeces [13].

The infant formula as earlier stated did not contain much bound and free sialic acid. In Cerelac, result showed no presence of bound sialic acid. It could thus be suggested that there are no glycoconjugates present in cerelac. By implication, breast milk is more beneficial to the infant. Age of the mother and parity did not appear to affect the concentration of sialic acid present in the breast milk.

7. Conclusion

We conclude that breast milk contains greater concentration of sialic acid than infant formula food and so the newborn infant should be breast fed for at least the first six months of life. If for any reason the infant cannot be breast fed, the infant formula food that best similates breast milk should be used. It is recommended that the values obtained in this study should be used as a guide in establishing the normal values for the concentration of sialic acid in human breast milk using larger population.

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Conflicts of Interest

All the authors do not have any possible conflicts of interest

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