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Protein and Lactose Content of Breast Milk of Lactating Mothers Attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki. Ebonyi State. Nigeria

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Abstract

Human breast milk (HBM) is a food designed to fulfill the energy and nutritional requirements of babies. This study was designed to determine the protein and lactose concentration in breast milk. The lactose and protein concentration were estimated using Phenol-Sulphuric Acid and Biuret method respectively. Statistical analyses were done on data and expressed as mean ± S.E.M. using Graph Pad Prism software. The level of significance was placed at $p<0.05$. The protein and lactose concentration were categorized based on age of the mother, age of infant and parity of the mother. A total of 200 breast milk samples were analyzed. The study subjects consisted of women aged ≤20 (11), 20-24 (73), 25-29 (64), 30-34 (39), 35-39 years (13); based on age of lactating infant ≤4months (131), 5-8 months (53), 9-12 months (16); and based on parity one (34), two (74), three (57) and four and above (35). The protein and lactose concentrations of the breast milk samples were determined. The mean protein concentration of all the samples was 19.16 (± 13.02) g/l, while the mean lactose concentration of the all the samples was 10.09 (± 4.07) g/l. From this report, it would appear that the protein and lactose content of HBM correlate negatively in relation to the mothers' age, parity and age of infant. Younger mothers had more protein while older ones had more lactose. Primiparous mothers had more lactose while multiparous ones had more protein and protein concentration of HBM increased with age of infant while lactose decreased.

Keywords: *human breast milk, lactose, milk protein, parity, lactation*

Introduction

Mothers' own milk is the best source of nutrition for nearly all infants and it is meant to fulfill the energy requirement of the babies. It contains both specific and non-specific immune factors that help newborn infants to consolidate their immature immune systems.[1-2] Bioactive proteins in human breast milk (HBM) are likely to contribute to the advantages of breast-feeding through enzyme activities, enhancement of nutrient absorption, growth stimulation, modulation of the immune system and defense system against pathogens. The main components of HBM are water, protein, carbohydrates and fats. It also contains an inorganic substance in the form of mineral and organic substances such as vitamins, acids, antioxidants, pigments and flavor components.[3-7].

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All kinds of macro-nutrients are contained in HBM, including carbohydrates, proteins and fats. The main type of carbohydrate found in breast milk is lactose. HBM contains lactose as much as 8% and its content depends on the mother and breastfeeding state. The stable concentration of lactose is important in maintaining a constant osmotic pressure in breast milk. Lactose also aids the absorption of minerals and calcium. In breast milk, many carbohydrate-based bioactive compounds, such oligosaccharides, are attached to lactose.[8-12]

The composition of breast milk is the biologic norm for infant nutrition. As a dynamic, bioactive fluid, breast milk changes in composition and varies within feeds, diurnally, and between mothers.[13]It is known that exclusive breastfeeding for the first 6 months of age lowers mortality against infectious diseases by 88% and lowers the possibility of death compared to partial breastfeeding as a dose dependent effect.[14] During the first six months of infant life, providing optimal nutrition is critical as the consequences of inadequate nutrition can be very severe hence the significance of this study to determine the lactose and protein content of breast milk of mothers of Igbo extraction.

Materials and Methods

This work was done in the Evangel University Akaeze laboratories. The population of the study is the lactating mother attending Post Natal Clinics of Alex Ekwueme Federal University Teaching Hospital Abakiliki. Samples were collected from lactating women who were practising both exclusive and partial breastfeeding. Pregnant and non-lactating women were excluded. Sample size (222) was calculated using the t-distribution formula ($N=Z^2$ p (1-p) $\div d^2$) and the estimated prevalence rate of breastfeeding in Nigeria of 17.5. Samples were collected after the purpose of the study was explained to the lactating mother and informed consent obtained. Ethical Clearance with registration number NHREC/16/05/22/252 was obtained from Health Research Ethics Committee (AEFUTHA-HREC) of the Teaching Hospital. 5mls of breast milk were collected from the subjects into sterile universal containers and the containers were promptly screw-capped to avoid spillage or bacterial contamination of the sample. The samples were estimated for lactose and protein using phenol-sulphuric acid and Biuret methods respectively.

Lactose Estimation Using Phenol-Sulphuric Acid Method[15]

Procedure

A 1 in 20 dilution was obtained by using 20 µl of the sample and 180 µl of distilled water. 200 µl of phenol solution was added to the test tubes. 1000 µl of 96% sulphuric acid was added to each test tubes and it was mixed vigorously and allowed to react for 10minutes at room temperature. The absorbance was read at 490nm in a spectrophotometer.

Protein Estimation Using Biuret Method [16]

Procedure

Into a clean test tube containing 1000µl of biuret reagent 20 µl of the sample was added, mixed and incubates at room temperature for 10minutes.the colour was read at 540nm.

Results were analysed according to the age, parity of the mothers and age of lactating infant.

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Results

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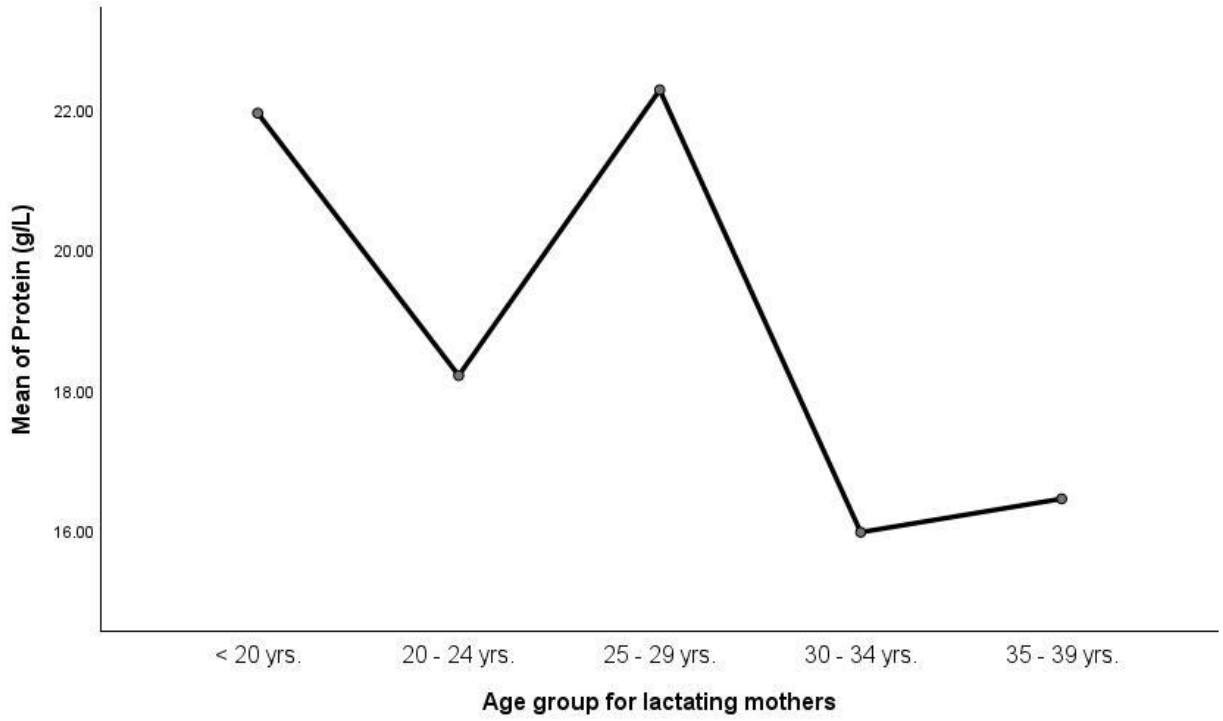

Figure 1: Comparison of breast milk protein of mothers according to age of lactating mothers

There was no significant age-dependent difference in breast milk protein concentrations between mothers (p = 0.11). However, the highest concentration of breast milk protein occurred within the ages of 25 to 29 years and declined with age. Younger mothers tended to have higher breast milk protein concentrations than older ones.

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Figure 2: Comparison of breast milk lactose of mothers according to ages of lactating mothers

Lactose concentration was significantly lower for mothers aged 25-29 years than mothers aged 30-34 years. (p = 0.01) and mothers aged 35-39 years (p = 0.001). There was positive correlation between breast milk lactose and mother's age, (r = 0.22; p = 0.001) and a negative correlation between age of lactating infant and lactose concentration, (r = -0.30; p = 0.001).

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Figure 3: Comparison of breast milk protein of mothers according to age of lactating infant
Significant differences (p = 0.004) existed in the breast milk protein concentrations of the samples collected at different times after the start of lactation by mothers. Highest concentrations were recorded between 5 to 8 months of lactation. Protein concentrations rose and decreased sharply after 6 months of lactation. There was positive correlation between breast milk protein and length of period of lactation, which is same as age of the lactating infant (r = 0.25; p = 0.001).

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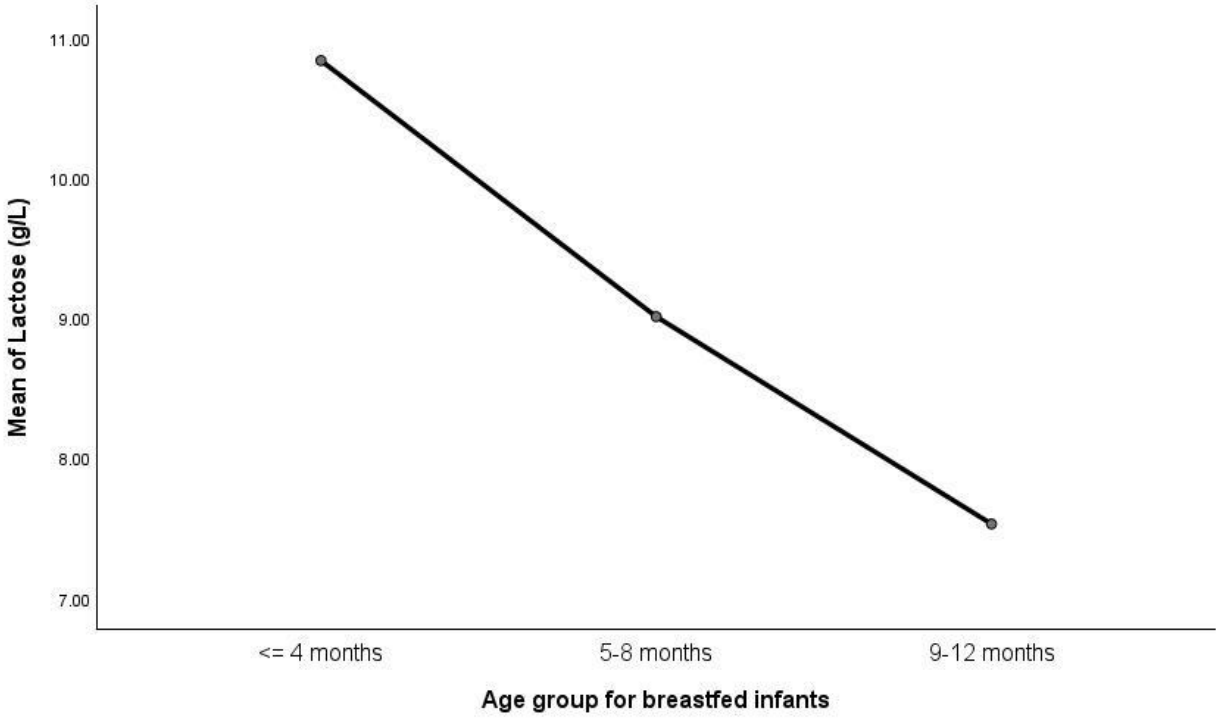

Figure 4: **Comparison of breast milk lactose of mothers according to age of lactating infant**

Lactose concentration of breast milk samples declined continuously as the age of the infant increases and was found to be highest in mothers breastfeeding for less than 4 months, and lowest in those breastfeeding for between 9 and 12 months (p = 0.001). At 6th month of lactation, the lactose concentration had declined by 50%.

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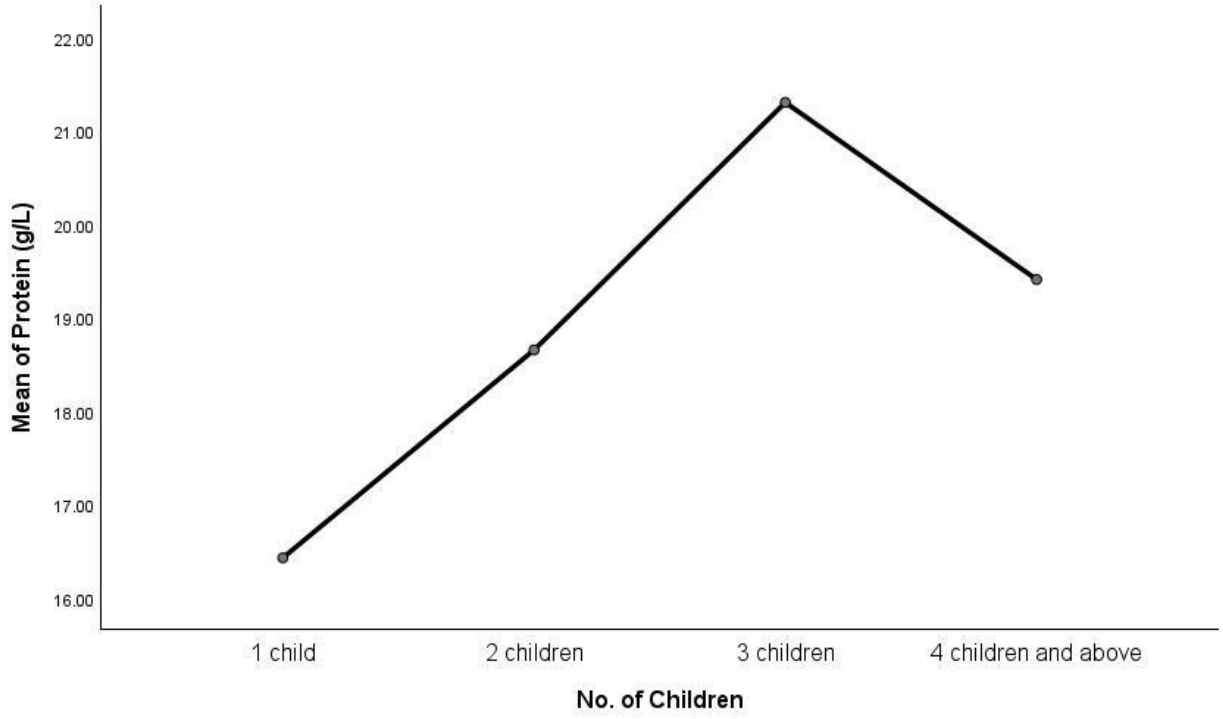

Figure 5: Comparison of breast milk protein among mothers by parity
Mothers with the 3rd pregnancy had the highest concentration of protein in their breast milk samples although there was no significant difference between primiparous and other groups of multiparous mothers (p = 0.369).

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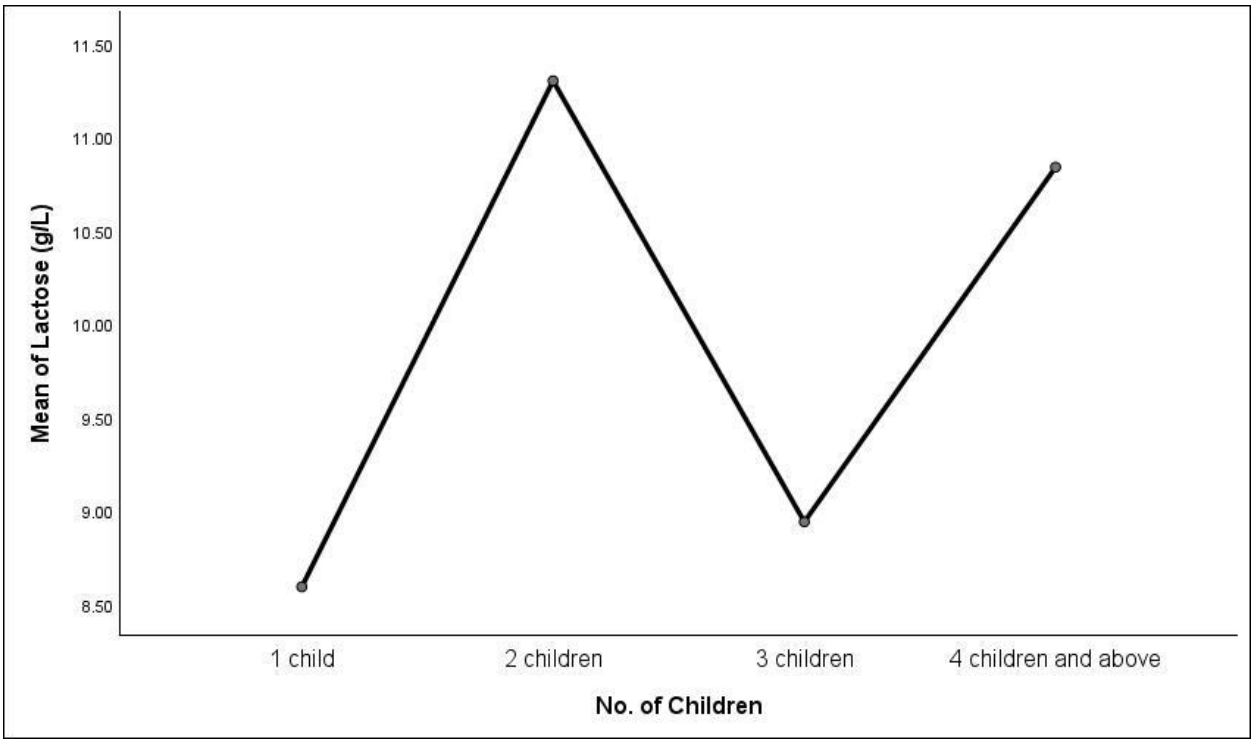

Figure 6: Comparison of breast milk lactose among mothers by parity

Lactose concentrations differed significantly with number of pregnancies (p = 0.001). Lactose level was lowest in primiparous mothers.

Discussion

Human breast milk, (HBM), lactose and total protein contents vary among mothers as well as with age of mother, parity and age of lactating infant. Lactose content correlated negatively with the protein concentration in respect of age of lactating mothers. While younger mothers had higher protein concentrations, older ones had higher lactose concentrations. These differences could be as a result of the socio-economic status of these women, length of period of lactation, parity and hormonal (prolactin and oxytocin) status of mothers. It is known that women within the age group of 25-29 are fresh out of school, trying to adapt to a new job, newly married, primiparous etc., and these could influence the lactose concentration in their breast milk. The composition of HBM is dynamic and changes over time, adapting itself to the changing needs of the growing child.[17] The milk that is expressed first (foremilk) is thinner with a higher content of lactose, which satisfies a baby's thirst, and following the foremilk, hindmilk is creamier with a much higher content of fat but less lactose for the baby's needs. The fat content of HBM was not investigated in this work but was said to increase with age of child in order to give taste to milk. Variations also occur with the stage of nursing (age of infant), maternal diet, maternal health, and environmental exposure.[18] In

this study, protein concentration was seen to be highest in samples from lactating mothers within the age group of 25-29. There was a positive correlation between the lactose concentration and the age of the mothers.

Changes in HBM content according to the lactation stage, race, diet of the nursing mother, region-specific environmental chemicals, storage and treatment have been demonstrated. The composition of breast milk is widely believed to be specifically tailored by each mother to precisely reflect the requirements of her infant.[18] Early milk or colostrum has lower concentrations of fat but higher concentrations of protein and minerals than mature milk. This relationship reverses as the infant matures with the possibility of the introduction of other types of food. Lactose concentration of HBM was found to be highest in samples of mothers whose infants were less than 4 months of age, and lowest in those between 9 and 12 months of age. This is consistent with the reason for exclusive breastfeeding for the first 6 months of infant life and alternate breast feeding after that. It is revealed that the best time for the infant to receive carbohydrate in the form of lactose which would provide energy, control intestinal flora, enhance the absorption of magnesium and calcium etc., is in the first 6 months after birth.[19]

Comparison of lactose concentration based on parity showed that lactose level was lowest in primiparous women although there was no significant difference between primiparous and other divisions of multiparous mothers. This is consistent with previous reports that parity does not significantly affect milk composition.[20] Comparison of protein concentration based on parity showed no significant variation though higher for multiparous than for primiparous mothers.

Conclusion

The concentrations of protein and lactose in breast milk varied across age of mothers, age of infant, but not necessarily the parity of the mother. From this report, it would appear that the protein and lactose content of HBM correlate negatively in relation to the mothers' age, parity and age of infant. Younger mothers had more protein while older ones had more lactose. Primiparous mothers had more lactose while multiparous ones had more protein and protein concentration of HBM increased with age of infant while lactose decreased.

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