



A Study on Microbial and Macronutrient Composition of Breast Milk Varies with Lactation Duration

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Authors' contributions

This work was carried out in collaboration among all authors. Author UD conceived the study project. Authors UD, MD, AD and YT designed experiments. vUD, MD and AD performed experiments and analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

Complementary feeding and continuation of breast feeding is directed and advised by the national and international child health agencies. Breast milk, a complex biological fluid, shows changes in its cellular, microbial and nutrient composition as lactation progresses. In a cross-sectional study, hospital-based pilot project showed the composition of microbial and macronutrients and compared their differences before and after first six months of lactation. *Staphylococcus aureus* was the predominant bacterium found in breast milk, and 45% (15/33) of samples within first six months and only 13% (3/22) from those more than six months of lactation showed any bacterial growth ($p=0.013$). The protein content was less in breast milk samples after six months compared to those within six months of lactation (mean difference, 95% CI: 0.33 (0.10, 0.55) gm/dL, $p=0.005$). After controlling the lactation period, bacterial culture positivity in breast milk was associated directly with

the breast milk protein content. Our pilot study observations indicate the need to study microbial and nutrient changes in breast milk as lactation advances, in a longitudinal study with larger sample size, and investigate its associations with maternal factors, infant growth, establishment of infant gut microbiota and possible role in environmental enteric dysfunction.

Keywords: *Bacteria; breast milk; infant; lactation; macronutrients; micro biome.*

1. INTRODUCTION

Breast milk is the only source of nutrition to the new born baby for the first six months of its life. Strong epidemiological evidence shows the advantages of breast milk for infants morbidity, including decreased risk of atopic dermatitis, asthma, necrotizing enter colitis, gastroenteritis and respiratory tract infections, obesity and type 2 diabetes [1].

Breast milk includes a variety of physiologically active components like lipids, oligosaccharides, immunological micro RNAs, hormones and cellular components. Milk along with its microbes, is very essential and the growth of microbes may be altered by the geographic locations, mode of birth, post-partum period, style of feeding, social networks, environment and motherly diets [2].

The breastfeeding skin for mothers, the mouth of her child, and some transfers from the maternal gastrointestinal system are known to cause the breast milk micro biome (entero-mammary path). *Staphylococcus* Sp., and *Streptococcus* Sp., are mainly detected bacterial taxa in human milk, but are also other anaerobic bacteria such as *Lactobacillus*, *Bifid bacterium*, and *Bactericides*. The reported bacterial content of human breast milk ranges from 10^3 to 10^6 bacterial cells/mL, which may vary based on the assessment methods (culture-based methods or molecular methods). The breast milk bacteria are believed to influence health of the mammary glands, colonizing infants gut, and maturation of gut immune system. Infants gut microbiota is now shown to influence morbidity outcomes such as gut and respiratory tract infections, asthma, metabolic syndrome and inflammatory bowel disease [3].

Most of the investigators have studied human milk composition from parturition till four to six months and the feeding continues after six months to twenty four month in India. Microbial, cellular and nutrient compositional factors are not much studied in Indian lactating women. Our objective was to study the amount of

macronutrient and microbial composition of breast milk at two different stages of lactation, and to investigate its association with different maternal-infant factors [4].

2. METHODS

This pilot study was performed with the help of Pediatrics in Government Medical College, Akola, Maharashtra, India. Datas were collected after an informed, written consent from apparently healthy, lactating mothers whose baby was admitted to the neonatal or childrens ward. Mothers delivery details, weight, height, and infants weight (where possible) were recorded. Infants birth weight was recorded by verbal recall from any of the parent or from the birth documents. Postpartum period (or lactation period) was calculated by subtracting date of data collection from the date of delivery, and considered as low birth weight (LBW) [5].

2.1 Breast Milk Collection and Microbial Assay

About 10 ml of breast milk sample was collected following a standard protocol of asepsis, by manual expression. The samples were approximately collected between 10 am to 12 pm and the collected Samples were streaked in Mac Conkey agar plates, within 3 hours of collection, and then transported to the laboratory at room temperature. After 24 hours of incubation, if bacterial growth was identified, colony characteristics were studied and subjected to bacterial identification [6].

2.2 Breast Milk Macronutrient Analysis

The fat, carbohydrate was analysed using Human Milk Analyzer (Miris, Upsala, Sweden), equipment based on mid-infrared transmission spectroscopy, calibrated to human milk standards. Each breast milk sample was first maintained at 40° C using MIRIS solicitor, according to the instructions in manual. The Human Milk Analyzer (HMA) requires 2mL of breast milk and provides the obtained values present in fat, carbohydrate, total solids, true

protein, and energy contents. Total solids and energy are derived from the Miris HMA measurement results. We could not measure macronutrients in 3 breast milk samples as the sample volume was insufficient [7].

2.3 Statistical Methods

Descriptive statistics (means and standard deviation or counts or percentage) were calculated for maternal age, height, weight, BMI, parity, mode of delivery, lactation duration, breast milk macronutrients and culture positivity. These maternal and infant characteristics were compared between two groups of lactation period: **Group A**, lactation period up to six months from delivery, and **Group B**, lactation period above six months to 2 years. For continuous data *t* tests were performed, and chi square tests were used for categorical data. Pearson correlation was used to study relation of macronutrients and maternal-infant factors using IBM Statistics SPSS 21.0 software [8].

3. RESULTS AND DISCUSSION

3.1 Morphological Characters

Datas were collected and analyzed from 55 lactating women, aged 23.8 ± 4 y, BMI 21 ± 3 kg/m², with lactation period varying from 2 days to 19 months. Thirty women had delivered vaginally and 25, by cesarean section. Twenty-two women were primiparous, 18 had one previous child and 15 had two previous children. The index infants mean birth weight was 2362 ± 767 g, and 26 were female. The infants number below the age of 6 months was 33, and 22 infants were more than 6 months age.

3.2 Identification of Bacterial Growth

Breast milk samples from 18 (33%) lactating women showed some bacterial growth, whereas 37 (67%) samples did not show any microbial

growth. Out of these 18 samples who tested positive for bacterial growth were further isolated and identified the organisms as *Staphylococcus aureus* in 14 samples, *Kleibseilla oxytoca* in 3 samples [9], and both the bacteria, *Staphylococcus* and *Kleibseilla* in one sample. Similar study suggests that as the infant microbiome establishes in the initial weeks after birth, human milk may show changes in the cellular composition. Almost all positive breast milk cultures showed growth of *Staphylococci*, which is also well described in other studies using culture dependent as well as culture-independent techniques [10].

The composition of human milk bacteria is observed to be influenced by bacteria from the maternal gastrointestinal tract, her skin and from infants mouth. *Staphylococcus aureus*, traditionally considered harmful, is now considered to favorably influence the evolving immune system of infant [11]. *Staphylococcus* species which contributes to normal skin flora and to nosocomial infections is reported as a predominant strain in human milk. Preliminary data also provides the evidence in breast milk, may promote growth of *Staphylococcus* bacteria in the lactating mammary gland.

3.3 Macronutrient Composition

Breast milk macronutrient composition was not associated with maternal age, BMI, parity, mode of delivery or infants gender. Breast milk protein content was inversely associated with breast milk carbohydrate content ($r=-0.335$, $p=0.016$), and it also showed an inverse association with birth weight ($r=-0.426$, $p=0.002$), infants age ($r=-0.368$, $p=0.008$), and infants present body weight ($r=-0.758$, $p<0.001$) [10]. The Macronutrient composition of Breast milk in total sample described in the following Table 1. There was no occurrence of microbial culture in fat and carbohydrate content of breast milk, but protein concentration present in breast milk culture showed the positivity of microbial growth.

Table 1. Macronutrient composition of breast milk in total sample

| S. No | Macronutrients in breast milk (n=52) | Mean \pm sd |
|-------|--------------------------------------|-------------------|
| 1 | Fat, g/dL | 3.33 \pm 1.46 |
| 2 | Carbohydrate, g/dL | 2.68 \pm 0.53 |
| 3 | True protein, g/dL | 1.35 \pm 0.42 |
| 4 | Total solids, g/dL | 7.82 \pm 1.57 |
| 5 | Energy, kcal/dL | 48.57 \pm 13.64 |

Table 2. Maternal and infant characteristics below and above six months of lactation period

| CHARACTERS | Group A, Postpartum period less than six months, N=33 | Group B, Postpartum period more than six months, N=22 | P value | Mean difference (A- B), and 95% Confidence Interval |
|---|--|--|--------------|---|
| Postpartum period, d | Mean 41 (Min 2, max 177) | Mean 365 (Min 183, max 569) | | |
| Maternal age, y | 23.6 ± 4.1 | 24.2 ± 3.7 | 0.614 | |
| Maternal parity, n | | | | |
| 1 | 15 | 7 | | |
| 2 | 10 | 8 | | |
| 3 | 8 | 7 | 0.346 | |
| Maternal BMI, kg/m ² | 21.0 ± 3.0 | 21.3 ± 3.3 | 0.788 | |
| Mode of delivery, n | 19 Cesarean | 6 Cesarean | 0.026 | |
| Infant gender, male, n | 18 | 11 | 0.478 | |
| Birth weight, kg | 2.1 ± 0.7 | 2.8 ± 0.7 | 0.001 | -0.7 (-1.10, -0.31) |
| Number of low birth weight (LBW) infants | 21 | 6 | 0.008 | |
| Culture positive breast milk samples, n | 15 | 3 | 0.013 | |
| Breast milk fat, g/dL | 3.30 ± 1.5 | 3.37 ± 1.4 | 0.854 | -0.08 (-0.91, 0.76) |
| Breast milk | 1.48 ± 0.4 | 1.15 ± 0.38 | 0.005 | 0.33 (0.10, 0.55) |
| Breast milk carbohydrate, g/dL | 2.69 ± 0.37 | 2.68 ± 0.73 | 0.945 | 0.01 (-0.30, 0.32) |
| Breast milk energy, kcal/dL | 49.16 ± 14.0 | 47.7 ± 13.4 | 0.710 | 1.45 (-6.36, 9.26) |

3.4 Comparison of Maternal and Infant Characters during Lactation Period

As described in the methods section, we compared maternal-infant and breast milk characteristics in two groups based on the postpartum days i.e. lactation period, **Group A**, below six months and **Group B**, more than six months of lactation. These characteristics are shown in Table 2.

Maternal age, parity, BMI and infant gender were similar in both Groups A and B. Birth weight of infants, and number infants was higher in Group A [11]. Breast milk protein content was higher by an average 0.33 g/dL in first six months of lactation compared to that after six months (Table 2). Within Group A, breast milk true protein content was ($r=0.436$, $p=0.014$) with the lactation duration. Also, within Group A, positivity of bacterial growth was associated directly with breast milk protein content, controlling for lactation duration (aOR=97, $p=0.009$). The significant differences between LBW observed Group A is attributable to the fact that the study was conducted in a tertiary health centre where complicated, high risk pregnancies are referred for further management, and so the frequency of cesarean section and LBW infants were higher in

Group A [12]. The macronutrient of milk varies from the degree of prematurity, stage of lactation, to mode of delivery, maternal nutrition and infant gender. Breast milk composition is widely believed to be specifically tailored to reflect the infants requirements. Our data shows that there is significant reduction in breast milk proteins as the lactation duration increases. Two observations from study of macronutrients in lactating women in India one from Austria have also shown that the protein content in term as well as in preterm breast milk decreases from first to four weeks of lactation period [13].

3.5 Sites of Bacteria and Its Percentage of Contribution in Infants Gut

Recent report on the association of bacterial species from breast milk is found to be present in areolar skin and infants gut. It was observed that in the first month after birth, in predominantly breastfed infants, bacteria from breast milk contributed 27.7 ± 15.2 % and bacteria from areolar skin contributed 10.3 ± 6 % to the infant gut microbiome. Also, daily breast milk intake influenced the bacterial diversity and composition changes of breast milk in a volume proportionate manner, which remained so even after complementary food introduction.

Our study also showed that even after controlling the lactation duration, frequency of positive bacterial growth was associated with the protein content of breast milk [14]. A few observations are reported about positive or negative relationships between breast milk nutrients and specific bacterial species. In the study by Boix A, breast milk protein content correlated positively with the proportion of *Bacillus*, *Anaerococcus* and *Peptoniphilus*, and the fat content correlated negatively with proportion of *Staphylococcus* in the breast milk. These findings suggested the potential prebiotic and/ or antagonistic interactions of breast milk nutrients and bacterial cells [15].

4. CONCLUSION

Our observations in lactating mothers are important in view of the present national and international policy of infant and young child feeding (IYCF) which advises after birth. Mode of infant feeding, i.e. direct breastfeeding could influence breast milk microbiota. Expressing human milk for feedings may introduce potentially pathogenic bacteria in the process of collection, storage, and handling. In case of good bacteria in mother milk and the child, the microbe of breast milk eventually boosts the immune system of the infant. The composition of baby gut microbiota is significantly related to exclusive breastfeeding and lactation time. These data highlights the necessity for further characterization and connection of breast milk along with infant intestinal myrobiomas with early childhood growth and environmental enteric dysfunction in 12 to 24 months of breastfeeding.

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ETHICAL APPROVAL

Institutional Ethics Committee, Government Medical College, Akola, Ethical Clearance Certificate GMCA/EC/185/2018.

CONSENT

Datas were collected after an informed, written consent from apparently healthy, lactating mothers whose baby was admitted to the

neonatal or childrens ward in Government Medical College, Akola, Maharashtra, India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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