The Microbiological Makeup of Human Mother Milk and Its Influencing Factors

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Abstract

In addition to its nutritional value, human milk contains a lot of bacteria. These microbes enter the baby's stomach during breastfeeding, where they may colonize it temporarily or permanently. Thus, the microbiological makeup found in human milk may have a significant role in forming the gut microbiota of infants, which in turn affects their health and illness. Our goal observing the mother gestational age, reproductive history and maternal age on anthropometric growth of new born will be made easier with an understanding of the factors that influence the makeup and function of the human milk microbiota. In this research was to find out how much weight term and preterm babies that were nursed mother milk and powder milk from birth to first six months old weighed. Babies were measured for length (once every week), weight (daily in the hospital and subsequently once every week), and head circumference (once every month) until they were six months old. Baby weight who are bottle fed gaining weight more as compare to baby on exclusively on mother feed.

Key Words: - Mother milk microbiota, gestation age, infant body weight

1. Introduction

The risk of developing noncommunicable diseases (NCDs), such as diabetes, cancer, heart disease, and chronic respiratory conditions, is linked to early life nutrition. Evidence has demonstrated that breastfeeding can benefit both the mother and the child, even if NCDs are currently thought to be the world's leading cause of death. For example, breastfeeding may offer long-term cardiovascular health benefits for moms and protect them against diabetes, breast cancer, and ovarian cancer. Conversely, low average blood glucose levels in infants delivered to women with gestational diabetes mellitus have been linked to prolonged nursing for at least eight months. Human mother milk is a complexes biological fluid which is adapted to satisfy the nutritional requirements of the rapidly growing infant: additionally, it educates the infant immune system and provide certain degree of protection against all types of diseases. [1]. Human milk is the gold standard for infant nutrition as it contains multiple biologically active components, including lipids, oligosaccharides, mRNA and hormones among others [2].

Traditionally a few years back it was thought human breast milk was sterile. During the last few decades, studies in the subject microbiological sciences were focused and restricted to the identification of potential pathogen bacteria in stored milk [3]. The first description of the bacterial diversity of human breast milk from healthy women was based on *in vitro* culturing methods .

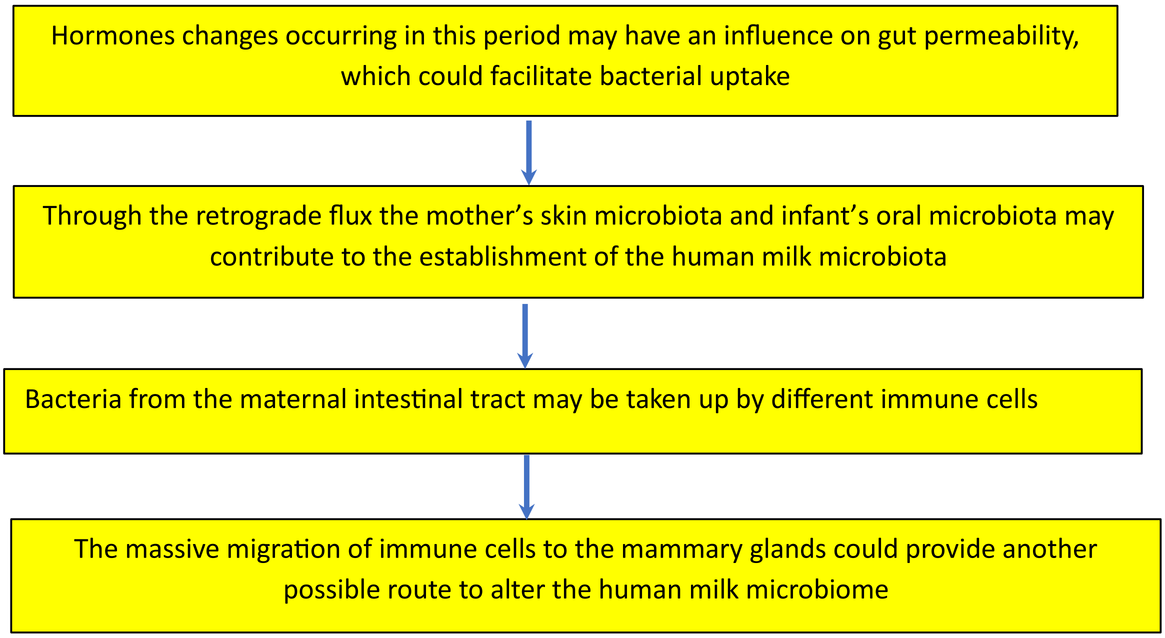

Figure 1.1 The potential mechanism of the human milk microbiome facilitates the migration of bacteria to the mammary gland

Bacterial translocation is the movement of live bacteria across intact intestinal mucosa. Though Serratia marcescens was initially described as having "translocated" from the rat duodenum, where it had been injected, to the lymph, this event had been proposed more than 60 years earlier. Subsequently, the transit of viable bacteria from the gastrointestinal tract into the lamina propria, followed by the mesenteric lymph nodes and other extra-intestinal organs such as the spleen, liver, kidneys, peritoneal cavity, or bloodstream, was characterized as bacterial translocation. Since gut bacterial translocation has historically been linked to pathogenic circumstances, it has mostly been examined in patients who have pathogenic bacteria, such as those with severe burns, transplants, pancreatitis, cardiac disorders, or AIDS.

Pregnancy and breastfeeding cause several temporary anatomical and physiological changes that set up the fetus's and the newborn's proper development frameworks [4]. Almost every system is impacted by these modifications, including the digestive, genitourinary, respiratory, and cardiovascular systems. Fascinatingly, these changes could promote more bacterial translocation in the latter stages of pregnancy and the early stages of nursing. Pregnancy-related hormonal changes to the mouth's pH and microbiome lead gums to become hyperemic, edematous, and more susceptible to bleeding.

The displacement of the abdominal organs by the uterus's increasing expansion and reduced motility-possibly due to progesterone's influence on smooth muscle contractility—are the principal consequences of pregnancy on the gastrointestinal system. This leads to a longer period for the stomach to empty, while less gastric secretion makes the pH of the stomach more basic. Problems with constipation and hemorrhoids can result from reduced gastrointestinal motility and peristalsis, especially in the latter trimester of pregnancy, along with increased uterine pressure. The amount of cells in breast milk varies greatly across and among individuals, from about 10,000 to 13,500,000 cells per milliliter of milk [5-6].

FACTORS AFFECTING HUMAN MOTHER MILK MICROBIOTA

Human milk, a natural, renewable meal that provides all of a baby's nutrients for the first six months of life, makes breastfeeding another advantage to the environment. Moreover, there are no packaging materials needed, unlike baby formulae and other human milk alternatives that need to be packaged and may wind up in landfills. 150 million formula containers are used for every million newborns fed formula; some of those containers may be recycled, but the majority wind up in landfills. Furthermore, baby formula need to be carried from the manufacturing facility to retail establishments like supermarkets for families to be able to buy them. While nursing may need moms to take in a small number of extra calories, it usually requires.

MATERIALS AND METHODS

SAMPLE COLLECTION

The breast milk samples were collected from a healthy volunteer mother who did not use any antibiotics for at least three months prior to sample collection and without any diagnosed disease from different hospitals of Mohali. The sample collection procedure was done by

1) By hand expressed method

The general information was checked from donors like her medical history, physical examination and mode of delivery. After collecting information, donors were made comfortable in the collection area. The donor breast and hands were washed properly with Lukewarm water to remove any traces of dirt or debris. Dry breast nipples were dried with a neat and clean hand towel. The breast milk sample expressed manually, while the sterile vial was placed under breast for proper collection. Lab number id, collection time, date and collection of milk from left or right breast or both were noted down. All this information was recorded at the same time. After this procedure the breast milk sample vial was tightly closed to prevent leakage. If a sample could not be delivered to the laboratory at the same time then it was kept in the refrigerator until delivered. The sample was ready to be delivered to the laboratory, for that place's sample in a cooler box with a cold pack or ice pack to keep the sample free from other pathogenic bacteria.

Statistical analysis

Statistical analysis was executed with the SPSS 17.0 software package (SPSS, Chicago, IL, USA). Friedman's test compared microbial groups throughout time (paired samples). Mann–Whitney U-test was used for comparisons between two groups. The $\chi 2$ test was applied to investigate the differences in bacterial prevalence between the studied groups. A P-value o0.05 was considered statistically significant.

MATERNAL AGE AND REPRODUCTIVE HISTORY

The data presented in Table 1.1 encapsulates a comprehensive analysis of perinatal characteristics among fifteen study participants, delineated by their unique Sample IDs. Each entry meticulously documents crucial parameters integral to understanding prenatal and postnatal health outcomes. Gestational periods, ranging from 37 to 40 weeks, illuminate the temporal progression of pregnancies within the cohort, with the majority falling within the term category. Correspondingly, infant weights at birth, spanning from 2202 to 3847 grams, exemplify the diverse spectrum of neonatal anthropomorphic observed. Concurrently, maternal age at childbirth, showcasing a breadth from 26 to 34 years, underscores the multifaceted demographic landscape shaping perinatal health trajectories. Gr-avidity and parity distributions elucidate the intricate reproductive histories of participants, encompassing primigravidous and multigravidous instances. The variability in the number of samples collected per participant, ranging from one to nine, coupled with postpartum spans spanning from mere days to several weeks, signifies the nuanced temporal dynamics characterizing postpartum recovery and maternal health transitions also showing in Table 1.2.. Noteworthy instances of twin pregnancies punctuate the dataset, accentuating the complexity inherent in multifetal gestations. Collectively, these findings offer a panoramic view of perinatal dynamics, facilitating nuanced insights into the multifactorial determinants shaping maternal-fetal health paradigms.

Table 1.1 Impact of Gestational Period, Maternal Age, and Reproductive History on Perinatal Health Outcomes

Sample Id	Gestation	Infant Weight, Grams	Age of Mother, Years	Gravida 1	Para 2	Number of Samples	Postpartum Span 3
Term 01	38 weeks	3101	29	1	1	4	–
Term 02	37 weeks 5 days	3209	33	2	2	4	2 weeks
Term 03	37 weeks	2202	32	1	1	9	4–12 days
Term 04	38 weeks	3212	27	3	3	5	1–5 weeks
Term 05	38 weeks	3200	28	1	1	7	4 days–7 weeks
Term 06	39 weeks + 2 days	3514	31	2	2	1	4 days
Term 07	40 weeks	3847	32	1	1	4	1–4 days
Term 08	39 weeks	3496	34	2	2	2	5–14 weeks
Term 09	38 weeks + 4 days	2918	32	1	1	3	2–5 weeks
Term 10	39 weeks + 2 days	3012	27	1	1	2	4–5 days
Term 11	39 weeks + 4 days	2989	28	6	5	2	4–6 days
Term 12	38 weeks + 4 days	3301	28	4	3 + 2 4	2	9–10 days
Term 13	40 weeks	2923	26	–	–	3	6 days–87weeks
Term 14	39 weeks + 3 days	2815	29	–	–	1	11 weeks
Term 15	38 weeks	3102	31	–	–	1	6 weeks

1 Number of pregnancies, including the current pregnancy; 2 number of times the mother has given birth; 3-time range indicates days postpartum of first and last samples from that donor; 4 indicates twin pregnancy.

Table: 1.2 Clinical parameters of the study cohort

	Term Birth (TB, n = 30)	Preterm Birth (PTB, n = 18)	p-values
Age at conception in years; Median (IQR)	23.5 (21–26.8)	21.5 (20–24.5)	0.3628
Height at conception in cm; Median (IQR)	151 (149–153)	154 (150–156)	0.136

Weight at conception in kilograms: Median (IQR)	48 (43–55)	48 (42.2–48.9)	0.3696
BMI at conception; Median (IQR)	20.9 (19.4–23.5)	20.1 (18.2–20.4)	0.1506
Outcome EGA (days); Median (IQR)	280 (270–284)	254 (242–255)	**9.277E-09**
Infant birth weight in Kg: Median (IQR)	3.07 (2.96–3.3)	2.26 (1.98–2.44)	**6.893E-08**
Infant Head circumfrence in cm: Median (IQR)	33 (32.4–33.6)	31 (30–31.5)	**2.219E-07**
BMI Categories n (%):			
Under weight	5 (27.5)	4 (13.3)	0.2145
NORMAL weight	11 (61)	17 (56.6)	0.7624
Over weight	1 (5.5)	7 (23.3)	0.1096
Obese	1 (5.5)	2 (6.6)	0.8776
Delivery (%):			
Vaginal	29 (96.6)	18 (100)	0.2731
Caesarean section	1 (3.3)	0 (0.0)	0.8776
Maternal Ethnic Group (%):			0.1587
Karen	22 (73)	9 (50)	0.1018
Burmese	8 (26.6)	8 (50)	0.2059
Gravida (%):			
1	11 (36.6)	8 (44.4)	0.5937
2	9 (30)	8 (44.4)	0.3111
3	5 (16.6)	1 (5.5)	0.2598
≥ 4	5 (16.6)	1 (5.5)	0.2598
Parity (%):			
0	12 (40)	9 (50)	0.499
1	10 (33.3)	8 (44.4)	0.4414
2	5 (16.6)	0 (0)	0.06725
≥ 3	3 (10)	1 (5.5)	0.5896
Maternal Antibiotic Exposure (%):	5 (16.6)	11 (61.1)	**0.004891**
1st trimester pregnancy	0 (0)	1 (5.5)	0.192
2nd trimester pregnancy	1 (3.3)	0 (0)	0.4337
3rd trimesterpregnancy	1 (3.3)	2 (1.1)	0.2812
Delivery	5 (16.6)	8 (44.4)	**0.03603**

The significant p-values are in bold. The p-values where calculated using chi-square test.

Immune systems in preterm infants exhibit developmental immaturities. They are therefore vulnerable to necrotizing enterocolitis (NEC) and harmful infections. It's interesting to note that preterm babies who are fed human milk rather than infant formula have a lower risk of developing NEC. This finding could be explained by the possibility that certain advantageous human milk bacteria could colonize the baby's gut, aiding in the immune system's development and offering defense against illnesses. Significant variations in the HMM by gestational age have been documented, nevertheless, with preterm mothers' milk showing lower levels of Bifidobacterium and higher levels of Enterococcus than moms who gave birth on the scheduled date. *Bifidobacterium, Lactobacillus, Staphylococcus, Streptococcus,* and *Enterococcus* were all found in milk from moms who had given birth either at full term or preterm, despite variations in their relative abundances. The same study found that, when comparing the various levels of premature, the milk of moms of extremely preterm newborns had a generally lower bacterial load than that of mothers of late preterm infants.

2. Exclusive Breast Feeding versues formula mlk

During the first six months of a baby's existence, breastfeeding provides a comprehensive supply of nutrients. All of a baby's nutritional needs for physical growth and development are met by breast milk. Infant health outcomes are improved, including weight gain, reduced mortality, infectious illnesses, and respiratory tract infections. Better motor skills and cognitive development are also linked to exclusive breastfeeding. Infant. The growth measurement tools included digital weighing scales to measure weight, and a World Health Organization growth chart used to compare the anthropometric index of weight. It has been observed baby on formula milk gaining weight in first six as compare to baby exclusively on breast feeding.

Table: - 2.1 Effect of mother milk over formula milk on Infant Body Weight

S.NO	Baby Exclusively on Mother Milk	Baby Exclusively on Formula Milk
1	2970.50±44.68 a	2983.60±38.60 a
2	2771.30±594.44 a	3133.10±54.65 a
3	2884.70±514.97 a	2983.60±68.81 a
4	222.10±502.52 a	3233.10±101.95 b
5	2145.60±503.31 a	3333.10±175.89 a
6	2789.30±510.92 a	2750.60±212.12 a
7	2784.30±171.98 a	2356.10±236.69 a
8	2986.20±501.76 a	3514.60±233.64 a
9	2189.70±482.38 a	3567.80±286.86 a
10	269.10±519.12 a	3261.40±154.73 b
11	2300.10±505.38 a	4109.10±261.85 b
12	3216.60±514.49 a	3962.10±322.71 a
13	2789.30±150.42 a	2648.10±355.53 a
14	2627.90±97.84 a	2549.30±282.91 a
15	2879.40±47.76 a	2569.10±64.93 b
16	2789.10±47.76 a	2456.60±53.08 b
17	2897.60±34.57 a	2269.10±241.96 a
18	2789.20±34.91 a	2456.90±247.03 a
19	2970.50±137.00 a	3623.50±237.18 a
20	2831.50±174.02 a	2983.60±189.23 a

21	2204.60±174.52 a	2983.60±585.21 a
22	2126.90±128.46 a	3213.40±571.77 a
23	2269.10±157.47 a	333.90±715.79 a
24	2196.10±180.93 a	3456.10±709.30 a
25	2978.40±161.94 a	289.30±684.26 b
26	2876.20±145.63 a	2879.30±590.01 a
27	2786.10±55.27 a	2879.60±551.75 a
28	2970.50±62.93 a	3456.80±164.08 a
29	3116.30±72.15 a	2648.50±189.44 a
30	2781.30±75.12 a	2489.40±231.99 a

Values with the same letter (a-a) in a row are not significantly different, while different letters (a-b) indicate a significant difference between the Vegetarian and Non-Vegetarian Mother values in that row at p<0.05.

Discussion

Typically, growth charts are used to monitor and evaluate an infant's development. When comparing the weight of exclusively breastfed infants in the current study to the World Health Organization growth chart, we discovered that all of the exclusively breastfed infants were within normal weight ranges but less weight gain as compared to the baby on formulated milk and that the majority (96.2%) of them weighed between the 10th and 90th percentiles.The mail reason of gained weight is may be due calculated amount of ingredients. But off-course formulated milk is not replaced benefits of mother milkOur results confirm the current United Nations International Children's Emergency Fund (UNICEF), American Academy of Pediatrics, and World Health Organization (WHO) advice that exclusive breastfeeding for the first six months of life is essential for a child's health.

Conclusion

Numerous factors affect the composition of the human milk microbiota and, consequently, the microorganisms that are passed on to the infant through breastfeeding, including the mother's health and diet, gestational age, infant gender, mode of delivery, lactation stage, feeding method, geographic location, and social network density. But there are still a lot of unsolved concerns about the composition and operation of the human mother milk. Last but not least, formula milk is frequently the best alternative when breastfeeding is not feasible. Therefore, more investigation is required to ascertain how formula milk related to human milk banking, impact the makeup of these microbial populations.

References

1. Morrow AL, Rangel JM. Human milk protection against infectious diarrhea: imlications for prevention and clinical care. Seminar in pediatric infectious Diseases 2004;15:221-8.

2. Hennet, T., and Borsig, L., (2016) Breastfed at tiffany;s Trends Biochem. Sci 41, 508-518.2016.

3. Burleigh, M. C., Rosier, B. T., Simpson, A., Sculthorpe, N., Henriquez, F., & Easton, C. (2023). The Probiotic *Streptococcus salivarius* M18 Increases Plasma Nitrite but Does Not Alter Blood Pressure: A Pilot Randomised Controlled Trial. *Applied Microbiology*, *3*(3), 774-785. https://doi.org/10.3390/applmicrobiol3030054.

4. Dinleyici, M., Pérez-Brocal, V., Arslanoglu, S., Aydemir, O., Sevuk Ozumut, S., Tekin, N., Vandenplas, Y., Moya, A., & Dinleyici, E. C. (2024). Composition of Microbiota in Transient and Mature Human Milk: Significant Changes in Large for Gestational Age Group. *Nutrients*, *16*(2), 208. https://doi.org/10.3390/nu16020208.

5. Dudgeon LS, Jewesbury RC. The bacteriology of human milk. J Hyg (Lond). 1924;23:64–76. doi:10.1017/s0022172400008470.

6. Mohandas S, Pannaraj PS (2020) Beyond the bacterial microbiome: Virome of human milk and effects on the developing infant. Nestle Nutr Inst Workshop Ser 94:86–93. https:// doi. org/ 10. 1159/00050 4997

7. Hunt KM, Foster JA, Forney LJ, Schütte UM, Beck DL, Abdo Z, Fox LK, Williams JE, McGuire MK, McGuire MA. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One. 2011;6(6):e21313. doi: 10.1371/journal.pone.0021313. Epub 2011 Jun 17. PMID: 21695057; PMCID: PMC3117882.

8. Hris Gale, Karen M Logan, Shalini Santhakumaran, James RC Parkinson, Matthew J Hyde, Neena Modi, Effect of breastfeeding compared with formula feeding on infant body composition: a systematic review and meta-analysis123, The American Journal of Clinical Nutrition,Volume 95, Issue 2012, Pages 656-669.

9. Sitelbanat Osman Mohamed Ahmed, Hawa Ibrahim Abdalla Hamid, Aruna Jothi Shanmugam, Murtada Mustafa Gabir Tia, Soad Mohamed Abdalla Alnassry, Impact of exclusive breastfeeding on physical growth, Clinical Nutrition Open Science, Volume 49, 2023, Pages 101-106,ISSN 2667-2685, https://doi.org/10.1016/j.nutos.2023.04.008.

10. Weli SM. Effect of Exclusive Breast Feeding on Infants Growth and Comparison with Other Types of Feeding in Sulaimani City, Kurdistan, Iraq. Iraqi J Pharm Sci 2021;30(1):76e80. https://doi.org/10.31351/vol30iss1pp76-80.

11. Wallenborn JT, Levine GA, dos Santos AC, Grisi S, Brentani A, Fink G. Breastfeeding, physical growth, and cognitive development. Pediatrics 2021;147(5). https://doi.org/10.1542/peds.2020-008029.

12. Beyrami HJ, Samadi-Afshar S. Comparison of Growth Index in Exclusive Breastfeeding and Infant Formula Feeding in SixMonth-Old Infants in East Azerbaijan Province. Depiction ofHealth 2018;8(4):252e9.

13. Koya S, Babu GR, Deepa R, Iyer V, Yamuna A, Lobo E, et al. Determinants of breastfeeding practices and its association with infant anthropometry: Results from a prospective cohort study in South India. Front Public Heal 2020. https://doi.org/10. 3389/fpubh.2020.492596.