

Maternal and Infant Microbiota: Identification and Correlations. Lactoferrin and Human Milk Oligosaccharides at Different Stages of Lactation

Abstract

Human milk is considered the optimal form of nutrition for newborns and infants, providing all essential nutrients required for the growth and development of breastfed children. In addition to nutrients, it contains numerous biologically active components with health-promoting effects, including immunoglobulins, cytokines, lactoferrin, vitamins, hormones, oligosaccharides, and viable microorganisms.

The aim of the study was to characterize the microbiota of human milk and the oral cavity of breastfed children, taking into account the sociodemographic characteristics of the study participants. In addition, lactoferrin (LF) concentration and the qualitative and quantitative composition of human milk oligosaccharides (HMOs) were determined. The study was approved by the Bioethics Committee of Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz (approval no. KB 196/2021). The study comprised two stages, during which human milk samples and oral swabs from children were collected from the same mother–child pairs, together with questionnaire data (N1 = 53, N2 = 32). The collected biological materials were inoculated using the surface plating method onto a set of microbiological media: agar supplemented with 5% sheep blood, MacConkey agar, Sabouraud agar, de Man, Rogosa and Sharpe (MRS) agar, and MYP agar. The microbiological media were incubated at 36°C for 48–72 h. The resulting colonies were identified using MALDI-TOF mass spectrometry. Lactoferrin concentration was determined using an enzyme-linked immunosorbent assay (ELISA), whereas oligosaccharides were analyzed using ultra-high-performance liquid chromatography (UHPLC).

The median estimated concentration of viable microorganisms in human milk was 7,100 CFU/mL at the first sampling and 9,200 CFU/mL at the second sampling. The predominant genera identified in human milk were *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Rothia*, *Enterococcus*, and *Acinetobacter*, together with members of the family *Lactobacillaceae*. In oral swab samples, the most frequently identified genera were *Streptococcus*, *Staphylococcus*, *Gemella*, *Neisseria*, *Rothia*, and *Acinetobacter*, as well as members of the family *Lactobacillaceae*. The most prevalent species in the human milk microbiota was *Staphylococcus epidermidis*, identified in 88.7% of participants at the first sampling point and 90.6% at the second. The next most frequently detected species were *Streptococcus mitis* (64,2% vs. 62,5%), *Streptococcus parasanguinis* (43,4% vs. 31,2%), *Streptococcus oralis* (41,5% vs. 21,9%), and *Streptococcus vestibularis* (41,5% vs. 56,2%). In oral swab samples collected from children at both sampling points, the predominant species were *Streptococcus mitis* (81,1% vs. 81,2%), *Streptococcus oralis* (67,9% vs. 53,1%), *Streptococcus*

vestibularis (49,1% vs. 56,2%), *Staphylococcus epidermidis* (47,2% vs. 56,2%), and *Gemella haemolysans* (45,3% vs. 28,1%).

The median lactoferrin concentration at the first and second sampling points was 2,535 mg/mL (range 1,240–14,268) and 2,805 mg/mL (range 1,376–14,454), respectively. The median total HMO concentration at the first and second sampling points was 2,49 mg/mL (range 0,85–100,48) and 2,43 mg/mL (range 0,77–72,78), respectively. The highest LF and HMO concentrations were observed in milk samples collected during the early stages of lactation, within the first month of the child's life.

The present study indicates that human milk is a microbiologically and biochemically dynamic system in which the stage of lactation is one of the major factors determining the levels of the analyzed components. Microbiota transmission between mother and child is associated with a shared core of bacterial species that may contribute to shaping the early oral microbiota of the infant.