Journal of Cytology & Histology

ISSN: 2157-7099

Patki, et al., J Cytol Histol 2014, S4:2
DOI: 10.4172/2157-7099.S4-009

Research Article Open Access

Cytology of the Human Milk in the First Post Partum Week - A Clinical Perspective

Satish Manohar Patki*, Vijay Tukaram Mali, Ujjwala Satish Patki and Shweta Satish Patki

Patki Research Foundation and Hospital, Kolhapur, Maharashtra, India

***Corresponding author:** Satish Manohar Patki, Patki Research Foundation and Hospital, Kolhapur, Maharashtra, India, Tel: 00-91-9823388858; Fax: 00-231-2656448; E-mail: satish.patki@rediffmail.com

Rec Date: Sep 01, 2014; **Acc Date:** Oct 27, 2014; **Pub Date:** Oct 29, 2014

Abstract

Human milk is a dynamic physiological fluid. The short term and long term benefits of breast feeding are already very well established. Apart from the nutritional components, the human milk contains many growth factors & different kinds of live cells. Present study is an observational study of the cytological evaluation of the 100 samples of human milk collected in the first post partum week of Indian women. The interesting observation of the present study is the rising counts of the immune cells in the human milk which peak on day 5. This finding probably correlates with the greater immunological needs of the neonate in the first week. These cells help the neonate in providing cell mediated immune responses and protection against respiratory and gut infections. They also help in the process of the neonatal gut maturation. The counts of the epithelial cells in the human milk are observed to be steady throughout the, first post partum week. The mesenchymal stem cells observed in the human milk could be myoepithelial in origin. The higher levels of the growth factors like hepotocyte growth factor and vascular endothelial growth factor in the human milk when compared with the umbilical cord or the maternal serum could be attributed to the paracrine secretions by the stem cells. The observations of the present study are correlating with the physiological needs of the neonate in the most important period of the first week after the birth. The study further strengthens the concept of exclusive breast feeding.

Keywords: Human milk; Macrophages; Mesenchymal stem cells; Immune cells

Introduction

Human milk is a dynamic physiological fluid which contains not only the necessary nutrients for the optimal growth of the infant, but also a lot of different kinds of live cells. The role of human milk in the overall development of the neonate is established beyond doubt. The short term as well as long term benefits of the human milk is already proven. However, there are a very few studies on the cytological evaluation of the human milk. Our own original study has documented the presence of the multipotent mesenchymal stem cells in the human milk [1]. Apart from these cells; the breast milk is thought to harbor epithelial cells and immune cells. Human colostrum contains a significant number of immune cells consistent with the higher immunological needs of the neonate in the early post partum period. However, within the first two weeks after birth, their number is decreased to 0-2% of the total cells, which is maintained throughout the lactation. Present study is an attempt to study the cytology pattern of the human milk in the first week of lactation. The importance of the present study is for the clinical correlation of the significance of the colostrums and its physiological role in the development of the neonate with the background of its cytological composition.

Materials and Methods

This is a prospective, observational study carried out at Patki Research foundation and Hospital in collaboration with D.Y.Patil Medical University at Kolhapur, India during the period of April 2013 to March 2014. The study was approved by the Institutional Ethics Committee and Institutional Review Board. The methods and the purpose of the study were explained to the parents and their informed written consent was obtained prior to enrolment.

Collection of breast milk samples

A total of 100 healthy patients, who had full term vaginal delivery were selected. None of them had any associated medical disorder like diabetes, hypertension or any infectious disease. The breast milk samples were collected everyday from day 0 to day 7, usually in the morning. The samples were collected by a sterile technique in sterile 15 ml falcon tubes (BD, Germany) in a volume of 7 to 10 ml, and were transported to the tissue culture laboratory for the cytological evaluation.

Cell analysis

The samples were centrifuged at 1500 RPM for 15 minutes and the supernatant was discarded. The volume of the pellet was 0.5 to 0.8 ml. The pellet of the cells was washed twice with phosphate buffered saline and then the smears were taken on clean glass slides. Usually, with one sample of 7 to 10 ml. of human milk, 3 to 4 slides were prepared. All the slides of each sample were stained and studied. The average of the cell counts of all the slides of one sample was considered. After fixing the smears, staining was done using hematoxyllin-eosin stains. The smears were observed through the phase contrast as well as the compound microscope. The composition of the cells and their features were noted. The differential and total cell count was done using Neubarch chamber. Using unpaired t test, the total and differential cell counts were compared.

Mesenchymal stem cells culture

For the study of the mesenchymal stem cells, the protocol of culture as on different days established in our earlier original study [1] was used. Breifly, the cell pellet was seeded in a 35 mm tissue culture dish (BD falcon) using Dulbeccos modified eagle medium (DMEM), containing 10% heat inactivated human umbilical cord blood serum (HUCBS), supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml). The dish was incubated at 37°C under 5% CO_2 and 95% humidity. Medium was changed every 48 hours. The cells were observed under phase contrast microscope at 400 x magnification. At 80% confluency, cells were passaged using trypsine ethylene diamine tetra acetic acid (EDTA).

Characterization of isolated cells using flow cytometry

The cells from early (2 to 6) passages were used for the characterization studies. The cells were dislodged using 0.05% trypsin, 0.02% EDTA in PBS and resuspended in DMEM. The cells were fixed in chilled 70% ethanol and incubated in mouse antihuman fluorescein isothiocynate (FITC) Phycoerythrine (PE) conjugated antibodies against CD33, CD34, CD44, CD45, CD73, CD90 and CD117 for one hour on ice. The cells were acquired using a flow cytometer laser 488NM (Becton Dickinson, New Jersey, NJ, USA) and data were analysed BD Cellequest pro software.

Results

Table 1 shows the clinical data of the study subjects. Table 2 shows the average cell counts and the percentage of the leucocytes and the epithelial cells observed on day 0.5 and 7. The total cell counts and the percentage of leucocytes are significantly higher on day 5 of lactation as compared to day 0. ($P<0.01$) However, the percentage of the epithelial cells is found to be steady throughout the week.

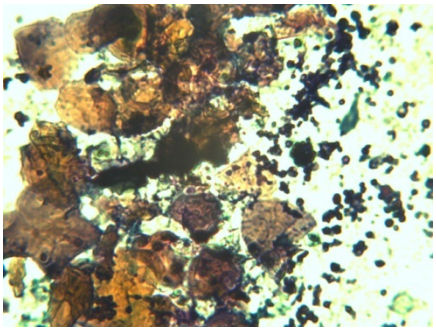

Figure 1: Clumps of epithelial cells on the postpartum day 5, stained with hematoxyllin – eosin stain.

Figure 1 shows the cluster of epithelial cells while Figure 2 shows the various types of leucocytes observed on day 5 of lactation. Figure 3 shows the mesenchymal stem cells (MSCS) adherent to the petridish with fibroblast like appearance. The adherent cell population is a mixture of epithelial cells from mammary gland alveoli and mesenchymal stem cells (MSCS). However, during the second week, the cells in the culture flask resembled a typical slender fibroblast – like cell phenotype. This could be because of epithelial to mesenchymal transition which is commonly observed in development and regeneration. The immunofluorescence study for specific cell surface marker clearly indicated that isolated human breast milk cells at passage four expressed mesenchymal stem cells (MSCS) markers, namely CD44, CD29, SCA1. These cells were found to be negative for CD33, CD34, CD45, CD73 by fluorescence activated cells sorting analysis confirming their identity as mesenchymal stem cells (MSCS).

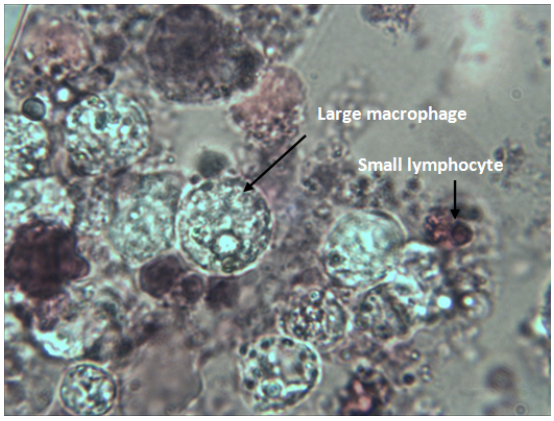

Figure 2: Large macrophages, small lymphocytes on the postpartum day 5.

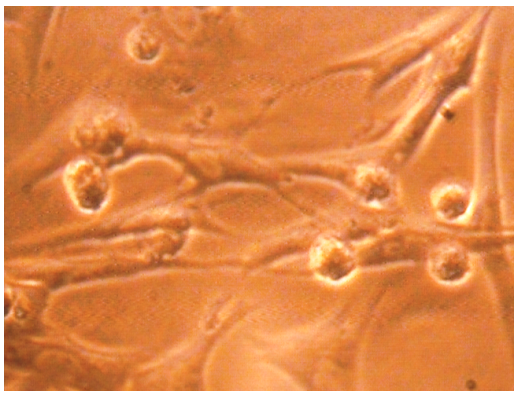

Figure 3: Mesenchymal stem cells observed at 400x phase contrast microscopy, on 7th day of culture. They are fibroblast like cells with plastic dish adherence.

S. No.	Parameter	Values
1	Maternal age	30.5 1.84 years
2	Gestational age of the neonates	37.0 1.20 weeks
3	Sex of the neonates M/F	55/45
4	APGAR Score at 1 and 5 min	8 and 10

Table 1: Clinical data of the study subjects.

Discussion

Composition of the human milk is indeed a topic of research over the years. Apart from the nutritional value, it provides many growth

factors like vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF) and many others as well as innumerable other bioactive substances. Our own original study has documented that the levels of these growth factors in the human milk are several folds higher than those observed in the umbilical cord blood [2]. It indicates the physiological phenomenon of switching over of the source of the growth factors from the placenta to the mother's milk. Breast milk has long been known to contain maternal cells. Typically, human milk is thought to harbor epithelial cells and immune cells. Researchers have shown that the immune cells selectively migrate to colostrum and milk [3]. Colostrum contains approximately 5×10^6 cells per ml. A breast fed baby ingests about 10^8 milk cells per day with breast feeding often continuing for several months [4]. The purpose of the present study is to throw light on the pattern of the cytology in the human milk throughout the first postpartum week and to see whether it follows the physiological needs of the neonate.

Parameter	Day 0	Day 5	Day 7
Cell count/ml	7×10^6	15×10^6	8×10^6
Leucocytes percentage	40.5	70.6	65.4
Epithelial Cells percentage	20.6	20.5	15.5

Table 2: Human milk cytology and day of lactation.

The immune cells in the human milk consist of macrophages and colostral corpuscles (large lipid – laden macrophages), neutrophils and lymphocytes of which the majority is T cells [4]. There is evidence to suggest that these cells survive passage through the infant's gut where they are absorbed and prime the gut for the process of maturation [5]. The relatively neutral pH of the stomach in early infancy together with the buffering capacity of the milk may allow the survival of the milk lymphocytes as well as cytokines during the gastrointestinal transit. These cells serve functions such as transfer of the cell mediated immune responses [6], protection against respiratory and gut infections and suppression of certain diseases such as atopy [7] and celiac disease [8]. Milk macrophages have been reported to contain engulfed sIGA, which they could release on contact with bacteria in gut.

In the present study of the cytology of the human milk in the first postpartum week, the cell counts are observed to rise from day 0 to day 5 and again found to fall to day 7. Interestingly, the percentage of the epithelial cells remained steady throughout the week, while the percentage of the leucocytes showed a significant peaking rise at day 5. Hassiotou et al. have also observed that 70% of the total human milk cells in the first two post partum weeks consist of immune cells [9]. They have further documented that their levels fall to a low baseline level of 0-2% of the total cells, which is maintained throughout the lactation. They have correlated the higher levels of the immune cells in the first two post partum weeks to the greater immunological needs of the neonate.

The functional unit of the mammary gland is the epithelium consisting of luminal epithelial cells and myocpithelial cells which are generated from self renewing stem and progenitor cells [10]. The epithelial cells are shed in to the milk during the lactation process. Most of them are viable and exhibit the characteristics of fully differentiated alveolar cells. Our study has concluded that the mesenchymal stem cells observed in the human milk could be myoepithelial in origin [1]. The stem cells are known to act through the paracrine mechanism by secreting bioactive substances and growth factors. We hypothesize that the stem cells in the breast milk could be the important contributing factor for the observation of the higher levels of the growth factors in human milk than in cord or maternal serum.

Thus, the observations of the present study correlate with the physiological needs of the neonate in the first week after birth. The cytological studies of the human milk during the entire duration of the breast feeding in the healthy as well as the diseased periods of the early infancy with larger umber of subjects are required in future to throw more light on the biological variations of the breast milk composition. The present study further strengthens the concept of exclusive breast feeding in the initial neonatal period.

References

1. Patki S, Kadam S, Chandra V, Bhonde R (2010) Human breast milk is a rich source of multipotent mesenchymal stem cells. Hum Cell 23: 35-40.
2. Patki S, Patki U, Patil R, Indumathi S, Kaingade P, et al. (2012) Comparison of the levels of the growth factors in umbilical cord serum and human milk and its clinical significance. Cytokine 59: 305-308.
3. Peroni DG, Chirumbolo S, Veneri D, Piacentini GL, Tenero L, et al. (2013) Colostrum-derived B and T cells as an extra-lymphoid compartment of effector cell populations in humans. J Matern Fetal Neonatal Med 26: 137-142.
4. Crago SS, Prince SJ, Pretlow TG, McGhee JR, Mestecky J (1979) Human colostral cells. I. Separation and characterization. Clin Exp Immunol 38: 585-597.
5. Malcolm G, Ellwood D, Devonald K, Beilby R, Henderson-Smart D (1991) Absent or reversed end diastolic flow velocity in the umbilical artery and necrotising enterocolitis. Arch Dis Child 66: 805-807.
6. Howie PW, Forsyth JS, Ogston SA, Clark A, Florey CD (1990) Protective effect of breast feeding against infection. BMJ 300: 11-16.
7. Businco L, Marchetti F, Pellegrini G, Cantani A, Perlini R (1983) Prevention of atopic disease in "at-risk newborns" by prolonged breast-feeding. Ann Allergy 51: 296-299.
8. Auricchio S, Follo D, de Ritis G, Giunta A, Marzorati D, et al. (1983) Does breast feeding protect against the development of clinical symptoms of celiac disease in children? J Pediatr Gastroenterol Nutr 2: 428-433.
9. Hassiotou F, Beltran A, Chetwynd E, Stuebe AM, Twigger AJ, et al. (2012) Breastmilk is a novel source of stem cells with multilineage differentiation potential. Stem Cells 30: 2164-2174.
10. Lochter A (1998) Plasticity of mammary epithelia during normal development and neoplastic progression. Biochem Cell Biol 76: 997-1008.