Cite as: Tomaszewska A, Królikowska K, Jeleniewska A, Porębska K, Aleksandrowicz K, Lipińska-Opałka A, Rustecka A, Zdanowski R, Kociszewska-Najman B, Kalicki B: Effects of maternal and child factors on the immunological composition of human milk. Pediatr Med Rodz 2023; 19 (3): 188–194. DOI: 10.15557/PiMR.2023.0034.

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Received: 16.08.2023
Accepted: 21.09.2023
Published: 05.12.2023

Effects of maternal and child factors on the immunological composition of human milk

Wpływ czynników matczynych i dziecięcych na skład immunologiczny mleka kobiecego

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https://doi.org/10.15557/PiMR.2023.0034

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Abstract

Introduction and objective: The relationship between the bioactive components of milk and maternal and child health is not well elucidated yet. The aim of this study was to determine whether maternal factors (diseases and drugs used during pregnancy, method of delivery, age) and child characteristics (age, length and body weight at birth) affect breast milk composition (leukocytes, stem cells, and epithelial cells). **Materials and methods:** Twenty-three breastfeeding mothers of infants aged 1–6 months were recruited for the study. All the mothers and children were healthy, with no symptoms of respiratory tract infection. Samples of breast milk (20 mL) were collected. Each sample was assessed for its cellular composition (epithelial cells, mesenchymal stem cells, leukocytes). Only a few studies available in the literature have evaluated both immune cells (leukocytes) and cells of non-immune origin (mesenchymal and epithelial cells) in human milk. In this regard, our analysis is innovative. **Results:** Women receiving pharmacotherapy during pregnancy had a significantly higher percentage of eosinophils in their breast milk compared to women who did not receive any medication when pregnant. The cellular composition of milk correlated inversely with the age of the mother and child. The more advanced the mother's age, the lower the number of monocytes in the milk. On the other hand, the older the child, the lower CD4 lymphocyte count in milk. **Conclusions:** The study suggests that the cellular composition of breast milk is influenced not only by the characteristics related to the mother but also by child-depended factors. Research in this area needs to be continued.

Keywords: flow cytometry, breastfeeding, child, mother, human milk

Streszczenie

Wprowadzenie i cel: Wciąż nieznana jest zależność między bioaktywnymi składnikami mleka a zdrowiem matki i dziecka. Celem pracy było określenie, czy cechy matki (choroby i leki stosowane w czasie ciąży, sposób porodu, wiek) i dziecka (wiek, wzrost i masa ciała w chwili urodzenia) wpływają na skład mleka kobiecego (leukocyty, komórki macierzyste i komórki nabłonkowe). **Materiał i metody:** Do badania zakwalifikowano 23 matki karmiące piersią dzieci w wieku od 1 do 6 miesięcy. Zarówno matka, jak i dziecko byli zdrowi, bez objawów infekcji dróg oddechowych. Pobrano próbki 20 ml mleka kobiecego. Każdą próbkę oceniano pod kątem składu komórkowego (komórki nabłonkowe, mezenchymalne komórki macierzyste, leukocyty). Tylko nieliczne badania w literaturze oceniają zarówno komórki odpornościowe (leukocyty), jak i komórki pochodzenia nieimmunologicznego (komórki mezenchymalne i nabłonkowe) w mleku kobiecym. Pod tym względem niniejsza analiza jest nowatorska. **Wyniki:** Kobiety otrzymujące farmakoterapię w czasie ciąży miały istotnie wyższy odsetek eozynofilów w mleku niż kobiety, które nie otrzymywały żadnych leków. Skład komórkowy mleka korelował z wiekiem matki i dziecka. Im bardziej zaawansowany wiek matki, tym mniejsza liczba monocytów w mleku. Z drugiej strony im starsze dziecko, tym niższe wartości limfocytów CD4 w mleku. **Wnioski:** Wyniki wskazują, że skład komórkowy mleka matki zależy nie tylko od cech matczynych, ale także od czynników zależnych od dziecka. Zagadnienie wymaga dalszych badań.

Słowa kluczowe: cytometria przepływowa, karmienie piersią, dziecko, matka, pokarm kobiecy

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INTRODUCTION

The infant immune system is functionally immature, so the immune response to infection may be ineffective. Protection is provided primarily by cells and non-specific mechanisms of the innate immune system, which are less precise in fighting microorganisms than specific mechanisms of the adaptive immune response[1]. In addition, the newborn is protected by maternal antibodies, transmitted by transplacental transfer, and by immunologically active (cellular and non-cellular) components found in breast milk[2].

Exclusive breastfeeding for six months is the gold standard for infant nutrition. In contrast to infant formulas, the composition of breast milk is dynamic and changes depending on the phase of feeding, time of day, and age of the infant. Immediately after birth, colostrum is secreted, between 5 and 15 days – transitional milk, and after the 15th day of lactation – mature milk[3]. Milk from different periods of lactation differs in energy value and content of nutrients. Colostrum is less caloric and contains more protein and fewer saccharides than mature milk. On the other hand, transitional milk contains more protein but less fat and lactose than mature milk[4]. Due to its unique composition and, as a result, preventive and health-promoting effects on the child's body, breast milk is considered a functional food. In addition to its nutritional value, it has a range of non-nutritional functions, including regulation of child development, protection against bacterial and viral infections, and functional maturation of the digestive tract[5].

Human milk is a biologically complex fluid, which, in addition to water (87%), also contains nutrients (13%) and a whole range of non-nutritive bioactive (cellular and non-cellular) ingredients[6]. Bioactive components include immune cells and multiple proteins directly and indirectly involved in immune processes, i.e. immunoglobulins, acute phase proteins, cytokines, hormones, and growth modulators. Some of them are produced in the mammary gland, while others are transported from the mother's blood serum[7].

The total content of immune system cells present in human milk is the highest in the first days after birth[8]. Multicolour flow cytometry analysis has shown that among all immune cells present in milk, the percentage of leukocytes depends on the lactation phase. It declines from 20% in colostrum to 2% in transitional and mature milk[9]. After reaching the child's digestive system, the leukocytes contained in milk pass through the intestinal barrier and enter the infant's bloodstream[10].

Among milk leukocytes, macrophages are the largest group (about 60%). Their main function is the phagocytosis of pathogens. Additionally, milk macrophages contain engulfed IgA, which they can release on contact with bacteria in the gut[11,12]. Neutrophils are the second largest population of milk leukocytes (approximately 25%)[11]. Most likely, they do not play an important role in the child's immune response. Their main function is to protect the mother, as they have limited functional capacity after secretion into milk[13].

The smallest population of immune cells in breast milk are lymphocytes (about 10%). The main fraction of lymphocytes consists of T cells (80%)[14]. Activated T cells of maternal origin compensate for functionally immature neonatal T cells and promote their maturation[15]. B cells, which make up about 20–25% of lymphocytes, undergo differentiation in the mammary gland into plasma cells secreting the IgA class antibodies[16].

In addition to immune cells, breast milk is a source of growth factors (e.g. G-CSF, M-CSF, GM-CSF), cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-16, IL-18, IL-10, TNF-α, IFN-γ) and immunoglobulins[17]. Breast milk also contains non-immune cells: epithelial cells, myoepithelial cells, and lactocytes (milk-secreting cells). Epithelial cells derive from the epithelium covering the secretory vesicles and milk ducts of the mammary gland. Some of the epithelial cells differentiate into lactocytes. Lactocytes absorb nutrients directly from the mother's blood, and synthesise and secrete milk[18]. Recent studies have shown that human breast milk also contains stem cells (human breast-milk stem cells, hBSCs). There are two types of stem cells – mammary gland stem cells (MaSCs) and mesenchymal stem cells (MSCs). They are activated during pregnancy and lactation[19,20], and are pluripotent, i.e. they can differentiate into other cell types[18]. *In vitro* studies have confirmed that these cells are able to differentiate into mammary gland cells, neurocytes, hepatocytes, osteoblasts, chondrocytes, and cardiomyocytes[21].

All immune cells contained in breast milk interact with one another, affecting the immune status of the breastfed child. However, the relationship between the active ingredients in milk and the health of the mother and child is still unknown. Understanding the factors affecting the cellular composition of human milk is an important tool in the infant feeding strategy. In this study, we analysed whether the cellular components of breast milk changed under the influence of specific maternal and child's factors.

AIM OF THE STUDY

The aim of the study was to determine whether maternal factors (diseases and drugs used during pregnancy, method of delivery, age) and child characteristics (age, length and body weight at birth) influence breast milk composition (leukocytes, stem cells, and epithelial cells).

MATERIALS AND METHODS

Study group

A total of 23 mothers breastfeeding 1- to 6-month-old infants were included in the study. At the time of entry into the study, both mothers and children were healthy, with no fever or symptoms of respiratory tract infection. The mothers whose children had ever been supplemented with infant formula or who were fed breast milk from a bottle were excluded from the study.

 DOI: 10.15557/PiMR.2023.0034

Inclusion criteria	Exclusion criteria
Exclusive breastfeeding	Partial breastfeeding
Age of child: 1–6 months	Supplemental feeding with infant formula
No chronic diseases in the child	Feeding breast milk from a bottle
No symptoms of respiratory tract infection in the baby	Symptoms of respiratory tract infection in the baby
No symptoms of respiratory tract infection in the mother	Symptoms of respiratory tract infection in the mother
Consent to participate in the study	Current pharmacotherapy in the mother
	Child's age <1 month or >6 months
	Chronic disease in the child
	Prematurity
	No consent to participate in the study

Tab. 1. Inclusion and exclusion criteria for the study

The study inclusion and exclusion criteria are presented in Tab. 1.

Following inclusion in the study, the mothers and children underwent body temperature check, medical history interview, and physical examination. Medical interviews considered the following factors:

- maternal: mother's age, number of pregnancies, diseases during pregnancy, medications taken during pregnancy;
- perinatal: method of delivery (spontaneous vs. caesarean section);
- child: child's age, body length, body weight.

Breast milk samples were collected from the mother using a breast pump, and the cellular composition of milk was determined by assessing for the presence of leukocytes (neutrophils, lymphocytes, monocytes, eosinophils), mesenchymal stem cells, and epithelial cells.

Collecting milk samples

The mothers participating in the study collected milk at least 2 hours after the last feeding, from the breast used to feed the child (to eliminate milk composition variability during feeding). Consecutive samples were always collected at the same time (in the morning) to eliminate diurnal variability in milk composition. The milk was expressed under the same conditions – in a specially prepared room for parents, ensuring an intimate atmosphere, using an electric breast pump (Medela, Symphony). 20 mL samples were collected into sterile, disposable containers and transported in an isothermal bag to the Laboratory of Molecular Oncology and Innovative Therapies of the Military Institute of Medicine – National Research Institute.

The milk samples were analysed by flow cytometry. Tab. 2 presents the analysed cellular composition of milk with CD profile.

Immunolabelling of cells in the breast milk

For extracellular staining, DPBS 10X (Corning), 4% PFA (Merck), and appropriate antibody-fluorochrome tandems (Beckman Coulter) were used. To determine the general phenotype of leukocytes – Leukocyte Common Antigen: anti-CD45-KRO was used. For the characteristics of individual subpopulations among leukocytes (CD45+ cells), the following antibodies were applied: anti-CD3-FITC, anti-CD8-PC5, anti-CD4-APC for T lymphocytes, anti-CD19-PC 7 for B lymphocytes, and anti-CD(16+56)-PE for NK cells.

Cell type	CD profile
All leukocytes	CD45
T cells	CD45/CD3
T helper cells	CD45/CD3/CD4
T cytotoxic cells	CD45/CD3/CD8
Natural killer T cells	CD45/CD5/CD16/56
B cells	CD45/CD19
Mesenchymal stem cells	CD73, CD105
Epithelial cells	CD326

Tab. 2. Cellular composition of milk analysed by flow cytometry with CD profile

CD45+CD14+ cells were qualified to the macrophages/monocytes population. CD45+CD16– with adequate SSC position cells were qualified to neutrophils. Eosinophils population were marked as CD45+ cells which high autofluorescence in empty UV channel.

In the study material, cells with a stem cell phenotype were also characterised based on the following markers: CD105-PC7, CD34-ECD, CD73-PE, CD45-AF700, CD44-AF750. For epithelial cells, anti-CD326-APC antibody was used.

20 mL of cold breast milk and 20 mL of cold 1X PBS were added to a falcon tube and centrifuged at 1,000 RCF for 20 minutes at 4°C. The pellets were rinsed and recentrifuged twice. After the last centrifugation pellets were resuspended in 1X PBS. 100 μL samples were dispensed into panels (phenotype, stem cells) and then incubated for 30 minutes in the dark at room temperature (RT). After labelling, the cells were fixed with 4% PFA and washed with 1X PBS. Samples labelled by all panels were resuspended in 1X PBS. Stained samples were assayed on a CytoFLEX, Beckman Coulter flow cytometer, and by FCAP Array software.

Statistical analysis

The results were analysed statistically using the StatSoft software (STATISTICA 2014). The analyses were initially verified using the diagram of normal distribution (Smirnov and Liliefors test). The Student's *t*-test was used to evaluate variables with normal distribution. For variables inconsistent with the normal distribution, the non-parametric test was used (Mann–Whitney *U* test). A correlation was calculated using Spearman's test (variables lacking the normal distribution) or Pearson's correlation factor (variables with the normal distribution). The p-value <0.05 was considered as statistically significant.

DOI: 10.15557/PiMR.2023.0034

RESULTS

Characteristics of the study group

Mothers

Twenty-three women (mean age 32 years, standard deviation, *SD* ±3) were enrolled in the study. Primiparas accounted for 48% (11/23) of all subjects.

Most women (17/23) declared history of diseases during pregnancy, of which infectious diseases accounted for 29% (5/17). Fourteen out of 23 mothers used pharmacotherapy during pregnancy; in 8 cases, it was polypharmacy (>2 drugs).

The dominant method of delivery was spontaneous delivery (13/23). In 7/13 women giving birth naturally, anaesthesia was used during childbirth: nitrous oxide (1/7), epidural (2/7), and spinal anaesthesia (4/7).

Among the women giving birth by caesarean section, only 2/10 caesarean sections were elective (maternal indications – abnormalities in the structure of the pelvis). In the remaining patients (8/10), an emergency caesarean section was performed due to lack of labour progress (5/8), premature discharge of amniotic fluid (1/8), polyhydramnios (1/8), and elevated blood pressure (1/8).

Infants

The average age of infants at the time of eligibility for the study was 3 months (*SD* ±1). All infants were born at term (average at 40 ± 1 week of gestation) with an AGA (appropriate for gestational age) weight – an average of 3,735 g ±510. Almost all infants (22/23) were rated at birth with an Apgar score of 10.

Most infants did not present any adaptive disorders at birth. However, jaundice not requiring phototherapy was observed in 4/23 infants.

Variable	Mothers (*N* = 23)
Age (years) [mean ± *SD*]	32 ± 3
First pregnancy (*n*)	11 (48%)
Subsequent pregnancy (*n*)	12 (52%)
Diseases during pregnancy (*n*):	17 (74%)
• hypothyroidism (*n*)	6
• diabetes (*n*)	3
• anaemia (*n*)	2
• hypertension (*n*)	1
• infection (*n*)	5
• urinary tract infection (*n*)	3
Medications during pregnancy (*n*)	14 (61%)
Childbirth:	
• spontaneous delivery (*n*)	13 (57%)
• caesarean section (*n*)	10 (43%)
Variable	**Infants (*N* = 23)**
Age (months) [mean ± *SD*]	3 ± 1
Birth weight (g) [mean ± *SD*]	3,735 ± 510
Body length (cm) [mean ± *SD*]	56 ± 2.6
Apgar score:	
• 10 points (*n*)	22
• <10 points (*n*)	1
Adaptive disorders (*n*)	4 (17%)
Kangarooing (*n*)	19 (83%)
Attachment to the breast within 2 hours after birth (*n*)	18 (78%)

Tab. 3. Characteristics of the study group

Variable	Spontaneous delivery	Caesarean section	*p*
CD3 [%]	73.97 ± 13.4	72.03 ± 15.8	0.75
CD4 [%]	10.52 ± 10.8	14.04 ± 4.8	0.35
CD8 [%]	13.54 ± 12.1	19.94 ± 6.2	0.14
CD19 [%]	9.53 ± 16.6	8.00 ± 9.2	0.80
CD16/56 [%]	9.72 ± 6.5	9.14 ± 4.6	0.81
CD45 [%]	10.96 ± 14.5	5.47 ± 1.9	0.25
Lymphocytes [%]	9.23 ± 7.1	11.26 ± 7.4	0.51
Neutrophils [%]	14.32 ± 17.4	14.87 ± 16.5	0.94
Monocytes [%]	35.91 ± 22.0	24.43 ± 9.7	0.14
Eosinophils [%]	1.31 ± 1.4	1.15 ± 1.2	0.77
CD105 [%]	8.19 ± 7.3	8.22 ± 7.8	0.99
CD73 [%]	0.80 ± 0.6	0.84 ± 0.4	0.83
CD326 [%]	78.83 ± 15.0	77.17 ± 16.7	0.80

Tab. 4. Effect of delivery route on breast milk composition

Tab. 3 shows the characteristics of the study groups.

Effects of maternal factors on the cellular composition of human milk

There were no statistically significant differences in the cellular composition of breast milk between women giving birth naturally and women giving birth by caesarean section (Tab. 4).

There were no statistically significant differences in the cellular composition of milk between the women who declared history of diseases during pregnancy and women who denied any disease. However, women receiving pharmacotherapy during pregnancy had a significantly higher percentage of eosinophils than women who did not receive any medications (Tab. 5).

A moderate statistically significant correlation was observed between the percentage of monocytes in milk and the mother's age. The more advanced the maternal age, the lower the monocyte percentage (Fig. 1).

Effects of child-dependent factors on the cellular composition of breast milk

There was no statistically significant correlation between the cellular composition of breast milk and the child's birth weight and length (Tab. 6).

However, a weak but significant correlation was observed between the age of the child and the percentage of CD4+ lymphocytes – the older the child, the lower the CD4+ percentage (Fig. 2).

DISCUSSION

Human milk is a source of many cell types, such as leukocytes, epithelial cells, stem, and progenitor cells.

 DOI: 10.15557/PiMR.2023.0034

Variable	Diseases in pregnancy			Pharmacotherapy in pregnancy		
	Yes	No	*p*	Yes	No	*p*
CD3 [%]	70.52 ± 14.5	80.51 ± 11.1	0.14	69.37 ± 15.2	78.96 ± 10.8	0.12
CD4 [%]	13.09 ± 9.3	9.11 ± 6.6	0.35	12.42 ± 7.6	11.47 ± 10.7	0.81
CD8 [%]	18.13 ± 10.6	11.20 ± 7.7	0.16	17.82 ± 8.9	13.99 ± 12.4	0.40
CD19 [%]	5.93 ± 6.7	17.19 ± 23.6	0.08	6.32 ± 7.4	12.84 ± 19.8	0.27
CD16/56 [%]	8.76 ± 4.8	11.48 ± 7.8	0.32	8.12 ± 4.2	11.55 ± 7.2	0.16
CD45 [%]	7.92 ± 12.6	10.43 ± 5.8	0.65	9.42 ± 13.8	7.26 ± 5.5	0.66
Lymphocytes [%]	10.40 ± 7.6	9.31 ± 6.0	0.75	8.74 ± 7.0	12.25 ± 7.2	0.26
Neutrophils [%]	12.67 ± 13.6	19.93 ± 24.1	0.37	15.44 ± 18.8	13.19 ± 13.4	0.76
Monocytes [%]	30.57 ± 18.6	31.89 ± 19.3	0.88	30.70 ± 20.9	31.26 ± 14.6	0.94
Eosinophils [%]	1.25 ± 1.3	1.22 ± 1.2	0.96	0.77 ± 0.8	1.96 ± 1.6	**0.03**
CD105 [%]	8.07 ± 15.0	78.93 ± 5.6	0.89	8.47 ± 8.4	7.78 ± 5.8	0.83
CD73 [%]	0.80 ± 0.5	0.86 ± 0.5	0.79	0.76 ± 0.5	0.91 ± 0.5	0.47
CD326 [%]	77.81 ± 8.0	78.93 ± 17.9	0.88	77.31 ± 14.8	79.34 ± 17.2	0.77

Tab. 5. Effect of pharmacotherapy and diseases during pregnancy on the composition of breast milk

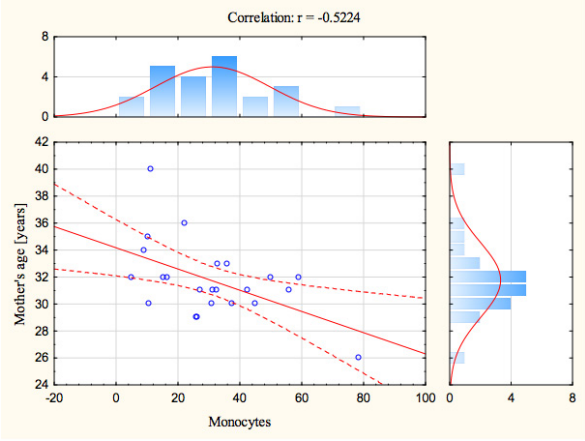


Fig. 1. Relationship between maternal age and monocyte percentage

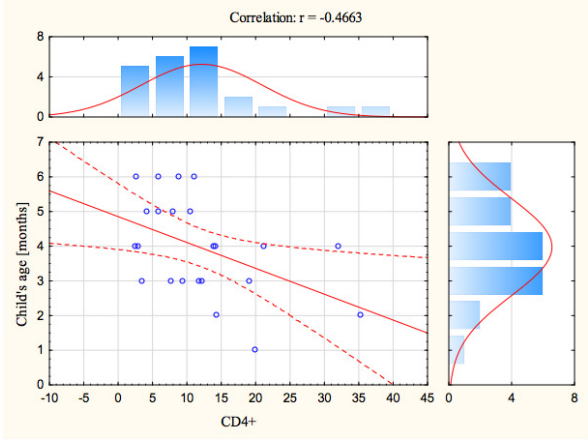


Fig. 2. Relationship between the child's age and CD4 percentage

Variable	Body length	Body weight
CD3	0.21	0.13
CD4	0.15	0.00
CD8	0.07	–0.01
CD19	–0.12	–0.04
CD16/56	–0.22	–0.14
CD45	0.05	0.07
Lymphocytes	0.15	0.06
Neutrophils	–0.04	0.04
Monocytes	0.02	–0.06
Eosinophils	0.17	0.08
CD105	0.08	0.08
CD73	0.01	0.11
CD326	–0.19	–0.29

Tab. 6. Correlations between breast milk composition and child's length and weight at birth

In our study, the leukocyte population, stem cells expressing CD105 and CD73 antigens (mesenchymal cells), and epithelial cells expressing CD326 antigen were analysed. Only a few studies in the literature have evaluated both the immune cells (leukocytes) and the spectrum of cells of non-immune origin (i.e. mesenchymal and epithelial cells) in human milk. Our analysis is innovative in this regard.

The function of cells in breast milk has been intensively studied over the past few years. The mammary sacs start producing milk between the 16th and 22nd week of pregnancy[22]. Based on that finding, the influence of pregnancy and perinatal factors on the composition of breast milk was analysed in our study. We evaluated mature milk and the possible effect of factors dependent on maternal and child characteristics (such as the mother's and infant's age, diseases and medications during pregnancy, method of delivery, infant's length and body weight), on the cellular composition of milk. Our analysis showed that the type of delivery (spontaneous delivery vs. caesarean section) and diseases during pregnancy did not affect the composition of breast milk. However, our results indicate a potential impact of drug use (pharmacotherapy used during pregnancy) on the number of eosinophils in milk. Previous studies on the cellular composition of human milk have focused on the impact of infection, either of the mother or the child, on lactation[23,24].

DOI: 10.15557/PiMR.2023.0034

Few publications evaluated the impact of non-infectious factors on the cellular composition of human milk. Dixon and Forsyth reported that the milk of mothers with asthma contained a higher percentage of polymorphonuclear cells and a lower percentage of lymphocytes than the milk of healthy mothers[25]. Piñeiro-Salvador et al. indicated a significant impact of obesity on the reduction of milk B cells. The authors hypothesised that this might translate into the quantity and quality of the passive immune response of infants. It has been suggested that the mother's health is linked to the infant's health[26]. Similar observations were made by Olivares et al.[27]. Significantly fewer IgA antibodies and lower level of TGF-β cytokines, were found in the milk of mothers with celiac disease, which indirectly reduces the protective effect of breastfeeding on the future risk of developing celiac disease in the child. Some researchers believe that the immune composition of breast milk is also influenced by factors to which a woman was exposed during early childhood. Amoudruz et al. observed that immigrant women from developing countries had statistically higher levels of IL-6, IL-8, and TGF-β in milk than native Swedish women. The authors suggest that the mother's country of birth could potentially be important for the risk of her offspring developing an immune-mediated disease[28].

Leukocytes are a group of cells that are not endogenous to the mammary gland but migrate from maternal blood vessels, acquiring new surface markers[29]. It has been proven that the leukocytes in human milk differ from those in the blood. Tuaillon et al. observed that the phenotype of B cells in breast milk is more like the phenotype of B cells in intestinal lymphoid tissue[30]. On the other hand, T cells in milk are more mobile than their blood counterparts[31]. Eosinophils make up a small percentage of all leukocytes found in milk. In our study, we observed higher eosinophil counts in the milk of mothers receiving pharmacotherapy during pregnancy. An increased number of these cells may correlate with the development of cow's milk allergy in the offspring. Järvinen and Suomalainen evaluated the relationship between the cellular composition of human milk and the development of allergy to cow's milk protein in breastfed infants. An increased percentage of eosinophils was observed in the milk of women whose children had allergies[32].

The nutrient content in breast milk is constantly changing during lactation. However, little is known about quantitative changes in the immunological components of human milk. In the current study, we found an inverse correlation between the child's age and the cellular composition of milk. This result should be interpreted with caution due to the moderate strength of the correlation. Most likely, the observation does not result strictly from the age of the child, but from the length of lactation. This proves the potential influence of the duration of lactation on the cellular composition of human milk. Changes in milk composition in the first month of life are well documented. However, the dominant view is that the composition of mature milk does not fluctuate substantially. Our results indicate that the number of immune cells present in mature milk also changes. Trend et al. found that the cellularity of mature milk is also affected by gestational age. The concentration of cytotoxic T cells and NK cells in mature milk was significantly lower in extremely premature infants (<28 Hbd) compared to premature infants born between 28 and 32 Hbd[33]. Significant differences were also observed in the number of B cells: their number was higher in extremely premature infants[33].

Until now, it was believed that the age of the mother did not significantly affect the composition of her milk[34]. These observations were mainly related to nutrients. In the current study, we found an inverse correlation between maternal age and the cellular composition of milk; the more advanced the mother's age, the lower the monocyte count in milk. This result should be interpreted with caution due to the moderate strength of the correlation. Monocytes are present in human milk in low numbers[35]. However, they perform an extremely important function of antigen presentation and participate in T cell activation[36]. An immunomodulatory effect of maternal age on milk production was also observed by Burch et al.[37]. The authors showed a negative correlation between maternal age and TGF-β concentration.

Although the current study provided new information on the immunomodulatory effects of various factors on milk composition, it had some limitations. The main issue was the small sample size due to difficulties in recruiting exclusively breastfeeding mothers. Any supplemental feeding with infant formula (also in the neonatal ward) was an exclusion criterion from the study. According to research, only 6% of mothers in Poland exclusively breastfeed in the first six months of child's life[38]. Another issue was the lack of supervision by medical staff when the mothers expressed milk by a breast pump. This may have affected the sampling standardisation process.

CONCLUSIONS

The findings of the study suggest that the cellular composition of breast milk is influenced not only by the characteristics of the mother, but also by child-depended factors. Research in this area needs to be continued.

Conflict of interest

The authors declare no conflict of interest.

Funding/Support and role of the sponsor

The research was financed from a grant implemented as part of the statutory activity of the Military Institute of Medicine (No. 572).

Availability of data and materials

The datasets used and/or analysed during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research project received approval from the Bioethics Committee of the Military Medical Chamber (consent No. 221/22).

The authors confirm that informed consent was obtained from all donors of milk. Milk sampling is described in the Materials and Methods section. The authors state that all methods were carried out in accordance with relevant guidelines and regulations.

The subjects enrolled in the study gave written informed consent to participate, and were informed that they were free to withdraw at any time without giving a reason.

The parents gave written informed consent to their child's participation in the study.

Author contributions

Original concept of study: AT, KK, AJ, ALO, AR, RZ, BKN, BK. Collection, recording and/or compilation of data: AT, KK, AJ, KP, KA, ALO, AR, RZ, BKN. Analysis and interpretation of data: AT, KK, KP, KA, BK. Writing of manuscript: AT, AJ. Critical review of manuscript: RZ, BKN, BK. Final approval of manuscript: AT, BK.

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DOI: 10.15557/PiMR.2023.0034 PEDIATR MED RODZ Vol. 19 No. 3, p. 188–194