Wydawnictwo UR 2024
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2024.1.22

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2024; 22 (1): 117–120

ORIGINAL PAPER

The content of lactoferrin and interleukin-8 in breast milk of patients with lactational mastitis

Nataliia Demianchuk [1], Viorika Akimova [1], Larysa Soyka [2], Mariia Shchurko [2], Lyubov Lapovets [1], Nataliia Lutsiv [1], Serhii Tkachuk [1], Olha Buchko [1], Svitlana Zubchenko [3], Ihor Hayduchok [3]

[1] Department of Clinical Laboratory Diagnostic, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[2] Communal Institution of Higher Education of Lviv Regional Council "Andrey Krupynsky Lviv Medical Academy", Lviv, Ukraine
[3] Department of Clinical Immunology and Allergology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

ABSTRACT

Introduction and aim. Information concerning lactoferrin and interleukin-8 (IL-8) local levels in breast milk are not numerous and requires further research. The aim of this study was to determine the content of lactoferrin and interleukin-8 in the breast milk of patients with lactational mastitis, and to identify new potential markers for assessing the activity of the inflammatory process in the mammary gland.

Material and methods. This study analyzed the breast milk of 30 women with lactostasis (group I), 37 women with lactational mastitis (group II) and 30 healthy lactating women (age 26±5 years old). The milk content of lactoferrin and interleukin-8 (IL-8) was determined by enzyme-linked immunosorbent assay.

Results. The average value of lactoferrin in breast milk of healthy women was 4.78±0.47 mg/mL, exceeding levels in group I 1.8 times ($p<0.05$). The level of lactoferrin in group II exceeds the control values 3.1 times ($p<0.05$). The content of IL-8 in breast milk of women in group I was 7.3 times higher than the control (3.63±0.12 pg/mL, $p<0.05$). In lactational mastitis, the concentration of IL-8 in breast milk exceeded the group I 13.9 times ($p<0.05$) and was 1.9 times higher than group I ($p<0.05$).

Conclusion. The analysis has revealed an increase of lactoferrin and IL-8 in breast milk of the test groups, which indicates the activation of non-specific protection.

Keywords. breast milk, interleukin-8, lactational mastitis, lactoferrin, lactostasis

Introduction

An urgent problem in the postpartum period of childbirth is the development of lactational mastitis, which begins with stagnation of milk (lactostasis), infection (in 70-80% of cases, the causative agent is *Staphylococcus aureus*), and a decrease in the overall stability of the body. Lactational (postpartum) mastitis is inflammation of the parenchyma of the mammary gland, which is associated with the lactation process. Lactational mastitis most often develops as a result of long-term pathological lactostasis. Lactostasis is a dysfunctional state of the lactating mammary gland, where there is a discrepancy between the processes of milk formation and milk yield.[1]

An insufficient immune response causes protracted disease, periods of disability enlarge, and the risk of chronic pathological process increases.[1-3] An important

Corresponding author: Viorika Akimova, e-mail: viorikakimova.kld@gmail.com

Received: 16.07.2023 / Revised: 21.11.2023 / Accepted: 9.12.2023 / Published: 30.03.2024

Demianchuk N, Akimova V, Soyka L, Shchurko M, Lapovets L, Lutsiv N, et al. The content of lactoferrin and interleukin-8 in breast milk of patients with lactational mastitis. *Eur J Clin Exp Med.* 2024;22(1):117–120. doi: 10.15584/ejcem.2024.1.22.

issue is the preservation of lactation, since breast-feeding provides not only harmonious physical and psycho-emotional development of the child, but is also considered an integral part of the reproductive health of a woman.[4,5]

Breast milk is a natural unique biological product that contains a wide range of biologically active substances and protective factors, which include immunoglobulins, lysozyme, lactoferrin, oligosaccharides, immunocompetent cells, as well as cytokines/chemokines (TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-10, IFN-γ, etc.).[6,7] There is an assumption that anti-inflammatory cytokines protect a child's body, and that inflammatory cytokines, especially interleukin-8 (IL-8), play a role in protecting the mammary gland against infection. The focus of inflammation is provided by interleukin-8, which activates adhesion and degranulation of neutrophils, and enhances the phagocytic effect.

Lactoferrin (LF) plays a special role in non-specific protection, has bacteriostatic and bactericidal effects mainly on gram-positive flora, and performs fungicidal, antiviral, immunomodulatory and other functions.[8] Currently, information on the local level of LF and IL-8 in the development of the inflammatory process in the mammary gland is not numerous and requires research.

Aim

To determine the content of lactoferrin and interleukin-8 in the breast milk of patients with lactational mastitis, and to identify new potential markers for assessing the activity of the inflammatory process in the mammary gland.

Material and methods

The selection of women for the examination was carried out in the surgery departments of the Clinical Emergency Medical Hospital of the city of Lviv in Ukraine. Women who gave birth for the first time, and in whom inflammatory process in the mammary gland occurred within three or four weeks after childbirth, participated in the study. The results of the anamnesis, clinical examination, special instrumental studies (ultrasound of mammary glands), and data from bacterial analysis were used to make the diagnosis. The women in this study were chosen based on convenience criteria. Breast milk was collected in the morning on an empty stomach on the first day of the patient's admission to the hospital.

Laboratory investigations were performed between 2016 and 2020 in the diagnostic laboratory of the Department of Clinical Laboratory Diagnostics of Danylo Halytsky Lviv National Medical University, Lviv, Ukraine. Research was performed with safety measures for the health of patients, respecting their rights, human dignity, and moral and ethical norms in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and the relevant Laws of Ukraine. This study was approved by the Ethical Committee of the Danylo Halytsky Lviv National Medical University, Ukraine (meeting minutes No. 2 dated February 15, 2016) and written informed consent was obtained from mothers if they agreed for breast milk sampling.

Breast milk was collected by extracting 5 mL into a sterile test tube. Immediately after sampling, the milk was centrifuged at 2000×*g* for 10 min.[9] From the middle layer of the centrifuged sample (the upper layer contains the supernatant fats), 1 mL of liquid was taken and 500 μL was placed into Eppendorf tubes. LF and IL-8 content in the obtained milk was analyzed by an enzyme-linked immunosorbent assay (ORG 284, Lactoferrin by Organtec; Human IL-8 ELISA Kit by Diaclone) using the STAT FAX 303 plus analyzer according to the manufacturer's guidelines. Statistical processing of the obtained data was based on the method of variation statistics, using the STATISTICA 6.0 (Statsoft, Tulsa, Oklahoma, USA) program.

Results

The study analyzed the breast milk of 97 women aged 18 to 36 (average age: 26±5 years old). Patients were divided into two comparison groups. Group I included 30 women with lactostasis. Group II consisted of 37 women who developed lactational mastitis. The control group included 30 clinically healthy lactating women.

The results of the LF content in breast milk of women with a dysfunctional breast condition are presented in Fig. 1.

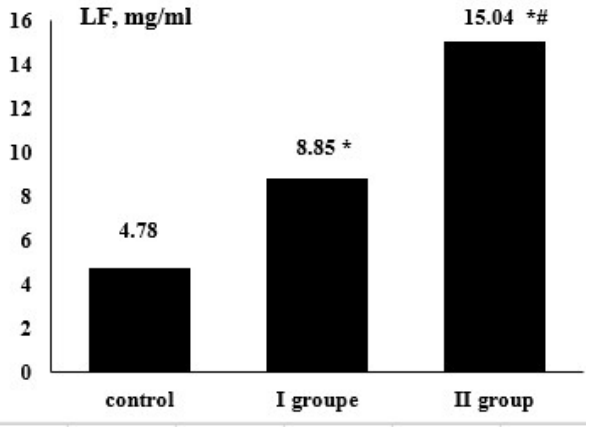

Fig. 1. The content of LF in breast milk of women in groups I and II, (mean), * – probability of difference in indicators compared to the control group ($p<0.05$); # – probability of difference in indicators compared to group I ($p<0.05$).

The average LF value in breast milk of practically healthy women who made up the control group is

4.78±0.47 mg/mL. In group I, the concentration of this marker is 8.85±0.3 mg/mL, which is 1.8 times higher than the control value ($p<0.05$). The level of LF in breast milk of women in group II is 15.04 ±0.53 mg/mL, which exceeds the control values by 3.1 times ($p<0.05$).

The study of breast milk of patients with lactational mastitis indicates changes in IL-8 production. The obtained results are presented in Fig. 2.

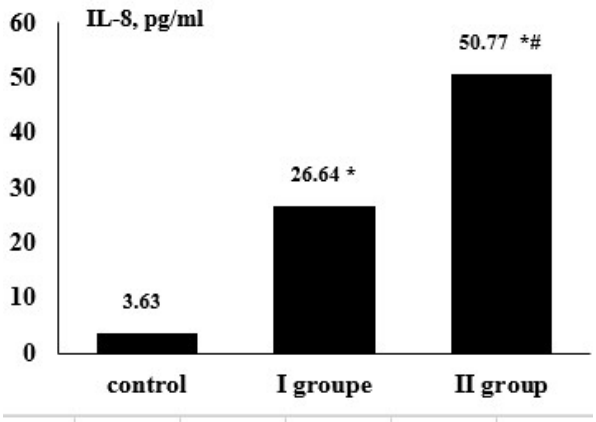

Fig. 2. The content of IL-8 in breast milk of women in groups I and II, (mean), * – probability of difference in indicators compared to the control group ($p<0.05$); # – probability of difference in indicators compared to group I ($p<0.05$)

The content of IL-8 in breast milk of women in group I is 26.64±1.68 pg/mL, which is 7.3 times higher than the control values (3.63±0.12 pg/mL, $p<0.05$). In breast milk of women in group II, the level of IL-8 (50.77±1.58 pg/mL) is 13.9 times higher than in the control group ($p<0.05$). The test group analysis reveals a 1.9-fold increase of IL-8 in breast milk with the development of lactational mastitis as compared to the indicator of women who developed lactostasis ($p<0.05$).

Discussion

Lactoferrin iron-binding glycoprotein, which is synthesized by mucous membrane epithelial cells, is contained in the secondary granules of neutrophils, the secretions of almost all exocrine glands. The highest LF content was found in breast milk, and its expression is controlled by prolactin.[8,10] Early functional changes in the mammary gland in lactostasis are accompanied by an increase in LF expression, which is likely to reveal the protective reaction of epithelial cells. The comparison of the LF content in the test groups reveals an increase in the production of this marker with the development of lactation mastitis by 1.7 times relative to the indicator of women in group I ($p<0.05$). The obtained results provide for the activation of local immunity, indicate pronounced inflammatory changes in the mammary gland.[11]

The non-specific protection of the body from infectious agents is known to activate various mechanisms and factors. As an anti-infective protection first line component, lactoferrin exhibits a bacteriostatic effect by sequestration of free iron. The lack of the necessary substrate inhibits the growth of bacterial colonies. The bactericidal effect of lactoferrin is due to the direct interaction of the protein molecule with the microorganism membrane, which leads to its destruction.[10,12,13]

It is worth noting a key role of lactoferrin in the modulation of the immune response. At the molecular level, in the presence of this glycoprotein, the expression of cytokines changes, in particular, pro-inflammatory interferon-γ, interleukins (IL-1β, IL-8, TNF-α), and the production of IL-5 and IL-10 is reduced. In addition, lactoferrin binds iron, which accumulates in damaged tissues, and catalyzes the formation of toxic hydroxyl radicals, that is, it has an anti-inflammatory effect.[14,15]

IL-8 is produced mainly by endothelial cells, monocytes, and macrophages under the influence of bacterial endotoxins and cytokines (IL-1, TNF-α, IL-6, etc.). IL-8 acts as a chemotactic factor for neutrophils, enhances their adherence to endothelial cells, promotes penetration from the vascular bed into infected tissue.[7,10] The analysis of the test group reveals a 1.9-fold increase in IL-8 levels in breast milk associated with the onset of lactational mastitis, as compared to women who developed lactostasis ($p<0.05$), indicating the involvement of the inflammatory process and indicating destructive processes in the breast parenchyma under the influence of activated neutrophils.[16]

High concentrations of the studied markers in lactostasis indicate the initial stages of the development of the inflammatory process. As a result of the absence of milk outflow, a favorable environment for the reproduction of bacteria is formed. Lactic acid fermentation occurs in milk, which leads to the destruction of the epithelium of the milk ducts and alveoli. When the pressure in the mammary gland increases, blood circulation is disturbed, venous congestion occurs, and this creates additional conditions for the development of infection. Untimely elimination of lactostasis leads to the development of mastitis.[1]

With the development of the inflammatory process, of paramount importance is the migration of neutrophils and other cells from the blood into breast milk through the epithelium of the milk alveoli. In the presence of IL-8, the processes of activation and degranulation of neutrophils occur, they are accompanied by the generation of reactive oxygen species, nitric oxide, the release of lactoferrin, lysozyme into the intercellular medium. Such mechanisms are likely to protect the surrounding tissues from destruction, contribute to the rapid completion of the inflammatory process.[7,8,16]

Conclusion

The study reveals an increase in the production of lactoferrin and interleukin-8 in breast milk of both groups as compared to healthy women. With the development of lactation mastitis, the level of lactoferrin exceeds the control values 3.1-fold, and 1.7-fold in women with lactostasis ($p<0.05$). The mean concentration of IL-8 is 13.3 times higher in the control group and 1.9 times higher in women with lactostasis ($p<0.05$). The established levels of lactoferrin and IL-8 in milk can serve as predictive markers for assessing the degree of inflammation. The results obtained in patients with lactation mastitis indicate the activation of non-specific protection, the ability to counteract the destructive effect of infectious factors.

Declarations

Funding

The study was conducted at the researchers' own expense.

Author contributions

Conceptualization, N.D. and I.H.; Methodology, N.D.; Software, S.T.; Validation, L.L., V.A. and N.D.; Formal Analysis, O.B.; Investigation, N.D.; Resources, N.L..; Data Curation, S.T.; Writing – Original Draft Preparation, V.A. Writing – Review & Editing, S.Z.; Visualization, L.S.; Supervision, L.L.; Project Administration, V.A.; Funding Acquisition, N.D.

Conflicts of interest

Authors declare no conflict of interest.

Data availability

The presented data are available from authors upon request.

Ethics approval

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Danylo Halytski Lviv National Medical University, Ukraine (meeting minutes No. 2 dated February 15, 2016).

References

1. Wilson E, Woodd SL, Benova L. Incidence of and Risk Factors for Lactational Mastitis: A Systematic Review. *J Hum Lact*. 2020;36(4):673-686. doi: 10.1177/0890334420907898
2. Kim SY, Yi DY. Analysis of the human breast milk microbiome and bacterial extracellular vesicles in healthy mothers. *Exp Mol Med*. 2020;52(8):1288-1297. doi: 10.1038/s12276-020-0470-5
3. Castro I, García-Carral C, Furst A, et al. Interactions between human milk oligosaccharides, microbiota and immune factors in milk of women with and without mastitis. *Sci Rep*. 2022;12(1):1367. doi: 10.1038/s41598-022-05250-7
4. Sayres S, Visentin L. Breastfeeding: uncovering barriers and offering solutions. *Curr Opin Pediatr*. 2018;30(4):591-596. doi: 10.1097/MOP.0000000000000647
5. Qiu R, Zhong Y, Hu M, Wu B. Breastfeeding and Reduced Risk of Breast Cancer: A Systematic Review and Meta-Analysis. *Comput Math Methods Med*. 2022;2022:8500910. doi: 10.1155/2022/8500910
6. Rio-Aige K, Azagra-Boronat I, Castell M, et al. The Breast Milk Immunoglobulinome. *Nutrients*. 2021;13(6):1810. doi: 10.3390/nu13061810
7. Ruiz L, Espinosa-Martos I, García-Carral C, et al. What's Normal? Immune Profiling of Human Milk from Healthy Women Living in Different Geographical and Socioeconomic Settings. *Front Immunol*. 2017;8:696. doi: 10.3389/fimmu.2017.00696
8. Hao L, Shan Q, Wei J, Ma F, Sun P. Lactoferrin: Major Physiological Functions and Applications. *Curr Protein Pept Sci*. 2019;20(2):139-144. doi: 10.2174/1389203719666180514150921
9. Moradkhani S, Jafarzadeh A, Bazargan-Harandi N, Baneshi MR, Mohammadi MM. Association of reduced count of interleukin-13-producing cells in breast milk with atopic dermatitis in infancy. *Indian J Med Res*. 2018;148(3):317-322. doi: 10.4103/ijmr.IJMR_1682_16
10. Czosnykowska-Łukacka M, Orczyk-Pawiłowicz M, Broers B, Królak-Olejnik B. Lactoferrin in Human Milk of Prolonged Lactation. *Nutrients*. 2019;11(10):2350. doi: 10.3390/nu11102350
11. Villavicencio A, Rueda MS, Turin CG, Ochoa TJ. Factors affecting lactoferrin concentration in human milk: how much do we know? *Biochem Cell Biol*. 2017;95(1):12-21. doi: 10.1139/bcb-2016-0060
12. Wang B, Timilsena YP, Blanch E, Adhikari B. Lactoferrin: Structure, function, denaturation and digestion. *Crit Rev Food Sci Nutr*. 2019;59(4):580-596. doi: 10.1080/10408398.2017.1381583
13. Avalos-Gómez C, Ramírez-Rico G, Ruiz-Mazón L, Sicairos NL, Serrano-Luna J, de la Garza M. Lactoferrin: An Effective Weapon in the Battle Against Bacterial Infections. *Curr Pharm Des*. 2022;28(40):3243-3260. doi: 10.2174/1381612829666221025153216
14. Dierick M, Vanrompay D, Devriendt B, Cox E. Lactoferrin, a versatile natural antimicrobial glycoprotein that modulates the host's innate immunity. *Biochem Cell Biol*. 2021;99(1):61-65. doi: 10.1139/bcb-2020-0080
15. Demianchuk NR, Belyavska BM, Lapovets LE, Akimova VM. The level of IL-8 in blood serum and breast milk in purulent lactational mastitis. *Med Chemistry*. 2015;1(62):92-94. doi: 10.11603/1681-2557.2015.v17.i1.4098
16. Kiełbasa A, Gadzała-Kopciuch R, Buszewski B. Cytokines--Biogenesis and Their Role in Human Breast Milk and Determination. *Int J Mol Sci*. 2021;22(12):6238. doi: 10.3390/ijms22126238