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Exploring the Potential of Lactoferrin in Neonatology and Obstetrics - Promising Advancements for Maternal and Infant Health

Kinga Bialic [1] bialic.kin@gmail.com ; https://orcid.org/0009-0009-4029-5919
Artur Bialic [1] abialic@op.pl ; https://orcid.org/0009-0008-9148-8801
Rafał Bakalarczyk [2] rmbak8@gmail.com ; https://orcid.org/0009-0008-8788-8503
Paweł Majewski [2] pawmaj7@interia.pl ; https://orcid.org/0009-0000-4624-3129
Lucjan Bednarz [3] lfbednarz@gmail.com ; https://orcid.org/0009-0001-3213-3508
Karolina Szala-Czerwonka [3] k.szala97@gmail.com ; https://orcid.org/0009-0001-8545-9237
Natalia Woś [3] natalia5wos@gmail.com ; https://orcid.org/0009-0002-9212-2664
Adrian Rejmer [4] ziomek00718@gmail.com ; https://orcid.org/0000-0002-1248-4941
Katarzyna Rojek [5] katarzyna1rojek@gmail.com ; https://orcid.org/0009-0004-3691-3669
Karolina Wijas [6] k.wijas21@gmail.com ; https://orcid.org/0009-0000-7776-8446

1. Kliniczny Szpital Wojewódzki Nr 2 im. Św. Jadwigi Królowej w Rzeszowie, ul. Lwowska 60, 35-301 Rzeszów, Poland
2. Wojewódzki Szpital Specjalistyczny im. Stefana Kardynała Wyszyńskiego Samodzielny Publiczny Zakład Opieki Zdrowotnej w Lublinie, Al. Kraśnicka 100, 20-718 Lublin, Poland
3. Uniwersytecki Szpital Kliniczny im. Fryderyka Chopina w Rzeszowie, ul. Szopena 2, 35-055 Rzeszów, Poland
4. Samodzielny Publiczny Zespół Zakładów Opieki Zdrowotnej w Kozienicach, Al. Gen. Wł. Sikorskiego 10 26-900 Kozienice, Poland

5. Samodzielny Publiczny Szpital Kliniczny Nr 4 w Lublinie, ul. Doktora Kazimierza Jaczewskiego 8, 20-954 Lublin, Poland
6. Samodzielny Publiczny Zakład Opieki Zdrowotnej w Świdniku, ul. Aleja Lotników Polskich 18, 21-040 Świdnik, Poland

Abstract:

Introduction and purpose:

Lactoferrin (Lf), a glycoprotein found in body fluids, plays a key role in many of the biological mechanisms that occur in the human body. Over the years, more and more studies have contributed to the expansion of the clinical applications of lactoferrin. In this article, we want to focus on using lactoferrin in Neonatology and Obstetrics. The applications of lactoferrin in obstetrics continue to expand, some of which include the prevention of preterm delivery (PTD) and anaemia in pregnant women. Lf also helps to reduce the incidence of necrotizing enterocolitis and sepsis in newborns by mobilising the immune system and participating in the defence against pathogens and has been under investigation as a potential therapeutic agent for genital infections. The aim of this review is to summarise the current state of knowledge, regarding lactoferrin's broad spectrum of action and to highlight its potential therapeutic applications in these contexts.

State of knowledge:

This article aims to review the current knowledge on the properties of the lactoferrin and its applications in medicine, especially neonatology and obstetrics. Studies available in the PubMed and Google Scholar databases were included in the analysis.

Conclusion:

While lactoferrin shows promise in improving neonatal and maternal health, additional research is required to fully understand its precise mechanisms of action and optimise its use as a therapeutic agent. There is also a need for further studies to assess the safety and efficacy of lactoferrin in a variety of patient populations. Despite the challenges, lactoferrin continues to be an intriguing subject of scientific research, with potential therapeutic benefits in various medical fields.

Keywords: lactoferrin, pregnancy, neonatology, inflammation, preterm delivery, iron deficiency anaemia

Introduction and purpose:

Lactoferrin (Lf) is one of the iron-binding glycoproteins belonging to the transferrin group. It has a molecular weight of approximately 80 kDa and is a key element in host defence against infection. It is one of the most important bio activators, produced by glandular epithelial cells in mammals, found in mammalian milk, as well as in many other biological fluids, including tears, vaginal secretions and neutrophil granules – in places where the body needs to be protected from outer threats. [1, 2, 3] Lactoferrin levels in milk vary at different stages of lactation. The highest concentrations of this protein are observed in colostrum (reaching up to seven times higher concentrations, about 7 g/L, than in later milk) and follow-on milk. [1]

Among its biological roles are the regulation of iron absorption, modulation of the immune response, microbiostatic and microbicidal activity against a broad spectrum of pathogens. [4, 5]. Bovine lactoferrin has a high degree of sequence homology with human lactoferrin and is found to function in the same way. Its ability to bind iron inhibits bacterial growth and biofilm formation, preventing microbial adhesion and penetration into host cells. [2,6] It also interferes with gram-negative bacteria proliferation by damaging the cell wall and releasing lipopolysaccharide. Furthermore, lactoferrin may enhance lysozyme's antimicrobial effect as a consequence of causing damage to the bacterial outer membrane and cell wall. [7]

Lactoferrin is capable of binding two ferric (Fe^{3+}) ions in a reversible way and has a much higher affinity for those ions than transferrin. It makes Lf one of the key modulators of iron homeostasis. [1] Lf binds iron ions strongly. It is also able to interact with two copper ions, zinc ions and manganese ions, but with a lower affinity. [8] This makes it a natural chelating agent that protects against the harmful effects of free iron. Too much iron may cause toxicity by producing reactive oxygen species such as superoxide anions. That precludes cellular membrane lipids from the destructive aspect of oxidative stress. Moreover, the binding of L-selectin to lipopolysaccharide is also intruded which prevents L-selectin-mediated neutrophil activation. [5, 8]

Furthermore, immunostimulation, the regulation of the body's immune response, is the most important role of lactoferrin in the body. Lactoferrin traps and binds iron in the body, preventing bacteria from accessing the metal ions they need for growth and development. What is more, recent studies have revealed a more direct way in which lactoferrin works. The N-terminal fragment of the protein has a strong positive charge. This allows lactoferrin to interact with and damage the bacterial cell wall, causing intracellular components to leak out and ultimately kill the bacteria. [1, 2, 5, 9]

Given its bioactive properties, lactoferrin has been investigated as a promising therapeutic in various clinical fields, including neonatology, genital infections and pregnancy.

Description of state of knowledge:

Potential use of lactoferrin in neonatology

The lactoferrin provided with milk is a key line of defence for the newborn's body against pathogenic microorganisms. The low efficiency of digestive enzymes, the alkaline pH of the stomach and the weakness of the intestinal barrier cause that much of this protein is absorbed into the bloodstream. Such absorption has not been observed in the adult population. [7, 10]

As early as the 1970s, human milk was shown to inhibit E. coli growth. [11] In human milk, the majority of iron is found in the complexes of the lactoferrin protein. Lactoferrin has a high iron-binding capacity, so the iron-saturated content of this protein in milk is less than 10%. This suggests that unsaturated Lf may be avidly binding iron, making it inaccessible to bacteria. [12]

Studies have shown that lactoferrin may protect against late-onset sepsis and necrotising enterocolitis in premature babies when added to enteral nutrition. Premature and low-birth-weight infants are at greater risk of developing sepsis as the use of formula milk does not ensure a sufficiently high concentration of lactoferrin. [13] Furthermore, formula milk is enriched with free iron and lactoferrin with an unknown level of iron saturation. As a result of this supplementation and the failure to ensure adequate protein concentration, newborns are exposed to a large amount of free iron, which becomes an ideal nutrient for bacteria. [7, 10]

Studies have also shown that activity of Myeloid-Derived Suppressor Cells (MDSCs), strongly immunosuppressive neutrophils and monocytes, increases due to lactoferrin. In mice, it was effective in the prevention of necrotising enterocolitis. [8, 14]

Between 4 and 9% of the amount of lactoferrin consumed in food was found in the stool samples of children who were breastfed. This amount has been shown in in vitro studies to be bacteriostatic and to have a positive effect on cell proliferation and differentiation. [12] In addition to lactoferrin, breast milk also contains lactoperoxidase, sIgA and lysozyme. [15] This help to create a bacteriostatic environment in the intestine of the infant. Such an environment does not allow pathogenic bacteria to overgrow and helps to eliminate unfriendly microorganisms from the small intestine without causing inflammation. Conditions such as these are conducive to the development of a healthy microbiome. [12]

Lactoferrin also plays an important role in modulating the immune response. Immune cells with receptors for Lf include macrophages, dendritic cells and lymphocytes. It is one of the inducers of dendritic cell maturation and increases the synthesis of immune system signalling proteins such as caspase-1 and IL-18. Studies have shown an increase in CD4, CD8 and NK cells in the lamina propria of the small intestine after oral administration of lactoferrin. [12, 16]

In 2012, Ochoa et al. published a review study in which they looked at 19 clinical trials that had been conducted in a population of children. These trials were designed to investigate the benefits of supplementing infants' diets with either human (hLf) or bovine lactoferrin (bLf). The trials showed varying degrees of the efficacy of such supplementation, but all emphasised the safety of its use. In a double-blind, randomised trial involving 79 infants, either a standard formula containing 102 mg/l of Lf or a formula fortified with 850 mg/l of Lf was given to babies from the age of 4 weeks to the age of 12 months. This study showed significantly higher haematocrit values at nine months of age in the group fed the fortified formula. A lower rate of lower respiratory tract disease was also observed compared with the standard diet group. [17]

Another study, conducted in China, looked at the effect of adding a small amount of Lf, 38 mg per 100 g of mixed formula. The study included 260 infants between the ages of 4 and 6 months. The results show a positive effect on iron metabolism parameters, including

transferrin, haemoglobin and soluble transferrin receptor, after three months of lactoferrin supplementation. [18]

A study of premature infants was also included. This tested the effect of oral supplementation with 100 mg of bovine lactoferrin alone or with a probiotic Lactobacillus strain, started on day 3 of life, on the risk of late-onset sepsis in very-low-birth-weight neonates. A significantly lower risk of sepsis was observed in infants receiving lactoferrin supplementation, 5.9% and 4.6% of infants, respectively, compared with 17.3% in the control group. This evidence is still considered to be weak, and further studies are necessary for confirmation of the above phenomena. [19]

Potential use of lactoferrin in pregnancy

Vaginal microbiota dysbiosis

Over the past year, research has demonstrated the potential of lactoferrin for the treatment and prevention of women's reproductive infections.

Inflammation of the reproductive tract in women is a common condition caused by bacteria and the most common symptoms are pain, bleeding and discharge from the genital tract. Traditional treatment involves using antibiotics, but alternative treatments are being sought due to the increased risk of bacterial resistance to antibiotics and side effects. [20]

A complex interplay between healthy microbiota and immune system cells and proteins is responsible for the defence of the genitourinary system. An important role in this defence system is played by vaginal lactobacilli and lactoferrin. [20] Lactobacilli are the predominant bacterial species in the vagina and play a crucial role in maintaining a healthy balance of microorganisms. They prevent the growth of harmful bacteria by competing for nutrients and adhering to the vaginal epithelium, as well as by reducing the vaginal pH by producing lactic acid, producing bioactive substances like bacteriocins and hydrogen peroxide, and regulating the local immune system. [21]

Lactoferrin is also one of the compounds found in cervicovaginal mucosa. With its microbicidal properties, it is capable of modulating inflammation. In response to microbial infections, the levels of vaginal lactoferrin and other antimicrobial peptides can change.

Research has shown that the levels of lactoferrin and defensins in the genital secretions of women with bacterial vaginosis, Trichomonas vaginalis, Candida spp., Chlamydia trachomatis, and Neisseria gonorrhoeae infections are elevated compared to healthy women. [22]

Female hormones and cytokine production affect both lactobacilli and lactoferrin.[23] When there is a microbial imbalance in vaginal mucosa, with lactobacilli reduced and anaerobic bacteria increased, lactoferrin can act as an immune modulator to restore balance taking on the role typically performed by the normal microbiota. Lactobacilli and lactoferrin can therefore serve as biomarkers of vaginal microbial dysbiosis, and using them directly in the vagina could be a potential therapeutic strategy to re-establish mucosal immune homeostasis in cases of recurrent and antibiotic-resistant bacterial infections. [20]

The results of research conducted by Bertuccini L. et al. showed that oral administration of a mixture of lactoferrin and lactobacilli resulted in substantial colonisation of the vagina by L. acidophilus GLA-14 and L. rhamnosus HN00. This colonisation led to the resolution of symptoms of vaginal microbiota dysfunction and normalisation of the Nugent score test results. [24]

Both hLf and bLf have antibacterial activities by sequestering iron, which is necessary for bacterial growth, and by inhibiting bacterial adhesion to the epithelial cells. Bovine lactoferrin can inhibit Chlamydia trachomatis infectivity by interacting with cell surface glycosaminoglycans and heparan sulphate proteoglycans, which are potential receptors for C. trachomatis adhesion. [20]

In vitro experiments, that mimic in vivo chlamydia infection showed that bLf added to infected cells resulted in an important reduction in interleukin-6 (IL-6) and interleukin-8 (IL-8), but did not affect the number of intracellular Chlamydia. [25]

The other research involved 7 of 176 pregnant women who tested positive for *C. trachomatis.* They were given 100 mg of bovine lactoferrin vaginally every 8 hours for 30 days. Notably, after one month, six of them got negative test results for C. trachomatis and had considerably decreased levels of IL-6 in their cervical-vaginal fluid. [20, 25] Lactoferrin seems to act as a protective shield that prevents early infection and safeguards host cells – it prevents Chlamydia's elementary bodies (EBs) from invading the mucosal cells of the host. [20]

In addition, the other study, in which sixty women with bacterial vaginosis were divided into two groups that received lactoferrin pessaries for 10 days, showed that administration of both 100 mg and 200 mg lactoferrin vaginal pessaries to women with bacterial vaginosis altered the composition of the vaginal microbiota by decreasing the abundance of BV-associated genera, like G*ardnerella*, *Prevotella*, *Lachnospira*, and increasing the abundance of Lactobacillus species. Furthermore, only women in the 200 mg pessary group maintained the microbiota balance for up to 2 weeks after therapy. [26] Further investigation is needed to elucidate the molecular mechanisms and immunological changes accompanying Lf's effects on the microbiota.

Iron deficiency anaemia

Anaemia is a common complication of pregnancy that, uncontrolled, can lead to serious health consequences for both mother and baby. Iron deficiency is a common problem, particularly among pregnant women in developing countries, where up to 52% of them suffer from iron deficiency anaemia (IDA). [27] The deficiency of iron during pregnancy can have adverse effects on both the health of expectant mothers and the well-being of their infants, elevating the chances of complications during childbirth and pregnancy, including preterm birth, low birth weight, intrauterine growth retardation and caesarean section. It also heightens the risk of perinatal infections, postpartum haemorrhage, and pre-eclampsia. Furthermore, IDA might lead to neurological disorders in the infant, causing neurobehavioral deficits. [28, 29] In addition, there is a higher risk of anaemia and increased susceptibility to infection in the offspring of mothers who have iron deficiency anaemia during pregnancy. [4]

Throughout pregnancy, the demand for iron rises as a result of foetal and placental growth, elevated haemoglobin levels, and an increase in uterine muscle mass. [4] Lactoferrin, a protein that binds to iron, can assist in averting iron deficiency and safeguarding against oxidative stress by regulating iron levels. Studies have shown that supplementation with Lafergin®, which contains lactoferrin, Ferric Sodium EDTA, Vitamin C and Vitamin B_{12}, may elevate levels of haemoglobin and ferritin, as well as promote higher birth weights in infants and extend the duration of pregnancy by an average of 1.5 weeks. [30] Non-haem iron supplements have poor bioavailability and are associated with adverse effects, especially in

pregnant women, such as nausea, diarrhoea, vomiting. Research suggests that lactoferrin is better tolerated than iron salts, with fewer side effects. [31]

Comparable results have been observed in studies of pregnant women who received either bLf or ferrous sulphate, where bLf improved haematological measures and stabilised iron homeostasis by reducing serum IL-6 levels and increasing the synthesis of prohepcidin. Iron sulphate, on the other hand, increased IL-6 levels and decreased prohepcidin levels without increasing haematological parameters. The efficacy of lactoferrin in the treatment of anaemia was also supported by another study which demonstrated its ability to reduce IL-6 and prohepcidin levels, restore iron transport from cells to the bloodstream and improve haematological parameters. [31]

In another study lactoferrin's efficacy in treating anaemia was also validated. Lactoferrin's reduction of IL-6 and hepcidin concentrations facilitated the restoration of ferroportin-mediated cell-to-blood iron transport, thereby improving haematological indices. [32]

Inflammation and the risk of preterm delivery

Inflammation is a natural part of pregnancy, but excessive inflammation during pregnancy can lead to negative outcomes. Interleukin-6 (IL-6) plays a key role in inflammation. Lactoferrin, a potent anti-inflammatory molecule, has been shown to regulate levels of pro- and anti-inflammatory mediators, potentially improving clinical outcomes and prolonging pregnancy. Studies have demonstrated that lactoferrin administration can reduce IL-6 concentrations and prostaglandin concentrations, leading to a decrease in cervicovaginal IL-6 levels and an increase in cervical length. Additionally, lactoferrin has been shown to downregulate pro-inflammatory cytokines and upregulate anti-inflammatory cytokines in amniotic fluid. Studies suggest that lactoferrin may have a therapeutic role in reducing inflammation and improving outcomes in pregnancy. [4, 33, 34, 35]

A study conducted by Locci M et al. in 2013 aimed to determine the effect of lactoferrin in reducing the risk of preterm labour in women with the shortened cervix and no symptoms. The study included 128 women with borderline cervical shortening (25 to 29 mm) and high levels of IL-6 in their vaginal fluids, divided into two groups. One group was given 300 mg of vaginal lactoferrin for 21 days, while the other served as a control group. The outcome of

the study revealed a reduction in IL-6 levels and prolonged cervical length in the group that received lactoferrin compared with controls. [4, 33]

In the other study, provided at Al Zahra University Hospital at the Department of Obstetrics and Gynecology, 50 women at the gestational age between 28 and 34 weeks with present sterile inflammation and singleton pregnancies were given 100 mg oral bLf. The blood samples were taken to assess the levels of IL-6 before and after the administration of bLf. [36]

Some studies have shown that the process of cervical ripening involves an inflammatory response, which is linked to increased levels of IL-6. Administering bLf orally to pregnant women with PTD threat has been found to have anti-inflammatory effects. By reducing serum IL-6 levels, which stimulate prostaglandin F2α, a significant contributor to uterine contraction, bLf can prolong pregnancy until corticosteroid administration become feasible. [4, 33, 34, 36]

Giunta G. et al. also conducted a prospective trial to evaluate the effectiveness of Lf in preventing preterm delivery. The study involved 21 pregnant women with iron deficiency anaemia, at the gestational age between 22 and 36 weeks. Fourteen of these women were administered 100 mg of recombinant human lactoferrin (rhLf) twice daily for one month, while the remaining seven received 520 mg of ferrous sulphate once daily. The researchers collected cervicovaginal fluid samples to measure IL-6 levels and evaluated cervical length and funnelling. The findings indicated that oral administration of recombinant human lactoferrin was linked to enhanced vaginal flora and reduced IL-6 levels in the cervicovaginal fluid of expectant mothers who were at risk of preterm delivery. [37]

Another clinical trial was performed on the group of 60 women undergoing genetic amniocentesis. Researchers aimed to assess the effect of intravaginal Lf on amniotic fluid cytokine, chemokine and growth factor concentrations. They were divided into three equal groups, two of them received 300 mg of vaginal lactoferrin 4 and 12 hours before amniocentesis, and one was untreated. The results revealed that lactoferrin had an impact on 24 out of 47 mediators tested, leading to a reduction in 17 pro-inflammatory mediators and an increase in 7 anti-inflammatory mediators in amniotic fluid. [38]

Summary:

Prematurity and obstetric complications pose significant risks to maternal and infant health. Lactoferrin, a naturally occurring protein found in various human secretions, has emerged as a promising therapeutic agent in this regard. This protein has been shown to possess immunomodulatory and antimicrobial properties, making it a potential candidate for preventing or managing infections and inflammation associated with prematurity and obstetric complications. Supplementing preterm infants with Lf has been discovered to enhance the development of the gut and the absorption of nutrients, leading to a decrease in the occurrence of sepsis and necrotizing enterocolitis. In iron deficiency anaemia supplementation of Lf has been shown to increase iron absorption and improve haemoglobin levels. It has also been studied as a potential preventive measure for preterm delivery due to its anti-inflammatory and immunomodulatory effects. However, the mechanisms of action and optimal dosing of lactoferrin in these contexts require further study. Overall, lactoferrin represents a promising way to improve the treatment for neonates and pregnant women.

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