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Case Report

Obstetrics and Gynaecology Section

Lactoferrin as a New Alternative for Prevention of Recurrent Preterm Delivery: A Case Report

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ABSTRACT

The upper female genital tract connects to the external world through the vaginal canal and can be a potential route of entry to pathogens into the reproductive organs. Lactobacilli exert their protective action by reducing the vaginal pH by producing lactic acid by degrading glycogen released by epithelial cells. Whenever a breach in this intricate defence mechanism or its balance is disturbed, the female genital tract becomes vulnerable to infections. A 30-year-old female reported with complaints of abdominal pain and vaginal discharge with poor obstetrical history and had three consecutive Preterm Premature Rupture of Membranes (PPROM) at the 20th, 22nd, and 25th week of gestation. All three pregnancies were natural conceptions. Three consecutive miscarriages were presumed to be due to recurrent vaginitis, cervical inflammation, and chorioamnionitis. The vaginal discharge culture examination was negative for Lactobacilli and positive for gram-positive bacteria. The patient was advised topical antibiotics, oral probiotics containing Lactobacilli species, and prebiotic Lactoferrin 100 mg. In a few weeks, Lactobacillus predominant vaginal flora was observed. During pregnancy, she used Lactoferrin and Lactobacillus combination orally until delivery. Cervical maturation was not observed before the term in this gestation. Lactobacillus had been the dominant vaginal flora during pregnancy, and the course of the pregnancy was good. She delivered vaginally at term a healthy male infant. Aetiopathogenesis of preterm delivery in humans has been hypothesised to be triggered by the inflammatory response caused by intrauterine infections. Lactoferrin inhibits the production of inflammatory cytokines and significantly prevents preterm delivery induced by infection.

Keywords: Antimicrobial activity, Intrauterine infections, Lactobacillus, Pregnancy, Vaginal flora

CASE REPORT

A 30-year-old, female patient reported with complaints of lower abdominal pain, cramping type not radiating to other parts, and vaginal discharge for two weeks. She had poor obstetrical history, and had three consecutive PPROMs at the 20th, 22nd, and 25th week of gestation [Table/Fig-1]. All three pregnancies were natural conceptions. During the first pregnancy, she had lower abdominal pain and leaking per vaginum for two days at about the 20th week of gestation, not seek any medical care, she miscarried after PPROM at 21 weeks of gestation.

Gravida	Cervical cerclage	PPROM
First	Not done	20th week
Second	Done	22nd week
Third	Done	25th week

[Table/Fig-1]: Three consecutive pregnancies with premature preterm rupture of membranes.

During the second pregnancy, at about the 10th week of gestation, she complained of bleeding per vaginum. She had a live foetus on sonography and was advised of bed rest. Vaginal discharge culture was also done. The culture was positive for Group B Streptococcus (GBS). She was prescribed parenteral antibiotics like 3rd generation cephalosporin (cefotaxime 1 gm) and metronidazole 500 mg for 14 days and after three weeks, Mc Donald's cervical cerclage was done. Around the 20th week of gestation, the vaginal swab culture was again positive for the same gram-positive bacteria. On sonography, cervical length was observed to be less than 2 cm. Blood neutrophil counts were raised along with C-reactive protein levels. She was given another course of parenteral antibiotics. In spite of active management, she had preterm PROM and underwent preterm delivery a week later, followed by early neonatal death.

Eight months later, in the subsequent pregnancy, the vaginal discharge culture was still positive for gram-positive bacteria. A course of parenteral antibiotics such as 3rd generation cephalosporin (cefotaxime 1 gm) and metronidazole 500 mg for 14 days were repeated at 12 weeks of gestation in the third pregnancy, and cervical cerclage was placed. Six weeks later, cervical length shortening was observed on sonography, and vaginal discharge culture was positive for gram-positive bacteria. She was advised a course of topical antibiotics such as clindamycin 100 mg and clotrimazole 100 mg (Clingen) vaginal pessary for seven days. She had a bout of fever at around 25 weeks of gestation and had preterm PROM. It was followed by preterm delivery followed by early neonatal death.

Three consecutive miscarriages were presumed to be due to recurrent vaginitis, cervical inflammation and chorioamnionitis. The vaginal discharge culture examination was negative for Lactobacilli and positive for gram-positive bacteria. The patient was advised topical antibiotics such as clindamycin 100 mg and clotrimazole 100 mg (Clingen) vaginal pessary and oral probiotics containing Lactobacilli species (manufactured by Life Space) and prebiotic Lactoferrin 100 mg and disodium guanosine 5 monophosphate 10 mg given orally for three months. In a few weeks, Lactobacillus predominant vaginal flora was observed. Lactobacillus gradually became dominant, and the patient achieved pregnancy three months later. She continued to use Lactoferrin. At the 13th week of gestation, cerclage was placed. During pregnancy, she used Lactoferrin and Lactobacillus combination orally until delivery. Cervical maturation was not observed before the term in this gestation. Lactobacillus had been the dominant vaginal flora during pregnancy, and the course of the pregnancy was good. She delivered vaginally at term, a healthy male infant. There were no foetal abnormalities.

DISCUSSION

Nature has provided defence against this invasion of harmful bacteria into the female genital tract by heavily colonising the epithelial mucosa of the vaginal by commensal micro-organisms, dominated by Lactobacillus species [1]. These commensal micro-organisms interact with epithelial cells, local macrophages, and proteins to defend against infections and other inflammatory processes. Lactobacilli exert their protective action by reducing the vaginal pH by producing lactic acid by degrading glycogen released by epithelial cells [1]. Change in vaginal microbiota for any reason leads to a reduction of lactobacilli and results in a clinical pathological condition called bacterial vaginosis [2].

Leitich H et al., concluded that this sensitive balance of vaginal commensals is disturbed, the female genital tract becomes susceptible to infections, and the woman's risk of suffering from pelvic inflammatory disease and tubal infertility increases [3]. In addition, the incidence of obstetric complications of late miscarriage and premature birth may increase.

Role of lactobacilli and lactoferrin in the mucosal cervicovaginal defence: Vaginal lactobacilli and Lactoferrin are the main pillars of the vaginal ecosystem [1]. Lactobacilli are the dominant bacterial species in the vagina of the adult female and restrict the growth of facultative and obligate anaerobes in the vagina, thus maintaining healthy microbial homeostasis. In this ecosystem, complex mechanisms underlie the protection provided by the dominant number of lactobacilli, like as a reduction of the pH to less than 4.5 and adherence to the epithelial cell membrane, thereby do not allow the competing pathogenic bacteria space to attach to the vaginal wall and nutrients to feed on [4].

Lactoferrin belongs to the transferrin family and is a multifunctional glycoprotein of about 690 amino acids and an MW of 80kDa. It comprises 1-4 glycan, but it depends upon the species [5]. Lactoferrin of bovine and human origin have similar amino acid sequences. It is one of the most important defence proteins of cervicovaginal flora. It is an iron-binding cationic glycoprotein with antibacterial, antifungal, antiviral, and antiparasitic activities. It also has immunomodulatory properties [6].

Antimicrobial activity: Lactoferrin exerts antimicrobial activity against many different pathogens, predominantly in the cervical mucosa [7]. Lactoferrin's antibacterial activity is attributable to a variety of modes of action. It primarily conceals free iron, removing a vital substrate for bacterial growth and exerting a bacteriostatic effect. It prevents bacterial metabolism from using iron [7].

In addition, it exerts antibacterial activity by binding to the lipopolysaccharide bacterial cell membrane and causes its lysis and inhibits bacterial adhesion to the epithelial cells of the vaginal wall, thereby inhibiting bacterial entry into host cells by competitively binding to host cells and/or to microbial surface [8].

Lactoferrin is present in a variety of secretions in the body and is mainly secreted by exocrine glands and many mucosal epithelial cells and also released by neutrophils during inflammation. In particular, the concentration of Lactoferrin in human vaginal fluid corresponds to 1-3 μg/mL, while it is quite high (100 μg/mL) in the cervical mucus plug [1]. A total number of 106 neutrophils release 15 μg of human Lactoferrin in sites of inflammation and infection [9].

Immunomodulatory: Similar to lactobacilli, bovine Lactoferrin affects the host immune system. It may inhibit inflammation and sometimes promotes both natural and adaptive immune responses. It acts in reducing Tumour Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1), and IL-6 production by immune cells (macrophages, neutrophils, and lymphocytes), as well as IL-8 release by endothelial cells [6].

Bovine Lactoferrin can alternatively be used to treat inflammation by ranging from proinflammatory macrophagic phenotypes M1 to regulatory/anti-inflammatory M2 phenotypes [6].

Antioxidant: Lactoferrin regulates the physiological balance of pro and antioxidants and hence helps protect the cells from oxidative stress and injury. Many researchers have proven that Lactoferrin is capable of modulating the adaptive immune response and that it has a crucial role in the uptake of vital antioxidant enzymes in the cells [6].

Clinical applications of Lactoferrin: Oral Lactoferrin can be of health benefit to the host. It is not considered to be absolutely necessary for mammalian life, and hence it is not classified as a vitamin but as a nutraceutical [10].

Pregnancy and preterm labour: According to report, pregnant women's mucus has higher lactoferrin concentrations than non-pregnant women's mucus [7]. This finding is in line with the protein's local function in mucosal immunity. Both preterm and term gestations experience markedly increased lactoferrin levels in the presence of intrauterine infection.

By regulating microbial development and the inflammatory response, protein is hypothesised to contribute to the host's defence against intrauterine infection. Due to its anti-inflammatory properties, particularly against the IL-6 axis, Lactoferrin has been shown to be a viable candidate in the treatment of preterm birth [7].

In the present case report, we had given prebiotic Lactoferrin 100 mg and disodium guanosine 5 monophosphate 10 mg given orally for three months before pregnancy and continued throughout pregnancy till delivery.

Talbert JA et al., conducted a clinical experiment, Lactoferrin was administered both orally and intravaginally to treat premature birth that was unrelated to cervicovaginal infection [7]. By reducing the main inducers of uterine contractions and membrane ruptures, Prostaglandin F2 (PGF2) and cervicovaginal IL-6, lactoferrin has been demonstrated to be an effective medication for preventing preterm delivery.

In vaginal dysbiosis, when the number of dominant lactobacilli is decreased, and endogenous anaerobic bacteria become dominant, the levels of Lactoferrin are increased and could act as an immune modulator in place of normally dominant lactobacilli. Vaginal dysbiosis due to acute infections can be treated by topical antibiotics, but chronic infections are difficult to eradicate by similar therapies and usually require a prolonged course [11].

Lactoferrin for preventing Intrauterine Growth Restriction (IUGR)/ premature delivery and associated brain injury: Aetiopathogenesis of preterm delivery in humans has been hypothesised to be triggered by the inflammatory response caused by intrauterine infections. It consists of various cytokines like IL-1β, IL-6, and TNF-α, which induce the production of cyclooxygenase-2, which in turn accelerates the production of PG E2 and F2α, causing the premature ripening of the cervical, uterine canal and causing the onset of the labour, leading to preterm delivery [12].

The proinflammatory profile observed in pregnancies and babies exacerbates CNS damage and poor neurodevelopmental outcomes caused by preterm delivery. Maternal infection, placental insufficiency leading to IUGR and sepsis, and Necrotising Enterocolitis (NEC) are all common in preterm deliveries. Because their antioxidant mechanisms are not fully matured and their brains have large amounts of free iron, preterm babies are also susceptible to brain injury due to disparity between the formation and scavenging of oxidative species. In this context, dietary interventions could be useful therapeutic tool for preterm infants, not only because of their direct effects in reducing preterm delivery, but also because of their potential to decrease upcoming direct brain damage and co-morbidities. Lactoferrin, a physiological compound produced by exocrine glands, is found in high concentrations in colostrum and maternal milk and performs a variety of biological

functions, including iron chelation, anti-inflammatory agents, immunomodulators, and antioxidants, and plays an important role in host-defense mechanisms. Lactoferrin, due to its iron-binding abilities, can exist in the Apo (iron-free) state or the holo state when saturated with iron, with a mixture of both observed in milk. This differential is significant because apo-lactoferrin may easily chelate iron, inhibiting bacterial development, whereas holo-lactoferrin is more effective at treating iron shortage [13].

CONCLUSION(S)

Preterm pregnant women were more likely to experience bacterial vaginosis than term pregnant women. Bacterial vaginosis, early in pregnancy, is a strong risk factor for preterm delivery and spontaneous abortion. Lactoferrin inhibits the production of inflammatory cytokines and plays a significant role in the prevention of preterm delivery induced by infection.

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