

Efficacy of Lactoferrin in Prevention of Premature Rupture of Membrane

Ali El-Shabrawy Ali, Manar Abdelhafeez Fathi*, Ahmed Ismail Mohammed

Department of Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt.

*Corresponding Author: Manar Abdelhafeez Fathi, Mobile: (+20)01002770634, Email: malikamahmoud21@gmail.com

ABSTRACT

Background: Prematurity is a leading cause of maternal and neonatal mortality and morbidity, complicating up to 10% of all pregnancies. Mortality and morbidities, including respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and sepsis, are inversely associated with gestational age at birth. **Objective:** This study aimed to decrease the morbidities and mortalities resulting from premature rupture of membrane (PROM).

Patients and Methods: This was a cohort study, which was conducted at Obstetrics and Gynecology Department, Faculty of Medicine, Zagazig University Hospital. Patients were divided into two groups; Group A: 24 patients received 100 mg of recombinant human lactoferrin (RHLF) twice a day before meals. Group B: 24 patients received placebo medicine. All patients were subjected to detailed history taking, general examination. Obstetric transvaginal ultrasound had been performed to evaluate cervical length and funneling.

Results: Mode of delivery in group (A) in 14 women (58.3%) was normal vaginal delivery (NVD) and in 10 (41.7%) was cesarean section (CS) while in group (B) 11 women (45.8%) was NVD and in 13 (54.2%) was CS. There was no statistically significant difference between groups where $P=0.564$. Lactoferrin had sensitivity of 62.5 with specificity 56.2 and positive and negative predictive value of 41.7 and 75 respectively, while the area under curve was 0.594 with p value of 0.222.

Conclusion: Supplementation with lactoferrin may be an option to reduce the risk of premature rupture of membrane.

Keywords: Lactoferrin, Premature, PROM.

INTRODUCTION

Premature rupture of membrane (PROM) refers to the disruption of fetal membranes before the beginning of labor, resulting in spontaneous leakage of amniotic fluid. PROM, which occurs prior to 37 weeks of gestation, is defined as preterm PROM while PROM that occurs after 37 weeks gestation is defined as term PROM⁽¹⁾. PROM occurs in approximately 5%–10% of all pregnancies, of which approximately 80% occur at term⁽²⁾. There are numerous risk factors for PROM, such as intrauterine infection at early gestational age, lower socioeconomic status of pregnant women, inadequate prenatal care and inadequate nutrition during pregnancy, sexually transmitted infections, vaginal bleeding, and smoking during pregnancy⁽³⁾.

PROM is linked to significant maternal and fetal morbidity and mortality. It has been shown to be the cause of 18%–20% and 21.4% of prenatal mortalities and morbidity respectively⁽⁴⁾. Complications of PROM for the fetus and newborn consist of prematurity, fetal distress, cord compression, deformation and altered pulmonary development leading to pulmonary hypoplasia and pulmonary hypertension, necrotizing enterocolitis (NEC) and neurologic disorder⁽⁵⁾. Infectious morbidities in mother, fetus and newborn have been related to both PROM and prolonged rupture of membranes⁽⁶⁾.

Maternal complications include intra-amniotic infection, which occurs in 13%–60% of women with PROM, placental abruption, and postpartum endometritis⁽⁷⁾.

Lactoferrin (Lf) is an approximately 80-kDa iron-binding glycoprotein, belonging to the transferrin family, with well-known bacteriostatic and bactericidal properties, that plays a significant role in iron homeostasis. Lf is produced and stored in specific (secondary) neutrophil granules and released during neutrophil activation and degranulation⁽⁸⁾.

It has been detected in secretory fluids, such as milk, tears, saliva, vaginal secretion, seminal fluid, and amniotic fluid. Therefore, it is considered an important component of host defense against microbial infection⁽⁹⁾. The aim of the present study was to decrease the morbidities and mortalities resulting from PROM.

PATIENT AND METHODS:

A cohort study was conducted at Obstetrics and Gynecology Department, Faculty of Medicine, Zagazig University Hospital from August 2020 to June 2021.

Inclusion criteria: Age from 20-40. Gestational age before 37 weeks with preterm premature rupture of membrane confirmed by ultrasound and clinical examination. Single pregnancy. Viable fetus.

Exclusion criteria: Twin pregnancy. ROM after 37 weeks. Comorbidity.

Patients had been divided into two groups; Group A: Patients received 100 mg of recombinant human lactoferrin (rhLf) twice a day before meals. Group B: patients received placebo medicine. At enrollment, and after 10 and 30 days of treatment, obstetric transvaginal



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ultrasound had been performed to evaluate cervical length and funneling.

All patients were subjected to detailed history taking, general examination including evaluation of vital signs (heart rate (HR), respiratory rate (RR), blood pressure, temperature, measurement of weight, height (Body mass index). Abdominal and local clinical examination to assess fundal level and gestational age, scar of previous operation, mass, tenderness or rigidity, any abdominal or pelvic clinically detectable pathology. Bimanual pelvic examination of both adnexa, and uterus for detection of any abnormality of female genitalia. Routine transvaginal examination. Ultrasound examination.

Technique:

Before the evaluation of the cervix with transvaginal ultrasonography, first of all, the patient should have an empty bladder and be placed in dorsal lithotomy position. A distended bladder can alter the shape of the cervix and compass the cervical canal in some cases preventing the detection of cervical incompetence. The vaginal probe should be placed in the anterior fornix without pressure. If the probe is

pressed too hard against the cervix, it can obscure cervical incompetence. Initial orientation is established by locating the sagittal view of the cervix. The cervical canal should appear as a hypoechoic groove. The junction between amniotic membrane and cervical canal is designated as the internal os. The external os is located at the lower end of the cervix. Cervical length (CL) is defined as the distance between the internal to external os along the endocervical canal. If the cervical canal is curved, the CL can be measured either as the sum of two straight lines that essentially follow the curve or by a straight line between internal and external os. A short CL is usually straight, and the presence of curved cervix generally signifies a CL greater than 25 mm and, therefore, is a reassuring finding (Figure 1).

The two groups had been followed up to detect premature rupture of membrane. PROM could be diagnosed via examination of the cervix (may show fluid leaking from the cervical opening), testing of the pH (acid or alkaline) of the fluid, looking at the dried fluid under a microscope (may show a characteristic fern-like pattern), Ultrasound.

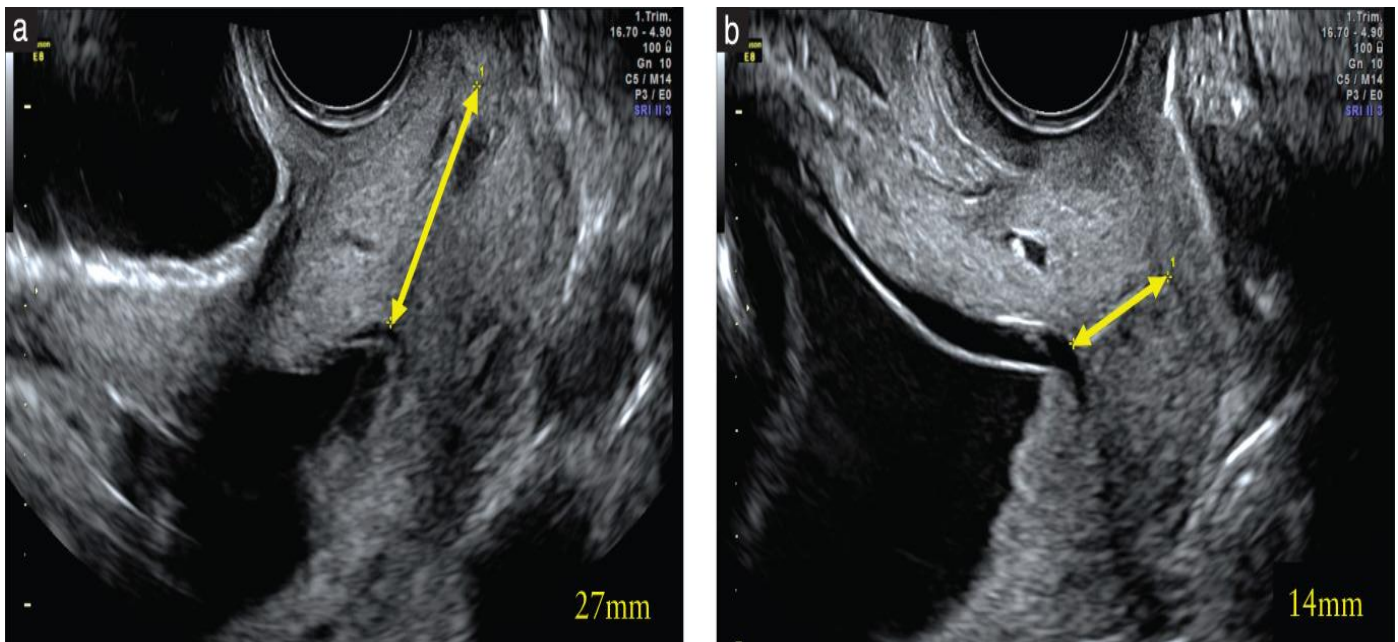


Figure (1): Transvaginal ultrasound measurement of cervical length in the same patient, with a full bladder (a) and with an empty bladder (b).

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Written informed consent was obtained from all patients, before starting the study with counseling about risk and benefit of study.

All patients were informed about the risk of intra-operative hemorrhage and postpartum hemorrhage (PPH), the need for blood products transfusion and the possibility of cesarean hysterectomy if needed to control severe bleeding. This work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical methods

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for the Social Sciences) version 20 for Windows® (IBM SPSS Inc, Chicago, IL, USA).

Data were tested for normal distribution using the Shapiro Wilk test. Qualitative data were represented as frequencies and relative percentages and were compared by Fisher’s exact test. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data), while Mann-Whitney test was used to compare nonparametric data. P value < 0.05 was considered significant.

RESULTS

As regard age there was no statistically significant differences between groups (Table 1).

Table (1): Comparison between two groups as regard to patient’s age (years)

Age	Group (A) “Lactoferrin” (n=24)	Group (B) “Placebo” (n=24)	P Value
Min.-Max.	20-39	20-40	0.223
Mean± S.D	29.63±5.807	31.67±5.990	

Regarding menstrual regularity, history of previous PROM, and mode of delivery there was no statistically significant differences between groups (Table 2).

Table (2): Comparison between two groups’ lactoferrin and placebo as regard menstrual regularity, history of PROM, and mode of delivery

	Group (A) “Lactoferrin” (n=24)		Group (B) “Placebo” (n=24)		P Value
	No.	%	No.	%	
Menstrual Regularity					
Regular	23	95.8	22	91.7	1.000
Irregular	1	4.2	2	8.3	
Total	24	100	24	100	
History of Previous PROM					
No	18	75.0	17	70.8	1.000
Yes	6	25.0	7	29.2	
Total	24	100	24	100	
Mode of Delivery					
NVD	14	58.3	11	45.8	0.564
Cesarean section	10	41.7	13	54.2	
Total	24	100	24	100	

PROM: Premature rupture of membranes, NVD: Normal vaginal delivery.

There was no statistically significant differences between groups as regard duration of PROM to delivery (Table 3).

Table (3): Comparison between two groups as regard to patient’s duration of PROM to delivery (hours)

Duration of PROM to delivery (hours)	Group (A) “Lactoferrin” (n=24)	Group (B) “Placebo” (n=24)	P Value
Min.-Max.	6-24	6-24	0.718
Mean± S.D	15.83±5.522	15.25±5.589	

PROM: Premature rupture of membranes

There was no statistically significant differences between groups as regard patient’s hospital stay (Table 4).

Table (4): Comparison between two groups as regard to patient’s hospital stay

Hospital stay	Group (A) “Lactoferrin” (n=24)	Group (B) “Placebo” (n=24)	P Value
Min.-Max.	1-7	1-6	0.254
Mean± S.D	2.42±1.767	3.12±2.028	

Figure 2 shows the ROC curve analysis of efficacy of lactoferrin on rupture membrane and it shows that lactoferrin had sensitivity of 62.5 with specificity 56.2 and positive and negative predictive value of 41.7 and 75 respectively while the area under curve (AUC) was 0.594.

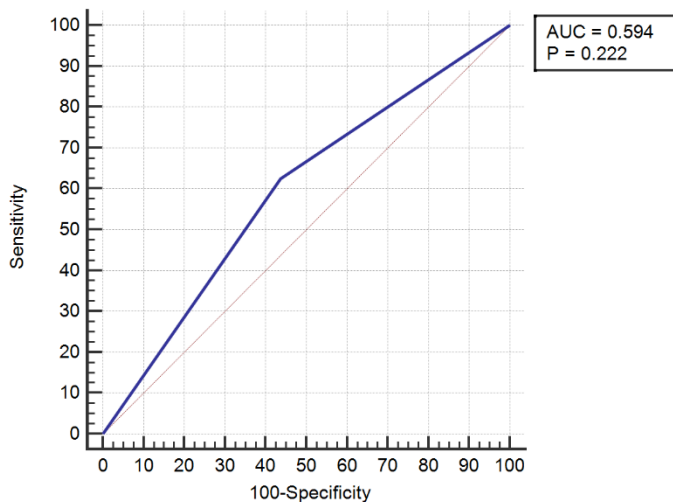


Figure (2): ROC curve analysis of efficacy of lactoferrin on rupture membrane

DISCUSSION

As regard demographic data, age in group (A) ranged between 20-39 while in group (B) it ranged between 20-40 years. There were no statistically significant differences between group. There were no statistically significant differences between groups where P=0.772 as regard residence. There were no statistically significant differences between groups as regard gravidity and parity. Our results were supported by study of **Giunta et al.** (9) as they reported that there were no statistically significant differences between their studied group as regard age and parity. Patients were divided in two groups: One group received 100 mg of rhLf (lattoferrina®; AG-pharma) twice a day before meals for one month and the other group (N=7) received a tablet of 520 mg of ferrous sulfate (Ferro-Grad®-Abbott Laboratories, USA) once a day, as suggested by the Italian standard treatments.

The present study showed that there were no statistically significant differences between groups as regard menstrual regularity, history of previous PROM, and mode of delivery. Several studies from USA, Sweden, India, Thailand, Egypt, Nigeria and Uganda revealed that previous PROM was a significant risk factor for premature rupture of membranes(10). The study

of **Assefa et al.** (11) also showed that previous PROM to be the strongest risk factor for premature ruptures of membranes. Women who had previous PROM were 4.45 more likely to develop PROM with adjusted odd ratio (AOR) 4.45 (CI: 1.87, 10.6). This might be due to untreated genitourinary infection and a short cervical length. In addition, obstetric problems are recurrent by nature. A study conducted by **Kaye**(12) in 2001 from Uganda revealed that CS was a significant risk factor for premature rupture of membranes. **Assefa et al.** (11) also found the caesarean section to be a significant risk factor. Participants with history of CS were 3.15 times more likely to develop PROM than who hadn’t history of CS. This might be due to the increased risk of rupture of CS scar in the subsequent pregnancy.

It is thought that Lf is capable of protecting against infection through different mechanisms of action, by regulating the iron needed for bacterial proliferation, specifically through its strong iron-binding power. Lf is also capable of causing cell membrane destruction by binding bacterial cell membrane proteins. Furthermore, it has an inhibitory effect on lipopolysaccharide (LPS)-induced production of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8 mRNA) and in monocytic cells by interfering with NF-κB activation. Additional functions of Lf have been reported, such as neutrophil and macrophage activation, regulation of specialization and function of lymphocytes, activation of the natural killer cells, and control of the oxidation injury(13).

The current study showed that as regard duration of PROM to delivery there were no statistically significant differences between groups. However, in the study of **Pacora et al.** (14), a significant inverse correlation was found between the amniotic fluid lactoferrin concentrations and the intervals from procedure to delivery in the preterm groups (intra-amniotic infection, r = -0.31; P = 0.02; vs no intraamniotic infection, r = -0.27; P = 0.03 by Spearman correlation). Spontaneous labor at term was associated with a significant decrease in amniotic fluid lactoferrin concentration. However, spontaneous preterm delivery was not associated with a significant decrease in amniotic fluid lactoferrin concentration. Spontaneous rupture of the membranes at term but not preterm was associated with a significant decrease in amniotic fluid concentrations of lactoferrin.

In the study in our hands, there was no statistically significant difference between groups as regard hospital stay, pulse rate, respiratory rate, Baby’s birth weight, ICU admission and Rupture membrane.

Our results were supported by study of **Miranda et al.**(15) as they reported that no between-group differences were noticed in the other outcomes, including chorioamnionitis, PPRM < 34 weeks, and neonatal outcomes. No cases of late miscarriage were reported in their cohort. Women who received supplementation with lactoferrin had a significantly lower rate of preterm birth (PTB) < 37 weeks (25.0 versus 44.6%; p 0.02), lower mean gestational age at

delivery (37.7 ± 3.2 versus 35.9 ± 4.1 weeks; p 0.01), and lower rate of admission for threatened preterm labor (PTL) (45.0 versus 70.8%; p 0.04). No cases of adverse events were reported. In a study conducted by **Russo *et al.***⁽¹⁶⁾, the primary outcome was to show that lactoferrin decreases the median risk of preterm birth by 30%.

According to **Ochoa *et al.***⁽¹⁷⁾, sepsis-associated death occurred in 22 infants (10.5%) in the bovine lactoferrin group vs 30 (14.6%) in the placebo group; there was no difference after adjusting for hospital and birth weight; hazard ratio was 0.73 (95% CI, 0.42-1.26). For infants with birth weights of <1500 g the hazard ratio was 0.69 (95% CI, 0.39-1.25). Growth outcomes and rehospitalization rates during the 2-year follow-up were similar in both groups, except for significantly less bronchiolitis in the bovine lactoferrin group (rate ratio, 0.34; 95% CI, 0.14-0.86). Also, **Ochoa *et al.***⁽¹⁸⁾ demonstrated that the cumulative sepsis incidence in the LF group was 12/95 (12.6%) vs 21/95 (22.1%) in the placebo group, and 20% (8/40) vs 37.5% (15/40) for infants \leq 1500g. The hazard ratio of LF, after adjustment by birth weight, was 0.507 (95% CI, 0.249 to 1.034). There were 4 episodes of culture-proven sepsis in the LF group vs 4 in the placebo group.

CONCLUSIONS

Lactoferrin (Lf) is the major whey protein in milk, with multiple beneficial health effects including direct antimicrobial activities, anti-inflammatory effects, and iron homeostasis. Oral Lf supplementation in human preterm infants has been shown to reduce the incidence of sepsis and necrotizing enterocolitis. Supplementation with lactoferrin may be an option to reduce the risk of PROM. Further studies are warranted to confirm data which provide valuable proof of concept for the potential use of Lf for the prevention of preterm delivery.

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