ROLE OF LACTOFERRIN IN PREVENTION OF PREMATURER UPTURE OF MEMBRANE AND ITS RELATION TO MATERNAL AND FETAL OUTCOMES

Ali El-Shabrawy Ali¹, Manar Abdelhafizee Fathi¹, Ahmed Ismail Mohammed¹
¹Department, Obstetrics and Gynecology, Faculty of Medicine, Zagazig University, Egypt.
Malikahmoud21@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. This study aimed to decrease the morbidities and mortalities resulting from PROM.

Methods: This was a cohort study was conducted at Obstetrics & 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. The duration of the study ranged from 6-12 months. All patients were subjected to detailed history taking, General examination, obstetric transvaginal ultrasound had been performed to evaluate cervical length and funneling.

Results: Baby's birth weight in Group (A) was ranged between 1364 – 3090 gm, while in Group (B) was ranged between 1366 – 3156 gm. Mode of delivery in Group (A) show that 14(58.3%) their mode of delivery was NVD and 10(41.7%) their mode of delivery was CS while in Group (B) 11(45.8%) their mode of delivery was NVD and 13(54.2%) their mode of delivery was CS. There was no statistically significant difference between groups where P=0.564. Rupture membrane in Group (A) show that 6(25.0%) had rupture membrane while in Group (B) 10(41.7%) had rupture membrane. 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.

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

Key words: Lactoferrin, PROM, Premature

I. 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, defined as preterm PROM as PROM that occurs after 37 weeks gestation defined as term PROM⁴. PROM occurs in approximately 5%–10% of all pregnancies, of which approximately 80% occur at term⁵.

There are multiple risk factors for PROM, including intrauterine infection at a young gestational age, pregnant women's poorer socioeconomic position, inadequate prenatal care and nutrition throughout pregnancy, sexually transmitted diseases, vaginal hemorrhage, and smoking during pregnancy⁶.

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 respectively⁷.

Prematurity, fetal discomfort, cord compression, deformation, and abnormal pulmonary development leading to pulmonary hypoplasia and pulmonary hypertension, necrotizing enterocolitis (NEC), and neurologic dysfunction

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are all PROM complications for the fetus and newborn (5). Infectious morbidities in the mother, fetus, and infant have been linked to both PROM and protracted membrane rupture (6).

Intra-amniotic infection, which occurs in 13%–60% of women with PROM, placental abruption, and postpartum endometritis are examples of maternal complications (7).

Lactoferrin (Lf) is a transferrin-family iron-binding glycoprotein with well-known bacteriostatic and bactericidal characteristics that plays an important role in iron homeostasis. Lf is synthesized and held in specialized (secondary) neutrophil granules before being released during neutrophil activation and degranulation (8).

Milk, tears, saliva, vaginal secretion, seminal fluid, and amniotic fluid have all been found to contain it. As a result, it is regarded as an essential component of host defense against microbial infection (9). The aim of the present study was to decrease the morbidities and mortalities resulting from PROM.

II. PATIENT AND METHODS:

A cohort study was conducted at Obstetrics & Gynecology Department, Faculty of Medicine, Zagazig University Hospital from August 2020 to June 2021. The duration of the study ranged from 6-12 months.


Viable fetus.


Written informed consent was obtained from all patients, before starting the study with counseling about risk and benefit of study. All patients was informed about the risk of intra-operative hemorrhage and PPH, the need for blood products transfusion and the possibility of cesarean hysterectomy if needed to control severe bleeding, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work was carried out for studies involving humans in accordance with the World Medical Association's Code of Ethics (Helsinki Declaration).

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 ultrasound had been performed to evaluate cervical length and funneling.

Method:

All patients were subjected to detailed history taking, General examination including Evaluation of vital signs (HR, RR, blood pressure, temperature), measurement weight, height (BMI).

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 Trans vaginal 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).

### III. STATISTICAL METHODS

Data from the history, basic clinical examination, laboratory tests, and outcome measures were coded, entered, and analyzed in Microsoft Excel software. The data was then imported into the Statistical Package for the Social Sciences (SPSS version 20.0) program for analysis. For significant results, the P value was set at 0.05, and for highly significant results, it was set at 0.001.

### IV. RESULTS:

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

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Group (A) “Lactoferrin” (n=24)</th>
<th>Group (B) “Placebo” (n=24)</th>
<th>U</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.-Max.</td>
<td>20-39</td>
<td>20-40</td>
<td>229.00</td>
<td>0.223</td>
</tr>
<tr>
<td>Mean± S.D.</td>
<td>29.63±5.807</td>
<td>31.67±5.990</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

U: Mann-Whitney test  
* p: p value for comparing between the two studied groups  
*: Statistically significant at P <0.05

Age in Group (A) was ranged between 20-39 years with mean±S.D. 29.63±5.807 years while in Group (B) was ranged between 20-40 years with mean±S.D. 31.67±5.990 years. There was no statistically significant differences between groups where P=0.223. **(table 1)**

**Table (2):** Comparison between two groups Lactoferrin and Placebo.

<table>
<thead>
<tr>
<th>Menstrual Regularity</th>
<th>Group (A) “Lactoferrin” (n=24)</th>
<th>Group (B) “Placebo” (n=24)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Regular</td>
<td>23</td>
<td>95.8</td>
<td>22</td>
</tr>
<tr>
<td>Irregular</td>
<td>1</td>
<td>4.2</td>
<td>2</td>
</tr>
</tbody>
</table>
Menstrual Regularity in Group (A) show that 23(95.8%) had regular menstrual and 1(4.2%) had irregular menstrual while in Group (B) 22(91.7%) had regular menstrual and 2(8.3%) had irregular menstrual. There was no statistically significant differences between groups where P=1.000.

History of Previous PROM in Group (A) show that 6(25.0%) had a history of Previous PROM while in Group (B) 7(29.2%) had a history of Previous PROM. There was no statistically significant differences between groups where P=1.000.

Mode of delivery in Group (A) show that 14(58.3%) their mode of delivery was NVD and 10(41.7%) their mode of delivery was CS while in Group (B) 11(45.8%) their mode of delivery was NVD and 13(54.2%) their mode of delivery was CS. There was no statistically significant differences between groups where P=0.564 (table 2).

Duration of PROM to delivery in Group (A) was ranged between 6-24 hours with mean±S.D. 15.83±5.522 hours while in Group (B) was ranged between 6-24 hours with mean±S.D. 15.25±5.589 hours. There was no statistically significant differences between groups where P=0.718. (Figure2).

<table>
<thead>
<tr>
<th></th>
<th>Group (A)</th>
<th>Group (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>15.83</td>
<td>15.25</td>
</tr>
</tbody>
</table>

Figure (2): Comparison between two groups as regard to patient’s duration of PROM to delivery (hours)
Table (3): Comparison between two groups as regard to patient’s Baby’s birth weight and hospital stay

<table>
<thead>
<tr>
<th></th>
<th>Group (A) “Lactoferrin” (n=24)</th>
<th>Group (B) “Placebo” (n=24)</th>
<th>U</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baby’s birth weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>1364 – 3090</td>
<td>1366 – 3156</td>
<td>0.468</td>
<td>0.642</td>
</tr>
<tr>
<td>Mean± S.D</td>
<td>2192.08±579.583</td>
<td>2269.25±563.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hospital stay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>1-7</td>
<td>1-6</td>
<td>234.50</td>
<td>0.254</td>
</tr>
<tr>
<td>Mean± S.D</td>
<td>2.42±1.767</td>
<td>3.12±2.028</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

U: Mann-Whitney testp; p value for comparing between the two studied groups

*: Statistically significant at P <0.05

Baby’s birth weight in Group (A) was ranged between 1364 – 3090 gm with mean±SD. 2192.08±579.583 gm while in Group (B) was ranged between 1366 – 3156 gm with mean±SD. 2269.25±563.012 gm. There was no statistically significant differences between groups where P=0.642.

Hospital stay in Group (A) was ranged between 1-7 days with mean±SD. 2.42±1.767 days while in Group (B) was ranged between 1-6 days with mean±SD. 3.12±2.028 days. There was no statistically significant differences between groups where P=0.254. (Table 4).

Rupture membrane in Group (A) show that 6(25.0%) had rupture membrane while in Group (B) 10(41.7%) had rupture membrane. There was no statistically significant differences between groups where P=0.359. (Figure 3)

Figure (3): Comparison between two groups as regard to patient’s rupture membrane

Figure 4: showed that the ROC curve analysis of efficacy of lactoferrin on rupture membrane and it show 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 was 0.594 with p value of 0.222.
Figure (2): ROC curve analysis of efficacy of lactoferrin on rupture membrane

V. DISCUSSION

As regard demographic data, age in Group (A) was ranged between 20-39 years with mean± S.D. 29.63±5.807 years while in Group (B) was ranged between 20-40 years with mean± S.D. 31.67±5.990 years. There were no statistically significant differences between groups where P=0.223. 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.

This results were supported by study of Giunta et al. 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 where P=1.000 as regard Menstrual Regularity. As regard History of Previous PROM in Group (A) show that 6(25.0%) had a history of Previous PROM while in Group (B) 7(29.2%) had a history of Previous PROM. There were no statistically significant differences between groups where P=1.000. As regard Mode of delivery in Group (A) show that 14(58.3%) their mode of delivery was NVD and 10(41.7%) their mode of delivery was CS while in Group (B) 11(45.8%) their mode of delivery was NVD and 13(54.2%) their mode of delivery was CS. There were no statistically significant differences between groups where P=0.564.

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. The study of Assefa et al. 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 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 from Uganda revealed that caesarean section was a significant risk factor for premature rupture of membranes. Assefa et al. also found the caesarean section to be a significant risk factor. Participants with history of C/S were 3.15 times more likely to develop PROM than who hadn’t history of c/s. This might be due to the increased risk of rupture of C/S 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 LPS-induced production of inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8 mRNA) and in monocyctic 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\textsuperscript{13}.

The current study showed that as regard duration of PROM to delivery in Group (A) was ranged between 6-24 hours with mean± S.D. 15.83±5.522 hours while in Group (B) was ranged between 6-24 hours with mean± S.D. 15.25±5.589 hours. There were no statistically significant differences between groups where P=0.718.

However, in the study of Pacora et al.,\textsuperscript{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, $r = -0.31; P = .02$; vs no intraamniotic infection, $r = -0.27; P = .03$ 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 Mirandaet al.,\textsuperscript{15} as they reported that no between-group differences were noticed in the other outcomes, including chorioamnionitis, PPROM < 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 PTB < 37 weeks (25.0 versus 44.6%; $p \leq .02$), lower mean gestational age at delivery (37.7 ± 3.2 versus 35.9 ± 4.1 weeks; $p = .01$), and lower rate of admission for threatened PTM (45.0 versus 70.8%; $p = .04$). No cases of adverse events were reported.

In a study conducted by Russo et al.,\textsuperscript{16} the primary outcome is to show that lactoferrin decreases the median risk of preterm birth by 30%.

According to Ochoa et al.,\textsuperscript{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 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.,\textsuperscript{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 ≤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.

VI. 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.

REFERENCES:


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