Lactoferrin Supplementation For Prevention Of Sepsis In Preterm Neonate

Pof.Nayera Ismail Attia[1], Dr.Ghada Ibrahim Gad[2], Dr.Hala Mahmoud Hafez[3], Dr.El Sayed El Sayed El Okda[4], and Mostafa Abd El-Latef El-Mokadem

[1]Professor of Pediatrics, Department of Medical Studies, Institute of Postgraduate Childhood Studies, Ain Shams University, [2]Assistant professor of pediatrics, Faculty of Medicine, Ain Shams University, [3]Assistant professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, [4]Assistant Professor of Community, Environmental and Occupational Medicine, Faculty of Medicine, Ain Shams University.

Abstract

Background: Sepsis related morbidity and mortality is a concern in neonatal intensive care units (NICUs) specially in preterm and Low birth weight (LBW) infants who are more vulnerable due to immaturity of immune defenses and protective barriers. Lactoferrin is an iron binding glycoprotein presents in mammalian milk and involved in innate immunity. Recent data suggest that bovine lactoferrin (BLF) might prevent late onset sepsis in preterm and LBW neonates.

Objective: To evaluate the effectiveness of oral bovine lacoferrin in prevention of neonatal sepsis in Egyptian preterm neonates.

Patients& Methods: A randomized clinical trial, double blind, placebo- controlled study was conducted on 135 preterm neonates (born before 37 weeks of gestation) admitted to the NICUs of of Ain Shams University and Manshiet El Bakry Hospitals from February 2013to January 2015. Infants were randomly sub- divided into two groups: Group 1 consisted of 45 infant received oral lactoferrin supplementation (100 mg/day) within a day of starting feeds for 4 weeks. Group 2 consisted of 90 infants matching group 1 neonates, received placebo in the form of distilled water in the same schedule. History and physical examination were carried out laying stress on signs of sepsis, severity (classified according to Töllner score), laboratory investigations were done: CBC with blood film (classified according to hematological scoring system), CRP, Blood culture upon admission and on suspicion of sepsis, other cultures and arterial blood gases when clinically indicated. Radiological investigation were done when clinically indicated, Data were analyzed using the Statistical Package for Social Sciences SPSS.

Results: Lactoferrin group (45 preterm neonates) with mean gestational age (33.11± 1.81 weeks), 32 males (71%) and 13 females (29%). Placebo group (90 preterm neonates) with mean gestational age (33.28± 1.89 weeks), 45 males (50%) and 45 females (50%). Lactoferrin group showed a significantly lower incidence of late onset sepsis according to Tollner score and Rodwell score and blood cultures (6.7%) compared to placebo group (17.8%). *E coli* and *S aureus* were the most common organisms found in septic neonates in the current study (28.6% for each).

Conclusion: BLF supplementation would be a suitable preventive tool for late onset neonatal sepsis in preterm neonates.

Keywords: Lactoferrin, neonatal sepsis, preterm.

تناول مكملات اللاكتوفيرين لمنع التسمم بالدم للأطفال حديثى الولادة المبتسرين

المقدمة: معدلات الوفيات والاعتلال المرتبطة بتسمم الدم تشكل مصدر قلق كبير فى وحدات العناية المركزة للأطفال حديثى الولادة، خاصا لدى الأطفال المبتسرين والاطفال ناقصى الوزن عند الولادة والذين يكونون اكثر عرضة نتيجة لعدم نضج الجهاز المناعى وخطوط الدفاع. اللاكتوفيرين هو جليكوبروتين قابل للأرتباط بالحديد موجود فى جميع البان الثدييات ويشارك فى الاستجابة المناعية للجسم. اشارت المعلومات الحديثة ان اللاكتوفيرين المستخلص من البقر قد يكون له دور فى الوقاية من حدوث تسمم الدم المتأخر بالأطفال المبتسرين والاطفال ناقصى الوزن عند الولادة

هدف الدراسة: تقييم مدى فعالية اللاكتوفيرين عن طريق الفم فى الوقاية من تسمم الدم المتأخر لدى الاطفال حديثى الولادة المبتسرين.

الحالات والاساليب: كانت هذه الدراسه مستقبلية، مختارة عشوائيا، مزدوجة التعمية للمجموعتين. أشتملت الدراسة على ١٣٥ طفل حديث الولادة مبتسرين محجوزين بوحدات الرعاية المركزة لحديثى الولادة بمستشفى جامعةعين شمس ومستشفى منشية البكرى العام خلال الفترة من فبراير ٢٠١٣ الى يناير ٢٠١٥. تم تقسيم الاطفال الى مجموعتين. المجموعة الأولى ٤٥ طفل تم أعطائهم اللاكتوفيرن البقرى عن طريق الفم بجرعة ١٠٠ ملجم/ اليوم خلال يوم من بداية الرضاعة ولمدة ٤ اسابيع. المجموعة الثانية ٩٠ طفل تم أعطائهم ماء مقطر بدلا من اللاكتوفيرن بنفس البروتوكول.

النتائج: مجموعة اللاكتوفيرين البقرى كان متوسط العمر الرحمى لهم (٣٣,١١± ١,٨١) أسبوع وكانوا ٣٢ (٧١%) من الذكور و١٣ (٢٩%) من الإناث. اما مجموعة البلاسيبو التى اخذت المياه المقطرة كان متوسط العمر الرحمى (٣٣,٢٨± ١,٨٩) أسبوع وكانوا ٤٥ (٥٠%) من الذكور و٤٥ (٥٠%) من اللإناث. وقد اظهرت النتائج ان معدل حدوث تسمم الدم المتأخر كان اقل بمجموعة اللاكتوفيرين من حيث علامات التسمم بالدم طبقا لدرجات تولنار وتحاليل صورة الدم طبقا لدرجات رودويل ومزرعة الدم (٦,٧%) بالمقارنة بمجموعة البلاسيبو التى تتناول المياه المقطرة (١٧,٨%)، كما اظهرت النتائج ان بكتريا الإشريكية القولونية وبكتريا العُنْقُودِيَّةُ الذَّهَبِيَّة كانوا اكثر نوعين من البكتريا المسببة لتسمم الدم المتأخر بالحالات (٢٦,٣% لكل منهما).

Introduction:

Sepsis related morbidity and mortality is a concern in NICUs. Regardless of the recent improvements in the quality of neonatal assistance, infections cause 1.6 million neonatal deaths annually worldwide and more than 50% of these deaths occur in preterm and LBW infants in NICUs (Manzoni et.al., 2011).

In developing countries, the incidence of neonatal sepsis is about (3.5- 4.3) cases per 1.000 live births (Fahmey, 2013). The most important neonatal factor predisposing to infection is prematurity or LBW. Preterm infants have a (3- 10) fold higher incidence of infection than full- term normal birth weight infants. (Stoll, 2011)

Classically neonatal sepsis has been divided depending on the time of onset of infection into EOS (≤72 hours of birth), and LOS (>72 hours) (Fahmey, 2013).

Lactoferrin is a glycoprotein, involved in the innate immune, devoted to capture ferric iron in order to be unavailable for pathogens growth when they try to colonize or invade the host. It is the major whey protein in mammalian milk being present in colostrum in higher concentration than mature milk, with a slower decrease in milk of premature neonates' mothers. (Lönnerdal, 2003)

Orally ingested lactoferrin has effects on promotion of growth and differentiation of the immature gut in a concentration dependent manner. At high concentrations as occur in the early days of life with colostrum, lactoferrin enhances proliferation, growth and maturation of the nascent enterocytes, promoting an increase in the number of gut cells and closing of enteric gap junctions. At lower concentrations as happens in mature milk, lactoferrin enhances differentiation of enterocytes and acquisition and development of their lactase and enzymatic activities (Buccigrossi et.al., 2007). Also, lactoferrin enhances the growth of the normal bifidogenic gut microflora with predominant healthy commensals such as Bifidobacteria and Lactobacilli (Rahman et.al., 2009).

Bovine lactoferrin (BLF) shares a 77% homology with the human isoform, and the same biochemical structure of its active site,. Both BLF and human lactoferrin (HLF) bind to the same specific receptors on enterocytes (Van der Does et.al., 2010).

The aim of lactoferrin supplementation is to restore and possibly even enhance the natural defensive system that ideally a neonate has if it has access to the adequate amounts of mother's fresh colostrum in the first weeks of life which usually do not all occur because of difficulties in instituting oral breast feeding from birth in immature infants (Manzoni et.al., 2012).

Aim of the study:

The aim of the study is to evaluate the effectiveness of oral bovine lacoferrin in prevention of late onset neonatal sepsis in preterm neonates.

Subjects And Methods:

- Type of the study: It is a randomized clinical trial, double blind, placebo-controlled study.
- Subjects: The present study was conducted on 135 preterm neonates admitted to the Neonatal Intensive Care Units of Ain Shams University Hospitals and Manshiet El Bakry Hospital from February 2013- January 2015.
- Inclusion Criteria: Neonates< 37 weeks of gestation, born in, or referred to the Neonatal Intensive Care Units of one of the participating hospitals in the first 48 hours of life free from infection and not fed. They were further randomly subdivided into two groups as follow:
  1. Group (1): Who received oral BLF supplementation (100 mg/day) within a day of starting feeds till age of 28 days old (Manzoni et.al., 2009).
  2. Placebo group: match gestational age and sex with group (1), received placebo in the form of distilled water with same protocol.
- Exclusion Criteria: Neonates with underlying gastrointestinal anomalies that prevent oral intake, suspected or proven early onset sepsis, predisposing conditions that profoundly affect growth and development (chromosomal, congenital, structural brain anomalies), family history of cow milk allergy, unable to complete the study time and whose parents refuse to participate.

Methods:

All neonates group 1 and placebo group were subjected to the following:

1. Full Medical History: Family history of Inherited diseases; Maternal history: age, gravity and parity, blood type and transfusions, bleeding disorders, recent infections or exposures, chronic maternal illness (diabetes, hypertension, renal disease, cardiac disease, ...), medications, drug abuse, alcohol, tobacco; Previous pregnancies: problems and outcomes (abortions, fetal demise, neonatal deaths, pre/ postmaturity, malformations); Current pregnancy: gestational age assessment using lat menstrual period, preeclampsia, bleeding, trauma, infection, poly/ oligohydramnios, PROM, glucocorticoids and antibiotics; Labor and delivery: presentation, rupture of membranes, duration of labor, fever, fetal monitoring, amniotic fluid (blood, meconium, volume), method of delivery, APGAR scores and resuscitation; Present history which included symptoms of sepsis, History of antibiotics given (type, number of doses, duration).
2. Clinical Examination:
   a. Gestational age assessment using last menstrual period date& extended Ballard score (Ballard et.al., 1991).
   b. Anthropometric measurements: Length (in centimeters), Head circumference (in centimeters), Body weight (in kilograms).
   c. Vital signs (pulse, temperature, blood pressure and respiratory rate).
   d. APGAR score at 1, 5 min.
   e. Thorough clinical examination laying stress on signs of sepsis (Richard and Joan, 2008).
   f. Disease severity were classified according to Töllner score (Töllner U., 1982).
3. Investigations:
   a. Laboratory: On admission and in case of suspected sepsis: Complete blood count with differential leucocytic count using automated coulter technique (to be repeated if needed). Blood film for hematological scoring system (Rodwell et.al., 1988). CRP quantitative assay using latex agglutination (to be repeated if needed). Blood culture upon admission and on suspicion of sepsis. Stool, urine, fungal and/or CSF culture when clinically indicated. Arterial blood gases when clinically indicated.
   b. Radiological investigation: As Chest x- ray, abdominal x- ray, pelvi-abdominal, cranial sonograghy or CT brain when clinically indicated.
4. Oral Lactoferrin Supplementation:

a. It was given to all neonates in group (1) in a dose of 100mg/day (Manzoni et.al., 2009) dissolved in 2 ml of distilled water within a day of starting feeds and continued till age 28 days old.
b. Placebo group received placebo in the form of 2 ml of distilled water starting feeds and continue for till age 28 days old.
c. Randomization was done using aliquots covered with opaque plaster.

Results:

Lactoferrin group included 45 preterm neonates with mean gestational age (33.11± 1.81 weeks) [32 males (71%) and 13 females (29%)], Placebo group included 90 preterm neonates with mean gestational age (33.28± 1.89 weeks) [45 males (50%) and 45 females (50%)], Demographic and clinical character of studied neonates showed in Table (1).

Lactoferrin group showed a significantly lower incidence of late onset sepsis according to Tollner score (2.2%, 14.4%) and Rodwell (6.6%, 17.8%) score and blood cultures compared to placebo group (6.7%, 17.8%) respectively.

Isolation of gram negative bacteria was higher than gram positive bacteria, *E.coli* and *S.aureus* were the most common organisms found in septic neonates in the current study (26.3% for each), followed by Staph. Epidermidis (15.7%), then Klebseila (10.5%), then acinetobacter spp, Enterobacter cloacae, Moraxella and Pseudomonas aeruginosa (5.3% for each) with non signifaicant difference between the three studied groups.

Table (1) Descriptive demographic data and examination upon admission of placebo and lactoferrin supplemental groups

Personal data and examination on admission		Placebo Group 90 Infants		Lactoferrin Group 45 Infants		P Value
		No.	%	No.	%	
GA Preterm	Nearterm	41	45.6%	16	35.6%	0.673
	Moderate	34	37.8%	21	46.7%	
	Severe	14	15.6%	7	15.6%	
	Extreme	1	1.1%	1	2.2%	
Sex	Male	56	62.2%	23	51.1%	0.217
	Female	34	37.8%	22	48.9%	
Single/ Multiple birth	Single	62	68.9%	29	64.4%	0.604
	Twins	28	31.1%	16	35.6%	
	Triple	0	0.0%	0	0.0%	
Mood Of Delivery	NVD	45	50.0%	32	71.1%	0.019
	CS	45	50.0%	13	28.9%	
Apgar 1 Minute	Median±IQ	7.3± 1		7.2± 1.5		0.88
Apgar 5 Minute	Median±IQ	9+ 1		9+ 0.8		0.92
Birth Weight (On Centile)	5th- 95th	82	91.1%	38	84.4%	0.497
	<5th	3	3.3%	3	6.7%	
	>95th	5	5.6%	4	8.9%	
Length On Birth (On Centile)	5th- 95th	79	87.8%	41	91.1%	0.836
	<5th	6	6.7%	2	4.4%	
	>95th	5	5.6%	2	4.4%	
Head Circumferance (On Centile)	5th- 95th	89	98.9%	43	95.6%	0.215
	<5th	1	1.1%	2	4.4%	
	>95th	0	0.0%	0	0.0%	
Temperature	Mean± SD	36.9± 1		36.6± 2		0.83
	Range	36.6- 37.3		35.9- 37.3		
Heart Rate (Per Minute)	Mean± SD	122± 5		125± 3		0.77
	Range	79- 165		77- 161		
Respiratory Rate (Per Minute)	Mean± SD	51± 2		50± 1.5		0.90
	Range	38- 87		38- 84		
Systolic Blood pressure	Mean± SD	61± 2		60± 1.6		0.78
	Range	42- 72		44- 87		
Diastolic Blood pressure	Mean± SD	40± 3		40.3± 2.6		0.81
	Range	23- 50		21- 56		

NVD: Normal vaginal delivery, CS: Cesarean section, GA: Gestational age, P >0.05: Non significant; P<0.05: Significant; P<0.01: Highly significant

Table (2) Comparison between placebo group and lactoferrin supplemental groups regarding occurrence of sepsis according to Tollner, Rodwell scores and blood culture

		Placebo Group		Lactoferrin Group		P Value
		No.	%	No.	%	
Tollner Score	No Sepsis	72	80.0%	41	91.1%	0.090
	Observation Range	5	5.6%	3	6.7%	
	Suspicison Of Sepsis	13	14.4%	1	2.2%	
	Mean± Sd	3.09± 2		1.1± 1.6		0.02
Rodwell Score	Very unlikely sepsis (≤ 2)	74	82.2%	42	93.3%	0.040
	Sepsis Is Possible (3 or 4)	0	0.0%	1	2.2%	
	Sepsis is very likely (≥ 5)	16	17.8%	2	4.4%	
Blood Culture	Positive	16	17.8%	3	6.7%	0.03

Table (3) Comparison between placebo and lactoferrin supplemental groups as regard blood, CSF, stool, urine and fungal cultures

Cultures		Control Group		Lactoferrin Group		P Value
		No.	%	No.	%	
	Positive Cultures	16	17.8%	3	6.7%	
Blood Culture No.= 90 Placebo Group (16 Positive), 45 Lactoferrin Group (3 Positive)	Negative	74	81.8%	42	93.3%	0.368
	Escherichia Coli	4	4.5%	1	2.2%	
	Staphylococcus Aureus	5	5.7%	0	0.0%	
	Staphylococcus Epidermidis	3	3.4%	0	0.0%	
	Acinetobacter Spp	1	1.1%	0	0.0%	
	Klebseila	1	1.1%	1	2.2%	
	Enterobacter Cloacae	1	1.1%	0	0.0%	
	Moraxella	0	0.0%	1	2.2%	
	Pseudomonas Aeruginosa	1	1.1%	0	0.0%	
CSF Culture	Negative	14	87.5%	3	100.0%	0.517
	Escherichia Coli	2	12.5%	0	0.0%	
Stool Culture	Negative	14	87.5%	3	100.0%	0.811
	Escherichia Coli	1	6.2%	0	0.0%	
Urine Culture	Negative	16	100.0%	3	100.0%	NA
	Pseudomonas Aeruginosa	0	0.0%	0	0.0%	
Fungal Culture	Negative	16	100.0%	3	100.0%	NA

Discussion:

In the current study, lactoferrin group showed a significantly lower incidence of late onset sepsis according to Tollner score and Rodwell score and blood cultures (6.7%) compared to placebo group (17.8%).

This goes in agreement with the study of Manzoni et.al. (2009) on 472 very low birth weight infants who received lactoferrin (100 mg per day), a statistically significant reduction in late- onset sepsis was found in the groups that received either lactoferrin alone (5.9%) or in combination with Lactobacillus (4.6%) vs 17% for placebo.

Ochoa et.al. (2012) study included 190 neonates weighing less than 2500 g at birth. Bovine lactoferrin (BLF) and maltodextrin (placebo) were turned over entirely at 200 mg/d in 3 divided doses over the first 4 weeks of life. There was decreasing trend in incidence of sepsis in the BLF group (12.6%) compared to the placebo group (23.2%).

Also this goes in agreement with Kaur et.al. (2013) who conducted a randomized controlled trial. They enrolled 121 low birth weight (less 2000 grams) neonates. BLF was supplemented daily from first to 28th day of life and the control group received placebo daily from first to 28th day of life. The incidence of culture proven LOS was significantly lower in the BLF group than in the placebo group (3.4%) versus (14.5%) (Sharma et.al., 2014).

The differences in sepsis incidence among the previous studies can be explained as incidence varies from NICU to NICU and within the same NICU at different time periods, the differences in incidence may be due to geographical, racial, socio- economic, cultural, technological, and differing definitions in making a diagnosis of neonatal sepsis (Kardana, 2011).

While, there was no statistical significance difference of culture proven nosocomial sepsis in Akin et.al. (2014) D5 study which included 50 infants (VLBW or born before 32 weeks), who were randomized to receive either placebo (25 infant) or BLF (25 infant) 200 mg per day. This may be attributed to the small sample size.

E.coli and *S.aureus* were the most common organisms found in septic neonates in the current study (26.3% for each), followed by Staph. Epidermidis (15.7%), then Klebseila (10.5%), then acinetobacter spp, Enterobacter cloacae, Moraxella and Pseudomonas aeruginosa (5.3% for each) with non signifaicant difference between the three studied groups.

This goes in agreement with Nair and Soraisham (2013) who reported that the most common organisms causing nosocomial infection in neonates included Staphylococcus, Escherichia coli, Klebsiella and candida. Coagulase negative staphylococcus was responsible for almost half of the LOS D2

Also, Aftab et.al. (2006) reported that bacteria commonly isolated in neonatal septicaemia included, Escherichia coli, Klebsiella pneumoniae Enterobacter spp, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus spp, Citrobacter spp, and coagulase negative Staphylococcus.

Afsharpaiman et.al. (2012) isolated Enterobacter (47.8%), coagulase negative Staphylococcus (26.1%), E. coli (8.7%) and Klebsiella (4.3%) in LOS.

While Gandhi et.al. (2013) found that coagulase negative Staphylococcus (19.4%), Klebsiella (16.7%), Escherichia coli and Staphylococcus aureus (13.8% for each), Pseudomonas aeruginosa (11.1%), Acinetobacter spp (8.3%), β- Hemolytic Streptococci, Citrobacter spp and Candida albicans (5.6% for each) in LOS.

The differences in percentages among the studies may be attributed to the fact that causative organisms in neonatal sepsis vary from place to place and the frequency of the causative organisms is different in different hospitals and even in the same hospital at different time (Shah et.al., 2012). In most of the developing countries gram negative bacilli remain the major cause of neonatal septicaemia (Ballot et.al., 2012).

In the current study, isolation of gram negative bacteria was higher than gram positive bacteria. These results were consistent with the findings of many previous studies which also reported gram negative bacteria to be more common in neonatal sepsis Shah et.al. (2012), Gandhi et.al. (2013), Aftab et.al. (2006) and Joshi et.al. (2000).

Conclusion:

Bovine lactoferrin supplementation would be a suitable preventive tool for late onset neonatal sepsis in preterm neonates.

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