Introduction

Neonatal sepsis (NS) related morbidity and mortality is a concern in neonatal intensive care units (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 or low birth weight infants in NICUs ***(Manzoni et al., 2011).***

Infections in preterm neonates increase their risk of death, cause prolonged exposure to antibiotics and may be associated with invasive fungal disease, feeding intolerance, necrotizing enterocolitis (NEC), growth failure, brain injury and cognitive or sensorineural disability, longer stays in NICUs, and greater hospital costs ***(Kaufman et al., 2004).***

Many risk factors account for the increased risk of infections in preterms: immaturity of immune defences, immaturity of the mucosal barrier in gut and airways, colonization with pathogens in peripheral sites, prolonged use of catheters and devices, disorders in the gut microflora and difficulties in enteral feeding. In addition, use of parenteral nutrition, acid inhibitors and steroids may enhance host susceptibility to infections ***(Manzoni et al., 2011).***

Immature or injured skin and impaired gut barriers allow dissemination of pathogens from various colonizing sites. Translocation from the gut reservoir, through impaired gut function barrier, is probably the crucial step generating most

disseminated infections in preterm neonates in NICUs ***(Stoll et al., 2002).***

The rationale for preventing rather than treating infections comes from several observations. As early diagnosis and successful treatment do not prevent prolonged stays in NICUs, greater costs and late neurodevelopmental impairment ***(Stoll et al., 2004).***

Prompt diagnosis in neonates is hampered by nonspecific clinical features, inadequate sensitivity of diagnostic tests and late recognition. Finally, most drugs in neonates are used in an off label way, and limited data is available regarding safety and interactions of antimicrobial agents in these settings ***(Bizzarro et al., 2010).***

Lactoferrin is a glycoprotein that in all mammals is involved in the innate immune response to sepsis. It is the major whey protein in mammalian milk being present in colostrum and mature milk and is highly concentrated in the former (~7 mg/ml) and more diluted in the latter (~1 mg/ml), with a decrease that is slower in milk of premature neonates' mothers ***(Lönnerdal B., 2003).*** Human milk processing affects survival and activity of the lactoferrin molecule depending on the techniques used ***(Akinbi et al., 2010).***

Orally ingested lactoferrin has effects on promotion of growth and differentiation of the immature gut, regulating maturation and functions of the nascent enterocytes 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).***

Lactoferrin has also immunomodulatory properties through contact with enterocytes and gut-associated lymphoid tissues (GALTs). By modulating cytokine production via the GALT cells, lactoferrin promotes the secretion of such substances into the systemic circulation and acts on circulating leukocytes ***(Actor et al., 2009).***

Bovine lactoferrin (BLF) shares a 77% homology with the human isoform, and the same biochemical structure of its active site, the N-terminal, 11-amino-acid peptide. Both bovine lactoferrin (BLF) and human lactoferrin (HLF) resist proteolysis through the infant's digestive tract, bind to specific receptors on enterocytes. Acid proteolysis of lactoferrin in the stomach yields peptides with increased anti-microbial activity called lactoferricins ***(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 very immature infants ***(Manzoni et al., 2012).***

Aim of the Study

The aim of the study is to:

- Evaluate the effectiveness of oral bovine lactoferrin in prevention of neonatal sepsis.
- Compare two dose regimen of lactoferrin supplementation.
- Study long term complications as broncho-pulmonary dysplasia, retinopathy of prematurity and necrotizing enterocollitis.
- Study the effect of lactoferrin supplementation on serum iron stores.

Prematurity

Moderate, late preterm, and early term deliveries represent a major and growing public health concern. These deliveries are associated with significant financial burden and pose serious risks to mothers and newborns. Women with preterm delivery at increased risk of preterm deliveries in subsequent pregnancy. Births in preterm gestational ages are associated with significant infant morbidity and mortality. Efforts to reduce preterm deliveries and interventions designed to ameliorate the problems in infants delivered at the gestational ages may be targets worthy of future investigation ***(Ananth et al., 2014).***

Definitions:-

Live born infants delivered before 37 weeks from the 1st day of the last menstrual period are termed premature by the World Health Organization ***(Carlo, 2015)***.

While preterm labor is defined as regular contractions accompanied by cervical change at less than 37 gestitional weeks ***(Ananth and Vintzileos, 2006)***.

Low birth weight are defined as those with a birth weight of ≤ 2.500g which may be due to prematurity, being born small for gestational age (SGA) or both ***(Carlo, 2015)***. Similarly, very low birth weight (VLBW < 1.500g) And extremely low birth weight (ELBW < 1,000g) ***(Smith, 2012).***

Three subgroups are distinguished by the World Health Organization (WHO):

- Moderate to late preterm (32 to <37 weeks).
- Very preterm (28 to <32 weeks).
- extremely preterm (<28 weeks)

(WHO, 2014).

Also, other classification of preterm births can be according to gestational age:

- Extreme prematurity: about 5% of preterm births, occurs at less than 28 weeks.
- Sever prematurity: about 15%, at 28-31 weeks.
- Moderate prematurity: about 20%, at 32-33 weeks.
- Late term: about 60-70%, at 34-36 gestational weeks.

(Shapiro-Mendoza and Lackritz, 2012)

Incidence and prevalence:-

More than 1 in10 of the world's babies born in 2010 were born prematurely, making an estimated 15 million preterm births (defined as before 37 weeks of gestation) ***(Blencowe et el., 2012)***.

Fortunately, the rate of preterm births appears to stabilized after a persistent increase over the period from 1990-2005, associated with the rising twin and triplet rate due to the use of fertility therapies. With advances in neonatal care, the number of critical ill very preterm infants who survive the neonatal period and are discharged from NICU has increased ***(Stewart and Joselow, 2012).***

In order to have the incidence of prematurity in Egypt, we should have a national survey and this was not conducted. However from a well documented survey, the incidence is approximately 11% in Menia Government and 50% in El Quliobia Government and much less in Cairo. The estimated percentage of LBW in Egypt ranged (5.9%-13%), LBW was frequent among females (13.2%) than males (11.2%) ***(El-Rafie, 2002) and (Monsour et al., 2002)***

Approximately 12.7% of all births in the United States are preterm, the distribution of this group is gradually shifted to a relatively older gestational age because of a 25% increase in late preterm infant (34 – 36 weeks) since 1990 to current rate of 9.1%. ***(Smith, 2012)***

More than 60% of preterm births occur in Africa and South Asia, but preterm birth is truly a global problem. In the lower-income countries, on average, 12% of babies are born too early compared with 9% in higher-income countries. Within countries, poorer families are at higher risk ***(WHO, 2014).***

Mortality and morbidity:-

Prematurity is the leading cause of death, inequalities in survival rates around the world are stark, in low income settings, half of the babies born at 32 weeks die due to a lack of feasible, cost-effective care, such as warmth, breastfeeding support, and basic care for infections and breathing difficulties. In high income countries, almost all of these babies survive ***(WHO, 2014).***

There is an increasing percentage of deaths in children <5 years old that occur in the neonatal period. Approximately 57% of deaths in this age group occur within the 1st month of life, of which approximately 36% are attributable to premature births ***(Carlo, 2015).***

Rates of survival to discharge increased with increasing GA (6% at 22 weeks and 92% at 28 weeks), with most early deaths occurring at 22 and 23 weeks (85% and 43%, respectively). Infants at the lowest GAs were at greatest risk for morbidities. ***(Stoll et al., 2010).***

Survival increases by 9.5% for each week if the baby is born at 23 weeks, and 16% per week if the baby is born at 25 weeks ***(Costeloe et al., 2012)***

Overall with extremely prematurity, 93% had respiratory distress syndrome, 46% patent ductus arteriosus, 16% severe intraventricular hemorrhage, 11% necrotizing enterocolitis, and 36% late-onset sepsis and more infants having bronchopulmonary dysplasia (68%). More than one-half of infants with extremely low GAs had undetermined retinopathy

status at the time of discharge. Center differences in management and outcomes were identified. ***(Stoll et al., 2010).***

Approximately 30% of premature infants <1,500g have IVH. The risk is inversely related to gestational age and birthweight, with the smallest and most immature infants being at the highest risk; 7% of infants 1,001-1,500 g have a severe IVH, compared with 14% of infants 751-1,000 g and 24% of infants ≤750 g. ***(Carlo, 2015)***.

The major factor in determining viability is gestational age, the outcome for infants born at the decreasing limit of viability has continually evoked anxiety in terms of the balance between poor survival with high rates of neuro-impairment and the burden of providing intensive care for many weeks or months, in 1980 this limit was 25 through 26 weeks, now it is 23 weeks, this issue is frequently described as an ethical dilemma as to whether intensive care should be instituted for them ***(Marlow, 2015).***

For a given gestational age female infant demonstrate a greater rate of survival than male infants and black neonate tend to do better than white neonate. Also neonatal mortality rates in general have declined over recent years largely because of improved neonatal intensive care ***(Ramsey and Goldenberg, 2006)***.

Pathogenesis:-

The pathogenesis of preterm labor is not well understood whether it is of normal labor process or pathologic mechanism, pathophysiological pathways leading to three main biological events: cervical ripening, formation and expression of myometrial oxytocin receptors, and myometrial gap junction formation which facilitated by Prostaglandins E_2 and F_2a. which lead to onset of preterm labor or and abortion ***(Koucký et al., 2009)***. Figure (1)

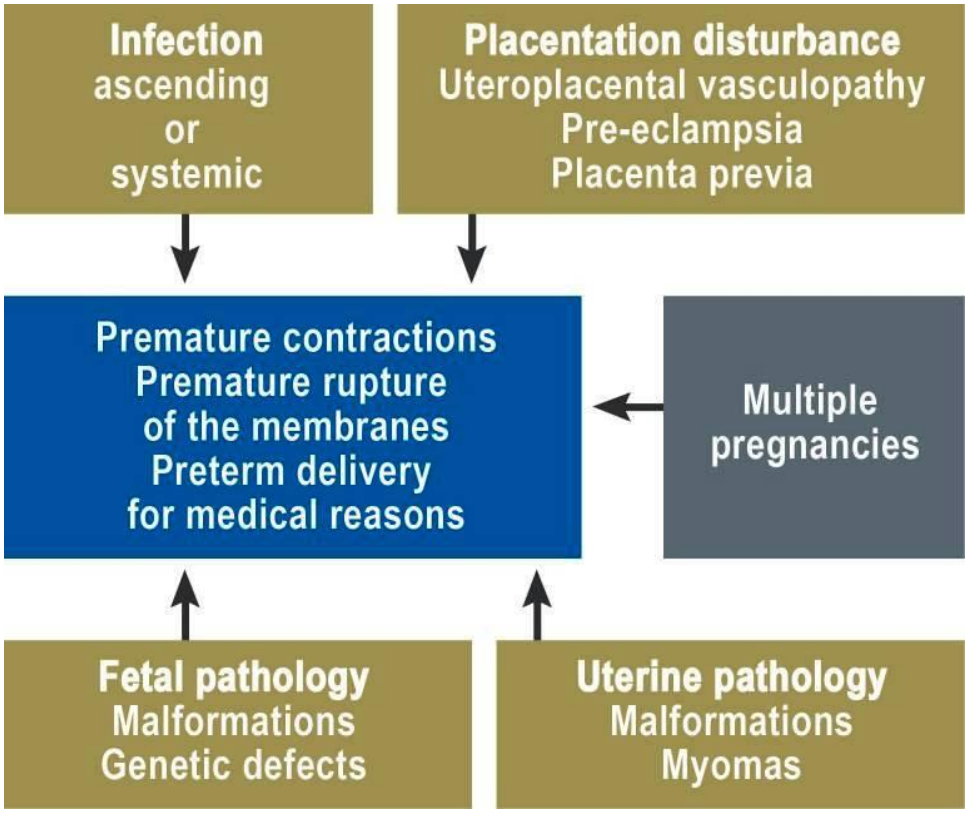

Fig. (1) The pathophysiology of premature labor (from: Schleußner E. (2013): The Prevention, Diagnosis and Treatment of Premature Labor. Dtsch Arztebl Int; 110(13): 227−236**.**

Risk factors:-

Approximately 25% of preterm births are indicated or iatrogenic due to medical or obstetric complications, the most common etiologies are preeclampsia (40%), non-reassuring fetal testing (25%), IUGR (10%), placental abruption (7%) and fetal death (7%). The remaining 75% are spontaneous in nature, approximately 40% of them due to PPROM ***(Loftin et al., 2010).***

There are several risk factors for preterm labor and premature birth, including ones that researchers have not yet identified. Some of these risk factors are "modifiable," meaning they can be changed to help reduce the risk. Other factors cannot be changed ***(Henderson et al., 2011)***

Often, the specific cause of premature birth isn't clear. Many factors may increase the risk of premature birth, including:

- Previous preterm labor or premature birth, particularly in the most recent pregnancy or in more than one previous pregnancy
- Pregnancy with twins, triplets or other multiples
- Certain problems with the uterus, cervix or placenta
- Certain infections, particularly of the genital tract
- Some chronic conditions, such as high blood pressure and diabetes
- Being underweight or overweight before pregnancy, or gaining too little or too much weight during pregnancy
- Anemia particularly during early pregnancy

- Too much amniotic fluid (polyhydramnios)
- Pregnancy complications, such as preeclampsia
- Vaginal bleeding during pregnancy
- Presence of a fetal birth defect
- Little or no prenatal care
- An interval of less than six months since the last pregnancy.
- For unknown reasons, black women are more likely to experience premature birth than are women of other races.

(*Mayo, 2014)*

Families of low socioeconomic status have higher rates of maternal under nutrition, anemia, and illness; inadequate prenatal care; drug misuse and obstetric complications which can cause reproductive inefficiency (abortions, stillbirths, premature or LBW infants) ***(Carlo, 2015)***.

Furthermore intrauterine inflammation/ infection, uterine overdistension, uteroplacental ischaemia / haemorrhage, and stress are risk factors. Mothers exposed to high levels of psychological or social stresses are at increased risk of preterm birth. Additionally, exposure to severe life events has also been linked to very and extremely pre-term births ***(Puri and Patra, 2012).***

Other associated factors, such as single-parent families, teenage pregnancies, short inter-pregnancy interval, and mothers who have born more than four previous children are also encountered more frequently in such families ***(Carlo, 2015)***.

Maternal age under 18 years or over 35 years ***(Schleußner, 2013).*** Also, Non Hispanic black women are more than 3 times as likely to deliver an extremely preterm infant (1.9%) compared with non Hispanic white and Hispanic women (0.6%). This disparity contributes to the substantial black-white gap in infant mortality ***(Smith, 2012).***

Clinical depression, possibly due to its associated increase in smoking, alcohol and drug use also plays a role in increasing preterm birth as mediated by these behaviours. Tobacco use alone increases the pre-term birth rate by almost 2 fold, due to the associated increased risk of small for gestational age and placental abruption ***(Misra and Pati, 2012).***

Having a short cervical length or the presence of fetal fibronectin - a substance that acts like a glue between the fetal sac and the lining of the uterus in the vaginal discharge has been linked to an increased risk of preterm labor **(*Mayo, 2014)***.

Bacterial products may stimulate the production of local inflammatory mediators (interleukin-6, prostaglandins), which may induce premature uterine contractions or a local inflammatory response with focal amniotic membrane rupture. Appropriate antibiotic therapy reduces the risk of fetal infection and may prolong gestation ***(Carlo, 2015)***.

Overt or asymptomatic bacterial infection (group B streptococci, Listeria monocytogenes, Ureaplasma urealyticum, Mycoplasma hominis, Chlamydia, Trichomonas vaginalis, Gardnerella vaginalis, Bacteroides spp.) of the amniotic fluid and membranes (chorioamnionitis) may initiate preterm labor ***(Carlo, 2015)***.

In contrast, viral infections. unless accompanied by a significant febrile response are considered not to be a major factor in relation to preterm birth ***(Goldenberg et al., 2008).***

Etiology:-

Preterm birth occurs for a variety of reasons. Most preterm births happen spontaneously, but some are due to early induction of labour or caesarean birth, whether for medical or non-medical reasons, common causes of preterm birth include multiple pregnancies, infections and chronic conditions, such as diabetes and high blood pressure; however, There is also a genetic influence ***(WHO, 2014).***

25% of preterm births are planned caesarean sections due to sever pre-eclampsia, kidney disease or because the baby is not developing properly, 20% of cases are due to premature rupture of the membranes, 25% of cases will be due to an emergency event, for example, placental abruption, infection, eclampsia or prolapsed cord, In 40% of cases, the cause is not known ***(Henderson et al., 2011)***. Table (1)